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

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

Policy on the Use of Animals in Neuroscience Research

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

Introduction

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

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

General Policy

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

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

Local Committee Review

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

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

Other Laws, Regulations, and Policies

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

Recommended References

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

POLICY ON THE USE OF HUMAN SUBJECTS IN NEUROSCIENCE RESEARCH

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

Recommended References

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

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

POLICY ON ETHICS

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

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

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

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

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

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

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

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

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

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

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

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433.	Long-term potentiation: physiology III	Poster			Tue AM		
434.	Long-term potentiation: physiology IV	Poster			Tue AM		
711.	Long-term potentiation: physiology V	Poster				Wed PM	
712.	Long-term potentiation: physiology VI	Poster				Wed PM	
788.	Long-term potentiation: physiology VII	Slide					Thu AM
680.	**Molecular Organization of the Postsynaptic Membrane**	SYMP				**Wed PM**	
777.	**Opioidergic Modulation of Long-term Potentiation in the Hippocampus: Insights Peptidergic Regulation of Synaptic Plasticity**	SYMP					**Thu AM**
333.	Pharmacology of synaptic transmission I	Poster		Mon PM			
615.	Pharmacology of synaptic transmission II	Poster				Wed AM	
616.	Pharmacology of synaptic transmission III	Poster				Wed AM	
240.	Postsynaptic mechanisms I	Poster		Mon AM			
241.	Postsynaptic mechanisms II	Poster		Mon AM			
242.	Postsynaptic mechanisms III	Poster		Mon AM			
243.	Postsynaptic mechanisms IV	Poster		Mon AM			
244.	Postsynaptic mechanisms V	Poster		Mon AM			
684.	Postsynaptic mechanisms VI	Slide				Wed PM	
209.	Potassium channel physiology, pharmacology and modulation I	Slide		Mon AM			
718.	Potassium channel physiology, pharmacology and modulation II	Poster				Wed PM	
719.	Potassium channel physiology, pharmacology and modulation III	Poster				Wed PM	

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

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

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

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

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

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

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

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

THEME H: OTHER SYSTEMS OF THE CNS

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

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

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

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
822.	Monoamines and behavior: norepinephrine	Poster					Thu AM
663.	Monoamines and behavior: serotonin	Poster				Wed AM	
183.	Motivation and emotion: animal models	Poster	Sun PM				
657.	Motivation and emotion: biochemistry and pharmacology	Poster				Wed AM	
378.	Motivation and emotion: primates including humans	Poster		Mon PM			
75.	Motivation and emotion: self-stimulation	Poster	Sun AM				
778.	**Neural Control of Breathing**	SYMP					**Thu AM**
20.	Neural plasticity	Slide	Sun AM				
73.	Neural plasticity: lesions and recovery	Poster	Sun AM				
181.	Neural plasticity: LTP	Poster	Sun PM				
74.	Neural plasticity: molecules and pharmacology	Poster	Sun AM				
182.	Neural plasticity: structural correlates	Poster	Sun PM				
72.	Neural plasticity: synaptic properties	Poster	Sun AM				
79.	Neuroethology: electroreception	Poster	Sun AM				
568.	Neuroethology: fish	Poster			Tue PM		
187.	Neuroethology: invertebrates	Poster	Sun PM				
186.	Neuroethology: other taxa	Poster	Sun PM				
381.	Neuroethology: songbirds I	Poster		Mon PM			
382.	Neuroethology: songbirds II	Poster		Mon PM			
304.	Neuropeptides and behavior I	Slide		Mon PM			
664.	Neuropeptides and behavior II	Poster				Wed AM	
823.	Neuropeptides and behavior III	Poster					Thu AM
309.	Psychotherapeutic drugs	Slide		Mon PM			
386.	Psychotherapeutic drugs: antidepressants	Poster		Mon PM			
191.	Psychotherapeutic drugs: antipsychotics I	Poster	Sun PM				
667.	Psychotherapeutic drugs: antipsychotics II	Poster				Wed AM	
827.	Psychotherapeutic drugs: other	Poster					Thu AM
281.	Stress: behavior	Poster		Mon AM			
207.	Stress: HPA axis	Slide		Mon AM			
764.	Stress: neurochemistry	Poster				Wed PM	
189.	Stress: neurotransmitter systems	Poster	Sun PM				
661.	Stress: preclinical and clinical studies	Poster				Wed AM	
583.	**What Is the Specific Role of the Cerebellum in Cognition?**	SYMP				**Wed AM**	
	THEME J: DISORDERS OF THE NERVOUS SYSTEM						
580.	Alzheimer's disease: ApoE	Poster			Tue PM		
400.	Alzheimer's disease: ApoE and genetics	Slide			Tue AM		
295.	Alzheimer's disease: chemical neuroanatomy	Poster		Mon AM			
773.	Alzheimer's disease: cholinergic neuropharmacology	Poster				Wed PM	
581.	Alzheimer's disease: genetics and clinical studies	Poster			Tue PM		
673.	Alzheimer's disease: mechanisms of degeneration I	Poster				Wed AM	
787.	Alzheimer's disease: mechanisms of degeneration II	Slide					Thu AM
108.	Alzheimer's disease: neurofibrillary degeneration	Slide	Sun PM				
674.	Alzheimer's disease: neuronal injury and death	Poster				Wed AM	
774.	Alzheimer's disease: neuropharmacology	Poster				Wed PM	
296.	Alzheimer's disease: tau and neurofibrillary degeneration	Poster		Mon AM			
507.	Beta-amyloid: aggregation	Slide			Tue PM		
109.	Beta-amyloid: animal models I	Slide	Sun PM				
579.	Beta-amyloid: animal models II	Poster			Tue PM		
9.	Beta-amyloid: ApoE I	Slide	Sun AM				
670.	Beta-amyloid: ApoE II	Poster				Wed AM	
671.	Beta-amyloid: cellular effects I	Poster				Wed AM	

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

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

327.3

A STUDY OF OPTIMAL CONDITIONS FOR HUMAN SCHWANN CELL HARVEST AND GROWTH IN VITRO. G.T.B. Casella, N. Kleitman*, R.P. Bunge and P.M. Wood. The Miami Project to Cure Paralysis., University of Miami School of Medicine, Miami, FL 33136.

The construction of cellular prostheses containing Schwann cells (SC) has been proposed as a future therapeutic approach in the repair of injury to neural tissue. To establish optimal conditions for SC harvest and growth, human SC were isolated from explanted adult (20-26 years old) peripheral nerve fragments after various time *in vitro*. The growth of SC in the presence of double (forskolin and heregulin) or triple (forskolin, cholera toxin and heregulin) mitogens in serum containing medium was compared at different concentrations of heregulin, and on different substrates (plastic, polylysine, collagen, fibronectin and laminin). Maximal cell yield was obtained by dissociating explants after a 2 week period *in vitro* ($12.05 \pm 2.43 \times 10^4$ cells/mg fresh weight of nerve, 2 donors).Maximal growth was observed on collagen substrata in double mitogen (2 μM forskolin and 10.0 nM heregulin). These conditions maintained the purity of the SC at 95.3 $\pm$3.2%. These results differ from previously reported results which described maximum cell proliferation with the triple mitogen mixture and indicate that cholera toxin is not required for maximal SC proliferation. Initially labelling indices following a 24 hour exposure to tritiated thymidine were 44.5 $\pm$ 2.1%. This high rate of SC proliferation was not maintained and SC did not increase in number after 6-8 doublings. A total increase of 50-70 fold was observed. Thus a substantial expansion of adult derived human SC can be obtained for use in promoting repair of neural tissues. (This research is supported by NIH grants NS09923 and NS28059, the Hollfelder Foundation, and The Miami Project to Cure Paralysis. Heregulin was generously provided by Genentech, and the human nerves by the University of Miami transplant team.)

327.4

POSTTRANSCRIPTIONAL REGULATION OF IGFBP-5 BY IGF-I IN CULTURED SCHWANN CELLS. **H-L. Cheng* and E.L. Feldman, Department of Neurology, University of Michigan, Ann Arbor, MI 48109.**

The insulin-like growth factors (IGFs) are trophic factors whose growth-promoting actions are mediated via the IGF-I receptor and modulated, in part, by six structurally related proteins, the IGF binding proteins (IGFBPs).

In the current study, we determined the profile of IGFBP gene and protein expression in transfected MT4H1 rat Schwann cells (SC). These cells resemble untransfected secondary SC in morphology, and the expression of P0 and glial fibrillary acidic protein. We speculated that SC secret IGFBPs which modulate IGF actions for SC growth. In the current study, IGF-I promoted SC growth and DNA synthesis in a dose-dependent manner. IGF-I treatment of SC increased the intensity of IGFBP-5 expression in SC conditioned media measured by Western immunoblotting. Des(1,3)IGF-I, an IGF-I analog with low affinity for IGFBP-5 but normal affinity for the IGF-I receptor, had no such effect, suggesting direct extracellular interaction between IGF-I and IGFBP-5 is essential for IGF-I regulation of IGFBP-5. Additionally, IGF-I treatment had no effect on IGFBP-5 gene expression as measured by Northern blotting and quantitative autoradiography. These observations suggested that IGF-I could protect IGFBP-5 from protease degradation. To test this concept, recombinant human IGFBP-5 was incubated in SC conditioned media at 37°C in the presence or absence of IGF-I. In the presence of IGF-I, IGFBP-5 remained intact at 34 kDa; however, in the absence of IGF-I, IGFBP-5 was degraded into 23-kDa fragments. Collectively, these data support a role for IGF-I in SC growth and imply that IGF-I treatment protects secreted IGFBP-5 from proteolytic degradation. This protection, in turn, could allow secreted IGFBP-5 to target IGF-I to SC and enhance IGF-I's biological effects.

This work was supported by R29 NS32843.

327.5

INTRACRANIAL GRAFTS OF GLIOMA CELL LINES EXPRESS THE CNS SPECIFIC HA-BINDING PROTEIN, BEHAB. D.M. Jaworski*, G.M. Kelly, J.M. Piepmeier#, and S. Hockfield. Section of Neurobiology and #Department of Neurosurgery, Yale Univ. School of Medicine, New Haven CT 06510.

Glial cells contribute to the microenvironment of the CNS. They express a number of extracellular matrix molecules, including hyaluronan (HA) and the HA receptor CD44. HA can organize water and create hydrated spaces, and thereby foster cell migration. HA levels are highest in the embryo during the period of rapid cell proliferation and migration. Postnatally, HA levels decline. Tumor invasion is accompanied by the synthesis and deposition of a pathologic matrix that contains high levels of HA and resembles the embryonic matrix. The increase in HA in tumorogenesis has led to the suggestion that HA may play a role in tumor invasion.

Previously, we reported that the expression of the CNS specific HA-binding protein, BEHAB, is dramatically increased in glioma compared to normal brain (Soc. Neurosci. 20:475). However, tumor-associated BEHAB expression is not maintained in vitro. No BEHAB was detected in seventeen glioma cell lines maintained under standard culture conditions. To determine whether BEHAB expression requires intercellular signals present in the intact brain, but missing in culture, the rat glioma cell lines C6 and 9L were grown as subcutaneous or intracranial grafts. Neither the C6 nor the 9L cell line expresses BEHAB when grown in culture or when grafted subcutaneously. When grafted into the brain parenchyma, the 9L cells do not express BEHAB; however, the intracranial grafts of C6 cells do express BEHAB. This difference in BEHAB expression correlates with differences in tumor growth characteristics: 9L tumors grow as circumscribed masses, while C6 tumors display invasive potential. These results show that the cellular environment can influence the molecular phenotype of a brain tumor cell line. They further suggest that BEHAB may be an important element in tumor invasion. Supported by EY06511(SH) and EY06451(DJ).

327.6

SEROTONIN RECEPTORS ON SCHWANN CELLS IN RAT SCIATIC NERVE. **E. Yoder*¥, H. Tamir±, and M. H. Ellisman¥. ¥San Diego Microscopy and Imaging Resource, Department of Neurosciences, UCSD School of Medicine, La Jolla CA 92093 and ±Division of Neuroscience, New York State Psychiatric Institute, New York NY 10032.**

We previously reported that Schwann cells cultured from rat sciatic nerve express 5-HT_{2A} receptors (Yoder *et.al., NS Abstract* 299.5, 1994; Yoder *et.al., NS Abstract* 460.18, 1993). In order to determine if these observations *in vitro* reflect the characteristics of Schwann cells *in situ*, we examined healthy and injured sciatic nerves using immunohistochemistry with an anti-idiotypic antibody that recognizes 5-HT receptors. Nerves were also labeled with α-S100β, a Schwann cell marker. Nerves from the operated and contralateral legs of adult rats were removed at 1, 2, 3, and 5 weeks following nerve crush (regenerating nerves) and 2, 3, and 5 weeks following nerve transection (degenerating nerves). Nerves from nonsurgical animals were also examined.

In a preliminary study, S100β labeling was observed in Schwann cells on all nerves examined. 5-HT receptor labeling was also observed on Schwann cells in injured nerves, both degenerating and regenerating. Additionally, weaker 5-HT receptor staining was often seen on Schwann cells in sciatic nerves from unoperated contralateral control legs. However, 5-HT receptor labeling was not observed on Schwann cells in sciatic nerves from nonsurgical animals. Thus, the 5-HT receptors expressed by cultured Schwann cells seem to correlate with Schwann cells *in vivo*, especially those responding to nerve injury. These results suggest that Schwann cells may be a target of 5-HT released from endoneural mast cells responding to nerve injury. The function of 5-HT receptors on Schwann cells remains to be determined.

327.7

TARGETED EXPRESSION OF INTERFERON-γ TO THE CNS OF TRANSGENIC MICE

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Much circumstantial evidence has implicated the immune cytokine interferon-gamma (IFN-γ) as a key mediator in the pathological changes that are observed in many demyelinating disorders, including the most common human demyelinating disease, multiple sclerosis (MS). Previous in vitro experiments in our laboratory have shown that application of IFN-γ to the MOCH-1 oligodendrocyte cell line results in dramatic molecular and morphological changes that may reflect the in vivo response of oligodendrocytes to perturbations that occur in demyelinating disorders. To produce an animal model with which to study the effects of IFN-γ on the central nervous system, we have generated transgenic mice in which the expression of IFN-γ has been placed under the transcriptional control of the myelin basic protein (MBP) gene. Transgenic mice generated with this construct have a shaking/shivering phenotype that is similar to that observed in known animal models of hypomyelination (e.g. *shiverer, jimpy, quaking*). Moreover, histological and immunohistochemical analyses have revealed that the CNS of these animals have dramatically less myelin than that which is present in non-transgenic control animals. Reactive gliosis as well as a large induction of MHC class I and class II mRNA in the CNS of MBP/IFN-γ transgenic mice was also observed. Additionally, development of the internal granule cell layer of the cerebellum was disrupted. These results strongly support the hypothesis that IFN-γ is a key mediator in immune-mediated demyelinating disorders and suggest that the MBP/IFN-γ transgenic mice will serve as a good model with which to further explore the effects of this cytokine in CNS disorders.

327.8

THE PERIPHERAL NERVE AS A TARGET FOR GENE THERAPY

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Injury to the peripheral nerve elicits a complex interaction of neurotrophic factors, leading to nerve regeneration. If neurotrophic genes responsible for neuronal survival and neurite growth can be transferred to Schwann cells, it may be possible to enhance peripheral nerve regeneration. Adenoviruses can efficiently transfer genes to dividing and non-dividing cells which makes this vector useful to transfer DNA to Schwann cells. In this study we test the ability of adenovirus to infect Schwann cells in normal and injured sciatic nerve using a replicative-defective adenovirus encoding the lacZ gene. Adenovirus (10^8-2×10^9 pfu's) was injected into sciatic nerve of female Sprague-Dawley female rats (N=40). Sciatic nerve was harvested 1-30 days after injection, cyro-sectioned and stained for beta-galactosidase (b-gal) by histochemical staining and immunofluorescence. Adenovirus (1×10^8 pfu's) is able to infect Schwann cells, as shown by double-labelled immunofluorescence using polyclonal antibody to b-gal and monoclonal antibody to S-100. In uncrushed nerve, Schwann cell staining was observed between 2 and 14 days after injection, with the highest levels seen after 7 days. The number of infected cells was increased after nerve injury. Adenovirus (1×10^8 pfu's) was injected 3 days after a sciatic nerve crush. The time of expression increased to 3 weeks, and the number of infected cells increased at each time point, as compared to infected normal nerve. Viral toxicity was evaluated by immunohistochemical analysis for cytotoxic T-cells (CTL), quantifing the number of myelinated axons, and muscle weights. At 1×10^8 pfu's no viral toxicity was observed. At 2×10^9 pfu's, infiltrating CTL's were observed after 7 days. The size and number of axons and muscle weight was also reduced. These results show that adenovirus transfer genes to Schwann cells of normal and injured nerve. Using this technique, it may be possible to enhance peripheral nerve regeneration by local overexpression of neurotrophic genes.

327.9

Transgenic mice expressing HSV-thymidine kinase under the control of the mouse GFAP promoter: for the targeted ablation of astrocytes.

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To investigate the role of astrocytes in a variety of processes it would be useful to have a means of their selective ablation. Towards this end, three lines of transgenic mice have been generated that have the herpes simplex virus thymidine kinase (HSV-TK) under the control of the mouse glial fibrilliary acidic protein (GFAP) promoter. HSV-TK is necessary to metabolise the benign substrate ganciclovir (GCV) to a toxic product inducing autonomous cell death.

Preliminary evidence suggests that in response to manipulations that upregulate GFAP expression in vivo, at least one transgenic line shows upregulation of HSV-TK immunoreactivity. The efficacy of astrocyte ablation after addition of GCV is under investigation both in vitro and in vivo.

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327.10

IMMUNOHISTOCHEMICAL STUDIES OF THE GLIAL RESPONSE TO PYRITHIAMINE-INDUCED THIAMINE DEFICIENCY IN THE RAT USING GFAP AND ED1 ANTIBODIES. D.K.Leong*, L.Oliva, R.F.Butterworth. Neuroscience Research Unit, Hopital St-Luc, University of Montreal, Montreal, Quebec, Canada, H2X 3J4.

Thiamine deficiency results in the Wernicke-Korsakoff Syndrome (WKS), which is characterized by symmetrical lesions in the diencephalon and brainstem. Experimental WKS can be produced in rats by the combined administration of pyrithiamine and a thiamine deficient diet. In a recent study, using quantitative receptor autoradiography (QRA), significant increases in densities of ^{3}H-PK11195 (a ligand for the "peripheral-type" benzodiazepine receptor; PTBR) binding sites were found in selective brain regions, including the thalamus, of thiamine-deficient rats compared to control rats (Leong et al., *J. Cereb. Blood Flow & Metab.* 14; 100-15, 1994). In the present study immunohistochemistry with antibodies to astrocytes (anti-glial fibrillary protein; GFAP) and macrophages (anti-ED1), was used to examine the cellular localization of PTBRs in the brains of rats at different stages of thiamine deficiency. Histological studies showed evidence of significant neuronal loss and reactive gliosis within the medial thalamus. QRA and immunohistochemical studies of this region revealed significant increases in densities of PTBR binding sites, and markedly increased numbers of GFAP-positive cells. A fewer number of ED1-positive cells were found within the medial thalamus. Thus, increased densities of PTBRs correlated principally with the distribution of reactive astrocytes in the medial thalamus of thiamine deficient animals. Positron Emission Tomography (PET) using ^{11}C-PK11195 offers a potentially useful tool in WKS in humans. (Funded by MRC Canada).

327.11

CHANGES IN GAP JUNCTION EXPRESSION AFTER PERIPHERAL NERVE INJURY. K.J. Chandross*[1], R. Dermietzel[2], E. Simburger[2], D.C. Spray[1], J.A. Kessler[1]. [1]Dept. Neuroscience, Albert Einstein Coll. Med., Bronx, NY and [2]Dept. Anatomy, Univ. Regensberg, Regensberg, Germany.

Following peripheral nerve injury the nonneuronal cellular components of the nerve undergo programs of phenotypic and functional changes in the distal region. Schwann cells dedifferentiate and divide within their original endoneurial tubes surrounding the degenerating axons. Macrophages and fibroblasts respond to injury by altering their phenotype and proliferating. We used molecular and immunocytochemical techniques to examine changes in connexin (Cx) expression that occur after peripheral nerve injury. In normal rat nerve, Cx32 was localized to Schwann cell nodes of Ranvier and Schmidt-Lantermann incisures (previously reported by Bergoffen et al., Science, 262:2039, 1993), and Cx43 was localized to perineurial cells. After crush injury, Cx32 mRNA and protein rapidly decreased within 24 hr, at and distal to the injury site, and returned to control levels after 20 d. In contrast, Cx43 expression dramatically increased in endoneurial fibroblasts in the crush and distal region by 3 d, coincident with macrophage infiltration, and decreased to control levels by 12 d. Schwann cells distal to the crush site are coupled as evidenced by intercellular exchange of Lucifer Yellow and calcium. Since the distal Schwann cells do not express either Cx32 or Cx43, this suggests that they express a different Cx after nerve injury. Molecular and immunocytochemical analyses of the injured region suggest that Schwann cells express a Group II Cx, possibly Cx46. Thus, injury responses in peripheral nerve include spatiotemporal patterns of Cx expression in nonneuronal cells that may coordinate degeneration and facilitate recovery of function.

327.12

Cx43/Cx32 GAP JUNCTION CHIMERAS PERMIT CALCIUM SIGNALING. M. L. Cotrina[1], C. C. G. Naus[2], S. L. Bond[2], S. A. Goldman[3] and M. Nedergaard[1]*. Depts. of [1]Cell Biology, New York Med. Col., Valhalla, NY, 10595, [2]Anatomy, Univ. of Western Ontario, London, ON, Canada N6A 5C1 and [3]Neurology, Cornell Univ. Med. Col., NY, 10021.

Cultured astrocytes can generate long-range calcium waves that travel from cell to cell by means of gap junctions. These calcium waves can modulate neuronal calcium levels, suggesting that astrocytes might play an active role in establishing neuronal firing patterns. However, it is not clear if gap junctions mediate calcium signaling between neurons and astrocytes, because different gap junction proteins are expressed in the two cell types. Neurons express connexin-32 (Cx32), while astrocytes express Cx43. We therefore asked whether chimeric gap junctions composed of Cx43 and Cx32 are competent to carry the intercellular calcium wave. To this end, we used C6 glioma cells, a cell line that expresses low levels of Cx43 and cannot propagate intercellular calcium waves (Charles, JCB 118: 195, 1992). C6 cells transfected with Cx43 cDNA were labeled with the membrane dye, DiIC18, then mixed with other C6 transfected with Cx32 cDNA, in a 1:250 or 250:1 ratio. Two days later, mechanically induced calcium waves were followed with the calcium indicator fluo-3. We found that signaling was transmitted bidirectionally between Cx43 and Cx32 transfectants. Although some signaling to non-transfected and nonsense-transfected controls was observed, the rate of communication was 20% higher in the direction from Cx43 to Cx32 than opposite. Our results suggest that 1) Cx32 and Cx43 are capable of forming functional pairs, 2) this coupling allows transmission of calcium signals, and 3) although coupling is bidirectional, transmission of a calcium signal seems facilitated in the direction from Cx43 to Cx32. Our data suggest that the unidirectionality of calcium signaling between astrocytes and neurons in mammalian forebrain cultures might reflect the transmission characteristics of the chimeric Cx32/Cx43 gap junction.

327.13

WIDE-SPACED MYELIN FORMATION IN SITU IN THE PRESENCE OF IGM-PRODUCING HYBRIDOMAS. J. Rosenbluth*, W.L. Liang, Z. Liu, D. Guo and R. Schiff. Dep't. of Physiology & Neuroscience and Institute of Rehabilitation Medicine, N.Y.U. School of Medicine, New York, NY 10016.

Implantation of the O1 hybridoma, which produces an IgM antigalactocerebroside, into rat spinal cord before or during myelin formation, results in some amyelination but also in the development of abnormal myelin in which most lamellae are separated by wide gaps, resulting in spacings of ~10nm (0 gap), ~19nm (~9nm gap) and ~32nm (~22nm gap). The gaps contain dense structures compatible with IgM molecules in single or multiple rows. The number of lamellae is often smaller than normal in wide-spaced sheaths, and the radial component is absent, as are tight junctions between adjacent paranodal loops, thus opening shunt pathways for nodal action currents that could compromise conduction. The paranodal axoglial junction does form, however. We conclude that abnormal CNS myelin is able to develop in the presence of the O1 antibody, even without direct contact between lamellae. Intercalated, multivalent IgM molecules may serve to anchor lamellae together, substituting for myelin constituents too widely separated to interact. Wide-spaced myelin also occurs after implantation of 04 or A2B5 hybridomas at comparable ages and has been reported in CNS myelin exposed to MS or EAE sera. Supp. by NIH and NMSS.

327.14

MODULATION OF ASTROGLIOSIS BY BASIC FIBROBLAST GROWTH FACTOR, HEPARAN SULFATE AND ß-ADRENERGIC BLOCKADE. J. F. Reilly*, L. Bair, and V. K. Menon Department of Cell Biology & Human Anatomy, School of Medicine, University of California, Davis, CA 95616.

Traumatic injury to the brain results in astrocyte hypertrophy and the formation of a dense scar at the site of the wound. This scar may present physical and chemical barriers to axonal regrowth and functional recovery. In the present study, we describe experimental modulations that increase and decrease astrocyte hypertrophy following traumatic injury to the adult rat cerebral cortex and hippocampus. Intralesion injection of 20 ng or 200 ng basic fibroblast growth factor (bFGF) significantly increased the area of GFAP-stained profiles in the vicinity of the wound, as demonstrated by quantitative image analysis. Co-administration of 48 µg heparan sulfate (HS) with 200 ng bFGF elicited a broader zone of astrocyte hypertrophy, compared to bFGF alone. This suggests that HS permits more extensive diffusion of bFGF from the site of injection. Systemic administration of DL-propranolol, a non-selective ß-adrenergic antagonist, resulted in a significant decrease in the area of GFAP-stained profiles. Administration of a ß-adrenergic agonist has been shown to increase bFGF mRNA expression in the non-injured rat brain, and it is possible that propranolol acts to reduce astrogliosis by reducing the synthesis and level of bFGF at the site of injury. These studies demonstrate that the astrocyte response to traumatic injury can be both enhanced and reduced, and provide useful models for future investigations. Supported by NIH AG 06159.

327.15

TUMOR NECROSIS FACTOR INFUSION IN ADULT RAT BRAIN CAUSES INFLAMMATION AND DEMYELINATION J.P.O. Hebb*, D. Sadi, T. Hagg
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TNF (tumor necrosis factor) is a cytokine thought to play a key role in demyelinating diseases such as multiple sclerosis. Here we have infused PBS or PBS containing recombinant human TNFalpha (1 or 3 µg/day; Preprotech) for 14 days into the cortex just above the corpus callosum of adult female Sprague-Dawley rats using Alzet pumps. With control PBS, essentially no tissue damage beyond the infusion-cannula tract was observed and infiltration of leukocytes was minimal and limited to the wall of the tract. In contrast, with 3 µg/d TNF, an area of tissue necrosis had developed with an abundant infiltration of leukocytes into a large area (2-3 mm diameter) of the cortex, corpus callosum and underlying striatum. In the same area, immunoreactivity for the MBP and MOSP markers for oligodendrocytes was absent, indicative of demyelination. Antero- and retrogradely labeled (choleratoxin B; List) axons of the corpus callosum were present in the area of demyelination but appeared to have undergone degenerative changes. CNTF (ciliary neurotrophic factor) is known to prevent TNF-induced oligodendrocyte death in vitro (Louis et al., Science 259:689-92). Our preliminary results suggest that recombinant human CNTF (3 µg/d; a gift from SCIOS Nova, Inc.) administered at the same time as TNF into the adult rat cortex has a modest protective effect against TNF-induced demyelination. CNTF itself caused the same limited extent of tissue damage and inflammation as control PBS infusion.

Thus, TNF appears to be useful for establishing in vivo adult CNS models for demyelination which can be used to test agents for oligotrophic and remyelination activities.

Supported by a grant to TH from National Multiple Sclerosis Society, USA.

327.16

A NOVEL ACTION FOR ADENOSINE IN THE CENTRAL NERVOUS SYSTEM SUGGESTED BY CELL DEATH IN RAT STRIATUM CULTURES M.P. Abbracchio[1,*], S. Ceruti[1], G. Burnstock[2], D. Barbieri[3], C. Franceschi[3], W. Malorni[4], H. O. Kim[5], D.K.J.E. von Lubitz[5], F. Cattabeni[1] and K.A. Jacobson[5]. [1]Inst. Pharmacol. Sci, Univ. Milan, Italy; [2]Dept. Anat. Dev. Biol., Uni. Coll. London, UK; [3]Inst Gen Pathol., Univ. Modena, Italy; [4]Ist. Sup. Sanita', Rome, Italy; [5]Mol. Recogn. Sect., Lab. Bioorg. Chem., NIDDK/NIH, Bethesda, MA, USA

We have previously shown that exposure of rat brain primary cultures to the adenosine analogue 2-chloro-adenosine (2-CA, 10^{-6}-10^{-4} M) results in a reduction of astrocytic cell number that was not antagonized by xanthine antagonists at A1 and A2 adenosine receptors. In the present study, we have characterized 2-CA-induced cell death and tested the possible involvement of the recently cloned xanthine-insensitive adenosine A3 receptor by using the two selective A3 agonists, N^6-(3-iodobenzyl)adenosine-5'-N-methyluronamide (IB-MECA) and its 2-chloro-derivate (Cl-IB-MECA). All compounds induced a concentration-dependent decrease of astrocytic cell number. However, agonists at the A3 receptor were effective at a much lower concentration range (0.1-10 nM) with respect to 2-CA. Maximal reduction of cell number was approx. 60-70% with 2-CA and 30-40% with the A3 agonists; these effects were also detected if agents were added 24 hours after cell plating, therefore ruling out any influence on cell adhesion. Both flow-cytometric analysis of propidium iodide-stained nuclei and light and electron microscopy demonstrated that 2-CA induced apoptosis. In contrast, no apoptosis was detected with the A3 agonists, but electron microscopy analysis suggested induction of cytoskeletal damage. These results suggest that adenosine may act as a negative regulator of astrocytic cell number in the brain; data with different adenosine analogues suggest that multiple mechanisms might be involved including apoptosis and necrosis.

327.17

EFFECTS OF HIGH-DOSE METHYLPREDNISOLONE AND 21-AMINOSTEROID ON REMOTE GLIAL ACTIVATION IN EXPERIMENTAL ACUTE SPINAL CORD INJURY. M.Mizuno, S.Shimosaka, T.Tanaka*, S.Waga, T.Kojima and Y.Kubo.
Department of Neurosurgery, Mie University School of Medicine, Tsu, Mie, 514, Japan

It is well known that high-dose methylprednisolone sodium succinate (MPSS) and 21-aminosteroid U-74006F are effective in the treatment of experimental acute spinal cord injury, but effect for the site away from the impact site is not known.

We studied the efficacy of high-dose MPSS and U-74006F on nucleus gracilis in rats with Th8 spinal cord injury. MPSS was administered intravenously in dose of 30mg/kg during a 15-minute period, followed by 45-minute pause and then a 23-hour maintenance infusion of 5.4mg/kg. U-74006F was administered intravenously in dose of 1.5mg/kg and then a 9-hour maintenance infusion of 0.5mg/kg. Control rats were treated with saline. Three days postlesion, the rats were sacrificed and the brain section was stained immunocytochemically with glial fibrillary acidic protein (GFAP) and OX-42. GFAP and OX-42 immunoreactivity of the nucleus gracilis in MPSS group (n=10) and U-74006F group (n=10) were higher than that in the control group (n=10). Astrocytes and microglia have been known as the source of neurotrophic factors and play significant roles in neuroprotective and regenerative responses, so the high density of astrocytes and microglia reaction in the nucleus gracilis in the MPSS and U74006F group may suggest neuroprotective and regenerative effect on the remote site. MPSS and U74006F may be effective on the distal site far away from the impact site.

327.18

EXPRESSION OF POLYPEPTIDE VARIANTS OF RECEPTOR-TYPE PROTEIN TYROSINE PHOSPHATASE (RPTP)β: THE SECRETED FORM, PHOSPHACAN, INCREASES DRAMATICALLY DURING EMBRYONIC DEVELOPMENT AND MODULATES GLIAL CELL BEHAVIOR IN VITRO. T. Sakurai, D. R. Friedlander and M. Grumet* Dept. of Pharmacology, New York University Medical Center, NY, NY 10016.

Glial cells express three splicing variants of a receptor-type protein tyrosine phosphatase called RPTPβ. Two are receptor forms that differ in a large extracellular domain that is absent in the deleted variant form dvRPTPβ and the third is a secreted proteoglycan called phosphacan that lacks the cytoplasmic phosphatase domains. We have now identified by immunoblotting proteins corresponding to these three forms of RPTPβ in rat C6 glioma cells and brain. Although dvRPTPβ is more prevalent than the full length receptor in C6 glioma cells, there is much less difference between them in rat brain. The secreted form, phosphacan, is much more abundant than either of the receptor forms in rat brain and increases progressively during development while the receptor forms show only moderate changes. In contrast to the long form of RPTPβ and phosphacan that contain a large alternatively spliced domain and were detected as proteoglycans, the dvRPTPβ form did not appear to be a proteoglycan. We recently showed that phosphacan binds to the neuron-glia cell adhesion molecule, Ng-CAM, and we now report that glia expressing RPTPβ adhered and extended processes on substrates coated with Ng-CAM. After one day in culture, however, the glia retracted their processes and often lifted off the substrate. Glial cells secrete large amounts of phosphacan which was found to inhibit adhesion of glia to Ng-CAM. Conditioned medium from glial cells inhibited glial adhesion, and depletion of phosphacan from the conditioned medium by immunoadsorption reduced the inhibitory activity. The results show that secreted forms of RPTPβ (phosphacan) increase dramatically during development and indicate that they can modulate glial cell adhesion and behavior.

327.19

STEROIDS MODULATE LEPTOMENINGEAL CELL INFILTRATION INTO CEREBRAL CORTICAL STAB WOUNDS. M.S. Li* and S. David.
Centre for Res. Neurosci., Montreal Gen. Hosp. Res. Inst. and McGill University, Montreal, Quebec, Canada, H3G 1A4

We have previously shown that topical applications of glucocorticoids can attenuate the lesion interface after cortical stab wounds in adult rats. In this study we have examined whether this is due to changes in the number of leptomeningeal cells that migrate into the wound. The surface of cortical stab wounds in adult rats were treated with topical applications of either 0.1% Halog or 0.05% Diprolene, and 3 weeks later cryostat sections through the lesion site were labeled with anti-laminin, anti-GFAP and Nuclear yellow. Because of the inavailablity of leptomeninges-specific antibodies, leptomeningeal cells were quantified by estimating the number of elongated nuclei within laminin-positive and laminin-negative areas of the wound. Laminin-negative areas of the wound contained fewer elongated nuclei (3/500µm) as compared to laminin-positive areas (13/500µm). These results were further confirmed by EM, which showed limited migration of leptomenigeal cells into the wound after steriod treatments. In vitro studies indicate that treatment of dissociated cultures of adult leptomeninges with these steriods at 10^{-5} M reduces proliferation assesed by ^{3}H-thymidine uptake. The attachment and spreading of these cells was also found to be better on laminin and fibronectin substrates. These results suggest that local steroid treatments may attenuate the lesion interface after penetrating wounds to the CNS by reducing the proliferation and possibly also the migration of leptomeningeal cells into the wound.

327.20

OLFACTORY GLIA: ARE SOME MORE EQUAL THAN OTHERS? AN EXAMINATION OF GLIAL SUBPOPULATIONS ALONG THE OLFACTORY PATHWAY. A.C. Puche*, K. Tisay and B. Key. Laboratory of Molecular Neurodevelopment, Dept. Anatomy and Cell Biology, Melbourne University, Parkville, Australia 3052.

The mosaic topographical projection of primary neurons in the olfactory system is a key determinant in the processing of olfactory information. The topographical projection formed during development is maintained in adult, despite continual neuronal turnover throughout life in the olfactory neuroepithelium. Growth cones and their axons continually grow along the olfactory nerve and form functionally correct synaptic connections within the bulb. Our laboratory has identified subpopulations of primary sensory olfactory neurons, distinguished by expression of specific cell surface carbohydrates, that self-fasciculate and sort into axons bundles only upon entering the olfactory bulb. We have hypothesised that local environmental cues in the olfactory bulb, generated by unique subpopulations of glia, may be involved in axon growth and/or sorting in the olfactory bulb. We analysed the expression of numerous antigens within the olfactory pathway by immunohistochemistry in order to identify specific markers of glial subpopulations. Three distinct subpopulations of glia were defined with antibodies against the low affinity nerve growth factor receptor (p75) and the cell surface carbohydrate Lewis^x (Le^x) , a ligand for the selectin receptors, and with a plant lectin from *Burhinia purpurea* (BPA), which exhibits specificity for N-acetyl-galactosamine. Peripheral Schwann cells ensheathing the olfactory nerve express only p75, both *in vivo* and *in vitro*. *In vitro* these glia support extensive olfactory neurite outgrowth. Ensheathing cells within the olfactory bulb, however, appear instead to only express Le^x. Astrocytes in the glomerular layer and deeper regions of the bulb selectively express N-acetyl-galactosamine. These results demonstrate that the olfactory system contains discrete subpopulations of glia which are spatially segregated to distinct regions of the pathway. These glial populations may be responsible for modulating axon fasciculation within the primary olfactory projection.

328.1

SYNCHRONIZED VISUAL ACTIVITY IN BOTH EYES DISRUPTS BINOCULAR MAP DEVELOPMENT IN *XENOPUS* OPTIC TECTUM. S. Grant, S.G. Brickley, E.A. Dawes and M.J. Keating (SPON: Brain Research Association). Dept. of Anatomy, Charing Cross & Westminster Medical School, London W6, and Div. of Neurophysiology, NIMR, London NW7, UK.

Activity-dependent rearrangements of axons and synapses are common processes underlying the refinement and alignment of topographic maps in the developing visual system. Temporal correlations in the activity of appropriately converging inputs - e.g., from neighbouring ganglion cells (GCs) in the retina - are believed to be essential for the success of these processes, by providing sufficient drive to activate voltage-gated, post-synaptic NMDA receptors. We have examined the effects of inducing temporal correlations in the activity of GCs across both retinae during development and after larval eye rotation, on the order and alignment of binocular maps in the optic tectum of the frog, *Xenopus laevis*.

Animals were reared in an environment of continuous 1Hz stroboscopic illumination. In control experiments, exposure to this environment was shown to produce synchronization in the firing of all 3 classes of retinal GC axon in the tectum, so that similar temporal correlations in afferent activity were present in all regions of both tectal lobes. Developmental refinement of the contralateral (retinotectal) map was unaffected by strobe-rearing, but ipsilateral (crossed isthmotectal) maps showed significant disorder and misalignment with the direct visual map from the retina, especially when its orientation had been rotated. These findings support conclusions from previous dark-rearing studies that precise rearrangements of retinotectal axons and synapses in developing *Xenopus* depend little on visually-evoked activity, and suggest that crossed isthmotectal axons are unable to find appropriate sites for synaptic convergence when all available targets are visually-activated in near-synchrony.

328.2

ON AND OFF EVOKED FIELD POTENTIALS IN THE SUPERIOR COLLICULUS OF NEONATAL RATS. S. Fortin, C. Allal, Y. Xu, S. Molotchnikoff, and S. Itaya*, Dept. Biology, Univ. de Montreal, Que., Canada, and Dept. Biomed. Sci., Univ. South Alabama, Mobile, AL 36688

In anesthetized young rats we have recorded on and off field potentials which were evoked by light on and off steps, respectively. These responses were analyzed in the dorso-ventral axis of the superior colliculus in order to investigate extracellular current sources. Furthermore, power spectrum analyses were performed on these potentials. Data were collected on animals from postnatal day 11 (first light evoked responses appear at day 12) to postnatal day 18. Adult rats were investigated as controls. The eyelids open at postnatal day 14. Results indicate that: (1) on and off responses appear at the same age; (2) their latencies are long and variable, but more importantly only slow waves are present; (3) on and off potentials are not distributed in the same collicular layers; (4) as the rat grows older, off field potentials exhibit more complex patterns, i.e., they contain oscillatory wavelets which are never seen in on-responses. Power spectral analysis reflects these characteristics. From day 12 to 18 the frequency increases from approximately 6 up to 30 Hz. We are also studying GABA immunohistochemical staining in the developing superior colliculus. GABA positive cells are present at birth, but their density and size appear to change with age. We are investigating the potential contribution of GABA circuitry development to the maturation of responses to light.

328.3

DL-2-AMINO-5-PHOSPHONOVALERIC ACID (APV) GREATLY SUPPRESSES SPONTANEOUS ACTIVITY IN GOLDFISH TECTAL NEURONS. Brad J. Kolls* and R. L. Meyer. Dept. Dev. & Cell Biol. University of California, Irvine, CA 92717

The NMDA antagonist, APV, inhibits activity-dependent synaptic refinement during the formation of connections in the retinotectal projection of goldfish. This has been interpreted to mean that NMDA receptors act as detectors of correlated activity at the primary synapse. However, other synaptic loci and roles for NMDA receptors remain possible. Here, we examine the possibility that NMDA receptors might regulate spontaneous activity, and thereby excitability, in the tectal cells of goldfish. Spontaneous multiunit activity as well as evoked optic field potentials were recorded in the isolated tectum of normal, regenerating, and enucleated adult goldfish. The tecta were removed, together with the optic nerves, and placed in a perfusion chamber into which drugs were infused during recording. Multiunit activity was recorded with extracellular metal electrodes inserted into the SFGS. Field potentials were elicited by optic nerve shock and recorded with pipettes in the SFGS. Exposure to 2.5-25μm APV resulted in a rapid and dramatic decrease in the background multiunit activity which was dose dependent and reversible. At 25μm, activity was reduced by >90%. Comparable effects were found in tecta from normal, enucleated and regenerating fish. In contrast, 25μM APV had relatively little effect on the field potential in both normal and regenerating tecta. No effects were seen on the height and latency to peak of the early component of the evoked potential. However, the width of the peak at half maximum was reduced on average by 32%. These data suggest that NMDA receptors may play an important role in regulating spontaneous background activity in tectal target cells which in turn might determine their overall level of excitability. The data do not strongly support a critical role for these receptors at the primary optic synapse. We suggest APV might prevent refinement by reducing the excitability of tectal neurons and thereby preventing regenerating optic axons from driving their postsynaptic targets. (Supported by EY6746)

328.4

RETINAL NMDA-TOXICITY ALTERS RETINO-TECTAL PROJECTIONS IN THE RAT J. Weise[1], M.R. Kreutz[1], T.M. Böckers[2], M.C. Humphrey[1]*, B.A. Sabel[1] Inst. of [1]Med. Psychology, Otto-v-Guericke University, Magdeburg, F.R.G.; [2]Anatomy, Westf. Wilh. University, Münster, F.R.G.

In the past decade various glutamate receptor subtypes have been reported in the retina and evidence was provided that the mammalian retina is highly susceptible to NMDA-toxicity. We have studied the topography of retino-tectal projections after NMDA-induced retinal lesions with intraocular injection of Fluorescein-isothiocyanat (FITC) and HRP. Anterograde tract tracing revealed that 6 weeks post lesion most of the fluorescent fibers were found in the rostro-medial part of the superior colliculus (SC) with a gradient of intensity in the rostro-caudal axis in comparison to control animals. In the lateral part of the SC the fluorescence signal was clearly diminished in NMDA treated animals. Changes in the dorso-lateral geniculate were less specific. Apart from a general but not dramatic loss of signal intensity only minor changes were observed. Axonal reorganization is suggested by the findings that 1 week after NMDA toxicity a general reduction of fluorescent fibers and HRP reaction products ocurred in all parts of the SC and that GAP 43 immunoreactivity was increased in the RGC layer after the lesion but not in controls. These data suggest that an axonal reorganization of surviving RGCs might be involved in recovery from visual deficits observed in this model of excitotoxicity.

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328.5

EYE IMPLANTS WITH PERIPHERAL NERVE BRIDGES TO THE SUPERIOR COLLICULUS: EFFECTS OF NGF AND DELAYED CONNECTION. B. H. Hallas*, P. Jacovina, S. Lehman, R. Galler, L. Guerra and M. Wells. Departments of Neuroscience and Anatomy, New York College of Osteopathic Medicine, Old Westbury, New York 11568.

Adult Wistar host rats were blinded in the left eye by the removal of the lens retina and vitreous body. Simultaneously, a 3-4 cm portion of the right sciatic nerve was removed for a bridge segment. Entire eyes from 3 day postnatal rats of the same strain containing an attached 2 mm segment of the optic nerve was removed from donor animals. The donor eye optic nerve was then inserted into the sciatic nerve and held in place with 10-O sutures. In one set of experiments the eyes attached to bridges were incubated in 1μg/ml NGF or saline for one hour. The eye was then implanted into the empty left orbit of the adult host and the attached nerve held in place with 10-O suture through the cornea. In other experiments agar containing either NGF or saline was also implanted in the orbit with the donor eye. The attached peripheral nerve bridge was placed in a subcutaneous pocket over the cranium. After two weeks, the bridge was exposed, the distal end trimmed and inserted into the superior colliculus through a hole drilled in the cranium. The peripheral nerve bridge was allowed to remain intact for three weeks at which time HRP was injected intra-ocularly or in the bridge. At the time of HRP injection, the implanted eyes appeared viable and healthy. The resulting tissue was processed histochemically and HRP label was demonstrated in the superior colliculus. Densitometric measurements of sections suggested that eyes and bridges incubated with NGF showed twice the amount of label transported to the superior colliculus compared to those incubated in saline alone.

328.6

QUANTITATIVE CYTOARCHITECTURE AND CYTOCHROME OXYDASE ACTIVITY IN THE SUPERIOR COLLICULUS OF THE VERVET MONKEY AFTER NEONATAL HEMISPHERECTOMY. [3]A. Pitto*[1,3]D. Boire,[1,2]M. Herbin, and [1,2,3]M. Pitto. [1]Neuropsychol. Unit and [2]CRSN, Université de Montréal and [3]MNI, McGill University, Montreal, CAN.

Quantitative morphometry was used to study the effects of early hemispherectomy on the superior colliculus (SC) in the vervet monkey. The SC contralateral to the lesion does not differ significantly from normals. The ipsilateral SC is significantly reduced in size, the superficial layers (I-III) undergoing a more important reduction than the intermediate layers (IV). The shrinkage of the SC results mainly from a reduction of its surface rather than of the thickness of each layer. The stratum opticum (layer III) is reduced, being less discernible on Nissl and cytochrome oxydase (CO) stained sections. There is a light reduction in neuronal density in the superfical layers of the affected SC and light gliosis is evidenced by an increase in the density of oligodendrocytes. CO histochemistry suggest that the superficial layers of the SC contralateral to the lesion exhibits an oxydative metabolism similar to what is observed in the normal SC. CO staining in the superficial layers is intense and similar to what is seen in the contralateral SC. These results show that the SC on the lesioned side of the brain is affected by the loss of cortical input but remains a functional subcortical endstation for the analysis of visual information. Cellular integrity and metabolism of the SC are much less affected than those of the dLGN in hemispherectomized monkeys and could better subserve the residual vision observed in the blind field of hemispherectomized subjects rather than the heavily degenerated dLGN.

328.7

DEVELOPMENT OF NADPH-DIAPHORASE ACTIVITY IN THE SUPERFICIAL LAYERS OF THE RAT SUPERIOR COLLICULUS (SC): EFFECTS OF EYE ENUCLEATION AND OF ACTIVITY. A. Vercelli*, S. Biasiol and S. Jhaveri (§). Department of Human Anatomy and Physiology, Torino Univ. Sch. Med., Italy; (§) Department of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

Nitric oxide (NO) is a gaseous neurotransmitter/neuromodulator possibly involved in the stabilization of active synapses in the developing CNS. NADPH-d is a NO synthase: it may be revealed in neurons and fibers of the superficial layers of the SC by histochemistry. To examine the regulation of NADPH-d levels in the SC by retinal axons, we studied its postnatal expression in normal Wistar rats, in animals with one eye enucleated (P0 or P5) and in rats injected with of tetrodotoxin (TTX, weekly intravitreal injection of 100 ± 25 nl of 1.6 x 10^{-3} M TTX starting on P0-Galli-Resta et al., J.Neurosci. 13:243-250). Animals were perfused at various postnatal ages and brain processed to visualize the NADPH-d in the superficial layers of the SC. The morphology, cell size and dendritic arborization of NADPH-d positive neurons were studied. NADPH-d activity was found in control rats at the end of the first postnatal week. NADPH-d positive neurons are first detected in the superficial layers of the SC on P8; they are abundant by P12. Following P0 enucleation, expression of NADPH-d is delayed in the deafferented SC; in adult rats the overall levels of enzyme are lower on the experimental side, enzyme-positive neurons can no longer be classified according to cell types or dendritic orientation. After P5 eye enucleation, the numbers of neurons of many cell types show a shift in distribution; cell sizes are smaller than normal and dendritic length and orientation are abnormal. TTX-treated rats also exhibit a delayed expression of NADPH-d in the silenced SC. These data suggest that NADPH-d expression in the superficial layers of rat SC is influenced by the presence or absence of retinocollicular fibers and also by the levels of activity in these afferents. (Supported by MURST grants to AV and NIH grant EY005504 to SJ)

328.8

OPTIC NERVE-DEPENDENT CHANGES IN ADULT FROG TECTAL CELL PHENOTYPES. E.A. Debski* and Q. Liu. School of Biol. Sciences, Univ. of Kentucky, Lexington, KY 40506.

Optic nerve activity plays a role in determining the placement of retinal ganglion cell terminals in the optic tectum of the frog (Cline et al., PNAS 84:4342-4345, 1987). We have begun to investigate whether input from the optic nerve might also help shape the neurotransmitter phenotype of cells within this neuronal target. The optic nerves of adult frogs were unilaterally transected and prevented from regenerating for 5 months after which the expression of substance P- and serotonin-like immunoreactivity of tectal cells was assessed.

The percent of substance P-like immunoreactive (SP-ir) cells was significantly higher in the tectal lobe to which direct optic nerve input had been eliminated than in the tectal lobe of the same animals in which optic nerve input had been maintained. Serotonin-like immunoreactive (5-HT-ir) cells were found in significantly greater percents only in medial regions of deafferented tectum. Measurements of the intensity of 5-HT-ir cells using a computer-aided confocal microscope showed that the intensity of cells in the deafferented tectal lobe was 156% of that of the afferented lobe. There were no differences in the intensity of the SP-ir cells. Our data suggest that activity and/or factors supplied by the optic nerve influence the neurotransmitter phenotype of tectal cells. Supported by NSF grant IBN-9209651.

328.9

DEVELOPMENT OF PROJECTION NEURONS CONTAINING SOMATOSTATIN (SS) IN THE RAT VISUAL SYSTEM. °G. Fontanesi, °°S.Thanos and °P. Bagnoli*. °Dept. of Physiology and Biochemistry, University of Pisa, 56127 Pisa, Italy and °°Dept. of Ophthalmology, University of Tubingen, 72076 Tubingen, Germany.

In the mammalian brain, SS immunoreactivity (ir) is generally confined to intrinsic neurons that project locally. To investigate the possibility that SS is expressed in long projection systems within either the visual cortex (VC) or the retina of the rat, retrograde tracing of nerve cell bodies through Fluoro-Gold (2% in PBS 0.1M) injections (1 µl) into the superior colliculus (SC) has been combined with SS immunocytochemistry (Mab S8, 1:750 in PBS 0.1M, FITC conjugated) at different postnatal stages. Early postnatal development of VC is characterized by the presence of SSir in immature bipolar neurons of deep cortical layers. Some SS-positive pyramidal cells can also be observed. In particular, at postnatal day (PD) 15, a small percentage of SS-containing pyramidal cells of layer 5 project to SC. Their number drastically increases during the third postnatal week when the adult pattern of SSir distribution in the different cortical layers is also reached. In the retina, early development of SSir is characterized by the presence of numerous labeled cells in the ganglion cell layer. Double labeling experiments show that about 10% of SS-positive cells of the temporal retina project to SC. At PD15, double labeled cells disappear and SSir is mostly confined to amacrine cells of the inner nuclear layer. These observations suggest that SS may play different roles in different visual regions, i.e. it may act as a neuromodulator in mature projections from VC to SC or it may be a factor regulating the establishment of retino-collicular projections in a restricted period of postnatal development.

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328.10

DEVELOPMENTAL CHANGES IN NMDA RECEPTOR INVOLVEMENT IN VISUAL RESPONSES IN SUPERFICIAL SUPERIOR COLLICULUS (SC). K.E.Binns* & T.E. Salt. Dept Visual Science, Inst. Ophthalmology, Bath St., London. EC1V 9EL, UK.

NMDA receptors may have a role in the maturation of sensory responses. In rat SC NMDA receptor density peaks at P20 [Hofer *et al* 1994, J Neurochem., 62, p2300]. Over a similar period, the expression of individual NMDA receptor subunits alters considerably in SC [Watanabe *et al* 1992, Neuroreport, 3, p1138]. Also Hestrin [1992, Nature, 357 p686] noted developmental changes NMDA receptor ion channel kinetics. We investigated the possibility that the changes in receptor number, subunit expression and channel properties may be reflected in the synaptic response to visual stimulation and the role of NMDA receptors in generation of the visual response.

In urethane anaesthetized hooded rats aged P14 to P45, multi-barrel electrodes were used for single neurone extracellular recording and iontophoresis of NMDA, AMPA and AP5. The response to a standard (300ms) LED visual stimulus was challenged with NMDA receptor selective currents of the antagonist AP5. At P14 the visual response was biphasic, consisting of a burst of action potentials followed by sustained discharge for up to 4sec. With time, the duration of the later phase shortened, and began earlier, until at P20 the response was monophasic and lasted 1-1.5sec. By P22 this had reduced to 0.5-1sec and the response resembled that of P45 and adult rats. At P14-P19 AP5 reduced the visual response of 37% of the neurones studied (mean = 23.6±4†). Between P20-P21 71% of neurones had visual responses which were reduced by AP5 (mean = 32±3†). In P22-P45 rats adult values were reached: 60% of neurones had AP5 sensitive visual responses (mean = 45±8†). The data indicate that in rat SC the visual response matures between P14 and P22. The proportion of neurones with AP5 sensitive visual responses peaked at P20-P21. However the involvement of NMDA receptors in the visual responses of AP5 sensitive neurones increased with age from P14 to adult.

† Values are mean reduction from control level ± SEM.

Work supported by the Wellcome Trust.

328.11

NITRIC OXIDE SYNTHASE IS EXPRESSED EARLY IN THE PRENATAL DEVELOPMENT OF THE CAT SUPERIOR COLLICULUS. C.A. Scheiner*, F.T. Banfro, R.D. Arceneaux, K.E. Kratz, and R.R. Mize. Department of Anatomy and the Neuroscience Center, Louisiana State University Medical Center, New Orleans, LA 70112.

Nitric oxide is a neuronal messenger which may play a role in synapse stabilization during development. We have used nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) histochemistry and brain NO synthase (bNOS) immunocytochemistry to examine expression of NO in the developing cat superior colliculus (sc). Prenatal, postnatal, and adult animals beginning at gestational day E41 were used. Labeled neuron distributions were mapped using a computerized neuron tracing system.

At E41, NADPH-d labeled cells intensely in the deep gray layer (dgl) of sc and in the upper-lateral aspect of the periaqueductal grey (pag). At E58, a similar pattern was observed excepting the intermediate gray layer (igl), where sparsely distributed but regularly spaced neurons were first observed at 300-350 µm intervals. These igl neurons increased in number by P1 and at P3 had formed regularly spaced clusters consisting of 3-5 closely apposed neurons. By P7, these clusters were more apparent, with as many as 5 clusters containing up to ten cells. In addition, fiber labeling was first visible at this age and increased markedly until it appeared adult-like at P57. While fiber labeling remained intense in adult animals, neuronal labeling was markedly reduced. bNOS immunocytochemistry showed a similar pattern of labeling as NADPH-d at P1, P7, P14 and later ages. Both NADPH-d and bNOS labeled cells had relatively mature morphologies in igl at the earliest times at which these substances were expressed.

The transient postnatal expression of NOS in the igl cluster cells suggests that NO is involved in synapse stabilization in these cell groups, in part because the choline acetyltransferase containing patches that overlap these clusters are also first visible during this early postnatal period. Supported by USPHS NIH EY-02973 and a LSU Neuroscience Center Incentive Grant.

328.12

EFFECTS OF NEONATAL AND ADULT ENUCLEATION ON THE SYNAPTIC ORGANIZATION OF THE SEROTONINERGIC PROJECTION TO THE SUPERFICIAL GRAY LAYER OF THE HAMSTER'S SUPERIOR COLLICULUS. E.A Arce, R.D. Mooney,* and R.W. Rhoades. Dept. of Anatomy and Neurobiology, Medical College of Ohio, Toledo OH 43699

We have previously shown that removal of one eye in either neonatal or adult hamsters results in a marked increase in the density of serotoninergic (5-HT) axons in the superficial layers (*stratum griseum superficiale* and *stratum opticum*) of the superior colliculus (SC) ipsilateral to the remaining eye. However, nothing is known about the synaptic organization of these altered projections. In the present study, immunocytochemistry for 5-HT and electron microscopy was used to compare the synaptic organization of the 5-HT projection to SC in normal animals and hamsters that sustained eye removal at birth or in adulthood. In normal adult hamsters, only 4.0% of 500 5-HT-positive profiles made synaptic contacts. Of these, 30% were axodendritic, 65% were axoaxonic, and 5% were axosomatic. In the animals that sustained eye removal at birth, 22% of 400 profiles made synaptic contacts; 26% were axodendritic, 73% were axoaxonic, and 1% were axosomatic. In the hamsters that sustained adult enucleation, 18% of 400 5-HT-immunoreactive profiles made synapses; 26% were axodendritic, 73% were axoaxonic, and 1% were axosomatic. The present results thus demonstrate that 5-HT axonal sprouting after enucleation in hamsters results in a significant increase in the percentage of 5-HT-positive profiles that make synapses in this nucleus, but that the relative proportions of synaptic types made by these axons remains normal.

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328.13

AUGMENTATION OF SEROTONIN IN THE DEVELOPING SUPERIOR COLLICULUS ALTERS THE NORMAL DEVELOPMENT OF THE UNCROSSED RETINOTECTAL PROJECTION. Tracy Crnko, R.D. Mooney, C.A. Bennett-Clarke, R.D. Lane, N.L. Chiaia, and R.W. Rhoades*

A previous study from this laboratory showed that sprouting of serotoninergic (5-HT) axons in the hamster's superior colliculus (SC) induced by a single subcutaneous injection of 5,7-dihydroxytryptamine (5,7-DHT) at birth (P-0) resulted in an abnormal terminal distribution of the uncrossed retinotectal projection such that these axons extended throughout the rostrocaudal extent of this nucleus and failed to form distinct clusters in the rostral *stratum opticum* (SO). The present study provided further evidence to support the role of increased SC 5-HT levels in this phenomenon by placing 5-HT-impregnated ELVAX chips over the SC on either P-1 or P-3 and assessing retinotectal projections via anterograde HRP transport when animals reached P>18. Analysis of ELVAX chips indicated that they released 5-HT for at least 14 days. Implantation of 5-HT-, but not vehicle-impregnated, chips resulted in abnormalities in the uncrossed retinotectal projection similar to those observed in the 5,7-DHT-treated animals. The patches that normally develop in the rostral SO were not present and uncrossed axons were distributed densely in this layer and the lower portion of the *stratum griseum superficiale* throughout the SC. These results suggest that the increase in 5-HT levels within the SC associated with the sprouting of the axons containing this amine in hamsters that received neonatal injections of 5,7-DHT is the most likely cause of the abnormalities in the uncrossed retinotectal projection in these animals.

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328.14

FACTORS UNDERLYING RESTRICTION OF THE EARLY UNCROSSED RETINOCOLLICULAR PROJECTION. Cara J. Snider & Leo M. Chalupa*, Neurobiology, Physiology & Behavior, University of California, Davis, CA 95616.

In all mammals the uncrossed retinocollicular pathway is restricted at maturity to the binocular segment in the rostral half of the superior colliculus (SC). Early in development this retinal pathway is widespread, innervating an expanded portion of the rostral-caudal aspect of the fetal SC (Williams and Chalupa, 1982). We have examined the organization of the early uncrossed retinocollicular pathway of developing cats and ferrets using retrograde as well as anterograde tracing methods. Our findings demonstrate two novel observations. First, the ipsilateral projection is topographically organized throughout development. At the time of the widespread retinocollicular projection, cells in temporal retina arborize in the rostral segment of the ipsilateral SC, while those in nasal retina terminate more caudally. Throughout development the contingent of nasal uncrossed cells is much lower in numbers and innervation density than uncrossed cells in temporal retina. Second, the normal restriction of the uncrossed retinocollicular pathway stems from multiple factors: the loss of uncrossed nasal RGCs, an elimination of a small number of widely misprojecting RGCs, as well as a reduction of retinocollicular terminal zones. (Supported by NEI-NIH 03991).

328.15

NITRIC OXIDE ENHANCES ACTIVITY IN THE DEVELOPING AMPHIBIAN TECTUM. M.-S. Rioult-Pedotti*, R.C. Rentería and M. Constantine-Paton. Department of Biology and Interdepartmental Neuroscience Program, Yale University, New Haven CT 06511.

In retinal ganglion cell axons extending from tadpole retinal explants, nitric oxide (NO) produces growth cone collapse in a cGMP-independent manner (Rentería and Constantine-Paton, 1994). To determine if this morphological observation might have a functional correlate in the intact retinal projection, we studied the effect of NO and its underlying mechanism in the developing tadpole tectum at a time when synaptic plasticity is prominent.

Spontaneous activity was recorded from layer VI neurons, the main recipients of retinal input, in slices from larval *Rana* optic tectum. Exogenous application of NO using S-nitrosocysteine (SNOC, 100 μM) reversibly increased the frequency and amplitude of spontaneous synaptic currents. Neither frequency nor amplitude were enhanced by exposure to NO-exhausted SNOC. This increase in amplitude was more prominent when the cell was held at depolarized potentials. The NMDA receptor antagonist APV (100 μM) did not prevent the NO effect on the frequency of spontaneous currents. Bath application of the membrane-permeable cGMP analogue 8-Br-cGMP (200 μM) increased the frequency and amplitude of spontaneous synaptic currents similar to SNOC application. These data suggest that the effect of NO on synaptic function is distinct from the effect of NO on retinal axon growth cone motility. The present data are consistent with the involvement of NO as a retrograde messenger in activity-dependent plasticity in the developing amphibian tectum. (Supported by NIH EY06039. RCR is a Howard Hughes Medical Institute Predoctoral Fellow.)

328.16

MATURATION GRADIENT OF THE CORTICOCOLLICULAR VISUAL PATHWAY. L. Martínez-Millán*, G. García del Caño and F. Jiménez Sampedro. Department of Neurosciences. Faculty of Medicine, University of the Basque Country, 48940-Lejona, Vizcaya, Spain.nilatera

Corticocollicular fibers of normal (P0-P30) albino rabbits and of visually deafferentiated rabbits (monocularly deprived at P1-P14) were labelled by extracellular iontophoresis of anterograde biotinilated tracers. Corticocollicular fibers first arrive to the stratum opticum (SO) and lower part of the stratum griseum superficiale (SGS) forming a broad plexus of tangential and oblique fibres. During subsequent days, an increasing number of fibers from this plexus ascend vertically to the stratum zonale (SZ), were they bend and follow a trajectory parallel to the collicular surface. As this projection maturates, the volume of the terminal field is reduced and increases are found in the density of fibers in all visual collicular strata as well as in the number of synaptic boutons and the number of axonal branches in the SGS.

The first visual cortical fibers arriving to the anterolateral part of the superior colliculus (SC) originate in the anterolateral part of the striate cortex and are already present in newborn animals. The posteromedial part of the striate cortex sends the last fibres to the SC and they reach to the posteromedial SO at P3. This maturation lag is also observed at later stages in the development of this projection. At P15, the cortical visual projection to the anterolateral part of the superior colliculus is mature whereas in the posteromedial collicular region the visuocortical terminal field still shows signs of immaturity such as a low density of fibers and long tangential fibers in the SZ.

This anterolateral to posteromedial gradient of maturation is also manifest in the sprouting capacity of this pathway. Unilateral visual deafferentiation at P14 does not induce sprouting of corticocollicular fibers originating in anterolateral striate cortex whereas, under the same circumstances, fibers coming from posteromedial strate cortex show a clear amplification of their collicular terminal field.

This work was supported by Fondo de Investigaciones Sanitarias grant F.I.S 95/1397 and University of the Basque Country grant UPV 212.327-EB072/93.

328.17

EFFECTS OF RETINAL LESIONS ON METABOLIC RESPONSES TO PHOTIC STIMULATION IN THE GOLDFISH TECTUM: A DEOXYGLUCOSE STUDY. M. K. Powers* and P. Melzer. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

We applied the autoradiographic deoxyglucose method to study the effects of unilateral retinal lesions on stimulus-related metabolic activation in the optic tectum of goldfish. Three types of lesions were carried out: *1)* enucleation of the right eye, *2)* injection of ouabain (1-2 μl; 0.4 mM) into the vitreal chamber of the right eye, and *3)* surgical removal of a small patch of retina (ca. 5% of the total area) near the vertical meridian in the dorsal half of the right eye. Eight days after the lesion, the fish were injected *i.p.* with 2-deoxy-D-[1-^{14}C]glucose (DG; 15 μCi/100 g b.w.) and exposed to moving black-and-white gratings. After one hour the fish were euthanized. The brains were frozen and cut transversely. The sections were autoradiographed with X-ray film and stained for Nissl substance or for cytochrome oxidase (CO) activity. In all fish the tectum contralateral to the intact eye showed the most prominent DG uptake. The metabolic activation was greatest in the *stratum fibrosum et griseum superficiale (sfgs), i.e.* the layer in which most retinotectal afferents terminate, and extended into the *stratum opticum*. A separate thinner band of high metabolic activity spanned the *stratum album centrale (sac)*. DG uptake in the other strata was low. *Sfgs* and *sac* contralateral to the enucleation showed reduced DG uptake, though their metabolic activity remained discernible. Injection of ouabain led to even more drastically reduced DG uptake in the two layers. The partial retinal lesion resulted in a diminished DG uptake in the whole contralateral *sfgs* and *sac*. However, DG uptake was reduced even further in the tectal portion corresponding to the site of the lesion. Staining for CO activity did not show any effects of the lesions. The autoradiographic DG method, therefore, is an effective tool to resolve subtle differences in tectal impairment caused by the three types of retinal lesions, and it may demonstrate the potential of the goldfish visual system to recover from the loss of function (support: NIH R01-EY08126 and P30-EY08256).

328.18

SEROTONERGIC FIBERS IN THE OPTIC NERVE OF XENOPUS TADPOLES ARE NOT FROM RETINAL GANGLION CELLS BUT TRANSIENT RETINOPETAL AXONS. Sen Huang* and S.A. Moody, Department of Anatomy and Neuroscience Program, The George Washington University Medical Center, Washington, D.C. 20037

Optic nerve fibers are believed to be retinofugal and the identification of serotonergic fibers in the optic nerve has been taken as evidence for the existence of serotonergic ganglion cells in the retina. Using immunofluorescence and confocal microscopy we studied the serotonergic nerve fibers in the optic nerve of early Xenopus tadpoles. Serial frozen sections from stage 26 (1½ days of development) to 48 (15 days) retina were examined. Serotonergic cells were first seen in the ventral hindbrain at stage 28, in the inner nuclear layer of the retina at stage 39 and in the ganglion cell layer at stage 42. Serotonergic axons were first seen in the optic nerve at stage 42 and started to disappear by stage 48. They are not observed in premetamorphic tadpole or adult Xenopus retina. In serial sections, the serotonergic nerve fibers could always be traced proximally to the site of the exit in the ventral forebrain, where they entered amongst a mass of labeled fibers in the ventral forebrain. Growth cones were often seen at the distal tip of the serotonergic fibers either in the optic stalk or just proximal to the optic nerve head. Only rarely were serotonergic fibers observed in the optic nerve layer or ganglion cell layer. These did not merge with a serotonergic cell body in the ganglion cell layer. Thus, the serotonergic fibers in the optic nerve are indeed transient retinopetal fibers, although we have yet to discover the identity of the cell body of origin. In the frog retina, serotonergic amacrine and bipolar cells were mostly located in the inner nuclear layer. A few serotonergic cells (<5%) were in the ganglion cell layer. Since there were no retinofugal serotonergic fibers detected, thus this study demonstrates that the serotonergic cells in the ganglion cell layer are displaced amacrine cells. Supported by NIH grant EY10096.

329.1

THE REGENERATION OF CNS NEURONS INTO PERIPHERAL NERVE GRAFTS IMPLANTED INTO THE THALAMUS OF ADULT MICE. G. Campbell, O. Avwenagha and A. R. Lieberman. Dept. of Anatomy and Developmental Biology, University College London. (SPON: Brain Research Association).

Previous studies have shown that the majority of neurons regenerating into a peripheral nerve graft implanted into the thalamus of adult *rats* lie in the thalamic reticular nucleus (TRN) with relatively few in dorsal thalamic and other nuclei. We are planning studies of the regeneration of CNS neurons into such grafts in genetically manipulated mice which either overexpress the cell adhesion molecule L1 (which we have recently shown is upregulated in regenerating neurons of the TRN) or in which the gene for the substrate adhesion molecule tenascin (the role of which in CNS regeneration is currently controversial) has been deleted. As a preliminary step to studying the transgenic mice we have assessed whether the normal mouse is a valid model for such studies. Segments of tibial nerve were implanted into the thalamus of 6 deeply anæsthetised normal adult ICR mice. After 1 month the animals were reanæsthetised and the distal end of the graft injected with a mixture of cholera toxin subunit B-HRP and WGA-HRP. 48 hr later the animals were again anæsthetised and perfused with fixative ; serial frozen sections of the brain were reacted with TMB.

Between 92 and 374 retrogradely labelled neurons were located in the TRN. 1-113 labelled neurons were present in dorsal thalamic nuclei, in hypothalamic nuclei and in other areas projecting to the thalamus/TRN including the basal ganglia and various nuclei of the caudal brainstem. The distribution of regenerating neurons was very similar to that previously found for the rat. These results show that the response to peripheral nerve grafts in the mouse thalamus is comparable with that in the rat, validating the use of mice in the planned studies. In addition, these results show that some forebrain and caudal brainstem neurons, axotomised in their terminal fields, a long distance from their perikarya, are nevertheless capable of a strong regenerative response in the presence of a nerve graft, and can regenerate an axon over distances far greater than their normal length.

329.2

STRUCTURAL AND FUNCTIONAL EVIDENCE OF RECOVERY FOLLOWING COMPLETE TRANSECTION OF THE ADULT RAT SPINAL CORD. H. Cheng†*, S. Almström, M. Cassarlöv and L. Olson. Dept. of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden; †Neurologic Institute, Veteran General Hospital-Taipei and Division of Surgery, National Yang-Ming University, Taiwan.

Methods allow meaningful functional regeneration across a complete spinal cord transection in man do not exist. To ensure absolute spinal cord discontinuity in the present rat experiments, 5 mm-long sections of the spinal cord were removed at the level of T10 in adult rats. The gaps were bridged by multiple, precisely neuroanatomically located peripheral nerve grafts using intercostal homografts and allografts to redirect between proximal white matter and distal grey matter in the stumps. aFGF-containing fibrin glue (2.5 µg/ml) was applied to stabilize the anastomoses in the grafted area. The thoracic spinal columns were rigidly fixed in dorsiflexion by compressive wiring of the posterior spinal processes. Rats subjected only to complete transection at T10 were used as controls. All operated animals were video-recorded monthly and rated on a blind basis for hind limb functional deficits for up to 13 months using standardized APA and CBS scoring. Treated animals improved significantly and progressively on both scores during the first 6 months, while controls did not change. Seemingly voluntary hindlimb movements and partial weight-bearing were noted in the treated group. Retrograde tracing studies with WGA-HRP demonstrated transport from low lumbar levels to neurons at many levels of the neuraxis proximal to the transection, including raphe regions, reticular nuclei, red nuclei, dorsal tegmentum of the midbrain, and remarkably, pyramidal nerve cell bodies in the hindlimb motor areas of cortex cerebri on both sides. The data demonstrate that it is indeed possible to obtain meaningful regeneration across a complete spinal cord transection in an adult mammal. To our knowledge, this is the first structural and functional evidence of recovery following complete transection of the adult mammalian spinal cord.

329.3

ELECTROPHYSIOLOGICAL IMPROVEMENT AFTER CO-IMPLANTATION OF CARBON FILAMENTS AND FETAL TISSUE IN THE CONTUSED RAT SPINAL CORD. L. S. Liu*, T. Khan, S. Sayers, M. Dauzvardis, C. Traush. Rehabilitation R&D Center, Hines VA Hospital, Hines, IL 60141.

Our previous studies showed that carbon filaments provide directionality and support to growing neurites *in vitro*, and that severed spinal cord axons grew on and between carbon filaments which were implanted into the completely transected rat spinal cord. The aim of the present study was to determine whether the co-implantation of carbon filaments and fetal spinal cord tissue would promote functional recovery following spinal cord injury as determined by somatosensory evoked potentials (SSEPs) and motor evoked potentials (MEPs).

Rats were anesthetized and subjected to a severe contusion injury at the T8 level by dropping a 10g weight from a heigth of 18cm. The rats were divided into five groups. Group One consisted of normal rats (n=7); Group Two consisted of rats which received carbon filament implants cultured with fetal spinal cord explants after contusion injury (n=5); Group Three consisted of rats which received fetal spinal cord tissue implants after contusion injury (n=4); Group Four consisted of rats which received carbon filament implants after contusion injury (n=4); and Group Five consisted of rats which sustained a contusion injury only (n=4). The SSEPs were recorded at L4, C6 and the cortex after stimulating the left sciatic nerve. The MEPs were recorded from the left and right tibialis anterior muscles after magnetic stimulation of the lumbar and thoracic spinal cord and the cortex. Both SSEPs and MEPs were recorded 8 weeks after injury.

The results showed that the most dramatic recovery of potentials was seen in Group Two. In Groups Four and Five, SSEPs and MEPs were completely abolished in 50% of the rats, and of a very low amplitude and prolonged latency in the remaining 50% of the rats. These results suggest that the co-implantation of carbon filaments and fetal spinal cord tissue play an important role in promoting spinal cord functional recovery after injury. (Supported by funds from the Veterans Administration, Rehabilitation R&D Center.)

329.4

MACROPHAGE-RELATED EFFECTS ON THE REGENERATION OF SENSORY AXONS INTO THE INJURED SPINAL CORD J.D. Houle* and C.J.M. Kane, Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205

To test for the possible involvement of macrophages in promotion of the regeneration of dorsal root axons, nitrocellulose strips treated with the macrophage activating factor lipopolysaccharide (LPS) or transforming growth factor beta (TGFβ) or macrophage inhibitory factor (MIF) were co-transplanted with fetal spinal cord tissue into an adult rat spinal cord lesion cavity. Cut dorsal roots were apposed to the nitrocellulose. Thirty days later animals were sacrificed and tissue sections processed for immunocytochemical detection of regenerated sensory axons containing calcitonin gene-related peptide (CGRP-ir) and for characterization of the non-neuronal cell types present at the nitrocellulose-transplant interface.

A graded macrophage response was observed, being most intense with LPS treatment and virtually non-existent with MIF treatment. TGFβ stimulated a macrophage response less intense than that induced by LPS. The extent of regeneration of CGRP-ir axons mirrored the intensity and distribution of the macrophage response, with axonal number and length of growth being greatest with the LPS-treated implant, in contrast to a minimal response with the MIF-treated implants. The non-neuronal cell layer between the nitrocellulose and the fetal tissue was the preferred site for axonal growth in all conditions tested, however, axonal association with a specific cell type was difficult to determine. The thickness of the non-neuronal cell layer was variable, but did not appear to influence the extent of axonal growth. These results implicate activated macrophages in the promotion of axonal regeneration from injured dorsal roots. It appears likely that the interaction of macrophages with non-neuronal cells positively influences these cells to provide an environment capable of support and guidance of axonal regrowth.

Supported by NIH Grant NS26380.

329.5

TRANSPLANTATION-INDUCED MYELINATION BY SCHWANN CELLS IMPROVES CONDUCTION PROPERTIES IN ADULT RAT DEMYELINATED SPINAL CORD. O. Honmou*, P. A. Felts, S. G. Waxman and J. D. Kocsis. Dept. of Neurology, Yale Univ. Med. Sch., New Haven, CT. 06510, and Neuroscience Res. Ctr., VAMC, West Haven, CT. 06516.

Transplantation of myelin-forming cells derived from the central and peripheral nervous system into dysmyelinating mutants or the area of demyelination in genetically normal host animals results in varying degrees of anatomically-defined myelination. We transplanted genetically marked (LacZ gene) glial cells from neonatal rats into adult rat spinal cord which had been demyelinated by prior spinal cord X-irradiation and injection of ethidium bromide and assessed the electrophysiological properties of the remyelinated adult dorsal column axons *in vitro*. Histological examination of the dorsal columns in the transplanted group demonstrated extensive myelin formation in over 99% of the region, although the region in the non-transplanted group did not show any remyelination. LacZ marking of Schwann cells indicated that the new myelin was formed by exogenous cells.

Stimulation of the dorsal columns was obtained with bipolar electrodes placed lightly on the dorsal surface of the spinal cord, and action potentials were recorded with glass microelectrodes attached to a high input impedance amplifier and stored on a digital oscilloscope. The conduction velocity in the region of remyelination increased to normal values. Moreover, impulses could propagate into and out of the transplant region. Conduction block which was observed in demyelinated axons was overcome by remyelination. The axons remyelinated by transplanted cells also improved frequency-response properties as compared to the demyelinated axons; the Schwann cell remyelinated axons could follow higher frequency than control myelinated axons. These results demonstrate that central remyelination by exogenously derived Schwann cells can lead to improved and secure conduction in the adult mammalian central nervous system.

Supported in part by the VA and the NMSS.

329.6

SCHWANN CELL SUSPENSION GRAFTS PROMOTE RECONSTRUCTION OF TRANSECTED POSTCOMMISSURAL FORNIX IN ADULT RAT C.C. Stichel*, K. Lips, G. Wunderlich and H.W. Müller Molecular Neurobiology Lab., Dept. of Neurology, Univ. of Düsseldorf, D-40225 Düsseldorf, Germany

During the past years glial cell transplantation has emerged as a powerful tool for promoting axonal regeneration in adult mammalian CNS. It has been shown that transplanted juvenile astrocytes as well as Schwann cells foster axonal regrowth. In a well defined lesion model, the transected postcommissural fornix, we have shown that astroblasts increase the number of regrowing axons while they are unable to guide the fibers across the lesion site (Wunderlich et al., 1994, Glia 10: 49-58). In order (1) to obtain a beneficial axotropic effect, (2) to bridge the impermeable lesion site and (3) to avoid extensive host tissue damage we implantated dissociated Schwann cells into the transected postcommissural fornix using a microtransplantation extrusion technique.

Schwann cells (SC) were prepared from sciatic nerves of neonatal rats, depleted of fibroblasts and prelabeled with the fluorochrome Hoechst 33342. Immediately after lesion cells were pressure injected (1.6 µl of a $1x10^8$ cells/ml suspension) into the unilaterally transected fornix. Implant characteristics and the spatio-temporal pattern of axonal regeneration were analysed using immunocytochemical and tracing methods.

The prelabeled SC integrated well into host tissue, exhibited extensive migration and proliferated only during the first week after implantation. They did not prevent axonal degeneration but induced extensive regeneration of transected fornix fibers. The regrowing fibers invaded the implant, crossed the lesion site and grew within their former pathway for approximately 1.2 mm up to their target area, the mammillary body. The regenerating axons were found to be myelinated at 4 weeks after implantation. The growth supporting effects were independent of the number of passages of the SC. Supported by the DFG (SFB 194/B5).

329.7

THE INFLUENCE OF PREDEGENERATED NERVE GRAFTS ON AXONAL REGENERATION FROM PRE-LESIONED PERIPHERAL NERVES. K.S. Bedi*, M.N. A-K E-S Hasan, M.M. Neumann, M.A. DeSouky#, and K-F. So+. Department of Anatomical Sciences, University of Queensland, St. Lucia, Australia, 4072 and #Department of Anatomy, University of Minia, Egypt and +Department of Anatomy, University of Hong Kong, Hong Kong.

Previous *in vivo* studies which have investigated whether or not predegenerated nerve segments used as grafts are capable of enhancing axonal regeneration have produced conflicting results. We have reinvestigated this question by using predegenerated nerve grafts in combination with preconditioning lesions of the host nerve to determine the optimal conditions for obtaining regeneration of myelinated axons. The right sciatic nerve of adult Dark Agouti rats was sectioned at mid-thigh level, and the distal portion was allowed to predegenerate for 0,6 or 12 days *in situ*. These distal nerve segments were then syngenically grafted onto the central stumps of sciatic nerves which had themselves received a conditioning lesion 0, 6 and 12 days previously, making a total of 9 different donor-host combinations. All surgical procedures were carried out under Ketamine/Xylazine anaesthesia. The grafts were assessed histologically 3 or 8 weeks after grafting. All grafts examined contained regenerating myelinated axons. The rats given a 3-week post-grafting survival period had a average of between 1,400 and 5,300 such axons. The rats given an 8-week post-grafting survival period had between about 13,000 and 25,000 regenerating myelinated axons. Analysis of variance procedures revealed significant main effects of both the donor and host conditions as well as Weeks (i.e. survival period post-grafting). These results indicate that both a conditioning lesion of the host neurons and the degree of predegeneration of peripheral nerve segments to be used as grafts are of importance in influencing the degree of axonal regeneration.

329.8

CELLULAR TRANSPLANTATION INTO A PARTIAL LESION MODEL TO STUDY AXONAL REGROWTH IN THE ADULT RAT SPINAL CORD. X.M.Xu[1], V.Guénard[2], N.H.Kabeer[1], P.Bates[3], and M.B.Bunge[2]. [1]Dept. Anat. & Neurobiol., St. Louis Univ. Sch. of Med., St. Louis, MO 63104; [2]ETH-Honggerberg, Neurobiology, Zurich, Switzerland; [3]The Miami Project, Univ. of Miami Sch. of Med., Miami, FL 33136.

Schwann cells (SCs) in guidance channels effectively promote propriospinal axons to regenerate in adult rat spinal cord transected mid-thoracically (J. Comp. Neurol. 351:145-160, 1995). When BDNF and NT-3 were infused, axons recruited from distinct brain regions also grew into these grafts (Soc. Neurosci. Abstr. 20:1111, 1994). As an initial step to explore regrowth of specific descending spinal pathways with neurotrophic factors, we developed a partial lesion-SC transplantation model. SCs were purified in culture from adult rat sciatic nerves, suspended in a 30:70 solution of Matrigel:DMEM (M/D), and placed in semipermeable channels (1.25 x 3 mm, MWCO=50K Da)(120 x 10^6 cells/ml). M/D channels served as controls. Adult Fischer rat spinal cord was hemisected at T8 on the right and a 2.5 mm gap created caudally. The SC-channel was placed into the gap with the rostral and caudal cord stumps closely contacting both openings of the channel. One month later, a blood vessel-rich cable bridged rostral and caudal hemicords. Cables through SC-seeded channels contained more myelinated axons at their midpoint when compared to control channels (mean=1054 vs mean=240). Electron microscopy revealed that the ratio between myelinated and unmyelinated axons was 1:8. When distally capped SC channels were implanted and animals were sacrificed 3, 6, 9, and 12 days after grafting, neurofilament positive axons were observed entering the graft 0.1 mm from the host-graft interface on day 3. By day 9, leading axons extended to the distal end of the cable, 2.5 mm from the interface. The present study shows the feasibility of using this model to investigate mechanisms underlying the regrowth of specific spinal pathways. (Supported by NS09923, NS28059 and The Miami Project to Bunge Lab. and Daniel Heumann Fund for Spinal Cord Research to XMX).

329.9

TRANSPLANTED SCHWANN CELLS ENGRAFT IN THE ADULT RAT CEREBELLUM AND PROMOTE REGENERATION OF INJURED OLIVARY AXONS. M. Bravin[1], T. Savio[2], F. Rossi*[1], and P. Strata[1]. [1]Dept. of Human Anat. and Physiol., University of Torino, I-10125, Torino, Italy; [2]Inst. of Anatomy, University of Genova, I-16132, Genova, Italy.

Axon regeneration in the adult central nervous system (CNS) is generally hampered by the presence of unfavourable environmental conditions. However, permissive conditions can be created in the CNS by grafting cells endowed with growth promoting properties, such as those from embryonic or peripheral nervous tissues. This study was aimed at assessing the capability of Schwann cells (SC) to promote the regeneration of injured olivocerebellar axons. Inferior olivary axons were transected in the white matter of adult rat cerebella and SC suspensions, obtained from P1-P2 aged rat pups, were immediately grafted into the lesion site. At different survival times (up to 20 days after lesion/grafting), grafted SC were labelled by anti-P75 antibodies, whereas olivary axons were traced by means of biotinylated dextran amine. In most of the examined cases, the transplanted SC filled the cavity produced by the injury bridging the host cerebellar tissue at the opposite sides of the lesion. In addition, numerous SC were able to migrate inside the recipient white matter and cortical layers where they acquired the typical fusiform shape with a small ovoid cell body and long thin processes. In the absence of the graft, the sectioned olivary axons invariably ended close to the lesion site with small terminal bulbs. By contrast, when the graft was apposed to the lesion site, bundles of labelled olivary axons, which could be followed for several hundred µm, were present inside the graft aligned to SC, indicating that regenerative phenomena have occurred in response to the transplant. These results indicate that grafted dissociated SC are able to bridge the gap produced by the injury, integrate within the host cerebellar parenchyma, and interact with injured olivary axons to promote their regeneration.

329.10

ACTIVATION OF SEROTONERGIC MECHANISMS ENHANCES LOCOMOTOR PERFORMANCE IN SPINAL + TRANSPLANT RATS. D. Miya[1]*, V. Adipudi[1], F. Mori[1], A Tessler[1], S. Giszter[1], K.J Simansky[2], S. Croul[3] and M. Murray[1]. [1]Departments of Anatomy/ Neurobiology. [2]Pharmacology, [3]Pathology. Medical College of Pennsylvania & Hahnemann University, Philadelphia, PA 19129.

Intraspinal transplants of fetal spinal cord permit development of useful locomotor function in rats transected as neonates (Miya et al., 1994). Transplants promote connectivity across the lesion, allowing increased weight support and fore-hind limb coordination. Since 5-HT axons originating in the brainstem regenerate through grafts in neonatal recipients, we investigated whether serotonin agonists would further enhance locomotion that develops as a result of the graft. The serotonergic agonists quipazine and DOI were administered to neonatally transected (n=3), transplanted (n=8), and normal (n=3) rats. The rats were trained daily starting at 3 weeks postsurgery on the treadmill, and wide and narrow runways, and were videotaped both before and after drug administration for kinematic and qualitative analyses. A dose response function was developed for quipazine (0.7, 1.0 and 1.4 mg/kg). Quipazine (1.0 mg/kg) increased weight support in transected rats with transplants but also produced pronounced hypermetria and tremors in these animals and in transected animals without transplants. DOI (0.3 mg/kg) improved weight support and other locomotor functions in rats with transplants without the hypermetria and tremors seen with quipazine. Our results indicate that activation of the serotonergic system improves overground locomotion in transected newborn rats with transplants and suggest that 5-HT_2 receptors are specifically implicated in the improved performance. Supported by grants NS 24707 from NIH, SCRF #300 from PVA and ASRI.

TRANSPLANTATION I

330.1

A HELPER VIRUS-FREE PACKAGING SYSTEM FOR HSV-1 PLASMID VECTORS THAT SUPPORTS EFFICIENT GENE TRANSFER IN THE RAT BRAIN. Alfred Geller*, Song Song, Filip Lim, Phung Lang, Linda Yu, Yaming Wang, and Cornel Fraefel. Div. Endocrinology, Children's Hosp., 300 Longwood Avenue, Boston, MA 02115.

Herpes simplex virus type 1 (HSV-1) vectors have potential for delivering genes into cells in the brain to study neuronal physiology and to perform gene therapy of neurological disorders. However, several unresolved problems with HSV-1 plasmid vectors have limited their potential utility. These unresolved problems include: i) acute cytopathic effects due to either gene expression from the helper virus or specific proteins in the HSV-1 particle, ii) potential interactions between the helper virus and endogenous latent viruses, iii) efficiency of long-term gene expression, and iv) reversion to wild type HSV-1 of the deletion mutant helper virus.

To essentially eliminate those problems associated with the helper virus, we have developed a helper virus-free packaging system for HSV-1 plasmid vectors. Crude, unconcentrated vector stocks produced by this procedure contain ~$4X10^5$ infectious vector particles (ivp) of pHSVlac/ml (the prototype vector which expresses the *Lac Z* gene), and plaque assays revealed no contaminating helper virus (< 1 pfu/ml). Upon concentration and purification a titer of ~$1X10^7$ ivp pHSVlac/ml can be achieved.

Infection of cultured cortical cells with helper virus-free pHSVlac resulted in expression of ß-galactosidase, and the number of positive cells remained relatively stable over a one week period. In contrast, using pHSVlac packaged using a helper virus, the number of positive cells was maximal at two days following gene transfer and decreased to ~30 % at one week. Following injection into the adult rat brain, pHSVlac packaged using a helper virus caused both significant cell damage and cell infiltration at the injection site. In contrast, injection of helper virus-free pHSVlac caused a level of cell damage that was similar to that observed following injection of saline. In the absence of helper virus, the number of positive cells was higher at both 4 days and 1 month after gene transfer compared to helper virus packaged pHSVlac, suggesting that the decreased cytotoxicity increased the efficiency of gene transfer.

330.2

ADENOVIRUS MEDIATED GENE TRANSFER TO ASTROCYTES FROM ADULT AND AGED ANIMALS. A METHOD FOR *EX VIVO* GENE THERAPY. S Ausim Azizi*, Dept of Neurology, Thomas Jefferson University, 1025 Walnut St., Philadelphia, PA 19107.

Astrocytes, because of their natural properties of migration and integration into the CNS and elaboration of growth factors, as well as their availability may serve as ideal candidates for cell replacement therapy. In the present study, using dispase enzyme and shaking methods, primary cultures of astrocytes (> 90% pure) from adult and aged rats (>6 mos.) were obtained. Beta Galactosidase (*Lac-Z*) gene transfer was achieved using a replication-defective recombinant adenovirus vector with a CMV promotor. Immunofluorescence and X-gal histochemistry techniques were used to characterize the cells and visualize *Lac-Z*.. The cultured cells uniformly express Vimentin, and depending on the stage of growth, 20 - 40% express GFAP activity. Viable astrocytes and stable gene transfer was achieved using 1 - 5 X 10^3 particles/cell. Two populations of infected cells were identified: adherent and non-adherent. Adherent cells maintain their mophological characteristics, wheras non-adherent cells become rounded and float in the culture medium, though remain viable and express *Lac-Z*. Infected cells remained viable in culture for at least 8 weeks. These cells were transplanted into the striatum of adult rats. *Lac-Z* positive cells were found in the brain for at least six weeks after grafting. Currently, we are in the process of cataloguing the patterns of migration, integration and long term survival as well as the host reaction to the grafted transformed astrocytes. It appears that intial reaction to the graft in the brain is marshalled by the glia. We believe that these techniques will provide a convenient method for *ex vivo* gene therapy in treatment of neurodegenerative diseases.

330.3

FUNCTIONAL ASSESSMENT OF ADENOVIRAL VECTOR FOR GENE TRANSFER OF DOPAMINE D_2 RECEPTOR cDNA INTO RAT STRIATUM. H. Ikari*, H. Umegaki, L. Zhang, J.M. Chernak, H. Kuo, A. Mastrangeli, R.G. Crystal, G. S. Roth, D. Ingram, Gerontol. Res. Ctr., NIH, Baltimore, MD 21224 and Div. Pulmonary & Critical Care Med., Cornell U., New York, NY 10021.

Dopamine D_2 receptors (D_2R) in the striatum (STR) show declines in normal aging and in neurodegenerative disorders, such as Huntington's disease and late-stage Parkinson's disease. To assess the feasibility of gene transfer of D_2R, we have constructed two vectors: (1) AdCMV.DopD_2R as an Ad5 E1$^-$ E3$^-$ replication-deficient recombinant adenovirus vector containing the rat D_2R cDNA driven by the cytomegalovirus (CMV) immediate early promoter, and (2) AdCMV.null, an identical vector without D_2R cDNA as a control. By injecting AdCMV.DopD_2R unilaterally by cannulae into rat STR, we have accomplished *in vivo* gene transfer of D_2R cDNA into brain. Using receptor autoradiography of tissue-mounted brain sections obtained several days after treatment, we observed markedly increased binding of the D_2R ligand, [^{3}H]spiperone, at the injection site of AdCMV.DopD_2R but not near contralateral sites receiving the control AdCMV.null. To begin assessing if the AdCMV.DopD_2R can produce functional receptors, we are using apomorphine (APO)-induced rotational behavior as a model. Behavior was assessed following unilateral injection of AdCMV.DopD_2R into intact STR or kainate-lesioned STR of rats. In kainate-lesioned rats, APO-induced (1 mg/kg) rotational behavior was not attenuated by injection of AdCMV.DopD_2R into the lesioned STR when assessed up to 12 wk after vector injection and compared to controls receiving injections of AdCMV.null or vehicle alone. In contrast, beginning about 5 weeks after injection of AdCMV.DopD_2R into intact STR, we have observed the initiation of APO-induced rotational behavior in a number of rats. These results must be confirmed in additional studies to determine whether the AdCMV.DopD_2R can produce D_2R functionally coupled to a behavioral response in this model.

330.4

LACZ GENE TRANSFER TO RAT RPE CELLS *IN VITRO* USING HERPES AND ADENOVIRAL VECTORS. B.V. Castillo[1]*, M. del Cerro[1], B.L. Davidson[2], H.J. Federoff[3], M.D. Geschwind[3], and M.C. Bohn[1]. ([1]Dept. of Neurobiol. & Anat., U. of Rochester Sch. of Med., Rochester NY; [2]Dept. of Med., U. of Iowa Sch. of Med., Iowa City IA; [3]Dept. of Med. & Neurosci., Albert Einstein Coll. of Med., Bronx NY)

Genetic modification of retinal pigment epithelial (RPE) cells has the potential of providing trophic support to photoreceptor cells. Objective: Our goal was to determine and compare the efficacy of virally-mediated gene transfer into rat RPE cells *in vitro* using herpes and adenoviral vectors carrying lacZ. Methods: RPE from P7 rat pups were isolated with 2% dispase (Chang et al. '91, Curr. Eye Res. 10:1081) and grown in DMEM low glucose + 10% FBS at $1x10^4$ cells/well (48 well plate). After 4 d in culture, cells were infected with different titers of a defective HSV-1 lacZ vector (0.02, 0.2, 2, 20 infectious particle (i.p.)/cell) or a recombinant adeno lacZ vector (0.1, 1, 10, 100 i.p./cell) for 2h at 37°C. The HSVlac vector is driven by a HSV IE 4/5 promoter whereas the adeno lacZ vector is driven by a RSV promoter and contains a nuclear localizing signal. RPE cells were fixed 2 d post-transfection for X-gal histochemistry. The percentage of blue X-gal reactive cells was determined from 9 pre-determined areas of the well. Results: Infection with adenovirus (100 i.p./cell) resulted in lacZ expression in 38.6 ± 7% of the cells. Percentage of blue cells decreased to <1% at lower viral titers. Infection with HSVlac resulted only in 11.1 ± 3% transfection efficiency at the maximal titer used (20 i.p./cell). The percentage of blue cells decreased ten-fold to 1.25 ± 0.6% at 2 i.p./cell and <.01% for lower HSV titers. No significant difference in cell survival was seen after infection with HSV by comparing the total number of cells in infected and uninfected groups. However, total cell number increased two-fold after adenoviral infection compared to uninfected controls. Conclusions: Rat RPE cells can be transformed *in vitro* using high titers of adeno or herpes viral vectors. No cytotoxicity was seen with either viral vector at the maximal titers used.

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330.5

GROWTH PROPERTIES OF NEURAL CELLS IMMORTALIZED WITH THE TSA58 ALLELE OF SV40 LARGE T ANTIGEN. O. Dillon-Carter*, M.E. Truckenmiller, H. Kulaga, C. Tornatore, W. J. Freed , NIMH Neuroscience Center at St. Elizabeths Hospital, Washington DC, 20032*; NINDS, NIH, Bethesda, MD 20892

The temperature-sensitive tsA58 allele of the SV40 large T antigen is frequently used to immortalize cells for transplantation studies. The tsA58 protein is reported to be active at 33°C, thereby permitting cell propagation. At 39.5°C, it is presumed that a conformational change in tsA58 occurs resulting in loss of binding activity and growth arrest. Effects of temperature on growth were studied in three rat cell lines: a striatal cell line (M213-20) and a mesencephalic cell line (J30a) immortalized with tsA58, and the A7 cell line from rat optic nerve immortalized with wild-type SV40 large T. At low density, growth arrest was not observed when the cells were shifted from 33°C to 39.5°C. Moreover, cells maintained at 39°C for more than 100 divisions continued to grow at the same rate as cells maintained continuously at 33°C. When, however, cells were grown to confluency at 33°C and then shifted to 39°C, there was 80-90% rate of cell death as measured by propidium iodide exclusion. BrdU incorporation and cell cycle analysis by FACS did not reveal growth arrest or significant differences in the percent of cells in S-phase at the "non-permissive" temperature. Thus, at the "non-permissive" temperature, cells immortalized with the tsA58 form of SV40 large T continue to be immortal. Growth arrest at the non-permissive temperature in these cells may require additional inhibitory conditions, such as confluency or serum starvation.

330.6

TEMPERATURE-SENSITIVITY OF THE P53 AND DNA BINDING FUNCTIONS OF SV40 LARGE T TSA58 C. Conejero*, C. Tornatore and W. Freed. SPN, NPB, NIMH Neuroscience Center at St. Elizabeths, Washington D.C. 20032, and Laboratory of Molecular Medicine and Neuroscience, NINDS, Bethesda, MD, 20892.

SV40 large T antigen is commonly used to immortalize cells in vitro. The immortalization is thought to be due in part to the binding of p53 by T protein. Cells immortalized by the temperature-sensitive tsA58 mutant of SV40 large T express an immortal phenotype at the permissive temperature (33° C). When tsA58-immortalized cells are grown at 39.5° C, the T protein is thought to be inactivated, p53 is released, and the cells lose the immortal phenotype. To study this phenomenon, we used the pG-13 Luc reporter plasmid described by El-Deiry et al. (Cell, 75, 817-835, 1993). This construct has a p53-responsive enhancer element cloned upstream from the luciferase reporter gene. When transfected into cells, this construct can be used to test p53 activity under different growth conditions. This plasmid was transfected into a rat striatal cell line immortalized with tsA58 SV40 large T (M213-20 cells), and luciferase activity was measured at both the permissive and nonpermissive temperature. A peak of p53 activity was observed approximately 24 hr following a shift to the non-permissive temperature. In addition, at the permissive temperature there was a 200-fold increase in luciferase activity over 48 hr from the baseline reading, suggesting that plasmid replication and DNA binding occurred at 33°C. Paradoxically, at the non-permissive temperature a 70-fold increase in luciferase activity was seen over the same period. These data suggest that both the DNA and p53 binding functions of the tsA58 allele of the SV40 large T antigen are temperature sensitive.

330.7

PHARMACOLOGICAL NEUROPROTECTION (NP) AND EARLY VIABILITY OF INTRASPINAL FETAL GRAFTS. T. E. White, G. W. Schrimsher , D. K. Anderson , D. P. Theele, and P. J. Reier*., Univ. Florida, Gainesville, FL. 32610.

There is a rapid attrition of intraspinal fetal grafts that is evident by 4d after grafting (pt). Thereafter, surviving cells give rise to expanding grafts that span lesion sites. The early loss of graft tissue may limit the extent of host-graft neuritic interactions. We have thus explored whether pharmacological interventions aimed at limiting possible graft susceptibility to secondary pathology can enhance early transplant viability. Adult S-D rats received E14 SC grafts in hemilesion cavities made at C1-C2. The animals were then divided into three groups: graft-only controls, rats receiving fetal tissue previously exposed to the synthetic glucocorticoid, methylprednisolone (MP), and rats treated with MP after grafting. Donor tissue treatment with MP entailed injecting the mother with MP (30mg/kg, i.v.) 1hr before the fetuses were removed. Subsequent fetal SC tissue preparation was done in Hank's Balanced Salt Solution containing MP (100µM). In the third group, recipients received injections of MP (30mg/kg, i.v.) at 5 min., 2hrs, and 4hrs after grafting. At 4d pt, the SCs were prepared for light microscopy. In controls, the transplants consisted of small islands of cells apposed to either host gray matter or pia, and there was extensive tissue necrosis surrounding these foci of donor tissue. MP-treated grafts in the second group of rats showed similar results except that the clusters of tissue were slightly larger. However, grafts in MP-treated hosts were substantially larger than in either of the other groups. There also was a suggestion of reduced inflammation and less host tissue loss. It thus seems that the addition of pharmacological NP enhances early SC reconstruction with fetal cell grafts, particularly if treatment is directed at the host. These findings also agree with reports of better fetal dopaminergic cell survival after exposure to 21-aminosteroids. Future studies will determine whether connectivity is better as a consequence of NP improved early graft survival. Support: Florida Brain & Spinal Cord Injury Trust Fund , NS 27511, and McKnight Foundation.

330.8

FUNCTIONAL OUTCOME OF RATS SUBJECTED TO SPINAL CORD CONTUSION TREATED WITH METHYLPREDNISOLONE, ISOGENIC TRANSPLANT OF PERIPHERAL NERVE, OR BOTH. G. Guizar-Sahagun*, I. Grijalva, H. Salgado-Ceballos, A. Gómez, A. Martínez, A. Ibarra, P. García-López, S. Méndez, A. Feria-Velasco, R. Franco-Bourland and I. Madrazo. Proyecto Camina, A.C., and U. Clin. Res. Neurological Diseases, IMSS. Mexico City, 14050, Mexico.

To compare the effect of several treatments for acute spinal cord (SC) injury, adult female inbred Fisher 344 rats were subjected to SC contusion with the method of Allen at T9 level, with an intensity of 45 g/cm. All rats received the corresponding management immediately after the injury. Five groups of 6 rats each were formed. Rats of a group were treated with methylprednisolone (MP), 3 doses (30 mg/kg IP) followed by 2 doses (60 mg/kg IM) during the first 24 h post-injury; other rats were transplanted in the SC injured area with dissociated cells (in Hanks solution) from sciatic nerve obtained from an inbred adult rat; in other rats a combination of MP and transplant as described before was used. Two groups were used as control: rats injected with Hanks solution in the SC injured area, and rats without any treatment (only contused). Rats were submitted to a battery of functional tests weekly during 8 weeks, then were killed to SC morphologic analysis. Comparing all groups, better functional outcome was observed in rats treated with MP only, even though this situation was reached from the second week to the end of the study. Rats of the control group without any treatment had the poorest functional score. Rats treated with transplantation only showed similar functional outcome compared with non-treated rats. Our hypothesis that the combination of MP and transplant of peripheral nerve will have the best functional outcome could not be sustained. In conclusion, under the paradigms used in this work, the best option to improve the functional outcome is the treatment with MP.

330.9

THE INFLUENCE OF CYCLOSPORIN A TREATMENT AND XENOTRANSPLANT IMMUNOGENICITY ON HOST MHC EXPRESSION IN THE RAT MIDBRAIN K.A. Czech*, J.W. Ryan, G.D. Pappas, and J. Sagen, Dept. of Anatomy & Cell Biology, University of Illinois at Chicago, Chicago, IL, 60612.

Previously, its been shown that a short course of cyclosporin A (CSA) is sufficient to achieve survival long-term survival of bovine adrenal chromaffin cells, BCCs, transplanted in rat brain. If immunosuppression is omitted before graft stabilization during these initial few weeks, the BCCs are rapidly rejected by the host. Since highly immunogenic passenger cells (e.g. endothelial, fibroblast, and smooth muscle cells) represent roughly 5% of the cell population, their role in graft rejection during this critical time needs to be assessed. To address this question, the influence of graft immunogenicity on host MHC I and II expression was determined. Rats were transplanted with either 1) isolated bovine chromaffin cells (approx. 95% pure) or 2) non-chromaffin cell constituents of the bovine adrenal medulla (NCC) or 3) isolated bovine adrenal endothelial cells. Half in each group were immunosuppressed with CSA for the duration of the experiment. Survival and MHC I and II were assessed using immunocytochemical staining at two weeks. Without CSA treatment, there was considerable graft degeneration in groups 2) and 3) that coincided with profound MHC I and II expression. In BCC transplants, graft rejection also occurred together with MHC I and II expression, but was less robust than groups 2) and 3). CSA treatment yielded healthy viable grafts with markedly decreased MHC I and II expression in all groups. In BCC transplants, no significant expression of MHC I and II was detectable in immunosuppressed animals. In summary, BCCs weakly induce host MHC expression as compared to NCC and endothelial cell transplants, and this can be completely eliminated by CSA treatment. Supported by NS25054.

330.10

XENOGRAFT REJECTION IN THE BRAIN: THE ROLE OF ASTROCYTIC AND NEURONAL CELLS. A. Pagenstecher*, P. Fisch, B. Volk, Dept. of Neuropathology, University of Freiburg, 79106 Freiburg, Germany.

Rejection of xeno- and allografts in the central nervous system is frequently variable and incomplete. The mechanisms of the immune response in the brain are yet not fully understood. To elucidate the role of astroglial and neural cells in graft rejection we transplanted cells from human glioma (U373MG) and human neuroblastoma (SH-SY5Y) cell lines into the rat striate body and into the skin. Furthermore, we determined the expression of surface antigens on the tumor cells by flow cytometry and the stimulation of rat lymphocytes in a proliferation assay.

While the SH-SY5Y cells proliferated in the host brain, the U373MG cells were rejected vigorously. This rejection was likely mediated by lymphocytes. In mixed U373MG/SH-SY5Y grafts, only the SH-SY5Y cells survived. In contrast, both cell types were rejected in the skin. FACS analysis revealed several antigens including MHC I and II on U373MG cells. IFN-γ stimulation increased the expression of MHC antigens. Also, SH-SY5Y expressed B7.2 after IFN-γ stimulation. Weak proliferative responses of rat lymphocytes to both cell lines were detected in vitro.

Our observations suggest an important role of astrocytes in the rejection of transplanted neural tissue. Expression of MHC I and II antigens by astrocytes is inducible by INF-γ, a cytokine which is secreted by microglia in the course of tissue damage. Moreover astrocytes produce several cytokines e.g. IL-1 and IL-6 which stimulate the rat lymphocytes. Thus astrocytes act together with microglial cells as propagators of the immune response.

Supported by the SFB 364

330.11

DIFFERENTIATION OF TRANSPLANTED NEURONAL PRECURSORS IS ALTERED IN THE LESIONED SPINAL CORD. S.M. Onifer[1,2], J.T. Pacheco[1], S.R Whittemore[1-3]. [1]The Miami Project to Cure Paralysis, Departments of [2]Neurological Surgery & [3]Physiology and Biophysics, University of Miami School of Medicine, Miami, FL 33136.

RN33B is a clonal neuronal cell line that was generated from dissociated, embryonic rat raphé nuclei following infection with a retrovirus encoding the temperature-sensitive allele of SV40 large T-antigen (Whittemore and White, *Brain Res.*, 615, 1993, 27-40). RN33B cells transplanted into the normal adult rat CNS differentiate with a morphology indistinguishable from that of endogenous neurons at the transplantation site (Onifer et al., *Exper. Neurol.*, 122, 1993, 130-142). These results suggest that RN33B cell differentiation in the adult CNS can be variably regulated by local epigenetic signals. The present study examines the differentiation of RN33B cells after transplantation into the adult CNS under different lesion conditions. Adult rats underwent unilateral lesion of interneurons and motor neurons in the cervical spinal cord by injecting 0.5% kainic acid into the grey matter of the C7 and C8 segments. Two weeks later, 2 X 10^5 *E. coli lacZ*-labeled RN33B cells were transplanted into the grey matter at each segment. At 2 weeks post-transplantation, ß-galactosidase immunohistochemistry revealed relatively undifferentiated RN33B cells throughout the grey matter. This observation contrasts with our previous finding that RN33B cells differentiated with a bipolar morphology similar to spinal interneurons 2 weeks after transplantation into the normal adult spinal cord grey matter. It also indicates that either the signal(s) which regulate RN33B cell differentiation is derived from interneurons, motor neurons, non-neural cells, and/or is suppressed by molecules released by the injured CNS tissue. To address this issue, experiments involving transplantation of RN33B cells into the selectively lesioned and chronically injured spinal cord are in progress. Supported by The Miami Project to Cure Paralysis, General Reinsurance, and NS26887.

330.12

DEVELOPMENT OF WHOLE EMBRYONIC RETINAL TRANSPLANTS ON BRUCHS MEMBRANE IN RABBITS. T. Grasbon, B. K. Siesjö* and B. Ehinger. Dept. of Ophthalmology, University of Lund, S-221 85 Lund, Sweden.

Ganglion cells are the only retinal cells which have not been convincingly demonstrated in embryonic retinal transplants to the eye. Such cells are needed if a transplant is to send axons to the brain. Therefore, trying to find a developmental window for ganglion cells, we examined transplants placed on Bruch's membrane. Such transplants mostly show good but inverted lamination. Several time points were examined.

Adult rabbits received a single 15 embryonic days old (E 15) rabbit neuroretina transplant. The transplantation tube was inserted into the subretinal space in such a way that the host pigment epithelium was scraped off, exposing the transplant directly to Bruch's membrane. Rabbits were enucleated at several transplant ages corresponding to E 26 up to postnatal day 26, and the eyes were fixed overnight in 4% paraformaldehyde. Cryostat section immunocytochemistry for ganglion cells was performed with an antibody against human protein gene product 9.5 (PGP 9.5, Ultraclone®).

Morphologically, the time course of the general transplant development was to a great extent similar to that of the normal retina. An inverted laminar organization was seen in the parts of the transplants facing denuded Bruch's membrane, particularly in the oldest transplants. A ganglion cell layer was apparent in the part of the transplant facing Bruch's membrane. However, there was no PGP immunoreactivity in the cells of the ganglion cell layer. It thus appears that the cells in this layer are mainly other cells than true ganglion cells and that the development of transplant ganglion cells is retarded and/or arrested before E26.

330.13

PROTEOGLYCAN AND GFAP EXPRESSION FOLLOWING SPINAL CORD INJURY AND INTRASPINAL TRANSPLANTATION. M.L. Lemons*, D.R. Howland and D.K. Anderson, VAMC and Dept. of Neuroscience, University of Florida, Gainesville, FL 32610.

Failure of injured axons to regenerate in the central nervous system is not fully understood. In an attempt to better understand the mechanisms that inhibit this neuritic outgrowth, we have examined an extracellular matrix molecule (ECM), chrondroitin-sulfate containing proteoglycan (CSPG), which can inhibit neuritic outgrowth and is associated with astrocytes. We have studied the expression of this ECM and glial fibrillary acidic protein (GFAP) in three groups: sham injury (laminectomy only), spinal cord contusion injury and contusion plus a suspension of E14 fetal spinal cord tissue transplanted ten days post-injury. The thoracic spinal cords of adult rats were contused using the New York University impactor device. Immunocytochemical techniques were utilized to identify CSPG and GFAP one week or forty days post-injury. One week post-injury, GFAP positive cells were enlarged and their processes were prominent along motor neuron axons. Their processes also appeared to form a glial boundary by one week. CSPG was generally co-localized with GFAP. At forty days post-injury, GFAP positive cells remained enlarged and radiated from the gray to the white matter. CSPG followed the GFAP radiating pattern in the areas close to the gray-white matter border. Although fetal tissue transplants did not appear to reduce hypertophy of GFAP positive cells, interruptions in the apparent glial boundary were observed. CSPG staining was reduced in the host but was still seen around the graft. To determine if axonal growth across the host-graft interface is greater in the absence or decreased expression of GFAP and/or CSPG, we are using immunocytochemical labeling of neurofilament protein, serotonin (to identify descending projections) and calcitonin gene related peptide (to identify segmental afferents). Supported by C.M. and K.E. Overstreet Foundation and Department of Veterans Affairs.

330.14

STUDY OF THE ROLE OF PSA-NCAM IN THE DEVELOPMENT OF INTRASTRIATAL GRAFTS OF EMBRYONIC DOPAMINERGIC NEURONS.

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Polysialylated form of the neuronal cell adhesion molecule (PSA-NCAM) has been implicated in various phenomena in the developing nervous system, such as axonal elongation and/or bundling or cellular migration. Similar processes take place during the development of intracerebral neural grafts. The present study aimed at testing the role of PSA-NCAM in the development of such grafts. A cell suspension prepared from embryonic mesencephalon, and containing dopaminergic (DA) neurons, was implanted into the striatum of rat pups, the nigrostriatal DA pathway of which had been destroyed previously using the neurotoxin 6-OHDA. Neonatal hosts were chosen because in such hosts, in contrast with adult host, there is an effective migration of implanted cells into the host parenchyma. For half of the animals, the cell suspension to be implanted was incubated before implantation with a phage-derived endoneuroaminidase that specifically cleaves the PSA moieties of PSA-NCAM. The absence of PSA does not seem to influence the development of implanted DA neurons, indicating that the role of PSA in cell migration and development of innervation is not universal. As a second approach to the same aim, we used NCAM knock-out mice as donors and/or hosts as developmental abnormalities observed in such mice have been attributed primarily to the absence of PSA. The development of grafted neurons in these conditions will also be presented.

330.15

THE REEXPRESSION OF TENASCIN AND CHONDROITIN SULFATE IN WOUNDS OF THE ADULT MOUSE STRIATUM, AND THEIR EFFECTS ON EMBRYONIC DOPAMINE CELL ATTACHMENT AND NEURITE GROWTH *IN VITRO*. MA Gates and DA Steindler*, Dept. of Anat. & Neurobiol., The Univ. of Tennessee, Memphis, TN 38163.

Failed regeneration in the injured brain has been attributed to the formation of an impeding glial scar. Extracellular matrix (ECM) molecules, such as tenascin and chondroitin sulfate proteoglycans (CSPGs), which are known to have effects on neuronal adhesion and neurite outgrowth during development, are upregulated in and around adult brain wounds. In the present study, striatal wounds from adult mice were used as *in vitro* substrates (after various survival times *in vivo*) for dissociated dopamine neurons from the embryonic ventral mesencephalon. By removing striatal wounds before or after the upregulation of tenascin and/or CSPG, it was shown that dopamine cell attachment increased (compared to unwounded controls) in relation to the reexpression of these ECM constituents, yet there was a concomitant decrease in neurite growth corresponding to the presence of tenascin. Dopamine cell attachment in this culture paradigm was totally abolished when wounds were exposed to the CS-56 antibody before being used as a substrate. Similar perturbations with a tenascin antibody revealed a decrease in dopamine cell attachment, but a significant increase in dopamine cell neurite outgrowth. These data suggest that CSPG facilitates embryonic dopamine cell adhesion, and that tenascin may augment this process while simultaneously inhibiting neuritic growth. This work has implications toward understanding the role of ECM during failed regeneration of the mature brain, as well as the influence that wound-related molecules may have on immature dopamine neurons that are sometimes grafted into the adult striatum. Supported by NIH/NINDS grant NS29225.

330.16

GRAFTS OF THE CHOROID PLEXUS INTO THE BRAIN PRODUCE A PERMANENT OPENING OF THE BLOOD-BRAIN-BARRIER (BBB). J.M. Rosenstein*, Departments of Anatomy and Neurological Surgery, The George Washington University Medical Center, Washington, D.C. 20037

Our previous studies have shown that when tissue containing permeable vessels such as autonomic ganglia or adrenal medulla is grafted to the brain it produces a permanent and focal opening in the BBB such that large proteins may enter the host parenchyme where normally they would not. Autonomic tissues, however either degenerate or become encapsulated by connective tissue or reactive astroglia. This report describes studies using the choroid plexus (CP) which is comprised of fenestrated vessels and epithelial cells as a viable tissue to produce a permanent opening in the BBB. Small (0.5mm^3) pieces of rat E18-19 choroid plexus or cografts of choroid and neocortex from the same donor fetus were placed in the host cortex or striatum. After survival periods up to 6 months, the host animals were examined immunocytochemically for antibodies to rat serum albumin (SA), the BBB glucose transporter (GLUT1) and GFAP. Some hosts also received a systemic injection of ^{3}H GABA which does not cross the BBB. By the latest time examined the CP grafts survived almost entirely intact. Immunostaining for SA showed a focal leakage of protein extending 0.5-0.8 mm from the graft into the host and co-graft neuropil at both light and electron microscopic level. GLUT1 staining showed that the graft vessels retained their non-CNS phenotype and there was only minor astroglial (GFAP) reactivity. Radiolabelled GABA was sequestered by some graft neurons. These results would indicate that the fetal choroid could be useful as a biological portal across the BBB and may provide a window for focal ingress of systemically administered bioactive materials. (NS-17468).

330.17

HOST NERVE FIBERS THAT REGENERATE AND RESIDE LONG-TERM IN A REJECTED NERVE ALLOGRAFT ARE NOT PROTECTED BY PERMEABILITY BARRIERS. N. A. Azzam*, A. A. Zalewski and R. N. Azzam. Lab. of Neural Control, NINDS, NIH, Bethesda, MD 20892-4120.

Host nerve fibers can regenerate through a short length of nerve allograft in a non-immunosuppressed recipient despite the fact that allogeneic Schwann, vascular and perineurial cells are rejected. This regeneration occurs because host axons and Schwann cells utilize the basement membrane tubes of rejected Schwann cells as pathways for growth. We investigated whether permeability barriers develop and protect host nerve fibers in a rejected nerve allograft. In order for barriers to form, host cells have to enter the allograft and differentiate into endothelial and perineurial cells that respectively form the impermeable endoneurial blood- and perineurium-nerve barriers that are present in normal nerve. A 2-cm long graft of peroneal nerve was taken from American Cancer Institute (ACI) or Fischer (FR) rats and transplanted to bridge a 2-cm gap between the cut ends of the peroneal nerve of other FR rats. Six months postoperatively, light and electron microscopic histology revealed that regenerated host nerve fibers in rejected ACI allografts were compartmentalized into numerous minifascicles by perineurial cells and that blood vessels were located outside the perineurial compartments rather than interspersed among the nerve fibers. Administration of the permeability indicator horseradish peroxidase (HRP) to allograft recipients (i.e., intravenously or topically to the graft in situ), revealed that HRP entered the endoneurium of microcompartments and spread among and into the nerve fibers. In contrast, no HRP reached nerve fibers in non-immunogenic FR nerve grafts which survived and were histologically normal. We concluded that host nerve fibers that regenerate and reside long-term in a rejected nerve allograft were not protected by permeability barriers.

330.18

DEPLETION OF MAJOR HISTOCOMPATABILITY COMPLEX (MHC)-BEARING CELLS FROM EMBRYONIC RAT SPINAL CORD. N.A. Pennell*, A.G. Rabchevsky, and W.J. Streit. Dept. Neuroscience, Univ. of Florida, Gainesville, FL 32610

It is common practice among researchers and clinicians to use immunosuppressive agents, such as cyclosporin A and FK 506, to prevent neural allograft rejection. However, immunosuppression causes increased susceptibility to secondary infection and the tissue is still subject to rejection should the agent be removed. While immunosuppression is designed to prevent the amplification of an already existing immune response, efforts would be better aimed at preventing the initiation of the immune response itself.

Major Histocompatability Complex (MHC) antigens are expressed on the surfaces of three types of cells in the CNS; microglia, perivascular cells or pericytes, and endothelial cells. These cells, when grafted into an allogeneic host, are recognized as foreign and instigate an immune response against the graft. Presumably, if these cells were removed from grafted neural tissue, the remaining cells would not be recognized as foreign and the immune response would be attenuated or absent.

The B_4-isolectin from *Griffonia simplicifolia* (GSL I) binds microglia and endothelial cells specifically among cells of the rat CNS. In this study, we have created highly viable single cell suspensions of E14 rat spinal cord tissue and tested the feasability of depleting these suspensions of GSL I-binding cells, thus removing the majority of MHC-expressing cells. The suspensions were incubated with GSL I-conjugated Dynabeads and the bound cells were pulled out utilizing a magnetic particle concentrator. Depleted and control suspensions were analyzed histochemically for GSL I expression. Freshly depleted suspensions showed significant lack of GSL I expression in comparison to non-depleted suspensions. In addition, depleted suspensions showed similar lack of GSL I expression after one week in culture, indicating that the depleted cell populations were not replaced by endothelial or microglial progenitors within the fetal tissue. Future studies will involve transplanting the depleted suspension into an allogeneic rat strain and analyzing the graft for strain-specific MHC expression.

330.19

PLASTICITY OF LOCUS COERULEUS NEURONS DURING AGING: EVIDENCE FROM INTRAOCULAR TRIPLE TRANSPLANTS. A.C.Granholm*[1,2], G.A. Gerhardt[2], and N. Srivastava[1]. Departments of Basic Science[1] and Pharmacology[2], Univ. Colorado HSC, Denver, CO 80262.

The age-related plasticity of central noradrenergic neurons was investigated, using intraocular transplantation of fetal brain tissue. Tissue pieces of fetal brainstem tissue (E17) and hippocampal CA1 tissue (E18) were placed adjacent to each other in the anterior chamber of the eye of 3 months old Fischer 344 hosts. The double transplants were allowed to mature for 16 months, after which a second fetal hippocampal tissue piece was placed next to the brainstem transplant. The triple transplants were then allowed to grow for an additional 2 months *in oculo*. Immunohistochemical studies of the triple transplants demonstrated that tyrosine hydroxylase (TH)-immunoreactive neurons in the brainstem transplant innervated both the young (2 months old) and the aged (18 months old) hippocampal transplants with a dense network of thin, varicose fibers. Furthermore, the aged double transplants exhibited a denser pattern of astrocytes than the young hippocampal grafts, as evidenced with glial fibrillary acidic protein immunohistochemistry. All three transplants displayed an intact blood-brain barrier, and contained significant densities of synaptic profiles, which were visualized with antibodies directed against synapsin. Electrophysiological and electrochemical properties of the noradrenergic innervation of the young and the aged hippocampal components of the triple grafts are currently under investigation. The present data demonstrate that aged locus coeruleus neurons maintain their ability to innervate appropriate targets, at least at 18 months of age in the rat. Thus, aging does not appear to impair the endogenous plasticity of these neurons. *Supported by USPHS grants AG12122 and MH49661.*

330.20

REGULATION OF L-DOPA PRODUCTION BY GENETICALLY MODIFIED PRIMARY FIBROBLASTS WITH RETROVIRAL VECTOR A.Ishida*, K.Yamashiro, J.Mukawa, M.Hasegawa. Dept.of Neurosurgery,Faculty of Medicine, Univ.of the Ryukyus, 207 Uehara, Nishihara-cho, Okinawa 903-01, JAPAN. DNABVEC Reserch Inc., 25-11, Kannondai 1-chome, Tsukuba-shi, Ibaraki 305, JAPAN

Intracerebral grafting is a new strategy of the treatment for Parkinson's disease. We focused on primary skin fibroblasts. Rat primary skin fibroblasts were transfected with a retroviral vector containing the cDNA (pLTHSNL) for human tyrosine hydroxylase typel (TH) or cytomegalovirus promoter (pCTHSNL) as a foreign promoter. These cells were measured by immunocytochemistry and high performance liquid chromatography with electrochemical detection (HPLC-ECD) analysis of L-DOPA production and release in vitro. When supplemented with biopterin (BH4;(6R)-L-erythrotetrahydrobiopterin) cofacters required for TH activity, these cells produced and released L-DOPA into the culture medium, especially fibroblasts containing a foreign promoter increased in releasing L-DOPA in time-dependent manner. This suggest that production of L-DOPA from genetically modified fibroblasts can be regulated by foreign promoter and BH4. This character was not affected by the number of cell-passage and freeze. Because retroviral vector including the foreign gene (THcDNA) integrate into chromosomal DNA of the target cells (fibroblasts). Primary skin fibroblasts can be obtained and cultured easily. And these cells did not produce tumors by transfection with a retroviral vector.

In conclusion, genetically modified primary skin fibroblasts transfected with THcDNA using retroviral vector must be a worthy graft for gene therapy in Parkinson's disease.

331.1

AN "IN VITRO" DYNAMIC MODEL OF BLOOD-BRAIN BARRIER D. Janigro and K. A. Stanness, Dept. of Neurosurgery, ZX-18 University of Washington, Seattle, WA 98104

Cell culture models have been widely used for screening of neurotoxicants and represent a viable alternative to direct *in vivo* experiments. We have developed a dynamic *in vitro* blood-brain barrier (BBB) model designed to allow for extensive pharmacological and physiological testing. Induction of blood-brain barrier properties in a tri-dimensional hollow fiber culturing apparatus (CellCo Inc.) were investigated by co-culturing a bovine aortic cell line and rat brain endothelial cell with rat brain astrocytes (or C6 rat glioma cells) under pulsatile flow conditions to mimic intraluminal blood flow. Cell growth was monitored over time by measuring glucose consumption and lactate production: these experiments confirmed that the hollow fiber cell culturing systems can maintain viable cells in culture for extended (>1 month) periods of time. Cells were visually inspected after culturing and dissociation from the hollow fiber cartridge and identified as endothelial (by fluorescent DiI-Ac-LDL uptake) or glial (by GFAP immunoreactivity). BBB properties were tested by intraluminal injection of horse-radish peroxidase (HRP, mol. weight 44,000), glucose (m.w. 180) or potassium. Either procedure demonstrated that BAEC co-cultured with astrocytes (or C6 cells) developed a selective barrier with an estimated electrical resistance of 2,900 Ω/cm^2. The electrophysiological and morphological properties of BAEC were also affected by the co-culturing process, suggesting that astrocytes induced CNS properties in these cells. These results demonstrate that the hollow fiber cell co-culturing system may be used as a dynamic model of mammalian blood-brain barrier for both pharmacological and neurotoxicological studies. Supported by NS 51614.

331.2

CHEMICAL INDUCTION OF FENESTRAE IN VESSELS OF THE BLOOD-BRAIN BARRIER. M. Kaya, L. Chang, A. Truong, M. Brightman*. LN, N.I.H., Bethesda, Md. 20892

Some brain microvessels, normally impermeable to circulating solutes, become fenestrated (FV) and permeable when they enter brain tumors. It is shown here for the first time, that retinoic acid (RA) and phorbol myristate acetate (PMA), both known to re-induce fenestrae in adrenal gland vessels in vitro, induce fenestrae in blood-brain barrier capillaries in vivo. When 100μM RA or 150ng/ml PMA are infused by an osmotic pump for 28 d into cerebral cortex, some brain vessels around the cannula site became FV. In 2 rats given RA, tissue identified as being near the cannula, contained FV. In 4 rats infused with PMA, about 1/3 of the total number of 136 were FV, with a range of 13 to 56. A total of 329 remained continuous, like those of the blood-brain barrier, with a range of 13 to 138. In one rat, there were 56 FV and 58 continuous microvessels. Only those vessels at the interface of brain and cannula, where the concentration of reagent should be highest during delivery, became fenestrated. Fenestra formation was irreversible for at least one month. One month after cannula removal, fenestrae persisted in some vessels and exogenous, circulating peroxidase tracer could exude from vessels.

331.3

EXPRESSION OF BLOOD-BRAIN BARRIER CHARACTERISTICS FOLLOWING NEURONAL LOSS USING A SUICIDE TRANSPORT AGENT. J.M. Krum*, K. Kenyon, and J.M. Rosenstein. Dept. of Anatomy, George Washington University Medical Center, Washington, D.C. 20037

Recent studies have suggested that astrocytes can induce certain endothelial blood-brain barrier (BBB) characteristics in vitro. There is also some evidence that neurons may influence BBB marker expression. The aim of this in vivo study is to determine whether the expression of certain BBB markers will change following neuronal loss in a localized area of the cerebrum. To effect neuronal death, adult rats were given intrastriatal injections (1-3 μl) of the immunotoxin OX7-SAP. OX7-SAP is a conjugate of a Thy-1 antibody and the ribosome inactivating protein saporin. Since Thy-1 antigen is expressed only on adult rat neurons throughout the CNS, OX7-SAP is an effective neurotoxic agent. At the injection site, neuronal degeneration, angiogenesis and gliosis occur in an area proportional to the volume of the injection, with the larger volumes forming a cystic area by two weeks postinjection. The immunocytochemical expression of BBB markers, including the brain endothelial glucose transporter (GLUT-1) and permeability to endogenous protein (rat serum albumin - RSA) were examined together with astroglial, macrophage and neuronal markers from one to six weeks after the injection. Preliminary results indicate that the barrier to endogenous protein had not reformed even after four weeks, despite dense astrocytic reactivity. In contrast, the vasculature maintained expression of GLUT-1 throughout the lesioned area, and most notably in regions of granulation tissue that lacked both astroglia and neurons. These results suggest that astrocytes may not be the sole regulating factor for BBB maintenance in situ. (Supported by NS-17468).

331.4

HISTOLOGICAL CHANGES DURING THE FIRST DAYS FOLLOWING INTRAPERITONEAL ADMINISTRATION OF GOLD THIOGLUCOSE. A. Czurkó [1] and F. Gallyas[2]. SPON: European Neuroscience Association. Inst. of Behavioral Sciences[1] and Inst. of Neurosurgery[2], Pécs Univ. Med. School, H-7643 Pécs, Hungary.

Histopathological changes in the mouse brain during the first days following intraperitoneal administration of gold thioglucose were examined. For the demonstration of blood brain barrier dysfunction anti mouse IgG immunocytochemistry was used. Diffuse neuronal damage was demonstrated by a special silver method selectively staining "dark" (collapsed) neurons.

At the 12th hour after gold thioglucose administration the blood brain barrier opened in two isodimensional hypothalamic areas next to the median eminence. Diffuse neuronal damage was observed only in a 200 μm thick hypothalamic zone bordering them. No morphologically damaged neurons were seen in any hypothalamic areas before the appearance of blood brain barrier disturbance. The size of the foci with opened blood brain barrier considerably increased with time while the "dark" neuronal damage did not "infiltrate" hypothalamic areas far from these foci.

These findings support the idea that gold thioglucose-induced disturbance of feeding behavior is caused by a parenchymal lesion having its origin in the opening of the blood brain barrier rather than by a putative selective vulnerability of certain hypothalamic neurons to gold thioglucose.

331.5

PHARMACOKINETICS AND BLOOD-BRAIN BARRIER (BBB) TRANSPORT OF [^{3}H]-BIOTINYLATED PHOSPHOROTHIOATE OLIGODEOXYNUCLEOTIDE COUPLED TO A VECTOR-MEDIATED DRUG DELIVERY SYSTEM. D. Wu, R.J. Boado, B. Howard*, and W.M. Pardridge. Depts. of Medicine and Biological Chemistry, UCLA School of Medicine, Los Angeles, CA 90024.

Phosphorothioate oligodeoxynucleotides (PS-ODN) are potential neuropharmaceuticals, should these highly charged anionic molecules be made transportable through the brain capillary endothelial wall, which makes up the BBB in vivo. The present studies examine the extent to which BBB transport of a PS-ODN may be enhanced by coupling to a vector-mediated drug delivery system. The vector is comprised of a conjugate of streptavidin (SA) and the OX26 monoclonal antibody to the rat transferrin receptor. The PS-ODN was an 18-mer complementary to the rev gene and nucleotides 5551-5568 of the human immunodeficiency virus (HIV)-1 genome. The PS-ODN was synthesized with a 3'-biotin moiety and 5'-amino group, which allowed for tritiation with [^{3}H]-N-succinimidyl propionate. In the absence of conjugation to the vector, the BBB transport of the PS-ODN was negligible. Following conjugation of the [^{3}H]-bio-PS-ODN to the OX26/SA vector, the BBB transport of the ODN was appreciable with a BBB permeability-surface area (PS) product of 5.2 μL/min/g, as measured with an internal carotid artery perfusion method. However, following intravenous injection, the BBB PS product of the [^{3}H]-bio-PS-ODN/OX26-SA was 30-fold lower, 0.17 ± 0.01 μL/min/g. The inhibition of BBB transport of the PS-ODN conjugated to the OX26/SA vector following intravenous injection was attributed to avid plasma protein binding of the PS-ODN. The inclusion of physiologic concentrations of plasma proteins to the internal carotid artery perfusate resulted in a reduction of the BBB transport of the PS-ODN. In summary, the advantages of metabolic stability conferred by the use of PS-ODN is offset by the avid plasma protein binding of PS-ODNs, and this binding retards the BBB transport in vivo of PS-ODNs conjugated to vector-mediated delivery systems.

331.6

KINETICS OF [D-PENICILLAMINE^{2,5}]ENKEPHALIN (DPDPE) ENTRY ACROSS THE BLOOD-BRAIN AND BLOOD-CSF BARRIERS. S. A. Williams, T. J. Abbruscato, V. J. Hruby and T. P. Davis*. Departments of Pharmacology and Chemistry, University of Arizona, Tucson, AZ 85724.

Previously in our laboratory we have found that intravenously administered DPDPE, a *delta* opioid-receptor selective enkephalin, can enter the CNS and produce analgesia (*J. Pharm. Exp. Ther. 259, 1109-1117*). Its route of CNS entry has also been investigated and has been shown to occur mainly through the blood-brain barrier (BBB), the blood-CSF barrier playing only a minor role. The aim of this present study was to further characterize the kinetics of the uptake of DPDPE into the CNS, by means of a vascular brain perfusion technique. Adult rats were anaesthetised and heparinised, the carotid arteries cannulated and perfusion started with an oxygenated, mammalian Ringer. The jugular veins were sectioned and [^{3}H]DPDPE, in the presence of unlabelled DPDPE (0-0.1mM), was infused into the perfusion medium for 20 minutes. A cisterna magna CSF sample was taken and the animal decapitated. Brain, CSF and perfusate samples were then taken for radioactive counting. These experiments revealed a saturable [^{3}H]DPDPE uptake into brain that followed Michaelis-Menten kinetics with a Km of 1.87±0.82 μM and Vmax of 3.34±0.31 $pmol.min^{-1}.g^{-1}$ (n=39, 5 concs; ±S.E.M.). These values are similar to those achieved with arginine-vasopressin (*Biochim. Biophys. Acta, 1025, 191-198*). However, in the presence of 0.1mM unlabelled DPDPE, uptake of [^{3}H]DPDPE into the CSF could not be inhibited. The non-saturable component, Kd, was significantly different from zero for both brain 0.88±0.10 and CSF 0.89±0.07 $\mu l.min^{-1}.g^{-1}$. We conclude that using this brain perfusion technique in the anaesthetised rat, [^{3}H]DPDPE entry into the CNS can occur by a high affinity saturable transport mechanism, which is likely to be found at the BBB. However, [^{3}H]DPDPE can also enter into brain and CSF by non-saturable mechanisms. This work supported by NIDA #DA-06284.

331.7

BLOOD-BRAIN BARRIER PERMEABILITY AND BIOAVAILABILITY OF A POTENT AND SELECTIVE MU OPIOID RECEPTOR ANTAGONIST, CTAP. T.J. Abbruscato*, S.A. Williams, V.J. Hruby, T.P. Davis. Departments of Pharmacology and Chemistry, University of Arizona, Tucson, AZ 85724

CTAP (D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2) is a cyclic octapeptide that is structurally related to somatostatin. It displays greater antagonist potency and a greater degree of selectivity for mu opioid receptors than the classical mu-selective antagonist CTOP (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2). These investigations attempt to characterize the ability of CTAP to cross the blood-brain and the blood-CSF barriers of the rat using a vascular brain perfusion technique. Adult rats were anaesthetized and the carotid arteries cannulated. Perfusion was performed with an oxygenated mammalian Ringer and outflow was through the sectioned jugular veins. Radiolabelled CTAP and sucrose were introduced into the perfusate via a slow-drive syringe. After a set perfusion time, a cisterna magna CSF sample was taken and the animal decapitated. The uptake of radiolabelled CTAP and sucrose into the brain and CSF was expressed as the percentage ratio of tissue to plasma activities (RTissue,mean±S.E.M. $ml \cdot g^{-1}$; n=4-6).

	[^{3}H]CTAP		[^{14}C]Sucrose	
Time	RBrain	RCSF	RBrain	RCSF
2.5min	0.023±0.003	0.005±0.001	0.014±0.003	0.003±0.011
10min	0.038±0.002	0.025±0.035	0.017±0.002	0.014±0.004
20min	0.044±0.003	0.052±0.012	0.018±0.001	0.012±0.002

Results show that the brain and CSF uptake of [^{3}H]CTAP was significantly greater than that of [^{14}C]sucrose. ($P<0.01$; Students t-test). In addition, protein binding studies revealed that [^{3}H]CTAP bound extensively (45.1%) to the 0.1% bovine serum albumin in the perfusion medium thereby limiting the bioavailability of CTAP. These data suggest that [^{3}H]CTAP can cross the blood-brain and the blood-CSF barriers of the rat. This work is supported by NIDA #DA-06284.

331.8

EXTENDED MODEL OF THE BINDING SITE OF THE CEREBROVASCULAR LARGE NEUTRAL AMINO ACID TRANSPORTER. Mitsuhiko Hokari and Quentin R. Smith*. Lab. of Neurosciences, National Institute on Aging, NIH, Bethesda MD 20892.

The structure of the binding pocket of the cerebrovascular large neutral amino acid transporter (System L1) was probed by competitive inhibition of L-[^{14}C]leucine uptake into brain using various cyclic aliphatic and aromatic L-amino acid analogs. Solute affinity was estimated as the inverse of the concentration that gave 50% inhibition of cerebrovascular L-[^{14}C]leucine transport into brain using the *in situ* rat brain perfusion technique. L-[^{14}C]leucine influx was shown to be mediated by a single, BCH-inhibitable mechanism (System L1) that exhibited strong preference for amino acids with hydrophobic side chains. The apparent linear relation between transport affinity and amino acid side chain hydrophobicity, which was first noted in 1987, was extended 2-orders of magnitude in the hydrophobic direction using various long-chain aliphatic and aromatic amino acids with octanol/water partition coefficients ranging from 0.1 to 300. Marked preference was noted for the meta-substituent position of L-phenylalanine, though the para position could be used with appropriate modification. Hydrophobic non-amino acid analogs were also identified that inhibited transport, thus demonstrating that the amino acid functional group was not required for binding activity. The minimum dimensions of the binding pocket cavity were probed with hydrophobic amino acids with fixed ring structures. From these data, a preliminary 3-dimensional model was developed of the L1 system binding site that will be of use in future studies of brain amino acid transport.

331.9

NITRIC OXIDE SYNTHASE INHIBITION BY BREFELDIN IN CEREBROVASCULAR ENDOTHELIUM IS REVERSIBLE. A. M. Morin*, A. Stanboli, A. Pham, F. Solamo and L. Te. V.A. Medical Center*, Sepulveda, CA and Dept. Neurology, UCLA School of Medicine, Los Angeles.

Nitric oxide synthase (NOS) can be visualized in paraformaldehyde-fixed cultured cerebrovascular endothelium(CVE) cells by NADPH diaphorase activity and immunostaining with anti-endothelial NOS antibody. CVE NOS is predominantly membrane-bound and appears to be associated with the Golgi complex(GC) in a unique juxtanuclear position(Morin A. & A. Stanboli,J. Neurosci. Res.36(1993)272-279). Exposure of the CVE to Brefeldin A (BFA), a mold metabolite which binds to specific ADP-ribosylation factor recognition sites on the Golgi complex, inhibits NOS activity. Decreased production of ^{3}H-citrulline from ^{3}H-arginine was observed when CVE were pretreated with BFA for 90min (↓ 78%) during which time the GC became disrupted and dispersed into the cytoplasm. NADPH diaphorase staining was also dispersed. BFA also inhibited NOS when present only during the 15min assay (↓ 44%). BFA inhibition of NOS was reversible after removal of the compound and 4hrs recovery. GC and NADPH diaphorase staining also returned to pre-BFA-exposure state. BFA inhibits the binding of specific ADP-ribosylation factors (ARFs) to the GC in brain (Tsai et al., J. Biol. Chem., 268(1993) 10820-10825). These ARFs bind GTP very tightly and are required for the integrity of the secretory processes of the GC. The role of NOS at the GC remains to be elucidated but appears likely to involve a membrane-mediated stimulation of ADP-ribosylation.

331.10

CHANGES IN THE EFFLUX OF ACIDIC AMINO ACIDS FROM BRAIN TO BLOOD WITH MATURITY IN RATS. H. Al-Sarraf, I.E. Preston*, M.B. Segal. *Physiology Dept., UMDS, London SE1 7EH, UK. *ACIOG, King's College, London.*

The acidic amino acids, aspartate (Asp) and Glutamate (Glu), are major excitatory neurotransmitters in the CNS. Their brain concentrations exceed plasma concentration by 20-100 fold (Price et al, 1990). In our earlier studies, we have shown a decline in the entry from blood to brain of Asp and Glu with age in the rats (Al-Sarraf et al, 1994). In this investigation using the bilateral in situ brain perfusion (Preston et al, 1995), the efflux of these amino acids from brain to blood was studied in adult and 2 week old rats. The brain was perfused with Ringer containing ^{14}C-Asp or ^{14}C-Glu for 10 min. The perfusion was then continued with ^{14}C-free perfusate for a further 20 min and samples of the jugular venous outflow taken at 30 sec intervals. The *ln* dpm in each sample was then plotted against sample time and the half time ($t1/2$) was measured (Preston et al, 1993). The $t1/2$ for 2 week old Asp was 16.16 ± 0.76 min, while that for the adult was 10.06 ± 0.46 min (means ± sem, n=3-4). Glu $t1/2$ was also smaller in the adult which was 50% of the 2 week old value. The above results, in addition to our previous studies, suggest that the systems involved in the influx and efflux of the acidic amino acids into and out of the brain, favour increased brain levels of amino acids in the developing brain.

331.11

PERMEABILITY AT THE BLOOD-BRAIN AND BLOOD-NERVE BARRIERS OF THE NEUROTROPHIC FACTORS: NGF, CNTF, NT3, BDNF Joseph F. Poduslo* and Geoffrey L. Curran, Molec. Neurobiol. Lab. Mayo Clinic and Foundation, Rochester, MN 55905

A comparison was made of the permeabilities of different neurotrophic factors at the BBB and BNB in normal adult rats by quantifying the permeability coefficient-surface area (PS) product after correction for the residual plasma volume (V_p) occupied by the protein in the capillary bed. The I.V. bolus injection technique was used in the cannulated brachial vein and artery using the same protein radioiodinated with a second isotope of iodine (^{125}I vs. ^{131}I) to separately determine the PS and V_p values. The plasma washout showed a decreasing plasma half-life in the order of BDNF < NT3 < CNTF < NGF. The PS at the BNB for NGF was $1.40 \pm 0.15 \times 10^{-6}$ ml/g/s ($\bar{x}$ ± SEM). The other neurotrophic proteins were all significantly higher than NGF (CNTF: 9.5X; NT3: 20.8X; BDNF: 18.9X). The V_p for NGF was 1.92 ± 0.12 µl/g and was not significantly different from the other proteins except for NGF vs. BDNF ($P < 0.05$). The PS at the BBB for NGF ranged from 1.5 to 2.7×10^{-6} ml/g/s for six different brain regions. The PS for CNTF ranged from 6.0 - 8.0 fold higher than NGF; NT3: 10.6 - 15.2 fold higher; and BDNF: 11.3 - 16.4 fold higher. The V_p values were not significantly different except for CNTF in the hippocampus and cortex ($P < 0.05$) (ANOVA). SDS-PAGE analyses of all the radioiodinated neurotrophic proteins after 60 min of uptake revealed intact protein in the endoneurium and in the six different brain regions with exposure times of 2-42 days. The quantification of the permeability of these neurotrophic proteins provides baseline values for comparison of different protein modifications that enhance the PS while still preserving the neurotrophic activity (e.g., protein glycation; see Molec. Brain Res. 23, 157, 1994).

331.12

REGIONAL DIFFERENCES IN BLOOD-BRAIN BARRIER TO ALBUMIN IN NORMAL AND SENESCENCE-ACCELERATED MICE. A.W. Vorbrodt*, D.H. Dobrogowska and M. Ueno. NYS Institute for Basic Research in Develop. Disabilities, Staten Island, NY 10314.

The blood-brain barrier (BBB) to endogenous albumin was studied in the cerebral cortex, hippocampus, pons and bulbus olfactorius of senescence-accelerated prone (SAMP8) and senescence-accelerated resistant (SAMR1) mouse strains in corresponding age groups (4-month-old and 12-month-old). Ultrathin sections of immersion-fixed brain samples embedded in Lowicryl K4M were exposed to anti-albumin antiserum followed by protein A-gold. The density of immunosignals (gold particles per μm^2) was recorded over four compartments: vascular lumen, endothelium, subendothelial space, and the neuropil. Morphometric and statistical analysis indicated that the barrier function of the capillaries located in the cerebral cortex and pons of SAMP8 mice was not significantly different from that in control (SAMR1) mice, although the percentage of leaking capillaries was higher in SAMP8 in both age groups. In the hippocampus, significantly higher extravasation of albumin in SAMP8 than in SAMR1 mice was observed, even in 4-month-old animal groups. Also, the labeling density of the hippocampal neuropil of both groups of mice was significantly higher than that in the cerebral cortex. These observations suggest that blood-borne albumin has access to the parenchyma of the hippocampus, presumably from the barrier-free areas such as the subfornical organ, and that this phenomenon is more pronounced during aging. Only in the bulbus olfactorius were some capillaries and larger microvessels (presumably venules) leaking in both control and SAMP8 groups, although in the latter group, older animals (12-month-olds) show an increased percentage of leaking vessels up to 35%, as compared to 10% in controls. These data indicate that the BBB in the olfactory bulb is not as tight as it is in other examined brain regions. (Supported by the National Institute on Aging, grant RO1-AG 10279-04).

331.13

CIS-ELEMENT/CYTOPLASMIC PROTEIN INTERACTIONS BETWEEN THE 3'-UNTRANSLATED REGION OF THE HUMAN GLUT1 GLUCOSE TRANSPORTER mRNA AND CYTOSOLIC PROTEINS IN HUMAN BRAIN TUMORS. H. Tsukamoto, R.J. Boado, C. Markham*, K.L. Black, and W.M. Pardridge. Depts. of Medicine, Neurology, and Surgery/Neurosurgery. UCLA School of Medicine, Los Angeles, CA 90024.

The principal glucose transporter at the blood-brain barrier is GLUT1, and mechanisms of GLUT1 gene expression may be evaluated in human brain tumors. High grade gliomas express the GLUT1 glucose transporter mRNA but have depressed levels of the immunoreactive protein, suggesting possible post-transcriptional mechanisms of GLUT1 gene expression. The interactions of cytosolic proteins isolated from human glioblastoma multiforme (GBM) with specific sequences within the 3'-untranslated region (UTR) of the GLUT1 mRNA were evaluated with RNase T1 protection assays. Full-length human GLUT1 mRNA was prepared from a transcription plasmid and, following protection by binding with GBM cytosolic proteins, was degraded with RNase T1. The protected RNA fragments were cross-linked to cytosolic proteins using ultraviolet (UV) light and separated by SDS-PAGE. These studies identified complexes of 120, 44, and 41 kDa molecular weight. The formation of the complexes was inhibited by high concentrations of unlabeled competitor GLUT1 mRNA. Cis sequences were mapped with RNase T1 to the region of nucleotides 2173-2224 of the GLUT1 mRNA. Competition studies with specific oligodeoxynucleotides further defined the location of the cis-regulatory region to nucleotides 2186-2203, which specifically bound to the 120 kDa protein. The 44/41 kDa complex was overexpressed in GBM compared to a human hemangioblastoma, a tumor that overexpresses GLUT1 immunoreactive protein. These studies support the hypothesis that GLUT1 gene expression in human brain tumors is subject to post-transcriptional regulation involving cytosolic proteins and specific cis sequences within the 3'-UTR of the GLUT1 mRNA.

331.14

IN VIVO PHOTOLABELING AND VISUALIZATION OF BLOOD-BRAIN BARRIER GLUT1 GLUCOSE TRANSPORTER. Nathan, M. Appel*, Mitsuhiko Hokari, Fran Maher, Ian A. Simpson, Quentin R. Smith and Susan J. Vannucci. FDA CDER, Laurel, MD 20708; NIDDK and NIA LNS, Bethesda, MD 20892; Pediatrics, Penn. State U., Hershey, PA 17033.

The 55 kDa form of the GLUT1 glucose transporter protein in cerebral microvessels mediates glucose transport from blood into brain. It appears to be asymmetrically distributed across endothelial cells with the greater proportion on the abluminal membrane. Acute and chronic changes in glucose transport rate into brain may be mediated by a redistribution of GLUT1 transporters. The impermeant photolabel 2-*N*-4-[^{3}H](1-azi-2,2,2-trifluoroethyl) benzoyl - 1,3-bis-(D-mannose-4-yloxyl) - 2 - propylamine ([^{3}H]ATB-BMPA) binds to exofacial glucose transporters. We used this to develop a method to quantitate and visualize GLUT1 present on luminal membranes of cerebral microvessels. Brains of anesthetized male Sprague-Dawley rats were perfused through a carotid artery with 1 mCi [^{3}H]ATB-BMPA in the absence or presence of 0.5 mM cytochalasin B. The brains were collected, cut into slabs and irradiated with high intensity UV light. Microvessels or 15 μm cryostat sections were prepared from the slabs. Binding to GLUT1 was quantitated from solubilized extracts of microvessels and by Western blot analysis. There was significant incorporation of [^{3}H]ATB-BMPA into 55 kDa GLUT1 and it was inhibited by cytochalasin B. Autoradiography revealed grains in registration with vascular endothelium. These data demonstrate the feasibility of quantifying GLUT1 glucose transporters on the blood brain barrier luminal surface *in vivo* and of assessing alterations in accessible GLUT1 under experimental and pathological conditions.

331.15

THE EFFECT OF AMMONIUM ON POTASSIUM ENTRY INTO THE BRAIN, CHOROID PLEXUS AND CSF OF THE RAT. K.A. Smart and M.B. Segal*. *Physiology Dept, UMDS, London, SE1 7EH, UK.*

During acute liver failure, it is well known that plasma NH_4^+ levels rise to in excess of three times their normal plasma concentration (39.9 ± 3.7μM). For this reason, NH_4^+ is believed to be one of the major toxins involved in the clinical symptoms of Hepatic Encephalopathy (Schenker et al, 1974). It has also been proposed that lipid insoluble NH_4^+ crosses the blood brain barrier (BBB) by competing with K^+ at channels and carrier molecules of the BBB (Cooper and Plum, 1987). Using the bilateral in situ brain perfusion technique of Preston et al (1995), and dextran density capillary depletion analysis, we have established a unidirectional uptake profile for K^+ ions using ^{86}Rb as a marker molecule. K_{in} values were calculated for brain tissue, choroid plexuses (CP), CSF, and capillary endothelial cells (CE), for ^{86}Rb alone, and ^{86}Rb in the presence of 1mM NH_4^+ ions :

K_{in}	Brain	CP	CSF	CE
^{86}Rb	4.10±0.113	75.45±1.3	5.75±0.54	0.38±0.18
^{86}Rb +NH_4^+	3.03±0.115	49.54±0.9	4.70±0.18	0.86±0.15

Values are means ± sem, in $\mu l.min^{-1}.g^{-1}$, (n=6).

The above results may suggest that NH_4^+ ions inhibit K^+ uptake at the abluminal face of the BBB, due to the accumulation of ^{86}Rb in the CE in the presence of NH_4^+. Seperate experiments using ^{22}Na as a permeability marker, have shown that the permeability and integrity of the BBB are not altered by NH_4^+.

331.16

IMMUNOHISTOCHEMICAL LOCALIZATION OF ARGININE VASOPRESSIN IN RAT CHOROID PLEXUS. J. Szmydynger-Chodobska*, S. Corsetti, N. Limthong, P.R. Monfils, C.E. Johanson, and A. Chodobski. Program Neurosurg., Dept. Clin. Neurosci. and Central Res. Labs., Brown Univ./R.I. Hospital, Providence, RI 02903.

Arginine vasopressin (AVP) has been shown both *in vivo* and *in vitro* to regulate the secretory function of the choroid plexus. In the present study, we demonstrate by immunohistochemistry the presence of AVP in rat choroidal tissue. Both polyclonal rabbit anti-AVP (Incstar) and anti-vasopressin-associated neurophysin (NPII) (provided by Dr. A.G. Robinson of University of Pittsburgh) antibodies were used. Rats were perfused transcardially with 4% paraformaldehyde. Choroid plexuses were subsequently dissected and postfixed for 2 h at 4 °C in paraformaldehyde solution. Free-floating tissue was incubated with primary antibodies (1:1000–1:2000) for 68 h at 4 °C. The immunopositive reaction was visualized by using secondary antibody (biotinylated donkey anti-rabbit IgG) and streptavidin-biotin-peroxidase complex. DAB-$NiSO_4$ was used as a chromagen. A distinct pattern of both AVP- and NPII-immunopositive staining was detected in the choroidal epithelium, with the immunoreactive product predominantly localized close to the apical (CSF-facing) membrane of epithelial cells. The AVP-ergic innervation was not found to be present in the choroid plexus. Similar to choroidal epithelium, the AVP-immunoreactive staining was also identified in the tracheal secretory epithelium. Our findings suggest that AVP plays a role in the regulation of epithelial secretory function. Supported by RIH RD Grant 7512-01-95 and by NIH Grants NS27601 and AM16166 (A.G. Robinson).

331.17

THE PRESENCE OF ARGININE VASOPRESSIN IN THE CHOROID PLEXUS OF THE BRATTLEBORO RAT. A. Chodobski*, S. Corsetti, J. Szmydynger-Chodobska, P.R. Monfils, and C.E. Johanson. Program Neurosurg., Dept. Clin. Neurosci. and Central Res. Labs., Brown Univ./R.I. Hospital, Providence, RI 02903.

Arginine vasopressin (AVP) has been shown to be produced in several peripheral tissues in the *diabetes insipidus* Brattleboro rats despite the lack of the central AVP synthesis in these animals. In the present study, we demonstrate by immunohistochemistry the presence of AVP in the choroid plexus of the homozygous (*di/di*) Brattleboro rats. Animals were perfused transcardially with 4% paraformaldehyde. Choroid plexuses were subsequently dissected and postfixed for 2 h at 4 °C in paraformaldehyde solution. Free-floating tissue was incubated with polyclonal rabbit anti-AVP antibodies (Incstar; 1:1000–1:2000) for 68 h at 4 °C. The immunopositive reaction was visualized by using secondary antibody (biotinylated donkey anti-rabbit IgG) and streptavidin-biotin-peroxidase complex. DAB-$NiSO_4$ was used as a chromagen. A distinct pattern of AVP-immunopositive staining was detected in the choroidal epithelium, with the immunoreactive product predominantly localized close to the apical (CSF-facing) membrane of epithelial cells. This AVP-immunopositive staining of choroidal tissue was similar to that observed in AVP-competent animals. As expected, no AVP-immunopositive neurons were found in both paraventricular and supraoptic hypothalamic nuclei of Brattleboro rats. The ability of choroidal epithelium to produce AVP in Brattleboro rats may be due to the presence of AVP synthetic pathways that are alternate to those operating in hypothalamic neurons. Supported by RIH RD Grant 7512-01-95 and by NIH Grant NS27601.

331.18

AGRIN EXPRESSION ON BRAIN CAPILLARIES DEVELOPS SIMULTANEOUSLY WITH THE BLOOD-BRAIN BARRIER. A.Barber* and E.Lieth. Dept. Neurosci. & Anat., Penn. State Med. Sch., Hershey, PA 17033.

Agrin organizes components of the post-synaptic membrane at the neuromuscular junction (NMJ), but is also found on brain capillaries, where it may function at the blood-brain barrier (BBB). Expression of vascular agrin on capillaries with or without selective barrier properties in various rat and chick tissues was therefore compared by double label immunohistochemistry using monoclonal antibodies to agrin and a specific endothelial cell marker.

In tissues with vascular diffusion barriers (brain, choroid plexus, retina, kidney and testicle) capillaries are agrin immunoreactive (IR) while in other tissues such as heart, skeletal muscle and liver agrin IR is not evident on microvessels. On adult brain microvessels agrin IR forms a continuous layer around the entire surface of both in situ and isolated brain capillaries and is indistinguishable from laminin IR, consistent with localization at endothelial cell basal lamina. Over the period of BBB development on rat microvessels weakly stained patches of agrin IR are present before embryonic day 14 and coalesce into bright, continuous tubes by postnatal week 2, while in chick brain vascular agrin IR is first observed at E9 and maximizes just before hatching.

The selective expression of agrin at the developing BBB and capillaries with barrier properties in other tissues suggests that agrin is a component of the BBB and may, by analogy to its function at the NMJ, organize the other components of the BBB.

331.19

EXPRESSION OF THROMBOMODULIN mRNA IN BOVINE BRAIN CAPILLARIES. L. Wang*, N. D. Tran, J. Goodman, J. G. McComb, M. H. Weiss, S. S. Schreiber and B. V. Zlokovic Dept. of Neurosurg. and Divn. of Neurosurg. CHLA, USC Sch. Med., Los Angeles, CA 90033.

Thrombomodulin (TM) is normally expressed at the luminal surface of endothelial cells from the systemic circulation. TM serves as a binding site for thrombin and the thrombin-TM complex activates protein C which shifts the hemostatic balance toward anticoagulation. The evidence for the presence of TM in brain microvascular endothelium, i.e., at the blood-brain barrier (BBB), is controversial. In this study we used isolated bovine brain capillaries as a model system to examine the expression of TM at the mRNA level. By using the reverse transcription-polymerase chain reaction (RT-PCR), we were successful in amplifying 707 basepair (bp) fragment of the TM coding sequence (primer pair from nucleotides 228-256 and 908-935) from cortical capillaries. TM mRNA was absent from capillary-depleted brains. Next, we determined whether there are regional differences in TM capillary expression. The amount of TM mRNA from capillaries obtained from the cortex, pons and cerebellum were compared by competitive quantitative PCR. A modified PCR mutagenesis technique was used to synthesize the TM competitor, a TM fragment of equivalent length containing a unique Sal I restriction site. Preliminary analysis revealed that the TM mRNA was most abundant in cortical capillaries, while its level of expression was significantly lower in cerebellar capillaries. Pons capillaries expressed only very little TM mRNA. It is concluded that the BBB may have a role in promoting the protein C anticoagulation pathway within brain microcirculation, and that this function may be region dependent.

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GENE STRUCTURE AND FUNCTION II

332.1

AN ALTERNATIVE PROMOTER IN THE MOUSE ACETYLCHOLINESTERASE GENE? E. Atanasova, S. Chiappa and S. Brimijoin* Dept. of Pharmacol., Mayo Clinic, Rochester, MN55905

Despite advances in the molecular neurobiology of acetylcholinesterase (AChE), regulation of the AChE gene in neurons is poorly understood. A minimal promoter has been identified, but alternative promoters may exist. We examined this possibility using RAGE (Rapid Amplification of Genomic Ends), a PCR method to amplify unknown genomic DNA next to known sequences. Mouse genomic DNA and Bluescript II SK(+) plasmid were digested in separate reactions with one of several restriction endonucleases and ligated to each other. Using the ligated samples as templates, two consecutive PCR amplifications were performed with nested pairs of Bluescript-specific primers and AChE primers targeted at the 5' end of the known genomic sequence. With XhoI digests, a 761 bp DNA product was obtained, which extended 5' from the cap site to position -1261. Digoxigenin-labeled RAGE DNA hybridized to the same clones in a mouse genomic library as full length AChE cDNA. Analysis of the RAGE sequence revealed potential binding sites for transcription factors including SP1, ATF/CREB, AP2, clustered Myo D and NFkB. One CCAAT box was found, but no typical TATA box. To test for promoter activity, RAGE-DNA was linked to the open reading frame of a luciferase reporter in a pGL2 vector and transfected into NIE.115 neuroblastoma. Cell lysates were tested for luciferase activity at 48 & 72 h. Compared with a positive control (luciferase gene + SV40 promoter & enhancer) the RAGE construct yielded 15% as much luciferase activity, over 4 times the yield from a promoterless construct. Therefore the RAGE sequence can serve as a weak AChE promoter in neuronal cell culture. Whether it does so in vivo remains to be determined.

332.2

YY-1 REGULATES THE TRANSCRIPTIONAL LEVEL OF HUMAN DOPAMINE β-HYDROXYLASE GENE BY TPA STIMULATION. H. Ishiguro*, K. Yamada, T. Nagatsu and K. Fujita Institute for Comprehensive Medical Science, School of Medicine , Fujita Health University, Toyoake, Aichi 470-11, Japan.

Dopamine β–hydroxylase (DBH) converts dopamine to norepinephrine and is specifically expressed in noradrenergic and adrenergic neurons. Cyclic AMP response element (CRE, TGACGTCC) of human DBH gene is an essential transcriptional element in the promoter region. In downstream of this CRE , there are three important DNA elements such as YY-1 binding sequence (CCAT), E-box (CANNTG), and AP-1 like sequence (TGTGTCA) that may regulate the transcription of many genes.

To identify the DNA binding proteins, 26 bp oligonucleotide (actgaTGACGTCCATGTGTCAttagt) was used as a probe for the electrophoretic mobility shift assay (EMSA). According to the site-directed mutagenesis, both CRE and YY-1 binding sequence formed complexes with proteins, but E-box and AP-1 like sequences did not. The addition of CRE oligonucleotides for tyrosine hydroxylase (TH) and somatostatin genes as competitors eliminated upper-shifted bands that were formed with CRE and CRE binding protein (CREB or ATFs). Furthermore, an excess amount of oligonucleotide that is the YY-1 binding sequence of *c-fos* gene as a competitor, completely eliminated the intermediate-shifted band. In order to assess the function of YY-1 binding to the CCAT motif, transient transfection experiments were performed in the DBH-expressing neuroblastoma cells (SK-N-SH). Site-directed mutagenesis experiments indicated that YY-1, a ubiquitously expressed zinc finger type DNA binding protein, positively regulated the transcriptional level of human DBH gene by the phorbol ester (TPA) stimulation. These data suggest that YY-1 is important in the transcriptional regulation of DBH gene.

332.3

MOLECULAR MECHANISMS OF INCREASED EXPRESSION OF DOPAMINE ß-HYDROXYLASE BY STRESS E.L. Sabban*[1], B. Nankova [1], R. Kvetnansky[2] and B. Hiremagalur[1]. [1]Dept. Biochem & Mol. Biol., New York Medical Coll., Valhalla, NY 10595; [2]Inst. Exp. Endocrinology, Bratislava, Slovakia.

Dopamine ß-hydroxylase (DBH) gene expression is elevated *in vivo* and *in vitro* by a number of physiologically relevant treatments. Cloning of the 5' upstream region of the gene (McMahon & Sabban, 1992, JNC, 59, 2042-2047) revealed several putative promoter elements which may be involved in regulation of DBH including 3 putative glucocorticoid regulatory sites. Another element termed DBH-1, was shown to be involved in induction of DBH expression by cAMP and phorbol esters in cell cultures (Shaskus et al., 1992, JBC, 267, 18821-30). Our previous studies found activation of DBH gene expression with immobilization stress. The aim of this study was to analyze the molecular mechanism of this regulation. Increased binding to adrenomedullary nuclear factors to DBH-1 was observed with repeated IMO by electrophoretic mobility shift analysis. Specific antisera and UV-cross linking indicated that the complex contains fos-related proteins, but not c-fos. Site directed mutation of DBH-1 showed that, the more distal of two adjacent putative cAMP-regulatory sites in DBH-1, is involved in the binding.

To test the possible additional involvement of glucocorticoid regulatory elements, the requirement for an intact adrenocortical axis and for neuronal inputs to the adrenal in the stress elicited changes in DBH gene expression was studied.

332.4

ADJACENT CRE-LIKE AND HOMEODOMAIN SITES IN THE PROMOTER OF THE RAT DOPAMINE B-HYDROXYLASE GENE CAN CONTRIBUTE INDIVIDUALLY TO SECOND MESSENGER RESPONSES. DJ Swanson, E Zellmer, Z Zhang, EJ Lewis* Dept. Biochemistry and Molecular Biology, Oregon Health Sci. Univ., Portland OR, 97201

Dopamine β-hydroxylase (DBH), the enzyme which converts dopamine to norepinephrine, is expressed in a cell-type restricted manner in neuroendocrine tissues which secrete norepinephrine and epinephrine. Studies have suggested that the regulation of DBH gene expression by activators of cAMP/PKA second messenger systems may contribute to the tissue specificity of gene expression. We have previously identified a pair of homeodomain (HD) core recognition sites (ATTA) within the proximal 230 bp of the promoter of the rat DBH gene. Adjacent to the 5' HD domain are two CRE-like domains separated by a single base pair. Through systematic deletion and mutational analyses of a wild-type DBH (-230/+14)-CAT construct we have examined the contribution of these recognition sequences in cAMP/phorbol ester-regulated induction of reporter gene expression.

Mutation or deletion of one of the pair of CREs and/or HDs had no effect on the cAMP/PMA induction of reporter gene expression. There was, however, a marked decrease (5-10 fold) in induction following the deletion or disruption of both CRE domains or both HD core sites separately. This effect was most severe when both CRE and HD sites are deleted. These findings suggest that either domain within the CRE-like pair or HD pair is functional in the 2nd messenger response from this proximal portion of the DBH promoter. Furthermore, it appears that the maintenance of at least one of each CRE and HD site is required for a normal 2nd messenger response.

Preliminary studies have also shown that cotransfection of the wild-type 5' DBH-reporter construct with a DNA binding protein which specifically recognizes either core HD site augments the reporter induction by cAMP. Taken together these findings suggest a possible interaction between these two adjacent promoter proximal regulatory regions of the DBH gene. This interaction may contribute to the tissue specificity of DBH expression.

332.5

PROMOTER ELEMENTS AND SECOND MESSENGERS MEDIATING FGF-2 INDUCTION OF TYROSINE HYDROXYLASE TRANSCRIPTION. H.Osaka*, A.Menezes and E.L. Sabban, Dept. Biochemistry & Molecular Biology, New York Medical College, Valhalla, NY 10595.

Basic fibroblast growth factor (FGF-2) play important roles in neuronal development and survival. Among the effects of FGF-2 in PC12 cells are induction of c-fos and neurite outgrowth. Treatment of PC12 cells with increasing concentrations of FGF-2 elevated tyrosine hydroxylase (TH) mRNA levels in a concentration dependent manner, maximal at 1-8 days of exposure. Promoter elements responsible for induction by FGF-2 were examined by transient tranfection of plasmids containing the chloramphenicol acetyl transferase (CAT) reporter gene under control of the TH promoter. Previous studies showed that mutation of the AP1-like site (-205 to -199) of the TH promoter did not prevent the induction of transcription by FGF-2. In this regard, inhibition of protein kinase C by bisindolylmaleimide prevented the rise in CAT activity by TPA, but not by FGF-2. A TH promoter construct spanning -60 to +27 was sufficient for induction of CAT reporter activity by FGF-2. This construct contains the CRE/CaRE and part of a putative AP2 site. Mutagenesis and gel-shift analysis were conducted to assess the contribution of these elements in TH mRNA induction by FGF-2.

332.6

An Ets-like Transcription Factor(s) Affects Cell-Type Specific Expression of the Rat Tyrosine Hydroxylase Gene in PC12 Cells. S. C. Wong*, M. A. Moffat, and K. L. O'Malley, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Previously we have shown that sequences between -505 an -575 of the rat tyrosine hydroxylase (TH) promoter contribute to cell-type specific expression when introduced into the rat PC12 cell line. Sequence analysis of this 75 bp region revealed the presence of a consensus interferon response factor (IRF-1) binding site (AAGTGG) and two consensus Ets-binding sites (GGAATA). Electrophoresis mobility shift assays (EMSA) using oligonucleotides with mutagenized IRF-1 and Ets-binding sites indicated that neither the IRF-1 site nor the 5' Ets-binding site contributed to the cell-type specific EMSA pattern observed in PC12 versus HepG2 cell lines. Instead the 3' Ets motif was critical for this response. UV crosslinking experiments showed the molecular weight of the putative transcription factor interacting with the 3' Ets-binding site to be approximately 52 kD, which is in the size range of the Ets-like protein family. We used oligonucleotides complementary to Ets proteins, Ets-1 and Ets-2, coupled with RT-PCR and cycle sequencing to show that only Ets-2 is present in PC12 cells. Co-transfection experiments were performed using -575 and -505 TH promoter/Luciferase reporter gene constructs together with Ets-1 sense, Ets-1 anti-sense and Ets-2 sense expression vectors. None of these Ets constructs affected luciferase activity in PC12 cells; however, co-transfection of Ets-2 with the -575 TH promoter construct increased reporter gene activity in HepG2 cells at least 5-fold. These data imply that Ets-2 or an Ets-like factor can regulate TH gene expression in immortalized cell lines. Ets proteins have been shown to play a role in cell growth and differentiation by forming heterodimers with other Ets family members or other non-Ets transcription factors. Conceivably other cell-type specific factors interact with the Ets-like molecule binding the GGAATA motif in the rat TH promoter to generate the observed pattern of expression.

332.7

ANGIOTENSIN II REGULATION OF TYROSINE HYDROXYLASE GENE PROMOTER ACTIVITY IS MEDIATED BY MULTIPLE ELEMENTS. **E. Kim, M.K. Stachowiak, Barrow Neurological Institute, Phoenix AZ 85013.**

In adrenal chromaffin cells (AMC) angiotensin II stimulates transcription of the TH gene by acting through cAMP- and Ca^{++}/PKC-signaling pathways. The roles of CRE- and TRE-like sequences in regulation of bovine TH gene promoter activity were examined by transfecting TH promoter-luciferase constructs into bovine AMC. Mutation of CRE did not affect basal promoter activity but it reduced stimulation with angiotensin from 2.9- to 1.75-fold. A similar reduction was observed when TRE was mutated. Mutation of TRE resulted in reduction of basal promoter activity (-60%) indicating a dual function of this element. The TRE site overlaps with dyad symmetry element (DSE) suggesting regulation by interacting transcriptional factors. DNA mobility gel shift assays detected protein binding to TRE but not to DSE. DSE was sensitive to digestion with S1 nuclease in supercoiled plasmid DNA. Pattern of S1 digestion is consistent with extrusion of cruciform-like structure in which the right arm of TRE is included in cruciform stem. Mutation that disrupts symmetry of DSE prevented secondary structure and enhanced angiotensin stimulation, from 2.2- to 4.4-fold. This enhancement was abolished by additional mutation of TRE. Together, our observations suggest that: (1) both CRE and TRE mediate angiotensin stimulation, and (2) the function of TRE in bovine TH gene promoter is modulated by an extrusion of secondary structure within overlapping DSE (supported by NIH).

332.8

HUMAN VGF PROMOTER: HOMOLOGY TO RAT AND CELL TYPE SPECIFIC EXPRESSION. N. Canu, R. Possenti*, A.M. Rinaldi, E. Trani, B. Giovannone and A. Levi, Institute of Neurobiology CNR, Via K. Marx 43, 00137 Rome, Italy.

VGF is a neuroendocrine specific gene which is transcriptionally induced by the nerve growth factor NGF in the rat PC12 cell line. To better understand the molecular mechanism involved in transcriptional regulation and tissue specific expression of VGF, we have isolated the human gene and its 5' flanking region by screening a human genomic library with a rat probe. We sequenced a 3 kb region upstream of the ATG and by primer extension analysis we localised the transcription initiation site of the gene 29 nt downstream the TATA box. Comparison with the rat sequence revealed striking homology, especially notable over 400 bp upstream the transcription initiation site. This region, previously shown to be the core promoter of the rat VGF gene, displays a high concentration of transcription factors binding sites including TATA box, CCAAT box and cAMP responsive element as well as AP-2 and Sp1 consensus sequences. Furthermore the overall structure of the gene is conserved since all these elements are localised to comparable distance with respect to the transcription initiation site, both in the human and rat gene. The 3kb genomic DNA fragment (- 2300+718) was fused in front of the CAT reporter gene and tested for transcriptional activity by transient transfection in several cell lines. This sequence was able to drive high expression of the reporter gene when transfected in neuronal cell lines. The region between -175+718 was both necessary and sufficient for cell specific expression. Deletions which affect the integrity of the cAMP responsive element, dramatically reduced the induction by NGF and cAMP of the recombinant plasmids transfected in PC12 cell line.

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332.9

SPECIES DIFFERENCES IN THE 5' FLANKING REGION OF THE RODENT OXYTOCIN RECEPTOR GENE L. J. Young* and T. R. Insel, Dept. of Psychiatry and Behavioral Sciences, Emory University, Atlanta GA 30322

Oxytocin and its receptor are involved in the control of complex, species-specific social behaviors such as sexual, maternal and pair bonding behaviors. The neuroanatomical distribution of oxytocin receptors differs greatly among species. These differences in oxytocin receptor distribution are thought to be responsible for species differences in behavior patterns. In order to better understand the molecular basis of the species differences in oxytocin receptor expression in the brain, we have cloned and sequenced the 5' flanking region of the oxytocin receptor gene from two species of microtine voles (*Microtus ochrogaster and M. montanus*) which differ extensively in oxytocin receptor distribution in the brain as well as in social behaviors. The two vole species share 99% nucleotide homology in the 1300 bp sequence upstream of the transcription start site. However, two potential cis regulatory elements in this region differ between the species. In contrast, the 5' flanking region of the oxytocin receptor in the rat differs considerably from that of the voles. Although the rat and vole share 90% nucleotide homology in the coding region, the homology drops to 60% in the first 500 bp of the 5' flanking region and is unrecognizable beyond that. As a result, the pattern of potential cis regulatory elements differs greatly between the species. These differences may be responsible for the species differences in oxytocin receptor gene expression in the brain and provide a potential mechanism for the evolution of species-typical social behaviors. We are currently testing this hypothesis using transgenic technology with an oxytocin receptor promoter construct.

332.10

BOTH UPSTREAM AND INTRAGENIC SEQUENCES OF THE HUMAN NEUROFILAMENT LIGHT GENE DIRECT EXPRESSION OF *lacZ* IN NEURONS OF TRANSGENIC MOUSE EMBRYOS. **L. Leconte, O. Semonin, A. Zvara, S. Boisseau, C. Poujeol, R. Kado* and M. Simonneau, Laboratoire de Neurobiologie Cellulaire & Moléculaire du CNRS, 91198 Gif sur Yvette & LIRBA, Ecole Normale Supérieure de Cachan, 94235 Cachan Cédex, France.**

Molecular mechanisms generating post-mitotic neurons from dividing neuroblasts remain largely unknown in mammals. One strategy is to identify DNA sequences which are sufficient to direct neuronal specificity during embryogenesis. During the development of the mouse embryo, initial expression of the neurofilament coincides with the appearance of post-mitotic neurons and axon extensions. To investigate the molecular mechanisms involved in neuron-specific gene expression during embryogenesis, we generated transgenic mice expressing the *lacZ* reporter gene under the control of various genomic regions of the human neurofilament light gene (hNF-L). We found that 2.3 or 0.3 kb of the hNF-L promoter region directs expression of *lacZ* specifically in neurons of transgenic embryos. Addition of the 1.8 kb of the hNF-L intragenic sequences (including first and second introns) to hNF-L promoter constructs gave a broader neuronal expression pattern of the transgene. Furthermore, the use of an heterologous promoter such as the mouse heat shock promoter allowed demonstration that the 1.8 kb hNF-L intragenic sequences alone are sufficient to direct neuron-specific expression of *lacZ*. We conclude that these hNF-L intragenic sequences contain cis-acting DNA regulatory elements that specify neuronal expression (Leconte et al.,1995 J. Molecular Neuroscience, in press). Taken together, these results show that the neurofilament light gene contains separate upstream and intragenic elements, each of which directs lacZ expression in embryonic neurons.

We are presently using these constructs to direct the expression of modified potassium channels in neurons.(Supported in part by FRM & DRET).

332.11

SIGNIFICANCE OF THE TRE (AP-1 BINDING SITE) IN THE GLUCOCORTICOID REGULATION OF THE GFAP PROMOTER ACTIVITY IN CULTURED ASTROCYTES. I. Rozovsky *, B. Teter, K.K. Krohn, T.H. Hogan and C.E. Finch. Andrus Gerontology Center, Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089.

Transcriptional regulation of GFAP by corticosterone (CORT) in primary cultured astrocytes is influenced by the duration of time in culture and by astrocyte-neuron interactions. In short-term cultures (3 weeks) CORT strongly increased GFAP transcription rate, mRNA, and protein. In contrast, in cultures maintained for 12 weeks, the direction of GFAP response by CORT switched to a decrease, the *in vivo* phenotype of response ("the switch"). The 1.9 kb of the 5'-upstream region of the rat GFAP promoter contains sufficient information to mediate the switch in the direction of GFAP response to CORT. Short- and long-term astrocytes *in vitro* were transiently transfected with the rat GFAP promoter nested-deletion constructs with luciferase reporter. Further analysis of the CORT responsiveness of these promoter constructs revealed a potential role for the TRE (AP-1 binding site) at -1501 in mediating the switch. We also showed synergistic effects of two glucocorticoid response elements in the GFAP promoter sequence that are involved in the regulation of GFAP transcription by CORT in short-term astrocyte cultures.

These findings are relevant to analysis of GFAP responses to neurodegeneration in transgenic mice models which use the human GFAP promoter constructs. (Supported by AG-07909 to CEF).

332.12

IDENTIFICATION OF A PHORBOL ESTER RESPONSE ELEMENT IN THE RAT GALANIN GENE PROMOTER. J.D. Corness, P.H. Burbach◊, A. Pramanik*, T.Hökfelt. Dept. of Neuroscience, Karolinska Institute, 17177 Stockholm, Sweden. ◊Dept. of Medical Pharmacology, Rudolf Magnus Institute for Neurosciences, Utrecht University, 3584 CG Utrecht, The Netherlands.

Expression of the neuropeptide galanin has been shown to be affected by a diverse range of conditions *in vivo* (e.g. salt loading, axotomy), yet the molecular mechanisms responsible for these changes are largely unknown. We have recently cloned a genomic fragment of the rat galanin gene for the purpose of studying the regulatory elements which confer transcriptional regulation of this gene to its promoter. The study of transcriptional control has been focused on the 5' flanking region of the clone, which totals 6.0Kb in length. Constructs of various lengths have been made from this region, which consist of DNA extending 5' from the transcriptional start site. In addition, PCR-generated mutant promoter constructs have been created with short-sequence deletions. Each of these fragments have then been coupled to a luciferase reporter gene and transfected into the placental cell line JEG-3. Following transfection, reporter gene expression was studied in response to 12-O-tetradecanoyl-phorbol-13 acetate (TPA), a direct activator of protein kinase C and a known stimulator of c-jun/c-fos complex-mediated transcription. As a result of these treatments, we have been able to identify a putative phorbol ester response element located within 100 base pairs of the transcription start site for the galanin-gene. Furthermore, gel shift mobility assays demonstrated specific binding of nuclear HeLa-cell extracts containing c-jun/c-fos protein complexes to a 31bp PCR-generated fragment corresponding to a sequence within this region of the promoter. We conclude that a functional phorbol ester response element is present in the 100bp proximal region of the rat galanin gene promoter.

332.13

TRANSCRIPTIONAL ENHANCEMENT BY ELEMENT(S) LOCATED IN THE FIRST INTRON OF THE MYELIN PROTEOLIPID PROTEIN GENE. P. A. Wight* and A. Dobretsova. Dept. of Physiol. & Biophysics, Univ. of Arkansas for Med. Sci., Little Rock, AR 72205.

The myelin proteolipid protein (PLP) gene is expressed primarily in oligodendrocytes, although low levels have been detected in Schwann cells and cardiac myocytes. We have previously demonstrated that transgenic mice expressing a PLP–lacZ fusion gene regulated the transgene in both a spatial and temporal manner, consistent with endogenous PLP expression. Given that: 1) PLP intron A sequences were maintained in the transgene, and 2) the structure of the PLP gene is highly conserved, with the first intron accounting for almost half of the gene, we hypothesized that important transcription regulatory element(s) might reside in PLP intron A. To test this hypothesis, deletion-transfection analysis was performed with PLP–lacZ fusion genes that contained 2.4 kb of PLP 5'-flanking DNA downstream to the ApaI site in exon 2 with various deletions of PLP intron A DNA, driving expression of the lacZ reporter gene. The PLP–lacZ fusion genes were used to transiently transfect an immortalized oligodendroglial cell line N20.1 or NIH3T3 cells. Reporter gene expression was lower in both N20.1 and NIH3T3 cells transfected with a construct that contained a PstI deletion in intron A sequences compared to cells transfected with a construct that contained all of intron A DNA, although the degree of diminution was greater in the N20.1 cells. To see if the diminution could be explained by the presence of enhancer elements contained within the deleted portion of PLP intron A DNA, various portions of PLP intron A sequences were tested in an "enhancer-trap" assay using constructs that contained the luciferase reporter gene driven by the basal HSV thymidine kinase (TK) promoter. With this system, we have identified a 541 bp fragment from PLP intron A that enhances the level of luciferase expression by the heterologous TK promoter.

332.14

DEFINING CIS-REGULATORY ELEMENTS THAT CONTROL EXPRESSION OF THE RAT SKELETAL MUSCLE TYPE 1 (SKM1) SODIUM CHANNEL GENE. S.D. Kraner*, R. Kleinfield, M. Rich, & R.L.Barchi, Dept.of Neuroscience, Univ. of Pennsylvania, Philadelphia, PA, 19104-6074.

A rat genomic library was screened with oligonucleotide probes directed against the SkM 1 5'untranslated region. A 3.1 kb clone encoding 222 bp of intronic sequence, the first exon (80-90 bp), and 2800 bp of 5'flanking sequence was selected for further study. Transcriptional start sites were determined by both RNAse protection and 5'RACE. A fragment from -2800 to +26 was inserted in front of the reporter gene for chloramphenicol acetyl-transferase (CAT) and the resulting construct, as well as nested 5'deletions thereof, were tested in transient expression assays using rat primary muscle cells and NIH 3T3 cells. The full-length construct and deletions up to -192 consistently exhibited 10-fold greater expression in primary muscle cells. Deletion to -82 resulted in a 12-fold increase in CAT activity in 3T3 cells with only a concomitant 2-fold increase in primary muscle cells. The activity of this repressor could be transferred to a heterologous promoter, although the repressor was more tissue-specific on its native promoter. In addition to this repressor, a positive element located within a 210 bp fragment encoding sequences +27 to +68 of the first exon and 168 bp of the first intron was discovered by similar analysis. This positive element can work in either orientation downstream of the promoter, but does not work in front of the promoter. Together the positive and negative element confer 18-fold higher CAT activity in primary muscle cells than in 3T3 cells. Supported by USPHS grant NS 18013.

332.15

A 5' FLANKING SEGMENT OF MOUSE ZIC GENE MEDIATES NEURAL TISSUE SPECIFIC GENE EXPRESSION IN TRANSGENIC MICE. J. Aruga[1]*, K. Shimoda[2] and K. Mikoshiba[1,3] [1]Mol. Neurobiol. Lab. RIKEN, Tsukuba, Ibaraki 305, Japan, [2]Sch. of Med., Keio Univ. [3]Inst. of Med. Sci., Univ. of Tokyo

Zic is a novel zinc finger protein which we identified as a nuclear factor expressed in the cerebellar granule cell lineage in a highly restricted manner. The gene shows a significant homology to the recently cloned *Drosophila* paired-rule gene, *odd-paired*. We found that *zic* is also expressed in dorsal half of neural tube at certain embryonic stage and human medulloblastoma, whose oncogenesis is involved in the cerebellar granule cell lineage. Recent our study indicates that there are several *zic* related gene in mouse genome.

To explore how *zic* expression is restricted, we determined the genomic organization and promoter elements of *zic* gene. We cloned and sequenced 8 kb region of mouse *zic* gene including the 5'flanking region. The mouse *zic* gene is a single gene composed of 3 exons, in which the zinc finger motif is divided into 3 exons. In the 5' flanking region. there are several regulatory elements which have been implicated in neural tissue specific gene expression. In order to define the regulatory region, we first produced transgenic mice bearing β-galactosidase gene linked to a 2.8 kb 5' flanking segment of the mouse *zic* gene. We observed that 2.8 kb of the 5' flanking is capable of restricting the transgene expression in nervous tissue that mimics the spatial and temporal expression of the endogenous gene. Transfection assays in a *zic*-expressing neuroblastoma cell line indicate that strong promoter activity is located in a proximal 246 bp segment while the upstream region contains a negative regulatory elements.

332.16

MOLECULAR CHARACTERIZATION OF THE PROMOTER REGION OF NEURON-SPECIFIC HEL-N1: EVIDENCE FOR ENHANCED TRANSCRIPTIONAL ACTIVITY IN NEURAL CELLS. P.H. King and J.N. Whitaker*. Dept. of Neurology, University of Alabama, Birmingham, AL 35294.

Hel-N1 (human *elav*-like neuronal protein) belongs to a family of neural-specific genes that are homologous to the *Drosophila* prototype, *elav* (*embryonic-lethal abnormal visual*). This gene family encodes highly conserved RNA-binding proteins which are expressed at the earliest stages of neuronal differentiation and play a vital role in development. Here I present the initial molecular and functional characterization of the promoter region of Hel-N1.

A 5.4 kbp fragment was isolated from a human genomic library and found to contain the initial exon of Hel-N1. The transcriptional start site was determined by primer extension using RNA from human occipital cortex and several neuroblastoma cell lines (SK-NSH, NBL-S, and 1691). Analysis of the sequence immediately upstream from this start site revealed several transcription-factor-binding sites including SP1, AP2 and two CCAAT boxes. Using a reporter-gene assay, analysis of various upstream segments in 1691 cells indicated strong transcriptional activity within the initial 530 bp (-1 to -529). When compared to U105 (glial) or HeLa (epitheloid) cells, this activity was significantly greater in 1691 cells. These findings suggest that *cis* elements for neural-specific Hel-N1 transcription reside within this segment.

332.17

TRANSIENT TRANSFECTION AND MUTATIONAL ANALYSIS OF THE DYNCRE3 ELEMENT OF THE PRODYNORPHIN PROMOTER. D.J. Kim, D.J. Messersmith, J. Gu, R. Dubner and M.J. Iadarola*. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Previous studies have shown that the DYNCRE3 sequence located at -1545 from the transcription start site participates in basal and cAMP-dependent expression of the rat prodynorphin gene. This element has a core heptanucleotide sequence (TGCGTCA) that is similar to the CRE (TGACGTCA) and AP-1 (TGAGTCA) consensus sequences and identical to the ENKCRE II sequence. We have compared the transcriptional efficacy of the DYNCRE3 to mutations that (a) convert it to consensus CRE or AP1 sites and (b) modify the core and flanking regions of a 41 bp oligonucleotide centered on the core and fused to a dynorphin minimal promoter (-135 to +65). Two base substitutions within the core reduced CAT activity to levels of the minimal promoter and nearly eliminated forskolin stimulation in transiently transfected PC12 cells. Mutation of the two bases immediately flanking the core produced a minimal effect on activity, whereas mutations further 5' and 3' yielded partial reductions in activity. Comparison between DYNCRE3 and its AP-1 and CRE "analogs" showed that it exhibited properties of both consensus elements. Under forskolin stimulation the DYNCRE3 showed an identical degree of inducibility as the CRE consensus (25-35 fold); the AP-1 element was less active (~7-fold). The three constructs were stimulated to a lesser degree (3 to 5-fold) by phorbol ester. All three were stimulated by co-transfection with a c-Jun expression vector with the CRE consensus being the least active. These data suggest 1) an essential role for the core heptanucleotide, 2) a partially stabilizing role for the flanking regions, and 3) that the DYNCRE3 site can display functional similarity to both AP-1 and CRE sequences depending on cellular context and experimental conditions.

332.18

CELL SPECIFIC BINDING OF PROTEINS TO THE ENKEPHALIN CRE-2 ELEMENT. L. MacArthur[1]*, A. Iacangelo[2], L. Eiden[2], D. Messersmith[1]. [1]Neurobiology Anesthesiology Branch/NIDR, [2]Laboratory of Cell Biology/NIMH, Bethesda, MD 20892.

Although a number of transcription factors up-regulate the enkephalin gene in recombinant systems, the factors that bind in a homogeneous tissue in which enkephalin is expressed have not been addressed. This study uses gel shift analysis to examine this question.

Chromaffin cells of the adrenal medulla are homologs of sympathetic ganglia neurons which express a variety of neuropeptide genes, including enkephalin, both in vivo and in primary culture. Gel shift analysis of three previously described enkephalin enhancers with extracts from chromaffin cells (CC) and PC12 cells, which do not express the enkephalin gene, demonstrate that all of the binding activity is to a single element, ENKCRE-2, a cAMP response-like element. CC and PC12 cells give different gel shift patterns and different competition profiles with oligos to consensus AP-1 and CRE sequences indicating different proteins bind to this site. Antibody supershifts demonstrate the majority of CC and PC12 cell binding can be shifted or competed with antibodies against Fos and Jun family members while only a small amount of binding is shifted by antibodies cross-reactive to CREB family members. Furthermore, distinct proteins are bound i.e. Jun D binds in CC whereas both Jun D and Jun B bind in PC12 cells, although not as heterodimers with each other. These data demonstrate that the ENKCRE-2 element is the major enhancer for enkephalin regulation in primary neuro-endocrine cells and is the site where CC and PC12 cells exhibit differences in the ability to bind specific proteins.

332.19

IDENTIFICATION OF *cis*-ELEMENTS AND *trans*-ACTIVATING FACTORS REQUIRED FOR THE GERM CELL-SPECIFIC EXPRESSION OF THE PROENKEPHALIN GENE Feng Liu, John P. Tokeson, Karl M. Ebert, Stephan P. Persengiev and Daniel L. Kilpatrick* Worcester Found. Exp. Biol, Shrewsbury MA 01545, & Tufts Vet. School of Med., Grafton, MA 01536

Rat and mouse spermatogenic cells use an alternate, germ cell-specific proenkephalin promoter. Previous studies have demonstrated that the rat proenkephalin germ-line promoter encompasses a TATA-less 500 sequence which is located downstream of the somatic promoter, within the first intron for the somatic transcript. To identify *cis*-elments required for expression of the germ line promoter, rat proenkephalin-CAT fusion genes containing different portions of the germ-line promoter were generated and analyzed in transgenic mice, using RNAse protection assays. It was found that deletion of the 5' flanking sequence of the germ line promoter almost completely abolished CAT expression in constructs RPKCAT0.4 and RPKCAT0.5-5'del containing respectively, 100bp and 121bp deletions of the 5' end. This suggests that the 5' flanking sequence is important in directing germ line-specific proenkephalin gene expression. To identify functional *trans*-activating factors, gel retardation assays were performed. Four DNA-nuclear protein complexes were identified using the 150bp 5' flanking sequence and germ cell nuclear extracts. Further competition assays revealed that all the four complexes can be depleted by a cold oligo competitor containing the 45bp sequence immediately upstream of the transcription initiation sites (GCP1), suggesting the exclusive involvment of this region in DNA-protein complex formation. A consensus binding site for TFII-I is present in the GCP1 sequence. Supershift, footprinting, and UV-crossing analysis are underway to further identify *trans*-activating factors that are required for the germ cell-specific expression of the proenkephalin gene. (Supported by NIH DK36468 and F32 DK09006)

332.20

Protein-DNA Interactions in the Promoter of the Cyclooxygenase (COX2) Primary Response Element in NG108-15 cells and in Rat and Human Brain. W.J.Lukiw*, V.L.Marcheselli, P.K.Mukherjee and N.G.Bazan, LSU Neuroscience Center, Louisiana State University School of Medicine, New Orleans, LA 70112 USA

The cyclooxygenase-2 (COX2, also TIS10, PGS-2, PTGS2) gene is a primary genetic response element that codes for the inducible form of prostaglandin synthetase. This enzyme, which catalyzes the oxidation of arachidonic acid derived from membrane phospholipids, represents the rate-limiting step in the synthesis of prostaglandins. These, in turn, are potent mediators of cellular damage induced by brain injury.

Using nuclear protein extracts (NPXTs) derived from platelet activating factor (PAF) induced NG108 cells, and the hippocampus of rats subjected to cryogenic injury (CI), electroconvulsive shock (ECS) or kindled seizures induced by kainic acid (KA), i.e. experimental protocols which are known to rapidly activate transcription of the COX2 gene, we have mapped protein-DNA interactions in the COX2 promoter during gene induction. As analyzed by electrophoretic mobility shift assay (EMSA) and automated DNA sequence analysis, transcription factor recognition sites for NFKB, AP2, NFIL6, PAF and several GC islands are present within the -463 to +30 bp region of the COX2 gene. As analyzed by EMSA, magnetic bead based purification of COX2 promoter binding proteins and SDS/PAGE/silver staining of NG108, rat and human brain hippocampal cell NPXTs, some 25 nuclear proteins were found to bind onto the -463 to -259 bp upstream region of the COX2 gene. This GC-rich upstream DNA contains a -371 to -370 bp region necessary for PAF-mediated COX2 gene activation. A ~35 Kd protein binding onto this DNA, present only in the uninduced (inactive) state, disappeared during induction of the COX2 gene. We speculate that one mechanism responsible for COX2 activation may be an alleviation of protein-mediated COX2 gene repression. This may represent one important endpoint in the cellular signal transduction pathway by which events initiated at the brain cell periphery are relayed to the nucleus to give primary genetic responses. Supported by NIH NINDS NS23002.

PHARMACOLOGY OF SYNAPTIC TRANSMISSION I

333.1

MECHANISMS OF ACTION OF A MUSCLE RELAXANT, CS-722, ON CULTURED HIPPOCAMPAL NEURONS. W. Marszalec*, J-H. Song and T. Narahashi. Dept of Mol. Pharmacol. and Biol. Chem., Northwestern Univ. Med. Sch., Chicago, IL 60611.

A recently synthesized compound, CS-722, reduces experimental muscular rigidity in rats, an effect accompanied by a depression of polysynaptic spinal reflexes (Tanabe et al., Neuropharmacol., 31: 1059, 1992). A further study of the mechanisms of action of CS-722 was undertaken with cultured rat hippocampal neurons using the whole-cell patch clamp technique. Bath perfusions of 100 and 300 μM CS-722 reduced the frequency of spontaneously occurring postsynaptic currents which were sensitive to 0.4 μM tetrodotoxin. This decrease was observed for both inhibitory and excitatory postsynaptic currents, with the latter being affected more. CS-722 produced no significant change in currents evoked by the direct application of GABA, glycine, or glutamate. Preliminary experiments suggested that CS-722 did not inhibit presynaptic transmitter release. However, CS-722 did significantly reduce voltage-gated sodium currents, suggesting that it may be acting like a local anesthetic on these cells leading to suppression of synaptic transmission.

333.2

CHARACTERIZATION OF GIANT MEPPS INDUCED AT NEUROMUSCULAR JUNCTIONS BY 2,4-DITHIOBIURET. Y. F. XU* and W. D. ATCHISON, Dept. Pharmacol. Toxicol., & Neurosci. Progrm., Mich. State Univ., E. Lansing, MI 48824

Rats treated with small daily doses of 2,4-dithiobiuret (DTB) develop a delayed onset neuromuscular weakness after 6 days of treatment. Evoked quantal release of ACh is impaired, and the frequency of giant miniature end-plate potentials (gMEPP) is increased dramatically in DTB-treated preparations. A comparison of the sensitivity of MEPP and gMEPP frequency to various treatments known to alter MEPP frequency was made using extensor digitorum longus preparations of normal and DTB-treated (1 mg/kg/day, 6 days) rats. Increasing extracellular Ca^{2+} from 2 to 8mM, depolarizing the nerve terminals with 30mM K^+ or addition of α-latrotoxin (α-LTx), or La^{3+} markedly increased MEPP frequency in normal muscles. In DTB-treated muscles, these agents were much less effective at increasing MEPP frequency than in controls. Similarly, the gMEPP frequency in normal preparations was increased dramatically by α-LTx, K^+ depolarization and La^{3+}, while in DTB-treated preparations, there was no significant effect of these treatments. gMEPPs are thought to result from addition of newly-synthesized ACh to quanta, as they are inhibited by treatment with vesamicol, which blocks active uptake of ACh into synaptic vesicles. Although vesamicol inhibited gMEPP frequency in DTB-treated preparations, the time necessary to block gMEPPs by vesamicol was longer than in controls. It is known that botulinum toxin A (BoTX) also induces gMEPPs, characterized by being insensitive to nerve terminal depolarization and to transmembrane Ca^{2+} fluxes. However, α-LTx is effective in increasing gMEPP frequency at BoTX-poisoned junctions. Taken collectively, the data indicate that the pharmacological sensitivity of gMEPPs induced by DTB is different from that not only of normal junctions but also that of BoTX-poisoned junctions. Supported by NINDS grant NS20683.

333.3

STRUCTURAL EFFECTS OF 2,4-DITHIOBIURET ON THE RAT MOTOR ENDPLATE RESEMBLE THOSE SEEN IN CHRONIC NEUROMUSCULAR DISORDERS. M.B. Rheuben*, D.M. Autio, and W.D. Atchison. Departments of Anatomy, and Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824.

Rats treated with daily doses of 2,4-dithiobiuret develop hindlimb weakness and a paralysis which is reversible upon cessation of treatment. Previous studies of treated animals have reported tubulovesicular profiles within the nerve terminal and abnormalities in the adaxonal glial wrappings, with long term treatment producing sprouting. A combination of light, transmission, and scanning electron microscopy was used to monitor the endplate region of the extensor digitorum longus in association with physiological studies (Xu and Atchison, Soc. Neurosci. Abstr. 1995). When paralysis was first evoked, after 5-6 days of treatment, we found a constellation of pre- and postsynaptic changes. The degree of abnormality varied both intraterminally and among terminals in the same muscle; one or more individual boutons might be swollen or the entire endplate could be deformed. Terminal Schwann cells responded systemically, with both changes in cytoplasmic organelles and changes in overall shape. The postsynaptic folds swelled and the interfold spacing increased. The synaptic cleft became wider. Some terminal regions appeared relatively normal and capable of release; other were retracted from the folds and many contained tubulovesicular profiles. Similar morphological responses have been found in association with both experimentally-induced and naturally occurring chronic dysfunctional conditions of the neuromuscular junction, and have broad functional implications. Supported by NIH Grant NS20683.

333.4

TWO TYPES OF TRAIN EPP CHANGES IN RAT'S ISOLATED DIAPHRAM AFTER EXPOSURE TO NEOSTIGMINE AND SOMAN

Chuan-gui Liu, Jun Li and Ming-yao Liu* Dept. of Neuropharmacol., Institute of Pharmacol. & Toxicol., Acad. Mil. Med. Sci., P.O.Box 130, Beijing 100850, China

It has been reported that in isolated rat diaphragm the indirect stimulation evoked train EPPs changed in two types after exposure to neostigmine(neo) or soman: the decreased amplitude with definite depolarization of membrane, which repolarized immediately after cessation of stimulation (Type I) and the disappearance of EPPs with sustained depolarization of membrane, which repolarized in definite period independent of stimulation duration (Type II, sustained or regenerative depolarization). In isolated nonuniform stretched muscle preparation of rat's diaphragm we were able to induce either Type I or Type II response (50 Hz, 1s) after exposure to neo or soman depending on different drug concentrations or different time schedule of stimulation. It was demonstrated that neo 0.3, 2.0 mM or soman 0.5, 5.5 mM could induce Type II response in 100% with no Type I, while increasing or decreasing the drug concentration both 'Type I and Type II responses with different percentages were observed. In preparations exposed to neo 2.0 mM or soman 5.5 mM given two successive indirect train stimulations with interval more than 20 sec., second stimulation induced Type II response in 100%, while with interval less than 5 sec. 100% of Type I responses were induced. When this interval was ranging 10-20 sec. the 60% or 80% of train EPPs were Type II response after exposure to neo or soman respectively. If the duration of indirect train stimulation was increased from 1s to 2s the response was transformed from Type II to Type I in the second half of the stimulation period in 100% or 80% preparations exposed to neo or soman respectively. It is suggested that in unrestrained rats the interval between two successive inspiratory contractions of diaphragm is much less than 5 seconds and the above mentioned Type I response rather than Type II response may be the main response of train EPPs after exposure to neostigmine or soman.

333.5

McN-A-343 (McN) INCREASES RENAL SYMPATHETIC NERVE ACTIVITY (RSNA) BY TWO MECHANISMS. J.R. Martin*, Dept. of Pharmacol., Kirksville Col. Osteo. Med., Kirksville, MO 63501

Intravenous injection of the muscarinic M_1 agonist McN into rats evokes an increase in mean arterial pressure (MAP) which correlates with an increase in RSNA. The present study shows that McN increases RSNA by two mechanisms. Male Sprague-Dawley rats (280-320 gm) were anesthetized with urethane (1.6 gm/kg), and implanted with a catheter in the left femoral artery and vein for monitoring MAP and drug administration. An isolated nerve branch arising from the coeliac ganglia and following the renal blood vessels was then placed on a bipolar hook electrode. All rats were pretreated with the nicotinic ganglionic antagonist pentolinium (10 mg/kg) to block baroreflex-mediated bradycardia. Methylatropine (MeATR; 0.25 and 2 mg/kg) and telenzepine (TLZP; .07 and .6 mg/kg) identically inhibited the McN-evoked increases in MAP and RSNA. Doses of McN greater than 0.3 mg/kg evoked increases in RSNA which could not be blocked by MeATR or TLZP but which still correlated with the increase in MAP. This increase in MAP, but not the increase in RSNA, involved catecholamines as it was attenuated by α-adrenergic receptor blockade. Benextramine (BNXT; 20 mg/kg) blocked the increase in RSNA evoked by the higher doses of McN without affecting the increase evoked by lower doses. Combining BNXT with MeATR (0.25 mg/kg) or TLZP (0.07 mg/kg) completely blocked the McN-evoked increases in RSNA and MAP. These results show that McN-evoked increases in RSNA occurs: (1) at low doses via an action on muscarinic M_1 receptors, and (2) at high doses via a BNXT-sensitive mechanism. (Supported by NIH HL44531.)

333.6

P_{2X} PURINOCEPTORS MEDIATE FAST SYNAPTIC TRANSMISSION IN MYENTERIC NEURONS OF THE GUINEA PIG ILEUM **K.J. LePard* and J.J. Galligan, Dept. of Pharmacology and Toxicology and the Neuroscience Program, Michigan State University, East Lansing, MI 48824.**

ATP is a fast excitatory neurotransmitter in numerous biological systems. Recent evidence suggests that purinergic P_2 receptors mediate fast excitatory actions of ATP in enteric neurons. The present studies characterize the P_2 receptor subtype mediating fast excitatory post-synaptic potentials (fEPSPs) recorded using conventional electrophysiological methods. In 23 of 66 neurons (35%), fEPSPs were cholinergic as they were blocked by hexamethonium (C6, 100 μM) [amplitude (mV), N=23: control, 19 $\pm$ 2; C6, 1 $\pm$ 0.2; P<0.001]. However, in 43 (65%) neurons, fEPSPs were only partially blocked (<67%) by C6. In this subset, 70% of fEPSPs were dose-dependently (1-30 μM) inhibited by the P_{2X} antagonist pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS, EC_{50} = 3 μM). In addition, superfusion of the P_{2X} agonist α,β-methylene adenosine 5'-triphosphate (α,β-Me ATP, 1 μM) desensitized PPADS-sensitive fEPSPs [amplitude (mV), N=6: control, 30 $\pm$ 6; C6, 16 $\pm$ 4; C6 plus PPADS (10 μM), 5 $\pm$ 1; C6 plus α,β-Me ATP, 4 $\pm$ 1]. PPADS and α,β-Me ATP had no effect on cholinergic fEPSPs or on responses to locally-applied acetylcholine. These experiments indicate that P_{2X} receptors mediate some fEPSPs in the guinea pig myenteric plexus. **(Supported by DK40210, NS01738.)**

333.7

SUBSTANCE P AND VIP POTENTIATE KETAMINE-INDUCED INHIBITION OF CALCIUM-ACTIVATED K-CONDUCTANCE IN GUINEA-PIG SYMPATHETIC NEURONS. W.H. Stapelfeldt*, J.M. Oleszewski. Depts. of Anesthesiology & CCM, Univ. of Pittsburgh, Pittsburgh, PA 15261, and VA Medical Center, Pittsburgh, PA 15240.

We recently demonstrated that the general anesthetic agent propofol augments substance P (SP)-mediated postsynaptic inward current responses of guinea-pig inferior mesenteric ganglion (IMG) neurons through synergistic interaction with a SP-invoked signal transduction mechanism to potentiate the inhibition of a $G_{K(Ca)}$-conductance which normally restrains SP excitatory responses and which also contributes to the afterspike hyperpolarization (ASH) of these neurons. Since the general anesthetic agent ketamine has recently been reported to specifically inhibit BK-channels, inhibition of ASH by ketamine was examined with regard to its possible analogous sensitivity to neuromodulation by SP- or VIP-evoked signal transduction mechanisms. Study parameters included baseline ASH amplitudes, neuropeptide-evoked inward current responses, and *associated changes in ASH amplitudes* of intermittently (0.5 Hz) evoked electrotonic action potentials before and *during neuropeptide responses* using single-electrode (manual) voltage clamp recordings from isolated perfused IMGs. Exogenous SP or VIP were applied by pressure microejection from nearby micropipets (Picospritzer). Ketamine (100 μM) caused significant ($p<0.05$) increases in the amplitude of inward current responses to both SP (32±16 %) and VIP (38±15 %) which were both associated and related in amplitude with more pronounced decreases in peak ASH amplitudes of intermittently evoked action potentials when compared to those produced by the neuropeptides alone or by ketamine alone. These findings suggest that the anesthetic sensitivity of the underlying $G_{K(Ca)}$-conductance to inhibition by ketamine in sympathetic neurons is subject to physiologic neuromodulation by signal transduction steps invoked by synaptic inputs utilizing the neuropeptide transmitters SP and VIP. Supported by UACCMF, VA RAG B, FAER & Syntex Laboratories.

333.8

INHIBITORY EFFECTS OF NITRIC OXIDE ON SYNAPTIC TRANSMISSION IN RAT INTRACARDIAC GANGLIA. S.W. Jeong, J.S. Walter*† and R.D. Wurster. Depts. of Physiology and Neurolog. Surg., Loyola Medical Center, Maywood IL 60153. † Hines VA Hospital, Rehab. Res. Dev.Ctr, Hines IL 60141

Some evidence has suggested that nitric oxide(NO) acts as a local neuromodulator in the central and peripheral nervous systems. Nitric oxide synthase(NOS) has recently been localized within parasympathetic intracardiac ganglia. However, the role of NO in this system is not well known. In the present study we investigated whether rat intracardiac neurons contain NOS and whether the NO produced modulates ganglionic synaptic transmission. NOS was localized using NADPH-diaphorase histochemistry. A large population of intracardiac neurons revealed NADPH-diaphorase activity. To test the effect of NO on synaptic transmission, intracardiac ganglia with intact preganglionic nerve fibers were isolated and superfused with the oxygenated Krebs solution at 36 °C. The orthodromic action potentials were evoked by the electrical stimulation of preganglionic nerve fibers using a concentric bipolar electrode. L-arginine, a substrate for NOS, reversibly decreased the amplitude of synaptic response. This effect was blocked by N^{ω}-nitro-L-arginine, a competitive NOS inhibitor. To confirm the inhibitory effect of NO on synaptic transmission, the well known NO donors, sodium nitroprusside and 3-morpholinosydnonymine(SIN-1), were used. These NO donors also depressed synaptic transmission similar to the results obtained with L-arginine. The NO scavenger, hemoglobin, prevented the inhibitory effects of NO donors. Methylene blue, a guanylate cyclase(GC) inhibitor, enhanced synaptic transmission and also prevented the NO donor-induced inhibition. In addition, a membrane permeable cGMP analogue, 8-bromo-cGMP mimicked the inhibitory effects of L-arginine and NO donors. We concluded that NO is produced endogenously in rat intracardiac ganglia and inhibits synaptic transmission via the activation of GC and a resultant increase in the cGMP level. Supported by NIH grant 27595.

333.9

NITRIC OXIDE POTENTIATES INHIBITORY POSTSYNAPTIC CURRENTS IN RAT SYMPATHETIC PREGANGLIONIC NEURONS. S. Y. Wu* and N. J. Dun. Dept. of Anat & Neurobiol., Medical College of Ohio, Toledo, OH 43614.

Whole-cell patch-clamp recordings were made from sympathetic preganglionic neurons (SPNs), the majority of which contain nitric oxide synthase (NOS)-immunoreactivity, from thoracolumbar spinal cord slices from 8-12 day old rats. Electrical stimulation of lateral funiculus evoked in a population of SPNs an inhibitory postsynaptic current (IPSC) that was sensitive to either strychnine or bicuculline. Superfusing the slices with the NO precursor L-arginine (L-arg, 300 μM) and the NO donor nitroprusside (SNP, 100 μM) increased the IPSCs. D-Arg was without effect. The potentiating effect was attenuated by bovine hemoglobin (100 μM). Pretreating the slices with the NOS inhibitors N^{ω}-Nitro-L-arginine (30 μM) or N^{G}-mono-methyl-L-arginine (100 μM) reduced the IPSCs by an average of 30%. Superfusing the slices with dibutyryl cGMP (750 μM) in the presence of the phosphodiesterase inhibitor IBMX (750 μM) potentiated the IPSCs as well. L-Arg and SNP had no significant effects on outward currents induced by pressure application of glycine. These studies raise the possibility that NO released endogenously from SPNs may be a retrograde messenger that enhances inhibitory transmitter release via a cyclic GMP-dependent mechanism. (Supported by NS18710).

ACETYLCHOLINE RECEPTOR: NICOTINIC—ACETYLCHOLINE-GENETICS: MOLECULAR

334.1

IDENTIFICATION OF NICOTINIC ACETYLCHOLINE RECEPTOR (nAChR) GENES FROM THE PARASITIC NEMATODE *ASCARIS SUUM*. H. L. Brooks [1] R. C. Foremen [1], J. F. Burke [2], L. Holden-Dye*[1] & R. J. Walker [1]. [1] Department of Physiology and Pharmacology, University of Southampton, SO16 7PX, [2] Department of Biochemistry, University of Sussex, BN1 9QG, UK.

Previous studies on the nematode *Ascaris suum* have pharmacologically characterized the native nicotinic receptors on somatic muscle (Colquhoun *et al*, *J. exp. Biol*, 158, 509-530 & *Mol. Neuropharm.*, 3, 11-16, 1993). The native receptor has pharmacological similarities to a mammalian neuronal subtype of nicotinic receptor. Previous studies on the nematode *Caenorhabditis elegans* have implicated a specific type of nicotinic receptor as the site of action for the anthelmintic levamisole. We are interested in isolating and characterizing the nAChR subunit genes from *A. suum* in order to compare the pharmacological properties of the subunits to those of the native receptor. PCR was used on genomic DNA to produce *unc-38* (Fleming *et al*, unpublished), an alpha-like nicotinic subunit from *C. elegans*. To identify the subunit genes in *A. suum* molecular cloning techniques have been employed to screen both genomic and cDNA libraries. PCR studies using degenerate probes to the conserved regions of other invertebrate AChR genes were performed. Genomic fragments have been isolated with homology to vertebrate and invertebrate neuronal nicotinic ACh receptors. RT-PCR on muscle tissue and the head region of *A. suum* identified cDNA fragments that hybridized to the *unc-38* gene from *C. elegans*. Evolutionary comparisons of the nicotinic receptor and cytoskeletal genes of *A. suum* to the corresponding invertebrate and mammalian sequences have been performed. Further characterization and expression of the *A. suum* nicotinic receptor genes is currently under investigation.

334.2

CELLULAR CONTROL OF NICOTINIC ACETYLCHOLINE RECEPTOR GENE EXPRESSION R.T. Boyd*. Department of Pharmacology and The Neuroscience Program, The Ohio State University College of Medicine, Columbus, Ohio, 43210.

The nicotinic acetylcholine receptor (nAChR) β4, α3, and α5 subunit genes are tightly clustered in the genomes of several species. The conserved organization of these nAChR genes implies a functional role for this arrangement. We have previously identified promoter elements in the region between the rat β4 and α3 genes and made luciferase reporter constructs containing DNA segments from this region. In this study we have examined the effects of increased adenylyl cyclase activity and decreased protein kinase C activity on transcription of the α3 nAChR gene in PC12 cells using these constructs. Increased cAMP levels or reduced protein kinase C activity both produced increased levels of α3 RNA and increased transcriptional activity of the α3 promoter. These promoter-luciferase constructs were also transfected into non-neuronal cells and the sequences required for neuronal-specific activity were identified. We have also mapped the organization of β4 and α3 transcripts in the intergenic region and have revealed that some β4 transcripts extend far into the intergenic region and overlap DNA sequences upstream of the α3 gene which have promoter activity in PC12 cells.

This work supported by AHA (Ohio Affiliate), Bremer Foundation (Columbus, Ohio), and NIH Grant NS29746.

334.3

SCIP IS A SPECIFIC ACTIVATOR OF THE ACETYLCHOLINE RECEPTOR α3 GENE IN PC12 CELLS BUT ACTIVATION IS NOT INFLUENCED BY POU-DOMAIN BINDING SITES POSITIONED UPSTREAM OF THE GENE Evan Deneris*, Dmitry Fyodorov, and Xiangdong Yang. Dept. Neuroscience, Case Western Reserve University, Cleveland, OH 44106.

The POU-domain factor, SCIP/Tst-1/Oct-6, can activate transcription by binding to A+T-rich sequences, such as the octamer motif. In PC12 cells, the nicotinic acetylcholine receptor α3 gene promoter is activated by SCIP. Each of the A+T-rich SCIP binding sites present in the α3 upstream region were analyzed for a contribution to activation. We found that elimination of these sites either singly or in combination did not affect the response to SCIP. Even when re-positioned near the α3 transcription start site region SCIP binding sites did not influence activation. This was paralleled by an inability of SCIP to activate octamer-dependent transcription from a simple octamer/TATA promoter. The α3 core promoter, which does not contain detectable SCIP binding sites, is strongly responsive to SCIP but not Oct-2. We analyzed the core promoter for sequences that might mediate the response to SCIP and were unable to find a region that was important for activation but not basal activity. SCIP domains important for activation were investigated by analyzing the activity of a set of amino-terminal and carboxy-terminal deletion mutants. We found that the activity of the POU domain alone was 50% of the activity of the full length protein. Restoration of wild type activity required two positively acting regions in the amino-terminal domain and an inhibitory region in the carboxy-terminal domain. Our results reveal distinct POU factor actions in PC12 cells and suggest that SCIP transactivates through specific protein-protein interactions with the basal transcription complex.

334.4

PURIFICATION OF RECOMBINANT α-BUNGAROTOXIN AND TWO MUTANTS USING A 7-RESIDUE HISTIDINE TAG. J.A. Rosenthal, O-L. Shi and E. Hawrot*. Dept. of Molecular Pharmacology and Biotechnology, Division of Biology and Medicine, Brown University, Providence, RI 02912.

We have previously demonstrated the production of biologically active, recombinantly produced α-Bungarotoxin (Bgtx) which was designed as a fusion protein with a highly soluble T7 coat protein. In an effort to maximize the recovery of recombinantly produced Bgtx, we have inserted a seven amino acid histidine tag N-terminal to the Bgtx sequence and C-terminal to the engineered thrombin protease site. Cleavage of the affinity-purified Histag-fusion protein with thrombin, results in a major ~9 kD protein as determined by SDS-PAGE. A final purification step using cation exchange chromatography separates fully active material from fractions with reduced activity. The fully active material is indistinguishable from native toxin under the solid-phase assay conditions used. We can produce ~600 µg of active toxin/L of starting LB culture, a 25% increase in active toxin produced in comparison to the non-Histag protein.

We further report the purification of two mutants of Bgtx, an R36A and a truncation mutant in which the carboxy-terminal 7 residues have been deleted. R36 was a target for mutagenesis as it is conserved in all toxins within the neurotoxin family and was proposed to be a critically important contact site in nicotinic receptor recognition. Substitution of the R36 in Bgtx with Ala results in a toxin with a <20-fold reduction in binding activity, suggesting that although this residue contributes to binding, it is not absolutely essential for toxin activity. We have similarly produced a shortened Bgtx protein to assess what, if any, role the carboxy-terminal tail plays in receptor recognition. We observe an 11-fold reduction in binding affinity. We are beginning to assess the physiological effects that these alterations may have on heterologously expressed mouse nicotinic acetylcholine receptors using two-electrode voltage clamp techniques.

334.5

A RAPID AND SIMPLE METHOD FOR SIMULTANEOUSLY LABELING TWO DIFFERENT MESSAGES *IN SITU*. K-P. Chiu, S.A. Berman, T. Sullivan, S. Bursztajn.* McLean Hospital and Psychiatry Dept., Harvard Medical School, Belmont, MA 02178.

AChRs subunits are expressed heterogeneously among the nuclei of a myotube. Does the nucleus which expresses one AChR subunit also express other AChRs subunits? To answer this question we have further improved our double label *in situ* hybridization procedure. For this purpose we have combined radiolabeled cDNA probes and conventional autoradiography with colorimetric detection methods using 4-chloro-2-methylbenzenediazonium-3-hydroxy-2-naphthoicacid2,4-dimethylanilide phosphate (Fast Red). This substrate is fully compatible with ^{35}S-labeled probe and generates a higher subcellular resolution and higher contrast than nitro blue tetrazolium chloride (NBT). The digoxigenin labeled probe is identified immunochemically with anti-digoxigenin antibody conjugated to alkaline phosphatase and the colorimetric reaction revealed with Fast Red. Cells are coated with NTB3 emulsion developed for an appropriate time period and the radioactive and colorimetric signals are readily detected and quantitated. The colorimetric signal is quantitated with a computer assisted densitometry program, permitting for the first time simultaneous detection of two different messages in the same muscle cell.

334.6

RECONSTITUTION OF A FUNCTIONAL HOMOMERIC NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR FROM C-ELEGANS. D. Bertrand, S. Bertrand and M. Ballivet*University of Geneva, 1211 Geneva, Switzerland

Eleven genes encoding neuronal acetylcholine receptor (nAChRs) subunits have been isolated from the vertebrate genome. There is now mounting evidence that the diversity of nAChR genes may be just as high in D. melanogaster and in C. elegans as it is in vertebrates. To take advantage of the powerful genetic tools available in C. elegans, we have cloned the cDNAs and genes of two putative nAChR subunits from this organism. One of these (Ce21) is an alpha-like subunit and shares 49% amino-acid identity with the vertebrate α7 subunit. Functional homomeric nAChRs are obtained in Xenopus oocytes upon nuclear cDNA injection of Ce21 alone. ACh-evoked currents were very robust in these experiments (in the μA range) and displayed physiological and pharmacological profiles typical of nicotinic receptors. Unexpectedly, however, the Ce21 receptor was not activated by the anthelminth levamisol, a full agonist of the nematode neuromuscular junction. In the 50-100 μM, levamisol efficiently antagonized ACh-evoked currents through the Ce21 channels with features compatible with an open channel block. Our data suggest that the Ce21 channel is a neuronal nAChR.

334.7

IDENTIFICATION AND MOLECULAR CLONING OF A FAMILY OF NICOTINIC ACETYLCHOLINE RECEPTOR GENES FROM THE LEECH. R. S. Allen*, M. Hartley and S. F. Heinemann. Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92037.

Acetylcholine, GABA, glycine and $5HT_3$ mediate distinct physiological effects via interaction with multiple functionally diverse but homologous receptor subtypes which share sequence and structural features. Studies in several invertebrate species reveal unusual and complex responses to many neurotransmitters, suggesting that novel receptor subtypes exist. The aim of this project is to clone and functionally characterize cDNAs encoding leech neuronal nicotinic receptor (nAChR) subtypes. This gene family contains at least 7 different members, as determined by an RT-PCR based strategy using degenerate primers to conserved nAChR sequences. Subtypes are designated as neuronal or muscle based upon Northern blot hybridization. DNA sequence analysis reveals 3 neuronal subtypes: 1) alpha-like, 2) beta-like and 3) a novel, intermediate subtype. A random-primed cDNA library has been constructed from leech ventral nerve cords, amplified from ~$3x10^6$ independent recombinants. The average (avg.) insert size is ~2 Kb. Screening of ~$1.5x10^6$ phage yielded 73 alpha-like, 29 beta-like and 28 intermediate-positive clones. ~20 of each have been further processed by phagemid excision, plasmid DNA purification and restriction analysis. 18 alpha-like clones have inserts ranging from ~1.1-4.3 Kb, (avg. ~2 Kb), 6>2 Kb. 16 appear to be independent, on the basis of size. 17 beta-like clones have inserts ~0.3-5.3 Kb (avg. ~1.7 Kb), 6>2 Kb, with 7 independent. 22 intermediate clones have inserts ~0.9-4.6 Kb (avg. ~2 Kb), 7>2 Kb, with 12 independent. Identification of full-length clones by sequence analysis will be followed by functional characterization using a *Xenopus* oocyte expression system.

334.8

CLONING AN *UNC-29* ACETYLCHOLINE RECEPTOR SUBUNIT FROM THE HUMAN PARASITIC NEMATODE *ONCHOCERCA VOLVULUS*. J.A. Lewis*, P. Gamez, A. Mendoza, P. Hernandez, and J. Sorrell. Division of Life Sciences, Univ. of Texas at San Antonio, San Antonio, TX 78249

We are interested in cloning acetylcholine receptor (AChR) subunit genes from the human parasitic nematode *Onchocerca volvulus* and expressing the genes in the laboratory nematode *C. elegans* to facilitate study of potential therapeutic drug targets. *Onchocerca* causes riverblindness in tropical regions of Africa and Central and South America. We have screened an *Onchocerca volvulus* cDNA library at reduced stringency with four different *C. elegans* hybridization probes corresponding to highly conserved region of the AChR subunit genes *unc-29*, *unc-38*, *lev-1*, and a new AChR alpha subunit located near *unc-74*. Hybridization probes were made by PCR using nonradioactively labeled digoxigenin dUTP and PCR primers that flanked highly conserved subunit sequences. Two strongly positive *Onchocerca* clones were identified and plaque-purified in screening 470,000 clones (47 plates, 94 filters) from the cDNA library. The two clones are different isolates of an *Onchocerca* homolog to the *C. elegans unc-29* nonalpha AChR subunit gene. The longer *Onchocerca* cDNA contains the sequence expected for the amino terminal two thirds of the AChR subunit, including the signal sequence. Over this region, the *Onchocerca* clone is 88% identical to *C elegans unc-29* at the amino acid level and about 75% identical at the nucleic acid level. The sequences of the first three (TM1-3) of the four highly conserved AChR transmembrane domains are virtually identical between *Onchocerca* and *C. elegans*. These results indicate that the function of the *unc-29* nonalpha subunit of the levamisole AChR has been highly conserved in evolution during the separation of free-living from parasitic nematodes. As only the 3' end of our clone is missing, reconstruction of a full-length *Onchocerca* clone should be possible, allowing tests of subunit functionality by substitution of the *Onchocerca* subunit for the *C. elegans unc-29* subunit in transgenic nematodes and in frog oocyte expressions assays.

334.9

THE RELATIONSHIP BETWEEN α7 GENOTYPE AND α-BTX LEVELS IS LIKELY DUE TO VARIATION IN NON-CODING PORTIONS OF α7. L.A. Stitzel*, D.A. Farnham, and A.C. Collins. University of Colorado, Boulder, CO 80309.

We have shown previously that inbred mouse strains differ in their sensitivity to nicotine-induced seizures and that this sensitivity significantly correlated with levels of hippocampal α-BTX binding. Classic genetic cross studies confirmed this relationship. However, seizure sensitivity was inherited with a significant dominance component whereas inheritance of α-BTX binding levels appeared to be additive. Recently, we have found that two strains (C3H and DBA/2) that differ in seizure sensitivity and α-BTX levels are polymorphic for the nAChR α7 subunit gene which codes for the α-BTX binding site in brain. A comparison of α7 genotype versus α-BTX levels and seizure sensitivity in a C3H-DBA-derived F2 generation demonstrated that α7 genotype is significantly correlated with levels of hippocampal α-BTX binding. Animals homozygous for the C3H variant of α7 had high levels of α-BTX binding and those animals homozygous for the DBA variant had low levels. Heterozygous animals exhibited intermediate levels of α-BTX binding. No significant relationship between α7 genotype and seizure sensitivity was observed. RNAse protection assays were conducted with RNA isolated from these two strains and results indicated that there is a nucleotide difference in the open reading frame of α7 mRNAs between these strains. Sequence analysis of several PCR-generated clones confirmed this nucleotide difference. However, the mutation was found to be silent; it did not alter the amino acid sequence. Therefore, differences in α7 genotype affect α-BTX levels in some fashion independent of the primary amino acid sequence of α7. These findings suggest that either differences in RNA stability or rates of transcription of the α7 strain variants are likely responsible for differences in α-BTX binding levels. Supported by DA-05131, DA-00197, and AA-07464.

335.1

PRENATAL EXPOSURE TO MORPHINE INDUCES AN ALTERATION IN THE ONTOGENIC EXPRESSION OF NMDA RECEPTOR G.C Yeh[1]*, C. H. Chen[2], and P. L. Tao[2], Taipei Medical College[1], National Defense Medical Center[2], Taipei, Taiwan, ROC

A transient increase of the N-methyl-D-aspartate (NMDA) receptor number during a postnatal period critical for the developmental plasticity of brain has been observed in both of animals and human . Lack of this normal ontogenic change may lead to a permanent sequel in learning , memory or other high cortical function. It has been recognized that child born to morphine-addicting mother have a higher percentage to suffer mild or moderate defect in psychomotor and intellectual activities. To determine whether prenatal exposure to morphine could induce an alteration in the ontogenicity of NMDA receptor, which may contribute to the long-term neuropsychological sequels, we examined the binding of [^{3}H]TCP, a ligand binding to the NMDA receptor-gated ion channel, in membrane preparation of whole brain tissues from different ages (5 day ,14 day and 30 day after birth) of rats born to mother- rats received daily injection of morphine after pregnancy. In naive rats, the [^{3}H]TCP binding affinity increase from 24 nM to 9.7 nM from day 5 to day 30. The number of TCP binding showed a transient increase at day 14 by a 31% (day-5: 0.70± 0.15 pmol/mg, day-14: 0.92 ± 0.02 pmol/mg, day -30: 0..5± 0.04 pmol/mg) . However, such transient increase in NMDA receptor binding number did not present in rats born to mother treated with morphine (day-5:0.58± 0.06 pmol/mg, day 14: 0.65± 0.05 pmol/mg, day-30 : 0.60 ± 0.02 pmol/mg). There is no significant difference in affinity of [^{3}H]TCP binding between naive rats and rats born to morphine-treated mother. This finding suggests that prenatal exposure to morphine alters the ontogenic change of NMDA receptor. Further studies in evaluating the long-term effect of this alteration will be required .

335.2

PHYSICAL AND FUNCTIONAL EVIDENCE FOR ASSOCIATION OF NMDA-R1 WITH PKC AND CAM KINASES IN THE POSTSYNAPTIC DENSITY (PSD) OF ADULT RAT HIPPOCAMPUS. P.C. Suen*[1,2], K. Wu[1,2], R.J. Wenthold[3], S.Y. Lin[1,2], and I.B. Black[1,2]. [1]Dept. Neurosci. and Cell Biol., Robert Wood Johnson Med. Sch.; [2]Program in Physiol. and Neurobiol., Rutgers-The State Univ. of NJ, Piscataway, NJ 08854; [3]Lab. of Neurochem., NIDCD, NIH, Bethesda, MD 20892.

N-methyl-D-aspartate receptors (NRs) play critical roles in synaptogenesis, neuronal degeneration and synaptic plasticity. The receptors are heteromeric complexes comprised of NR1 and NR2A-D subunits. We previously reported that NR complexes are intrinsic components of PSD and are phosphorylated by endogenous PKC and CaM kinases (CKs) in the density. The identity of specific protein kinases involved in the phosphorylation remains unclear. To begin examining this issue, we focused on PKCγ and CKs, enzymes that are intrinsic to the PSD. Immunoprecipitation and Western blot analysis, using anti-PKCγ antibodies or anti-αCKII antibodies which also recognize the major PSD protein (mPSDp), revealed that anti-NR1 antibodies immunoprecipitated PKCγ and/or CKs from hippocampal PSD solubilized in a buffer containing 50 mM Tris-HCl, 0.1% SDS, 1% CHAPS, 1% Triton X-100 and 0.5% NP-40 (pH 7.4). Antibodies that recognize PKCγ (≈ 70 kDa) and Cks (≈ 50 kDa) immunoprecipitated NR1/PKCγ and NR1/CKs complexes, respectively. When the PSD was solubilized under more stringent conditions (at 100 °C), none of these associations was detected, suggesting that the NR1 complexes with PKCγ and/or CKs through noncovalent interaction. NR1 in the NR1/PKCγ and NR1/CKs complexes was phosphorylated upon specific activation of PKCγ and CKs. Our findings indicate that NR1 in the PSD is closely associated with PKCγ and CKs, which may regulate NMDA receptors through phosphorylation. (Supported by NICHD 23315.)

335.3

COEXPRESSION OF CaM-KINASE II WITH IONOTROPIC GLUTAMATE RECEPTOR ACCELERATES ITS RECOVERY FROM DESENSITIZATION. V. Derkach, D. Brickey and T.R. Soderling*, Vollum Institute, Oregon Health Sciences University, Portland, OR. 97201

Desensitization of ionotropic glutamate receptors (GluRs) and their recovery are important parameters which may modulate synaptic transmission. We studied these paramcters using whole cell recording at -80 mV for GluR6 expressed in HEK 293 cells without or with co-transfected CaM-kinase II which is known to phosphorylate and regulate GluR6 (Yakel et al, PNAS 92: 1376-80, 1995). The onset of desensitization with glutamate (0.1-1 mM) and kainate (0.1-0.2 mM) was very fast and was fitted to a single exponential function with time constants of 17.7 ± 2.4 ms (n=9) and 16.6 ± 6.0 ms (n=4), respectively. Recovery from desensitization, determined by application of a second pulse of agonist, was slow with time constants of 2.64 ± 0.35 sec (n=9) and 18.1 ± 2.3 sec (n=5, $p<0.001$) for glutamate and kainate, respectively. These data suggest the rate of recovery depends on the rate of dissociation of agonist from the receptor. Co-transfection of GluR6 with a mutant of CaM-kinase II (His282Arg; Brickey et al, J. Biol. Chem. 269: 29047-54, 1994) which is 20% constitutively active decreased by 5-fold the time constant of recovery from kainate desensitization to 3.4 ± 0.6 sec (n=5, $p<0.001$). The time constant for onset of desensitization was not effected. This study indicates that phosphorylation of GluR6 by CaM-kinase II, both of which are co-localized in the postsynaptic density, may be important in determining the involvement of this GluR type in synaptic transmission. Supported by NIH grant NS27037.

335.4

THE ROLE OF NITRIC OXIDE IN THE MODULATION OF THE NMDA RECEPTOR BY INTERLEUKIN-1β AND PREGNENOLONE SULFATE. J.M. Fahey*, D.G. Lindquist and L.G. Miller. Department of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine, Boston, MA 02111.

Previously, this laboratory has demonstrated modulation of the NMDA receptor by both the proinflammatory cytokine interleukin-1β (IL-1β) and the neuroactive steroid pregnenolone sulfate (PS). NMDA-mediated increases in intracellular calcium are antagonized by IL-1β and potentiated in the presence of PS. The present study examines the role of nitric oxide (NO) in the modulation of the NMDA receptor by IL-1β and PS by examining the intracellular calcium concentration in cultured chick cortical neurons using the fluorescent dye Fura2. N^G-Monomethyl-L-arginine acetate (L-NMMA), a nitric oxide synthase (NOS) inhibitor, antagonized the decrease in intracellular calcium caused by IL-1β in the presence of both NMDA/glycine and the endogenous polyamine spermine. L-arginine, the precursor of NO, had no effect on intracellular calcium levels in cells treated with IL-1β. L-NMMA also inhibited the increase in intracellular calcium caused by PS in the presence of both NMDA/glycine and spermine. L-arginine, however, only antagonized the increase in intracellular calcium seen in the presence of NMDA/glycine. These data indicate that the mechanism by which IL-1β and PS modulate NMDA-mediated increases in intracellular calcium may involve the NO signal transduction system.

335.5

Inhibition of NMDA-activated current by dynorphin (1-13) is subunit-specific and procedes by a non-opioid mechanism
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Cellular and Molecular Neurobiology, NIH, Bethesda, MD 20892-8205

Heteromeric mouse *N*-methyl-D-aspartate (NMDA) receptors were expressed in *Xenopus* oocytes. The κ–opioid receptor agonist dynorphin (1-13) inhibited NMDA-activated currents of all heteromeric NMDA receptors in a subunit-specific manner. The EC_{50} for ε1/ζ1 was 17μM, and the respective potencies were: 1 : 0.32 : 0.32 : 0.17 for ε1/ζ1, ε2/ζ1, ε4/ζ1 and ε3/ζ1. The threshold for the dynorphin effect was about 0.3μM for ε1/ζ1 and ≥10μM for the other subunit combinations. Dynorphin inhibition of NMDA-activated currents was voltage-independent and not competitive with regard to NMDA. NMDA-activated currents were not affected by the synthetic κ–opioid receptor agonist trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzene-acetamide (U50488) or the specific κ–opioid receptor antagonist nor-binaltorphimine (NorBN1). Also, NorBN1 did not attenuate dynorphin inhibition when coapplied. These observations suggest that the interaction between dynorphin and the NMDA receptor is direct, rather than involving the κ–opioid receptor. There was also an interaction between dynorphin and the NMDA receptor that was dependent on glycine concentration. At low glycine concentrations (≤100nM) dynorphin potentiated NMDA-activated currents for all heteromeric NMDA receptors.

335.6

BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) POTENTIATES NMDA-EVOKED RESPONSES C.R. Jarvis*[1], J. Plant[1], D. Churchill[1], Z. Xiong[2], J. F. MacDonald[2] and B.A. MacVicar[1], [1]The University of Calgary, Calgary, Alberta and [2]The University of Toronto, Toronto, Ontario

Neurotrophins have been suggested to be potentially therapeutic in the treatment of neurodegenerative diseases and neurotoxicity because they promote cell survival, growth, and differentiation. BDNF receptors, which are in the hipocampus and striatum, possess intrinsic protein tyrosine kinase activity. Since NMDA receptors are modulated by protein kinases and phosphatases, we hypothesize that BDNF may modulate NMDA currents. Voltage clamp recordings from neurons cultured from the hippocampus or striatum in the presence of low glycine revealed that BDNF (0.1 to 10 ng/ml) reversibly potentiated NMDA currents by up to 600% in a dose dependent manner (n=66). BDNF's potentiation was voltage-independent at -70 mV and +60 mV, had a time course for onset and offset of <1 sec, but was not observed in saturating glycine (>1 μM). These results demonstrate that BDNF acutely enhances NMDA receptor-activated currents. Moreover, imaging of $[Ca^{2+}]_i$ in fura-2-loaded cells revealed that BDNF (10 ng/ml) potentiated NMDA-evoked $[Ca^{2+}]_i$ increases by 207% (n=44 cells). Enhancement of NMDA-activated responses may be important in the short-term actions of neurotrophins. Supported by NCE.

335.7

COCAINE MODULATION OF STRIATAL NMDA CURRENTS. J.R. Plant*, D. Churchill, C.R. Jarvis and B.A. MacVicar. Neurosci. Research Group, Univ. of Calgary, 3330 Hospital Dr. NW, Calgary, AB. T2N 4N1

Cocaine abuse is a pressing social and medical problem. Although cocaine's reinforcing and stimulant properties are thought to be mediated by inhibition of dopamine (DA) re-uptake, recent reports have linked NMDA receptors to some of cocaine's actions. Perforated patch-clamp recordings, and fura-2,AM imaging of NMDA-evoked increases in intracellular calcium ($[Ca^{2+}]i$) were used to investigate whether cocaine modulates NMDA receptor-mediated responses in cultured striatal neurons. Cocaine at low concentrations (0.1-100 μM) potentiated NMDA-evoked currents and calcium signals in a glycine-dependent manner, while higher concentrations (>100 μM) inhibited. Cocaine had no effect on AMPA or kainate-evoked currents. Procaine, a structural analogue of cocaine with similar psychoactive properties, was also tested for its ability to modulate NMDA-evoked responses. Like cocaine, procaine at low concentrations (0.1-20 μM) enhanced NMDA-evoked currents and $[Ca^{2+}]i$ increases, and inhibited at higher concentrations (>20 μM). Since procaine lacks cocaine's ability to inhibit DA re-uptake, our results suggest that their shared psychoactive properties might be mediated by their ability to directly and selectively modulate striatal NMDA transmission.

335.8

Mag-1, a novel group of peptide modulators for NMDA receptors.

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Several groups of peptides that specifically bind NMDAR1 have been identified from random peptide libraries. The purpose of this study is to characterize physiological function of one group of peptides, Mag-1, on NMDA receptors. The core sequence of Mag-1 peptides is CDGLRHMWFC. Human embryonic kidney (HEK) 293 cell line was used for transient coexpression of NMDAR1 and NMDA2A. At 36-72 hours after transfection, whole-cell recordings were carried out to study the function of the Mag-1 peptides. In the presence of 10 μM glycine, 50 μM NMDA-induced currents were significantly reduced by coapplication of Mag-1 peptides. The inhibition on NMDA-evoked current is dose-dependent and voltage insensitive. Increase of NMDA concentration, up to 500 μM, can not reverse the blockade of Mag-1 peptides, indicating the inhibition is non-competitive. In addition, the peptides were found to bind N-terminal extracellular domain of NMDAR1. The binding is not sensitive to Mg^{2+}, Zn^{2+}, Gly, Glu, MK-801, polyamines and Conantokin-G. These results suggest that the peptides recognize a new site and modulate the receptor activity.

335.9

THEANINE, A GLUTAMATE ANALOG, STIMULATES NMDA-RECEPTORS BUT SUPPRESSES EXCITATORY EFFECT OF CAFFEINE IN CORTICAL NEURONS A. Nozawa*[1,2], K. Umezawa[1], K. Kobayashi[1], K. Muramoto[1], M. Kawahara[1], A. Mizutani[1], T. Kakuda[2] and Y. Kuroda[1]. [1]Dept. of Molecular and Cellular Neurobiology, Tokyo Metropolitan Institute for Neuroscience, Tokyo 183, Japan, [2]ITOEN Central Research Institute, Shizuoka 421-05, Japan.

Green tea leaves contain 1-2%(W/W) theanine (L-glutamate γ-ethylamide) which makes up 60% of the total amino acid content. Since theanine can pass through blood-brain barrier, we have carried out an initial study on its effects on brain function, using cultured cortical neurons. When intracellular Ca^{2+} was monitored by fura-2 fluorometry (Kuroda et al. ; Neurosci. Lett., 135: 255, 1992), the addition of theanine produced a Ca^{2+} increase in the neurons. This effect was inhibited in the presence of D-APV, suggesting that theanine stimulates NMDA-receptors. Since it has been reported that theanine prevents caffeine-induced convulsions and behavioral activation in mice, we studied whether theanine could inhibit the effect of caffeine on cultured neurons. Cultured neuronal networks with high synaptic convergence fire spontaneously in synchronous oscillations which can be monitored by Ca^{2+} fluorometry(Robinson et al., J. Neurophysiol., 70: 1606, 1993). Caffeine increased the frequency of the Ca^{2+} oscillation, but theanine was able to inhibit the effect of caffeine. These results suggest that theanine is an interesting glutamate analog which has a dual function on neuronal activity, stimulatory at low concentration, but sedative at high concentration, suppressing excitation produced by caffeine, which is also a neuro-active constituent of tea leaves.

335.10

INSULIN POTENTIATES NMDA RECEPTOR ACTIVITY IN *XENOPUS* OOCYTES AND RAT HIPPOCAMPUS

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Growth factor signal transduction pathways have recently been shown to affect voltage-gated ion channel activity. In this study we report that insulin can modulate the activity of a ligand-gated ion channel, the N-methyl-D-aspartate (NMDA) receptor. In *Xenopus* oocytes, a 2 min. insulin exposure rapidly potentiated NR1a/NR2A and NR1a/NR2B receptor responses 2-3 fold and weakly potentiated NR1a/NR2C and NR1a/NR2D mediated-responses 20-40% above baseline. Peak enhancement was observed at 10 min. followed by a return to baseline within 30 min. The PKC inhibitor staurosporine more potently inhibited phorbol ester-enhanced NR1a/NR2A receptor responses than insulin potentiated-responses suggesting involvement of kinases other than PKC in insulin action. Insulin modulation of native NMDA receptors is suggested by the observation that insulin potentiated the NMDA receptor-mediated synaptic component in region CA1 of hippocampal slices by 26 ± 6% with peak enhancement observed at 20-30 minutes after initiating insulin exposure. Regulation of NMDA receptor activity by growth factors may account for previous observations of growth factor modulation of CNS excitotoxicity. This work was supported by NIH grant NS 28966.

335.11

REGULATION OF NMDAR1 PROMOTER BY NGF AND GROWTH FACTORS IN PC12 CELLS. G. Bai* and J.W. Kusiak. Mol. Neurobiol. Unit, Gerontology Research Center, NIA, NIH, Baltimore, MD 21224.

We previously analyzed the function of a 3 kb promoter of the NMDAR1 subunit gene of the glutamate receptor family (*JBC* 270:7737, 1995). In its proximal region, it has a GSG binding motif for NGFI zinc finger protein family of immediate early genes which can be rapidly induced by NGF in PC12 cells. To clarify whether NMDAR1 promoter is regulated by NGF, we transfected NMDAR1 promoter-linked luciferase reporter gene constructs into PC12 cells and measured luciferase activity after adding NGF and other growth factors. Our results showed that NGF treatment increased the luciferase activity in both transient and stable transfectants. This response is time and dose dependent. In stable transfectants, luciferase activity increased one hour after addition of NGF and reached a peak in 4 hours. Some growth factors, e.g., EGF and FGF, showed a similar potency, while phorbol ester had a greater effect than NGF. Either a cAMP analog or forskolin alone had weak effects, but cAMP strongly potentiated the NGF effect. 5' deletion studies indicated that the NGF response elements reside in the proximal 356 bp promoter fragment which we previously showed was sufficient for activity in PC12 cells. Adding 1 μg/ml actinomycin D abolished the NGF effect. Single mutations of GSG and two Sp1 sites in the 356 bp fragment slightly reduced the NGF effect, however, a double mutation of the two Sp1 sites greatly decreased the response. The endogenous NMDAR1 mRNA, measured with an RNase protection assay, in PC12 cells treated with NGF did not show any significant change, nor did mRNA turn-over rate change. Our studies suggest that NGF and some growth factors are able to up-regulate the NMDAR1 promoter. This promoter is subject to regulation by other signaling pathways, as well. The interactions of the basal promoter of NMDAR1 with multiple factors may control this up-regulation. In PC12 cells, the effect of NGF on endogenous NMDAR1 gene expression may be countered by strong, as yet unknown, inhibitory factors.

335.12

ANTAGONISM OF NMDA RECEPTORS BY SIGMA SITE LIGANDS V. Ilyin, E. R. Whittemore, and R. M. Woodward*. Acea Pharmaceuticals, a subsidiary of CoCensys Inc., 213 Technology Drive, Irvine, CA 92718.

Haloperiodol is a non-competitive subtype-selective antagonist of mammalian NMDA receptors [Ilyin *et al. Soc. Neurosci. Abstr.* 20:1143 1994]. Like ifenprodil, haloperidol selectively inhibits NR1 / 2B subunit combinations (IC_{50} ~3 μM). NMDA responses in cultured rat cortical neurons (E17, 4-10 days *in vitro*) are inhibitied at similar potency. A recent report proposes that modulation of NMDA receptors by haloperidol and other σ-ligands is due to actions at the σ1-site, mediated indirectly via a second messenger system [Yamamoto *et al. J. Neurosci.* 15:731 1995]. To investigate this idea we assayed a variety of σ-site ligands for inhibition of cloned NMDA receptors expressed in *Xenopus* oocytes, and also characterized inhibition of neuronal NMDA receptors by haloperidol at the single channel level. Carbetapentane, DTG, 4-IBP, 3-PPP, pentazocine, rimcazole and SK&F10047 inhibited NMDA responses with a wide range of potencies (IC_{50}'s ~0.1-300 μM) but did not have pronounced subtype-selectivity. We found no clear correlation between affinity at σ1-sites and antagonism of NMDA currents. Inhibition by DTG, pentazocine and SK&F10047 showed use- and voltage-dependence suggesting a PCP-like channel block. Single channel conductance of neuronal NMDA receptors in excised patches was ~50 pS. Mean channel open times were fit by two exponentials; τ_1 0.4-0.9, τ_2 2-6 msec. Haloperidol (3-30 μM) inhibited single channel currents at the same or greater potency as whole cell currents, arguing against involvement of a second messenger system. Inhibition was mainly due to reduction in mean open probability. Our results suggest that antagonism of NMDA receptors by σ-site ligands is through direct interactions at two types of site on the receptor; allosteric modulatory sites (e.g. haloperidol and ifenprodil), and sites in the channel pore (e.g. DTG, pentazocine and SK&F10047).

335.13

EFFECTS OF TRYPSIN AND PAPAIN ON *N*-METHYL-D-ASPARTATE (NMDA) ACTIVATED WHOLE-CELL CURRENT.

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We studied whether enzymes used to dissociate neuronal cells may affect the function of NMDA receptor-channels. Whole-cell patch-clamp recording was used to investigate the effects of trypsin (0.25%) and papain (14.1 U/ml) on NMDA-activated current in cultured mouse cortical neurons. Acute application of either enzyme generated a large inward current. Preapplication of pertussis toxin blocked the current generated by trypsin suggesting G_i protein involvement. Current generated by papain reversed near 0 mV and was not blocked by pertussis toxin. Application of trypsin for 150 sec did not cause a reduction of NMDA-activated currents. Application of papain for 40 sec. produced a >50% decrease of NMDA-activated current amplitude. Recovery from papain's effect was not observed over 20 min. Although papain is considered to be the gentler of the two enzymes, the results suggest that trypsin digestion would be better for studying NMDA-activated currents in acute preparations. DWF was supported by NIMH grant #5T32MH19547.

335.14

PHARMACOLOGICAL CHARACTERIZATION OF THE [^{3}H]SPERMINE BINDING TO THE POLYAMINE-SITE OF NMDA RECEPTOR IN THE RAT BRAIN.

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We investigated the [^{3}H]spermine binding which recognized the polyamine-site of NMDA receptor in the rat cortical membranes. [^{3}H]Spermine bound apparently to two binding sites, with Kd of 185 nM (high affinity) and 5.9 μM (low affinity). Displacement analysis of [^{3}H]spermine binding shows a pharmacological profile similar to that reported for [^{3}H]ifenprodil binding as a ligand for spermine-site of NMDA receptor. Polyamines displaced [^{3}H]spermine binding with the order of potency spermine > spermidine > diaminodecane > diethylenetriamine > putrescine. The binding of [^{3}H]spermine was also inhibited by arcaine, ifenprodil and neomycine. In addition, MK801, an antagonist of the NMDA receptor, inhibited the [^{3}H]spermine binding (IC_{50}=745 μM). However, glutamate, glycine and their antagonists, D-AP5, D-AP7 and 7-chlorokynurenic acid, did not affect the binding. These results show that [^{3}H]spermine has a relatively higher affinity than other polyamine ligands, such as spermidine, and is a useful ligand for investigating the polyamine-site of NMDA receptor.

335.15

ISOPROTERENOL POTENTIATES SYNAPTIC TRANSMISSION PRIMARILY BY ENHANCING PRESYNAPTIC CALCIUM INFLUX VIA P-TYPE CALCIUM CHANNELS.

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The effects of the selective β-adrenergic receptor agonist isoproterenol (Iso) on synaptic transmission was investigated in slices of rat amygdala using intracellular recording techniques. A 10-15 min application of Iso (15 μM) produced a long-lasting enhancement of EPSP without altering the resting membrane potential or neuronal input resistance. The sensitivity of postsynaptic neurons to AMPA was unchanged by Iso pretreatment . The Iso-induced potentiation was not affected by pretreatment with APV, or Kynuretic acid+APV. However, substituting Mg^{++} for Ca^{++} in the medium blocked the effect of Iso suggesting that Ca^{++} entry into presynaptic terminals, but not into postsynaptic cell via glutamate receptors, is required for the action of Iso.

Amygdala neurons fired rapid action potentials which accommodated in response to a long-duration depolarizing pulse. The end of depolarizing pulse was followed by an afterhyperpolarization (AHP). Superfusion of Iso reversibly blocked the AHP and accommodation resulting in more action potential firing. Intracellular applied PKI, a selective blocker of the catalytic subunit of PKA, blocked the effect of Iso on the AHP, whereas the Iso-induced potentiation was entirely normal in the same neuron. These results indicate that Iso-induced potentiation is not due to blockade of postsynaptic I_{AHP}. Iso-induced potentiation was not affected by nifedipine or ω-conotoxin-GVIA, but was blocked by ω-agatoxin-IVA or ω-conotoxin-MVIIC, suggesting that the mechanism underlying the potentiating effect of Iso is likely due to an increased Ca^{++} influx through P-type Ca^{++} channels presynaptically.

335.16

THE NMDA RECEPTOR NR2 SUBUNITS INTERACT WITH THE POSTSYNAPTIC DENSITY PROTEIN PSD-95.

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NMDA receptors mediate the slow component of excitatory postsynaptic currents and activity-dependent plasticity in the mammalian central nervous system (CNS). Different NMDA receptor subtypes have been molecularly characterized and these assemble from a common NMDAR1 subunit and a modulatory NMDAR2 (NR2A-D) subunit. The four NR2 subunits are uniquely equipped with long cytoplasmic C-terminal tails. Screening a rat brain cDNA library with the two-hybrid system we found that these tails interact with PSD-95, a major postsynaptic density protein. PSD-95 is a multidomain protein which consists of three PDZ domains (also termed GLGF or DHR domains), one SH3 domain and a region homologous to yeast guanylate kinase. Molecular and biochemical analyses showed that the interaction results from binding between the C-terminal domain common to all NR2 subunits and a particular PDZ domain in PSD-95. Immunohistochemistry documented subcellular co-localization of NR2B subunits and PSD-95 in cultured hippocampal neurons, and *in situ* hybridization revealed a wide-spread and largely congruent expression pattern of transcripts for PSD-95 and NMDA receptor subunits. Our data suggest that the NR2/PSD-95 interaction is involved in localizing NMDA receptors at postsynaptic sites in the CNS.

335.17

PURIFICATION OF A 20 kD PROTEIN WHICH ASSOCIATES WITH THE NR1 SUBUNIT OF THE NMDA RECEPTOR.

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NMDA receptors play important roles in synaptic plasticity, neural development and excitotoxicity. Molecular cloning studies have shown that NMDA receptors consist of two types of subunits: NR1 and NR2A-D. These subunits contain large COOH-terminal domains which may interact with cellular proteins. To identify proteins which interact with NMDA receptors, NR1 immunoprecipitates were examined for the presence of coprecipitating cellular proteins. A 20 kD protein was observed to specifically associate with the NR1 subunit. Moreover, this protein, termed RAP-20, preferentially associates with the NR1 subunit splice variants containing the C1 exon cassette at the COOH-terminus. We have utilized an NR1 COOH-terminus fusion protein affinity resin to purify this protein. RAP-20 is present in the brain, and coimmunoprecipitates with NMDA receptors from rat cortex. We are currently attempting to determine the identity and the functional implications of this potentially important protein.

336.1

NMDA NR1 SPLICE VARIANTS AND NR2A AND NR2B SUBUNITS: DIFFERENTIAL PROTEIN EXPRESSION IN RAT BRAIN REGIONS.

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NMDAR1 splice variants and NMDAR2 subunits show developmental and regional heterogeneity in rat brain resulting in physiological and pharmacological diversity of the NMDA receptor complex. To determine the nature of the association between NR1 splice variants and NR2 subunits, we developed antibodies specific for the alternatively spliced regions of NR1. These are: an antibody AbN1 recognizing N-terminus insert (insertion of 21 a.a. residues located between residues 190 and 191), antibodies AbC1 and AbC2 recognizing variants of the C-terminus (C1-a.a. residues 901 to 938, and alternatively spliced C2 composed of 22 a.a. residues), and an antibody C36 recognizing the sequence known as deletion 1 present in some variants (a.a. residues 864 to 900). Antibodies selective for the NR2 subunits, NR2A and NR2B, were also used.

Rat brains were dissected and regions solubilized under conditions maximally preserving the NMDA receptor complex for co-immunoprecipitation studies, or solubilized with SDS when studying relative amounts of each protein. Antibodies specific for the NR1 alternatively spliced regions were used for immunoprecipitation. These and an antibody recognizing all of the the NR1 variants, as well as antibodies to the NR2 subunits, were used for detection on western blots. This allowed us to study relative amounts of some of the NR1 splice variants.

Differences of NR1 splice variant expression were found both during postnatal development and between regions in the adult brain. For example, NR1 protein with C1 was more abundant in the cortex and the hippocampus than in the thalamus or the striatum, whereas the opposite was found for the NR1 variants with C2. NR1 subunits containing N1 were far more abundant in the thalamus, and less expressed in the cortex and the hippocampus. Expression of NR2A and NR2B was also found to vary between these regions; for example, NR2B was found to be expressed at higher levels in the striatum than in the thalamus, whereas the same levels of the NR2A subunit were expressed in both regions.

336.2

FOCAL CEREBRAL ISCHEMIA ALTERS NMDA AND AMPA RECEPTOR AND PREPROENKEPHALIN GENE EXPRESSION IN STRIATUM AND CORTEX.

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Activation of NMDA receptors by glutamate may play a role in neuronal cell death following middle cerebral arterial occlusion (MCA-O). NMDA and AMPA antagonists reduce the extent of infarction produced by MCA occlusion. *In situ* hybridization was used to measure glutamate and preproenkephalin (PPE), (precursor of enkephalin peptides) expression in the striatum and cortex of adult rats following 1.5 hrs of MCA occlusion and three recirculaion (R) periods. At 1.5 hrs R, autoradiography indicated pronounced but differential decreases in NR1, GluR2, GluR3, and PPE expression throughout the ipsilateral striatum. In the cortex more selective changes in expression were observed. NR1 was reduced in most of the ipsilateral cortex relative to control brain and the contralateral side. GluR2 was predominantly reduced in pariatal cortex. In contrast, cortical GluR1, GluR3 and PPE mRNAs were unaltered. At 3 hrs R, MCA-O animals showed a regional specificity in glutamate receptor gene expression in ipsilateral striatum; reduced expression was restricted to the dorsomedial striatal subfield. At 24 hrs R, reduced mRNA expression occured only in animals that exhibited some neuronal damage in ipsilateral striatum and cortex. PPE expression was reduced in the contalateral side of these animals. All mRNA changes were in mRNA content per neuron. Histological evaluation showed pallor in the absence of neurodegeneration in areas that showed marked changes in glutamate and PPE mRNAs at all three recirculation periods. Changes in mRNA expression occur prior to obvious neuronal cell damage or loss but persist in animals that exhibit cell damage. In addition, the region where PPE expression is reduced in the ipsilateral striatum is primarily innervated by the affected ipsilateral cortical region suggesting a relationship between glutamatergic and enkephalin synapses.

336.3

GLUR2 RECEPTOR PROTEIN EXPRESSION IS REDUCED IN HIPPOCAMPAL CA1 AFTER GLOBAL ISCHEMIA.

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Transient global ischemia results in delayed, selective cell death of hippocampal CA1 neurons. Reduced mRNA expression of GluR2 (the AMPA receptor subunit that limits Ca^{2+} permeability) occurs in CA1 neurons after induction of global ischemia and prior to neuronal cell death. The present study was undertaken to examine AMPA and NMDA receptor protein expression in ischemic and control (sham-operated) rats at the level of the hippocampus. Global ischemia was induced by four-vessel occlusion; cerebral circulation wasπ restored after 10 min. At 24 hrs, GluR2 immunolabelling (detected with a monoclonal Ab to GluR2) was decreased in CA1 neurons. Isolated CA1 neurons exhibited somatic staining in the absence of dendritic staining. At 48 hrs, GluR2 immunoreactivity in CA1 was virtually absent in 6 of 7 animals. Histological examination revealed little or no neurodegeneration at this time. In conclusion, changes in GluR2 receptor protein follow changes in GluR2 mRNA in the CA1 subfield prior to neurodegeneration. These findings provide further support that increased formation of Ca^{2+}-permeable AMPA receptors in CA1 neurons may enhance glutamate pathogenicity in this region.

336.4

EXPRESSION PATTERN OF THE KAINATE RECEPTORS STUDIED BY IN SITU HYBRIDIZATION IN THE BASAL GANGLIA AND VENTRAL MESENCEPHALON. S. BISCHOFF* and S. HEINEMANN, Salk Institute, Mol. Neurobiol Lab., La Jolla, CA 92037

Molecular cloning led to the identification of five kainate receptor subunits, GluR5-7, KA1 and KA2. To gain knowledge on their possible functions within the CNS, we performed a detailed mapping of the mRNA encoding each of them in the mouse brain basal ganglia. This circuitry plays a pivotal role in the translation of motivation into action and receives multiple glutamatergic inputs. mRNA transcripts were mapped by using in situ hybridization histochemistry with ^{35}S-labeled antisense riboprobes. The kainate receptor subunits were found to be expressed at various degrees in all aspects of the basal ganglia. However, they differ from each other in their relative expression levels as well as in their specific localization. GluR6 is expressed at highest levels in the striatum in the soma of the descending GABAergic pathways, in the nucleus accumbens and olfactory tubercles, and in the subthalamic nucleus. GluR7 is the major subunit in the ascending nigrostriatal and mesolimbic DAergic neurons. GluR5 is only expressed at high levels in the islands of Calleja and the substantia nigra pars compacta. KA2 usually followed the distribution of GluR6, though with a generally much lower level of expression. Finally, KA1 is not expressed at significant levels in any region of this neuronal circuitry. These data suggest that the kainate receptors in general might be involved in the functions associated with the basal ganglia such as the control of the motor programs. Often co-expressed with dopamine receptors, the kainate receptors may regulate the central dopaminergic transmission. Consequently, they might also be implicated in diseases associated with movement disorders, in neurodegenerative processes as well as psychiatric illnesses associated with impairment of the basal ganglia and limbic structures.

336.5

ANTISENSE OLIGODEOXYNUCLEOTIDES TARGETED TO GLUR-B mRNA ALTER THE CALCIUM PERMEABILITY OF AMPA RECEPTORS IN RAT CULTURED CORTICAL NEURONS

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We tested the role of GluR-B in controlling the functional properties of neuronal AMPA receptors. In cortical neurons cultured from embryonic day 17 rats, the calcium permeability decreased as the number of days *in vitro* (DIV) increased. Whole cell patch clamp, with 100 µM cyclothiazide and 100 µM AMPA, was used to obtain current-voltage relationships in external solutions containing either sodium or calcium as the sole external cation. Neurons at 9 DIV and 24 DIV had P_{Ca}/P_{Na} ratios of 0.13 ± 0.088 (n=7) and 0.0068 ± 0.0019 (n=7), respectively. Additionally, 9 DIV neurons obtained from postnatal day 1 rats exhibited a ratio of 0.010 ± 0.0041 (n=5).

In one experiment, cortical neurons obtained from postnatal day 0 rats were maintained in MEM plus N2 supplement. At days 3 and 5 *in vitro*, antisense and sense phosphodiester oligonucleotides targeted to the GluR-B mRNA translation start site were added to the culture medium at a concentration of 1.0 µM. At 7 DIV, the average P_{Ca}/P_{Na} ratio for 1 µM antisense was 1.29 ± 1.0 (n=3) and the ratio for sense treated cells was 0.018 ± 0.017 (n=4). Subsequent experiments have focused on increasing the efficiency and detection of oligonucleotide uptake. For example, liposome-mediated transfection of 0.3 µM antisense oligonucleotide increases average calcium permeability, producing a P_{Ca}/P_{Na} ratio of 0.54 ± 0.75 (n=2).

Treatment of cortical neurons with antisense oligonucleotides may decrease the expression of GluR-B and subsequently prevent the decline in calcium permeability that occurs during *in vitro* cortical neuron maturation.

336.6

USE OF ANTISENSE OLIGODEOXYNUCLEOTIDES TO DEFINE DEVELOPMENTAL CHANGES IN THE SENSITIVITIES OF NMDA RECEPTORS TO ANTAGONISTS.

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Antisense oligodeoxynucleotides were used to determine whether alterations in the expression of NMDA receptor subunit mRNAs are responsible for developmental changes in the sensitivities of the receptor to different NMDA receptor antagonists. *Xenopus* oocytes were injected with mRNA prepared from neonatal and adult rat cortex, and the affinities of the receptor for different NMDA receptor antagonists were determined under voltage clamp. NMDA receptors expressed from adult rat cortex mRNAs have higher affinities for glycine site antagonists like 7-chlorokynurenate and for glutamate site antagonists like CGP-39653 than those expressed from 1 day old rat mRNAs. The properties of receptors seen when adult brain mRNA was coinjected with antisense oligonucleotide against the NMDA receptor subunit NR2A were similar to receptors expressed following injection of 1 day old rat cortex mRNA in sensitivity to 7-chlorokynurenate and CGP 39653. We have previously reported the delayed expression of NMDA receptors having a low affinity for ifenprodil during development. Coinjection of antisense oligonucleotide for the NR2A subunit with adult mRNA selectively blocked the expression of receptors with a low affinity for ifenprodil. These data suggest that the sensitivity of NMDA receptors to various antagonists changes during development, and alterations in the expression of different subunits during development can account for those changes.

336.7

CHARACTERIZATION OF NMDA RECEPTOR SUBUNITS IN HIPPOCAMPAL AND CEREBELLAR PRIMARY CULTURES. M. Didier,*M. Xu, S.A. Berman, S. Bursztajn. Lab. for Molecular Neuroscience, McLean Hospital and *Dept of Psychiatry, Harvard Med. Sch. Belmont and Boston, MA, USA.

We characterized the molecular nature and the excitotoxic properties of NMDA receptors expressed in mouse hippocampal and cerebellar granule neurons. RT-PCR revealed that NMDAζ1 as well as NMDAε1-4 subunits were expressed in cerebellar cultures. In contrast, mRNA for NMDAε3 was undetectable in cultured hippocampal neurons. In cerebellar cultures, western-blot analysis showed that the amount of membrane-bound NMDAζ1, ε1 and ε3 subunits increased throughout the neuronal development in vitro. In contrast, the ε2 expression was already high after 3 days in culture and remained relatively stable thereafter. At 9 days in culture, a somatic and neuritic immunolocalization for all these NMDA subunits was clearly identified in most granule neurons but not glial cells. In hippocampal cultures, an increase in NMDAζ1, ε1 and ε2 proteins was observed during the neuronal developement. Most of pyramidal cells were immunoreactive for these NMDA subunits. The NMDAε3 subunit was never detected either by western-blot analysis or immunocytochemistry in hippocampal primary cultures. In both cerebellar and hippocampal neurons, an overactivation of NMDA receptor induced a dramatic neuronal cell death. The NMDA receptor subunits involvement in excitotoxcity was assayed using an antisense oligonucleotide (ODN) strategy. The antisense ODN treatment reduced significantly and specifically the amount of the respective NMDA subunits. In cerebellar cultures, antisense, but not sense, ODNs for NMDAζ1, ε1,2 and 3 rescued granule cells from NMDA excitotoxicty, wihtout protecting them from toxicity mediated by kainate or MPP+ exposure. In hippocampal cells, antisense ODNs for NMDAζ1, ε1 and 2 reduced NMDA-induced neurotoxicity. In many experiments, co-treatments with several antisense ODNs for NMDAε subunits did not improve the neuroprotection suggesting that several NMDAε subunits may be colocalized in the same NMDA receptor. Immunoprecipitation experiments indicated that at least NMDAζ1, ε1 and 2 can be present as heteromer complexes in both cerebellar and hippocampal cultured neurons. Further experiments are in progress to identify other NMDA receptor subunits which may mediate neurotrophic and neurotoxic glutamate properties in cerebellar and hippocampal cells.

336.8

TIME DEPENDENT CHANGES IN EXPRESSION OF NR1, NR2A AND NR2B NMDA RECEPTOR SUBUNITS IN RAT BRAIN AFTER MK801 X.M. Gao*, T. Hashimoto and C.A. Tamminga. Maryland Psychiatric Research Center, University of Maryland School of Medicine, Baltimore, MD 21228

A single dose of either PCP or MK801 in rat produces a dose-related increase in NMDA-sensitive ^{3}H-glutamate binding in CA_1 (29%) and DG (22%) of hippocampus 24 hours after a dose (Neurosci. Lett. 1994, J. Neuro. Transm. 1995). We have now examined the time-dependent action of MK801 on the expression pattern of NR1, NR2A, and NR2B NMDA receptor subunits in rat hippocampus across a MK801 dose range. The three mRNAs encoding the NMDA subunits NR1, NR2A and 2B were quantified at 1, 3, 6, 24 and 48 hours after MK801. In situ hybridization and quantitative autoradiography using specific oligonucleotide probes for each subunit showed that these 3 subunits were expressed throughout the brain although this analysis focuses on hippocampus. Quantitative analysis of film densities indicated that in hippocampal CA1, an increase of 26%, 32% and 35% expression of the mRNA coding for the NR1 NMDA subunit was observed at 3h, 6h and 24h respectively after MK801 ($P<.001$). Also, an increase in the expression of NR1 mRNA was found in CA_3 and DG between 3h to 24h ($P<0.05$). In addition, an increase in expression of NR2A mRNA was observed both in CA1 (16-23% , $P<.01$) and DG (15-21%, $P<.01$) between 3h and 24h. No change was found at 1h after MK801 in the expression of NR1 and NR2A in any hippocampal region. By 48 h after drug the expression of NMDA subunits had returned to control levels. This result indicates that time dependent increases in NMDA receptor binding after PCP or MK801 in CA_1 and DG of hippocampus are mediated by increased NMDA receptor subunit gene expression.

336.9

IMMUNOHISTOCHEMICAL LOCALIZATION OF GLUTAMATE RECEPTORS IN THE CAT BRAIN STEM. R. Ambalavanar, C. Ludlow, Y. Tanaka and R.J. Wenthold* NIH, NIDCD, Building 10, Room 5D38, 10, Center Drive MSC 1416, Bethesda, Maryland, MD 20892.

The glottic closure reflex in the larynx plays an important role in protecting the lower airway from invasion of foreign bodies. Excitatory amino acid receptors may play an important role in the laryngeal sensory-motor network. In the present study we investigated the distribution of AMPA-selective glutamate receptor subunits (GluR1-GluR4) and the NMDA receptor subunit NMDAR1 in the cat brain stem. Eight cats of both sexes were deeply anaesthetized with pentobarbital (100 mg/kg, i.p) and perfused transcardially with 0.1M phosphate buffer (PB, pH 7.4) followed by Zamboni fixative. Transverse sections (30μm thick) of the brain stem were incubated in antibodies to GluR1, GluR2/3, GluR4 and NMDAR1 (1-3mg/ml) and then with avidin-biotin complex (ABC-kit). Controls were carried out by substituting either PBS for primary antibody or preabsorbed antibodies with their corresponding peptide antigens. The controls did not show any evidence of nonspecific labeling. Generally, denser staining of perikariya and neuropil was observed with GluR2/3 and GluR4. GluR4 labeling was particularly clear in the cell bodies including their fine processes. In the nucleus tractus solitarius (NTS), which receives afferent input from the larynx, staining was found with all four antibodies. The solitary tract showed punctate staining. Cell body staining was more common for GluR1 and GluR2/3 than for NMDAR1 or GluR4 in different subnuclei of the NTS. Staining was observed with varying intensity in the reticular core and other sensory and motor nuclei in the brain stem including the nucleus ambiguus where laryngeal efferent neurons are located. The present results suggest that both NMDAR1 and AMPA receptor subtypes are involved in the laryngeal neural circuitry and that there may be an overlap in their distribution.

336.10

DISTRIBUTION OF NMDA AND NON-NMDA RECEPTORS IN THE FELINE PONS AND MEDULLA. Y.Y. Lai*, J.-P. Wu, J.S. Kuo and J.M. Siegel. Dept. Psychiat, Sch Med, UCLA, VAMC, Sepulveda, CA 91343 and Dept Med Res, Taichung Veterans General Hospital, Taiwan.

We have reported that microinjection of nonNMDA agonists produces muscle tone suppression, while NMDA agonists produce motor excitation in both pons and medulla of the cat. We have shown that glutamatergic neurons in brainstem nuclei project to both the pontine inhibitory area and nucleus magnocellularis. The present study identified brainstem neurons containing NMDA and nonNMDA receptors using immunohistochemical techniques. Alternate 50 μ sections were processed for NMDA receptors with NMDAR1 (PharMingen) and kainate with GluR5/6/7 (PharMingen). In both pons and medulla, the number of neurons labelling for NMDA was higher than the number labelling for kainate. The number of NMDA and kainate receptor immunoreactive neurons was found to be high in pontine gray, dorsal and ventral nuclei of the lateral lemniscus, tegmental reticular nucleus, trapezoid body, cochlear, superior and inferior olivary, hypoglossal, dorsal raphe nuclei, and motor neurons; moderate in laterodorsal tegmental, vestibular, spinal trigeminal, paragigantocellularis, and magnocellularis nuclei; low in gigantocellularis nucleus and pontine reticular formation.

336.11

IMMUNOCYTOCHEMICAL LOCALIZATION OF GLUTAMATE RECEPTOR SUBUNITS IN PURKINJE CELLS IN DEVELOPING AND ADULT RATS. R.S. Petralia*, H-M. Zhao, Y-X. Wang and R.J. Wenthold, Lab. of Neurochemistry, NIDCD, NIH, Bethesda, MD 20892.

Cerebellar Purkinje cells receive 2 major excitatory inputs: an extrinsic input from climbing fibers originating from inferior olive neurons, and an intrinsic input from parallel fibers originating from granule cells of the cerebellar cortex. Activity in both of these afferents may be necessary for plastic changes (i.e., long-term depression) needed to modulate the inhibitory response of the Purkinje cells. Previous *in situ* hybridization and immunocytochemical studies indicated that a number of glutamate receptor subunits are prevalent in adult Purkinje cells, with the most abundant including GluR2, GluR3, NR1, delta 2, and mGluR1. In this study, we used C-terminus peptide antibodies recognizing GluR1, GluR2/3, delta 1/2, NR1, and mGluR1α (the latter provided by Drs. C.D. Blackstone and R.L. Huganir) as well as an N-terminus fusion protein antibody to GluR5-7 (provided by Dr. J.H. Morrison). Light microscope studies of Purkinje cell development revealed that staining with GluR2/3, delta 1/2, NR1 and mGluR1α antibodies was light at 10 days postnatal (P10). By P15, staining was moderate to dense and was similar to adult levels. Since maximum synaptogenesis occurs between P10 and P15, expression of all of these subunits simultaneously may be necessary for function of Purkinje cell circuitry. Ultrastructural examination of adult Purkinje cell parallel and climbing fiber synapses revealed differing patterns of postsynaptic staining with the 6 antibodies, with dense staining in both types of synapses seen most commonly with mGluR1α antibody. In addition, some presynaptic staining was seen with GluR5-7, and staining in Bergmann glial wrappings of parallel fiber synapses was common with GluR1, as noted previously. Thus, a particular combination of glutamate receptor types at Purkinje cell synapses may be established late in postnatal development and be required for Purkinje cell responses.

336.12

QUANTITATION OF AMPA RECEPTOR SURFACE EXPRESSION IN CULTURED HIPPOCAMPAL NEURONS. R.A. Hall* and T.R. Soderling, Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

Protein and mRNA levels of the AMPA-type glutamate receptor subunits GluR1-3 are high in many brain regions, but it is not known how much of the GluR protein is expressed on the surface of neurons in the form of functional receptors. To provide insight into this matter, GluR1 and GluR2/3 immunostaining in primary hippocampal cultures was quantified via western blot before and after three different treatments: cleavage of surface receptors by chymotrypsin, cross-linking of surface receptors with the amine-reactive cross-linker BS^3, and biotinylation of surface receptors with the amine-reactive reagent NHS-ss-biotin. None of these three treatments affected immunostaining for the intracellular proteins actin, tubulin or CaM kinase II; immunostaining for the GluR1-3 subunits, however, was reduced by approximately 75% by all three methods, suggesting that at least three-quarters of the total receptor protein resides in the plasma membrane. The same three methods were also used to quantify [^{3}H]AMPA binding to membrane preparations derived from control vs. treated cultures; chymotrypsin reduces binding presumably by cleaving binding sites, while BS^3 and NHS-ss-biotin reduce binding presumably by reacting with lysine residues known to be crucial for agonist binding to AMPA receptors. Estimates of the percentage of surface [^{3}H]AMPA binding sites were quite different from the estimates of surface GluR protein, as less than 25% of total [^{3}H]AMPA binding could be inactivated by the three treatments; parallel studies with the biotinylated samples demonstrated that, even though only a small fraction of the binding sites were inactivated by the biotinylation, the majority of binding sites could still be immunoprecipitated with streptavidin beads. These data suggest that the majority of AMPA receptors in hippocampal neurons are located in the plasma membrane and are exposed extracellularly, but that the binding sites of most of these receptors are not accessible to the reagents used in the present study (Supported by NIH grant NS27037).

336.13

MK-801 DIFFERENTIALLY REGULATES AMPA RECEPTOR SUBUNIT GENE EXPRESSION IN LIMBIC AREAS AND HIPPOCAMPUS. D.J. Healy*, J. Frederick, H. Hamid, J.H. Meador-Woodruff Department of Psychiatry and Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

The NMDA receptor is blocked by a magnesium ion at rest. In order to extrude the magnesium ion, the cell membrane must be partially depolarized. Complete depolarization cannot occur unless preceded by this partial depolarization. AMPA and kainate receptors have been shown to be colocalized with NMDA receptors, and may facilitate NMDA function by partially depolarizing the membrane. Therefore, any change in NMDA function may cause a compensatory change in AMPA or kainate receptor subunit expression. We tested this hypothesis by treating rats with 0.3 mg/kg, 1.0 mg/kg, and 3.0 mg/kg of MK-801, an NMDA antagonist, daily for one week. Then we measured AMPA and kainate receptor subunit mRNA levels in cortical, subcortical, and hippocampal structures, using *in situ* hybridization. Preliminary data suggest differential regulation of AMPA subunit gene expression in limbic cortex and hippocampus, indicating that the NMDA receptor may be able to autoregulate it's activity via non-NMDA receptors. This may reflect a postsynaptic mechanism by which glutamatergic tone is self-regulated, in parallel with a presynaptic, autoreceptor-mediated mechanism.

336.14

DISTRIBUTION OF IMMUNOCYTOCHEMICALLY IDENTIFIED NMDA RECEPTOR SUBUNIT NMDAR1 IN THE FROG *RANA PIPIENS*. Z. Li*, C. L. Bengston and K. V. Fite. Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003.

NMDA receptors have been suggested to play an essential role in neural development, synaptic plasticity, and compensation following injury. In the present study, immunocytochemistry using a polyclonal antibody that recognizes NMDA receptor subunit NMDAR1 was used to assess the localization of NMDA receptors in the CNS of the adult frog *Rana pipiens*.

Observations revealed NMDAR1 immunoreactivity in the diencephalon, midbrain and brainstem. The densest stained perikarya and dendrites were observed in the anterior preoptic area (POa) and magnocellular preoptic nucleus (Mg) of the central diencephalon. In the pretectum, scattered perikarya in the posterior thalamic neuropil (PTN) were moderately stained. Weak NMDAR1 immunoreactivity was noted in the nucleus of the basal optic root (nBOR). Perikarya in the ocular motor nucleus as well as many reticular and motor nuclei in the caudal midbrain and brainstem were moderately to densely immunostained.

Currently, assessment of how behavioral training and surgical manipulation may affect the distribution of the NMDA receptors in the frog CNS is under investigation. (supported by NSF Grant # IBN 921157 to K.V.F.)

336.15

DISTRIBUTION OF NMDA AND AMPA RECEPTOR mRNA IN THE RHESUS MACAQUE TEMPORAL CORTEX. V.T. Garyfallou, S.G. Kohama and H.F. Urbanski*. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, Oregon, 97006.

To complement and extend data from previous immunocytochemical studies, we have examined the distribution of NMDAR1, GluR1, GluR2 and GluR3 mRNA in the temporal cortex of the rhesus macaque (*Macaca mulatta*). Using [35-S]-labelled cRNA antisense probes transcribed from rat cDNA templates, sections of temporal cortex at the level of the caudal hippocampus and lateral geniculate body were hybridized overnight at 60 C. Following various washes, RNAse treatment and a criterion wash at 70 C in 0.1X SSC, the sections were apposed to Hyperfilm (Betamax) for autoradiography. The hybridization patterns for the NMDA and AMPA receptors were similar in both distribution and intensity. High levels of hybridization were seen in the dentate gyrus and CA3, followed by slightly lower signal in the CA1, dentate hilus, entorhinal and surrounding temporal cortex. Despite high levels of NMDAR1 mRNA in the lateral geniculate body there was an apparent lack of GluR1, 2 or 3 mRNA in this region. Also of note was the differential expression of GluR1 mRNA in the temporal cortex. Unlike the other receptors examined, GluR1 expression was lacking in an intermediate layer of the cortex, but still present in apical and basal layers. The apparent colocalization of receptors in many areas is of integrative importance, but the lack of specific receptor subtypes may be equally important for regional function.

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336.16

DIFFERENTIAL EXPRESSION OF NMDA RECEPTOR SUBUNITS NR2A AND NR2B IN ADULT AND DEVELOPING RAT CNS REVEALED BY SUBUNIT SPECIFIC ANTIBODIES. DL Price*, LJ Martin, and C Portera-Cailliau. Neuropathology lab, The Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

N-methyl-D-aspartate (NMDA) receptors participate in physiological and pathological processes in developing and mature CNS and are probably assemblies of NR1 with NR2 subunits. We have generated two affinity-purified polyclonal subunit-specific anti-peptide antibodies that recognize NR2A and NR2B. In Western blots of rat, pig, monkey, or human brain homogenates, each antibody detects a single 172 kD protein corresponding to its homologous subunit. In adult rat CNS, NR2A and NR2B are enriched in cortex and hippocampus, but are present in other forebrain regions. In hindbrain, NR2A is present at low levels but NR2B is barely detectable. By immunocytochemistry, both subunits are enriched in neuropil and in some cell bodies of neurons and astroglia. These subunits are also differentially expressed in postnatal CNS development. In cerebellum and spinal cord, low levels of NR2A are present throughout development, whereas NR2B is only transiently expressed in the first weeks of postnatal life. In cortex and striatum, NR2A is absent at birth but levels progressively increase thereafter, while NR2B is expressed at nearly adult levels throughout cortical development, and its developmental increase in striatum is less than that of NR2A. These results suggest that some NMDA receptors may consist of assemblies of NR1 subunits with either NR2A or NR2B, and that transient changes in NMDA receptor function may occur during maturation of certain neuronal and/or glial populations.

336.17

TWO DIFFERENT EXPRESSION PATTERNS FOR THE *N*-METHYL-D-ASPARTATE RECEPTOR CHANNEL IN CEREBELLAR PURKINJE CELLS OF THE *REELER* MUTANT MOUSE. Masahiko Watanabe*, Shin Nakagawa and Yoshiro Inoue. Dept. of Anatomy, Hokkaido Univ. Sch. of Med., Sapporo 060, Japan.

The purpose of this investigation is to clarify the molecular-anatomical organization of the *N*-methyl-D-aspartate (NMDA) receptor channel in the cerebellum of the *reeler* mutant mouse, in which various categories of the Purkinje cells are present as to the cell position and synaptic connectivity. The ε1 subunit mRNA of the NMDA receptor channel was shown to be expressed in a subset of the adult *reeler* Purkinje cells. In the rostrocaudal extent, the Purkinje cells expressing the ε1 subunit mRNA were distributed preferentially in the rostral cerebellum, irrespective of the normal and heterotopic positions. In the mediolateral extent, they formed segregated cell clusters, interposed by the ε1 subunit mRNA-negative cell clusters. Hybridizing signals for the ζ1 subunit mRNA were found in all the Purkinje cells, whereas those for the ε2, ε3, and ε4 subunit mRNAs were not detected in the cells. Thus, it is clear that there are two different expression patterns in the *reeler* Purkinje cells, one expressing the ζ1 subunit mRNA alone, and the other expressing the ε1 and ζ1 subunit mRNAs. Developmentally, it was found that the reeler cerebellum were composed only of the former cells during 1-3 postnatal weeks, and that later and additional expression of the ε1 subunit mRNA in some of these cells led to appearance of the latter cells. These findings suggest that postnatal maturation of the *reeler* cerebellum has evolved the Purkinje cell heterogeneity, with respect to the gene expression for the NMDA receptor channel.

336.18

EXPRESSION OF KAINATE-PREFERRING GLUTAMATE RECEPTORS IN PRIMARY NEURAL PROGENITOR CELLS. S. Scherer* and V. Gallo, Lab. Cell Mol. Neurophysiol., NICHD, NIH, Bethesda, MD 20892.

Primary neural progenitor cells, derived from E10 rat neural tube and neural crest, provide an ideal system for the study of various developmental processes in the mammalian nervous system. These 100% nestin-positive cultures are comprised of approximately 60% GD3-positive neural progenitors and 20% of cells which coexpress antigens characteristic of both neural and smooth muscle progenitors. These cells are capable of differentiation along both neuronal and glial pathways, and stimulation of differentiation by the adenylate cyclase agonist forskolin, increases the size of neuronal and glial cell clusters, and degree of neurite extension and branching. Furthermore, plating the cells on polyamines as opposed to fibronectin has a marked effect on morphology and significantly increases the percentage of GD3-positive cells. Using RT-PCR analysis, we have found transcripts for all of the non-NMDA ionotropic glutamate receptor subunits with the exception of GluR2 and GluR5. Previous data has shown that the kainate-preferring subunits GluR6 and KA2 are capable of forming functional heteromeric channels (Wenthold et al., J. Biol. Chem., 1994, 269:1332, and Patneau et al., Neuron, 1994, 12:357) Indirect immunohistochemistry of our primary progenitor cells, using antibodies directed against these subunits, suggests expression in both mature neurons and immature neuroepithelial precursors.

336.19

EXPRESSION OF NON-NMDA GLUTAMATE RECEPTOR (GluR) SUBTYPES IS TIME- AND LAMINAR-DEPENDENT DURING CORTICOGENESIS. A Furuta, ME Blue*, RC Koehler, RJ Traystman and LJ Martin. Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

We tested the hypothesis that different molecular subtypes of GluRs have distinct patterns of expression during corticogenesis. Fetal lamb brains at 60, 71, 93, 110, and 136 days gestation (term = 145 days) and adult sheep brain were prepared for immunoblotting and immunocytochemistry to determine the neocortical protein expression of AMPA receptors (GluR1, GluR2/GluR3 [GluR2/3]), kainate (KA) receptors (GluR6/GluR7 [GluR6/7]), and a metabotropic GluR (mGluR5). Synaptophysin expression was used as an index of synaptic maturation. AMPA and KA receptors and mGluR5 are present at E60, at least 10 days before the onset of synaptogenesis. With maturation, AMPA receptor expression increases to reach adult levels near E136, whereas KA receptor and mGluR5 expression decreases. GluR subtypes have distinct localizations in the developing telencephalic vesicle. GluR1 is enriched in superficial cortical plate at E60-E93 and later is expressed in nonpyramidal neurons, whereas GluR2/3 is enriched early in deep cortical plate (layer 5) and later is localized to pyramidal neurons. GluR6/7 is highly expressed in marginal zone, mid-cortical plate, and layer 5 at E60-E71 and then is down-regulated. mGluR5 is highly expressed in marginal zone at E60-E93 and is down-regulated to minimal expression in layer 1 at E136. GluR1, GluR2/3 and GluR6/7 are expressed transiently in germinal matrix. AMPA and KA receptors are expressed in developing white matter. mGluR5 is not expressed in germinal matrix or white matter. We conclude that the expression of ion channel and metabotropic GluR subtypes is dynamic during corticogenesis, with subtype- and subunit-specific regulation. This laminar and temporal segregation of GluRs may be important for cortical pattern formation. (Supported by NS20020)

336.20

DISTRIBUTION OF AMPA-GLUTAMATE RECEPTOR B mRNA AND PROTEIN IN RAT HIPPOCAMPAL GABAERGIC NEURONES C. Racca[1], M.V. Catania[2,*], H. Monyer[2], and B. Sakmann[1], Max-Planck-Institut für Medizinische Forschung[1] and Zentrum für Molekulare Biologie der Universität Heidelberg[1], 69120 Heidelberg, Germany

The distribution of GluR-B mRNA and protein in adult rat hippocampal GABAergic neurones was studied by performing a cross-labeling study (GluR-B mRNA *in situ* hybridization with GAD67 immunohistochemistry, and GAD67 mRNA *in situ* hybridization with GluR-B immunolabeling), accompanied by the respective controls (GAD67 or GluR-B mRNA *in situ* hybridization plus GAD67 or GluR-B/C protein immunocytochemistry). Digoxigen-labeled cRNA probes against GluR-B mRNA subunit, or GAD67 and 65 enzyme mRNAs were used in *in situ* hybridization experiments. In immunocytochemical studies, we used an antibody recognizing an epitope conserved on GluR-B, -C, and 4c subunits and common to both flip and flop variants or a polyclonal antibody for GAD67 enzyme.

GABAergic hippocampal neurones expressed varying levels of GluR-B mRNA. Although the majority were GluR-B mRNA positive, the levels of GluR-B mRNA observed in GABAergic neurones were overall lower than in pyramidal and granule cells, which showed the highest hybridization signal.

The differential GluR-B mRNA expression levels in GABAergic versus principal neurones was also observed at the protein level. There was a paucity of GluR-B/C labeling in the vast majority of somata of GABAergic neurones which contrasted with the strong expression of GluR-B/C proteins in the hippocampal principal neurones. Only a few immunoreactive somata of inhibitory neurones were found in the hilus and stratum lucidum.

Our results illustrate the low expression of the GluR-B subunit mRNA and GluR-B/C proteins in GABAergic hippocampal neurones. Considering the dominant role of this subunit in determining the divalent ion permeability of the receptor, it can be predicted that most GABAergic neurones express calcium-highly permeable AMPA receptor channels.

336.21

ALTERED GENE EXPRESSION FOR NMDA RECEPTOR SUBUNITS IN CEREBELLAR NEURONS OF THE *STAGGERER* MUTANT MOUSE. Shin Nakagawa*, Masahiko Watanabe and Yosiro Inoue. Dept. of Anatomy, Hokkaido Univ. Sch. of Med., Sapporo 060, Japan.

The *staggerer* is an autosomal recessive mutation, resulting in a selective and almost complete absence of the parallel fiber-Purkinje cell (PC) synapses, where glutamate is the major neurotransmitter. In the present investigation, we have examined the gene expression of the NMDA receptor channel subunits in cerebellar neurons of the *staggerer* mutant mouse, with ^{35}S-labeled antisense oligonucleotide probes. At postnatal day 21 (P21), a subset of medium-to-large neurons (MLNs) in the cerebellar cortex, which compromise PCs and Golgi cells, showed strong signals for the ε 1 and ζ 1 subunit mRNAs and weaker signals for the ε 4 subunit mRNA, with regional variations. The MLNs expressing the ε 1, ε 4 and ζ 1 subunit mRNAs were aligned as monolayer in the midline region of the *staggerer* cerebellum, whereas in the lateral (hemisphere) region they were dispersed in various depths of the granular layer or just beneath the layer. The distribution of the MLNs was virtually consistent with that of neurons expressing the calbindin mRNA strongly, suggesting that they are, if not all but mostly, the Purkinje cells. The granule cells displayed marked signals for the ε 1, ε 2, ε 3 and ζ 1 subunit mRNA at P14 and P21. When compared to expression patterns in corresponding neurons in the wild-type mouse (Watanabe et al. 1994), it is evident that the *staggerer* PCs expressed the ε 1 and ε 4 subunit mRNAs additionally and anomalously, while down-regulation of the ε 2 subunit expression was retarded in the *staggerer* granule cells. These findings raise a possibility that time-dependent and neuron type-specific expression of the NMDA receptor channel subunits is not only regulated by genetic programs of individual neuron, but could be modified by certain epigenetic factors.

336.22

ANATOMICAL DISTRIBUTION AND POSTNATAL DEVELOPMENT OF ENDOGENOUS FREE D-ASPARTATE AND D-SERINE IN THE RAT BRAIN AND PERIPHERY. A. Hashimoto*, T. Oka and T. Nishikawa. PRESTO, Research Development Corporation of Japan, Saitama 332 Japan and National Institute of Neuroscience, NCNP, Tokyo 187 Japan.

We have investigated the anatomical distribution and postnatal changes in D-aspartate and D-serine in the rat brain and periphery using HPLC techniques. D-Serine was concentrated predominantly in the brain throughout postnatal life. A substantial quantity of D-serine was seen throughout the brain areas at birth. The cerebral D-serine content increased from birth to postnatal week (PW) 3 and remained constant thereafter, whereas the cerebellar D-serine content peaked at PW 1. In contrast, the transient emergence of D-aspartate was observed in almost all brain and peripheral organs. A substantial quantity of D-aspartate was also seen in all brain areas at birth, whereas the D-aspartate content in the cerebrum and cerebellum decreased dramatically by PW 1 and 7, respectively. Further, the D-aspartate content and the ratio of D- to total aspartate were highest in the adrenal at PW 3 (608 ± 70 nmol/g, 45.9%) and in the testis at PW 14 (221 ± 7 nmol/g, 57.8%), respectively. Because D-serine potentiates NMDA receptor-mediated transmission through the strychnine-insensitive glycine site and because D-serine exhibits an NMDA receptor-related distribution and development, D-serine may be a tenable candidate for an intrinsic ligand for the glycine site. In contrast, because the periods of maximal emergence of D-aspartate in the brain and periphery occur during critical periods of morphological and functional maturation of organs, D-aspartate could participate in the regulation of these developmental processes of organs.

EXCITATORY AMINO ACID RECEPTORS VII

337.1

CHARACTERIZATION OF [^{3}H]ANIRACETAM BINDING IN BRAIN MEMBRANES. F. Fallarino[1], A.A. Genazzani[2], S. Silla[1], M.R. L'Episcopo[2], F. Nicoletti*[1] and M.C. Fioretti[1]. *[1]Dept. Exp. Med. Biochem. Sci., University of Perugia, and [2]Institute of Pharmacology, University of Catania, Italy.*

[^{3}H]Aniracetam bound to specific and saturable recognition sites in membranes prepared from discrete regions of rat brain (specific binding was high in purified synaptosomes or mitochondria, and low in the myelin fraction). In cortical membranes, binding was Na^+-independent, was still detectable at 4°C, and underwent rapid dissociation. Scatchard analysis revealed a single population of sites with an apparent K_D value of 70 nM. Specific [^{3}H]aniracetam binding was displaced neither by various metabolites of aniracetam, nor by piracetam, oxiracetam, cyclothiazide, or GYKI 52466. Specific binding was abolished when membranes were incubated at 37°C under shaking and then washed (a procedure that reduces the amount of endogenous glutamate), and could be restored after addition of glutamate or AMPA, but not of kainate. The action of AMPA was antagonized by DNQX, which also reduced [^{3}H]aniracetam binding in unwashed membranes. It appears therefore that activation of AMPA receptors facilitates the access of [^{3}H]aniracetam to its specific binding sites in brain membranes.

337.2

SUBTYPE SELECTIVE ACTION OF THE AMPA ANALOG (RS)-2-AMINO-2-[3-CARBOXYMETHOXY)-5-METHYLISOXAZOL-4-YL] PROPANOIC ACID (AMOA) ON RECOMBINANT IONOTROPIC GLUTAMATE RECEPTORS. P. Wahl*, K.M. Houamed, P. Krogsgaard-Larsen, J.S. Rasmussen, J. Egebjerg. Novo Nordisk A/S, DK-2880 Bagsvaerd, and Royal Danish School of Pharmacy, DK-2100 Copenhagen, Denmark

The effects of AMOA on kainate- and AMPA-preferring glutamate receptor subtypes, expressed in *Xenopus* oocytes or mammalian cells, were investigated using electro-physiological methods. AMOA at concentrations up to 500 µM did not antagonize currents evoked by submaximal concentrations (3 - 5 µM) kainate on homomeric GluR6(Q) expressed in mammalian cells. At identical concentrations AMOA reduced the currents evoked by 100 µM kainate on homomeric GluR1, expressed in oocytes, by 70 to 80%. The actions of AMOA on GluR currents in oocytes injected with brain mRNA were complex, and agonist dependent. When AMPA was used as an agonist the effects of AMOA were biphasic; at low AMPA concentrations AMOA depressed the currents, while currents evoked by high AMPA concentrations were augmented by AMOA. In contrast, kainate currents were depressed by AMOA at all agonist concentrations tested. The mechanism(s) of AMOA action are currently being investigated. AMOA may turn out to be a useful tool for elucidating structure-function relations of GluRs, as well as being basis for novel therapeutics.

337.3

MODULATION OF FAST GLUTAMATERGIC EXCITATORY SYNAPTIC TRANSMISSION BY NBQX AND CYCLOTHIAZIDE IN THE CA1 REGION OF HIPPOCAMPAL SLICES. G. Rammes[1], C.G. Parsons[2]*, D. Swandulla[1], and G.L. Collingridge[3] Dept. Molecular Pharmacol., Univ. Erlangen, Germany[1], Dept. Pharmacol., Merz+Co., Frankfurt, Germany[2], Dept. Anatomy, The Medical School, Bristol, England[3]

We have previously shown that the AMPA receptor positive modulator cyclothiazide and the non-competitive AMPA receptor antagonist GYKI-52466 have very strong allosteric interactions when tested on agonist-induced currents in cultured cells and on synaptic transmission in hippocampal slices (Rammes et al., Soc. Neurosci. Abs. (1994) **20:** 1535). The purpose of the present study was to test the specificity of this effect by accessing possible interactions of cyclothiazide and the competitive AMPA receptor antagonist NBQX on isolated AMPA receptor-mediated EPSCs (AMPA-EPSCs) recorded in the CA_1 region of hippocampal slices. Cyclothiazide concentration-dependently slowed the decay of AMPA-EPSCs with an EC_{50} of 31μM (control τ=23ms, cyclothiazide 330μM τ=70ms) and produced a variable increase in the amplitude of AMPA-EPSCs (up to 133% of control). NBQX (1μM) almost abolished EPSCs (4% of control) but had little discernible effect on time constants. The effects of this concentration of NBQX were reduced (response amplitude 25% of control) in the presence of cyclothiazide (330μM). Moreover, NBQX (1μM) was able to partially reverse the effects of cyclothiazide in slowing decay time constants by about 50%. Further experiments are being performed to test the concentration-dependency of these effects. These provisional data indicate that cyclothiazide has allosteric interactions with both competitive and non-competitive AMPA receptor antagonists but do not lend support to the hypothesis that GYKI-52466 and cyclothiazide actually bind to the same site. As our previous data indicate that cyclothiazide increases and GYKI-52466 decreases the affinity of AMPA it may be that these interactions on response kinetics are mediated via such a mechanism. Furthermore, the cyclothiazide-induced changes in AMPA-EPSC response kinetics may be governed by changes in agonist affinity i.e. a slowing of τoff, rather than by desensitisation.

337.4

INHIBITION OF HIPPOCAMPAL FIELD POTENTIALS *IN VIVO* BY AMPA RECEPTOR-SELECTIVE ANTAGONISTS. J.C.R. Randle*, V. Borday, D. Briet and G.A. Böhme, Rhône-Poulenc Rorer Central Research, CNS Pharmaceuticals Research Program, 94400 Vitry-sur-Seine, France.

AMPA receptors are a promising molecular target for the development of neuroprotectant drugs. We have evaluated the blood brain barrier (BBB) penetration of AMPA receptor antagonists by recording their effects on central glutamatergic neurotransmission *in vivo* after intravenous administration. The excitatory post-synaptic potential (EPSP) and population spike (PS) evoked in the hilus of the dentate gyrus following stimulation of the perforant pathway (at 30 sec intervals) were measured (slope of EPSP, amplitude of PS) in urethane-anæsthetized male Sprague-Dawley rats (300 g ± 10%). The competitive AMPA antagonist, YM-900 [6-(1*H*-imidazol-1-yl)-7-nitro-(1*H*,4*H*)-quinoxaline-2,3-dione], dose-dependently inhibited the PS (ID_{50} = 9 mg/kg) and EPSP (ID_{50} = 13 mg/kg), when infused over 32 min at 2-16 mg/kg (0.0625-0.5 mg/kg/mn). In contrast, perfusions of NBQX (6-nitro-7-sulfamoylbenzo(*f*)quinoxaline-2,3-dione; 8-64 mg/kg) were virtually inactive unless preceded by an intraperitoneal injection (200 mg/kg) of the organic acid transport inhibitor, probenecid, suggesting rapid elimination upon slow infusion. Accordingly, NBQX (8-64 mg/kg i.v.) inhibited evoked potentials when administered as a bolus (ID_{50} = 25 mg/kg on PS, 15 min post-treatment). GYKI 52466, an allosteric AMPA receptor antagonist, partially inhibited hippocampal field potentials when perfused at doses of 8 and 16 mg/kg and, like NBQX, was more effective when administered as a bolus. These results confirm that all three compounds are able to penetrate the BBB to inhibit glutamatergic neurotransmission at doses at which they are anti-convulsant and neuroprotective. However, rapid elimination appears to limit their efficacy, particularly for NBQX and GYKI 52466 compared to YM-900.

337.5

HETEROMERIC NMDA RECEPTOR CALCIUM RESPONSES MEASURED USING AEQUORIN BIOLUMINESCENCE. E.R. Grant*, L. Kricka, D.E. Pleasure, and D.R. Lynch. Dept. of Pharmacology, Neurology, and Pathology, Univ. of Pennsylvania and Children's Hospital of Philadelphia, Philadelphia, PA 19104

The NMDA receptor mediates increases in intracellular calcium levels. These calcium changes in response to native NMDA receptor activation have been characterized in a variety of neuronal cell types. However, the receptor subunit requirements for eliciting such responses as measured in non-neuronal expression systems have not yet been determined. Thus, we measured the relative calcium changes mediated by heteromeric NMDA receptor subunit combinations transiently expressed in HEK-293 cells cotransfected with the calcium sensitive bioluminescent protein, aequorin. Aequorin emits blue light proportional to binding of calcium (wavelength=464 nm) in the presence of its chromophore cofactor *h*-coelenterazine. Upon addition of NMDA receptor agonists and antagonists, luminescence responses were measured in individual wells of 96-well microtiter plates using a luminometer. Addition of 100 μM glutamate, 100 μM glycine, and 2 mM Ca^{++} to the assay buffer elicited robust luminescence responses (10-100 fold over baseline) in cells expressing the NMDA 1a-2A or NMDA 1a-2B subunit combinations. Glutamate and glycine in the absence of extracellular calcium elicited no response, and calcium alone in the absence of agonists elicited no response. MK-801 inhibited NMDA 1a-2A mediated responses to 100 μM glutamate, 100 μM glycine, and 2 mM Ca^{++} with an IC_{50} of 93±21 nM. NMDA 1a-2A responses to quinolinic acid were dose-responsive. Cells expressing the NMDA 1a-2C combination consistently showed no substantial response to 100 μM glutamate, 100 μM glycine, and 2 mM Ca^{++} or to quinolinic acid (at best 1.5-2 fold over baseline), even though this combination's expression levels were similar to that of NMDA 1a-2A based on ^{3}H-glutamate binding and whole cell current amplitudes. These data suggest that NMDA receptor mediated changes in intracellular calcium are partly dependent on the receptor subunit composition. The source of these calcium changes in non-neuronal cells requires calcium influx through the receptor channel, but other intracellular factors may be involved. (NIH-DA07130)

337.6

DIRECT DEMONSTRATION THAT FUNCTIONAL N-GLYCOSYLATION SITES ENGINEERED INTO GluR1 TO PROBE TOPOLOGY ARE EXTRACELLULAR. M. Hollmann* and T. Morth, Glutamate Receptor Lab., Max-Planck-Inst. f. Experim. Medicine, D-37075 Göttingen.

The transmembrane domain (TMD) topology of glutamate receptors (GluRs) is currently hotly debated. Several epitope-tagging studies have concluded that a 3-TMD model featuring a non-membrane-crossing hairpin loop as the pore region best fits the available data (inter alia, Hollmann et al. *Neuron*, 1994, **13**: 1331-1343). Analysis of the utilization of native and engineered N-glycosylation sites by gel shift assays has been widely used to evaluate receptor topology. It has been contended, however, that N-glycosylation might occur at intracellular sites, thus invalidating gel shifts as topological evidence.

The goal of this study was to confirm that carbohydrates attached to ectopic sites are indeed located extracellularly in GluR1 expressed in *Xenopus* oocytes. Biotinylated concanavalin A (ConA) was reacted with intact oocytes expressing N-glycosylation mutants of GluR1. ConA-labeled receptors were solubilized, precipitated with streptavidin-sepharose beads, and analyzed on Western blots. It was found that all actively N-glycosylating mutants (which cause gel shifts) can be labeled and precipitated by biotinyl-ConA/streptavidin. Conversely, non-N-glycosylating mutants (which cause no gel shifts) cannot be precipitated. This shows that N-glycosylation in the oocyte expression system is a valid and reliable topological marker for extracellular sites. In addition, these data confirm the 3-TMD topology for GluR1.

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337.7

KAINATE INDUCED DOSE-DEPENDENT Ca^{2+} WAVES SIGNALING PATTERNS IN ASTROCYTES. A.H. Cornell-Bell*, W. T. Kim+, R.M. Villalba, Lion Imaging, Inc. Westbrook CT 06498 and +Yale University School of Medicine, New Haven CT 06510.

Time-lapse confocal scanning laser microscopy was used to study Ca^{2+} waves in rat hippocampal astrocytes exposed to increasing doses of kainate (KA= 1,10, 25, 50, 100 μM). There is a progression of wave patterns generated depending upon dose of agonist. When the astrocyte ionotropic glutamate receptor is bound with KA (100 μM) there is a sustained rise in cytoplasmic Ca^{2+} levels followed by a regenerative curvilinear intercellular Ca^{2+} wave that is driven by the Na^+/Ca^{2+} exchanger (Kim et al., 1994). In this ionotropic response Na^+ and Ca^{2+} do not flux through voltage-gated channels: a) TTX (10 μM) which blocks Na^+ channels had no effect on the wave b). Blocking the dihydropyridine-sensitive Ca^{2+} channels with Nifedipine (10 μM) or Nimodipine (1 μM) likewise had no effect on this wave. However, inhibiting the Na^+/Ca^{2+} exchanger with Benzamil (100 μM) completely abolishes the Ca^{2+} wave. Astrocytes were loaded with the fluoroprobe Fluo-3AM (4 mM with Pluronic acid). At low KA (1 μM) multiple target waves develop that involve 4-10 cells. These waves spread from a single site and then collapse back upon their point of origin. As KA dose increases the velocity of the spreading target waves increases from 7.5 ± 2.3 μM/sec (1-10 μM KA) to 8.8± 9 μM/sec (25 μM KA) to 15 ± 3.3 μM/sec (50 μM KA) to 25.7± 6 μM/sec (100μM KA). Target waves develop into regenerating spiral waves at a dose of 100 μM KA. With increased doses of KA (50-100μM) there is a significant increase in the number of individual cells involved in a wave. This data suggests that astrocytes near the site of neurotransmitter release will produce a Ca2+ wave that extends longer and faster than a wave initiated by astrocytes at site of lower neurotransmitter concentration.

337.8

NMDA RECEPTOR-MEDIATED REGULATION OF AMPA RECEPTORS IN ORGANOTYPIC HIPPOCAMPAL CULTURES. D. M. Gellerman*, X. Bi, and M. Baudry. Neurobiology Program, University of Southern California, Los Angeles, CA 90089.

Several laboratories have shown that activation of calpain, a calcium-dependent protease, is a necessary step in the formation of long-term potentiation (LTP) in the hippocampus. Specifically, it has been proposed that stimulation of NMDA receptors leads to an increase in intracellular calcium concentrations, calpain activation, proteolysis or cytoskeletal elements, and modification of AMPA receptor properties. We recently showed that calpain activation in frozen-thawed rat brain sections produces striking modifications of the immunological properties of the AMPA receptors, suggesting that calpain might produce a partial proteolysis of the AMPA receptors, thereby altering their properties. Organotypic hippocampal slices in culture have been shown to represent a useful tool to investigate mechanisms of synaptic plasticity. In particular, NMDA treatment has been shown to result in rapid calpain activation as evidenced by the accumulation of a specific breakdown product of spectrin (SBDP). In the present study, we evaluated the effect of NMDA treatment of cultured hippocampal slices on the properties of AMPA receptors. Slices were treated with NMDA (100 μM) for 10 minutes and [^{3}H]-AMPA binding to membrane fractions was measured. NMDA-treated slices exhibited a significant increase in [^{3}H]-AMPA binding. This increase was significantly reduced by incubation of slices with 100 μM calpain inhibitor I. Furthermore, we confirmed that under these conditions, NMDA treatment resulted in SBDP accumulation, an effect completely blocked by calpain inhibitor I. These results indicate that NMDA receptor stimulation activates calpain and produces modifications of AMPA receptor properties. Thus, our results support the hypothesis that changes in AMPA receptor properties underlie changes in synaptic plasticity. (Supported by grants from NIH and Sankyo.)

337.9

SELECTIVE BLOCK OF NATIVE Ca^{2+}-PERMEABLE AMPA RECEPTORS BY JORO SPIDER TOXIN IN RAT SPINAL CORD DORSAL HORN NEURONS J.G. Gu*, C. Albuquerque, and A. B. MacDermott. Dept of Physiology and Cellular Biophysics and Center for Neurobiology and Behavior, Columbia Univ., 1106 Black Building, 630 West 168th Street, New York, NY 10032

We have examined the relative distribution of Ca^{2+}-permeable AMPA receptors on cultured embryonic rat dorsal horn neurons with perforated patch recordings and Ca^{2+} imaging using Joro Spider Toxin (JSTX) (Blaschke et al., 1993). 100 uM kainate evoked sustained inward currents (0 - 400 pA) with apparent reversal potentials (Er) ranging from -35 to -100 mV in extracellular bath containing 10 mM Ca^{2+} as the only permeant extracellular cation. Kainate-evoked inward Ca^{2+} current was strongly inhibited by 1uM JSTX. The JSTX block occured in a use-dependent manner. The block was not reversible at holding potentials (Vh) ranging from -100 to +10 mV, but it could be reversed at +70 mV in the presence of kainate. Three types of dorsal horn neurons were identified according to changes in the I-V relationship and Er after using 1 uM JSTX. In neurons where Er was ~ -40 mV, JSTX decreased the slope of the I-V curve without significantly changing Er, suggesting that these neurons predominantly express Ca^{2+}-permeable AMPA receptors. In neurons where Er was ~ -70 mV, a large negative shift in aEr was evident in JSTX and the slope of the I-V curve was decreased, suggesting that both Ca^{2+} -permeable and -impermeable AMPA receptors were co-expressed on this type of neuron. The third group of neurons did not show any inward Ca^{2+} current indicating that no Ca^{2+}-permeable AMPA receptors were present. In these cells, JSTX did not significantly change the I-V relationships. Ca^{2+}-permeable AMPA receptors were determined to be predominantly localized on neurites. Taken together, these results suggest that JSTX is highly selective to native Ca^{2+}-permeable AMPA receptors and it may be useful for the study of functional role of Ca^{2+}-permeable AMPA receptors in synaptic transmission. (supported by NIH Grant NS29797).

337.10

CALCIUM SIGNALLING BY NON-NMDA GLUTAMATE RECEPTORS EXPRESSED IN HEK293 CELLS. R.E. Petroski*, Y. Stern-Bach, S.F. Heinemann. The Salk Institute, La Jolla, CA 92037

In neurons, intracellular calcium ($[Ca^{2+}]_i$) signals are generated by influx of calcium through voltage-dependent ion channels and neurotransmitter receptors as well as by release of calcium from intracellular stores. In order to examine the ability of non-NMDA glutamate receptors to induce calcium signals in isolation of other calcium-signal-generating channels and/or receptors, non-NMDA receptors were transiently expressed in HEK293 cells and calcium signals were optically detected using Fura-2.

Homomeric kainate receptors of GluR6(Q) or GluR6(R) did not produce calcium signals in response to glutamate or kainate. However, when HEK293 cells expressing GluR6(Q) were treated with ConA to block receptor desensitization, agonists rapidly elevated $[Ca^{2+}]_i$ by 50%-400% above resting levels. The calcium signals were abolished in Ca^{2+}-free bath and reversed when agonists were washed out. In contrast, ConA treated GluR6(R) failed to elicit a detectable calcium response suggesting that the edited receptor is less calcium permeable than the unedited receptor. Homomeric AMPA receptors of GluR3 flip or GluR3 flop did not produce calcium signals in response to glutamate or kainate. Co-application of agonist with cyclothiazide to block receptor desensitization resulted in elevated $[Ca^{2+}]_i$ in GluR3 flip but not GluR3 flop expressing cells. This is consistent with flop splice variants being less sensitive to cyclothiazide than flip variants. In order to examine the structural components required for calcium signalling, receptor chimeras constructed from GluR6(R) and GluR3 flop were tested.

337.11

CHARACTERIZATION OF HIGH AND LOW AFFINITY [^{3}H]-KAINIC ACID BINDING SITES IN RODENT FOREBRAIN, CEREBELLUM AND SPINAL CORD. C. B. Nelson, C. J. Spencer and R. D. Schwarz* Parke-Davis Pharmaceutical Research, Division of Warner Lambert Co., Ann Arbor, MI 48105

Pharmacologically, ionotropic non-NMDA glutamate receptors have been divided into two groups based on binding preference for either AMPA or kainate (KA), the KA group being further subdivided into high and low affinity populations. Molecular biology has supported this receptor diversity identifying at least five different subunits that in various combinations might contribute to such KA-preferring receptors.

Initial characterization of KA binding sites was carried out in extensively washed rat forebrain synaptosomal membranes in 50mM Tris-HCl buffer with and without 20mM calcium (Ca^{2+}). With Ca^{2+}, KA recognized a single binding site with a Kd of 33.2 nM and Bmax of 2,429 fmol/mg protein. KA binding without Ca^{2+} revealed a higher affinity site having a Kd of 2.8 nM and a Bmax of 849 fmol/mg. Variation in method of membrane preparation and degree of tissue washing may explain differences in the absolute values of dissociation constants when compared with previously reported numbers. However, the rank order of potency for agonist binding was in agreement with previous findings, with the high affinity assay showing KA> domoate> glutamate>> AMPA, and the low affinity assay having domoate> KA> glutamate>> AMPA. In general agonists had greater relative affinity for the high affinity site, with the exception of domoate which saw both sites equally. Next, in an attempt to ascertain if high and/or low affinity binding sites would show regional variation, binding was compared in membrane preparations from forebrain, cerebellum, and spinal cord. Preliminary results suggest that ligands differ in their ability to displace KA from different regions of the nervous system, suggesting that high and low binding sites are not uniform in all areas. This heterogeneity may be explained by differences in subunit type and stoichiometry contributing to receptors that comprise high and low affinity populations in different brain regions.

337.12

AUTOANTIBODIES TO GluR2 FROM A PATIENT WITH CEREBELLAR DEGENERATION ENHANCE GLUTAMATE RECEPTOR CURRENTS. R.E. Twyman*[1], L.C. Gahring[2] and S.W. Rogers[3]. Programs in Neuroscience and Human Molecular Biology & Genetics, Depts. of [1]Neurology, [2]Medicine and [3]Neurobiology & Anatomy, Univ. of Utah School of Medicine and [2,3]VA-GRECC, Salt Lake City, UT 84112

Autoantibodies to glutamate receptor (GluR) subunits modulate glutamate receptors. Anti-GluR3 antibodies found in association with Rasmussen's encephalitis (Twyman et al., Neuron 14:755-762, 1995) activate and anti-GluR5/6 antibodies found in association with some paraneoplastic degenerative syndromes (Gahring et al., Molecular Medicine 1:245-253, 1995) enhance glutamate receptor currents. We recently studied the serum from a 65 year old Asian male with a 4 year history of non-familial progressive olivopontocerebellar degeneration with pseudobulbar palsy. Specific immunostaining to GluR2 was demonstrated on transfected cells and to a fusion protein constructed from an extracellular domain of GluR2. Whole cell recording using cultured mouse cortical neurons indicate that the serum (1:8 dilution) did not activate non-NMDA glutamate receptor currents. The serum rapidly and reversibly enhanced glutamate-evoked (100 μM) non-desensitizing currents by 200-500% in a subset of neurons. Sera from healthy controls did not alter glutamate-evoked currents. Enhanced currents could be reduced by a GluR2 specific peptide. These results implicate autoantibodies to GluR2 in the pathophysiological processes of this patient's disease and also extend our observations that autoimmunity targeted against GluR epitopes present on some neurons may contribute to specific neurotoxicity in conditions such as epilepsy, paraneoplastic syndromes and neurodegenerative diseases.

337.13

RASMUSSEN'S ENCEPHALITIS PATIENTS' SERA ACTIVATE GLUR3 RECEPTORS EXPRESSED IN *X. LAEVIS* OOCYTES. **J. Eichten Gunther* & R. E. Twyman.** Programs in Neuroscience and Human Molecular Biology & Genetics, Depts of Neurology & Pharmacology, Univ. of Utah School of Medicine, Salt Lake City, UT

An autoimmune response to the GluR3 subunit has been associated with Rasmussen's encephalitis, a childhood epilepsy of intractable focal seizures. Whole cell electrophysiological studies of cultured mouse cortical neurons demonstrated that both rabbit antisera to the GluR3 subunit and Rasmussen's encephalitis patient antibodies have agonist properties on a subset of neurons. Application of these samples evoked non-NMDA sodium currents (Twyman, Neuron 14:755, 1995). We further studied the effect of these Rasmussen's encephalitis patients' sera on rat GluR3 homomeric receptors expressed in *Xenopus laevis* oocytes.

TEVC electrophysiology revealed that a sera dilution of 1:16 evoked sodium currents with an average response of 29.7% (S.E.M. = 8.0, n = 5) of the control kainic acid response (500 μM). Sera-evoked currents were blocked by 30 μM CNQX. Patient sera did not evoke currents in water injected oocytes. Control sera did not evoke sodium currents in GluR3 homomeric expressing oocytes. This study demonstrates that the GluR3 subunit alone is sufficient to bind patient antibody and to evoke a glutamate receptor response. This provides further evidence that antibodies to glutamate receptors may be involved in the pathophysiological mechanism of Rasmussen's encephalitis.

337.14

MODULATION OF AMPA/KA-INDUCED Ca^{2+} FLUXES IN BRAIN NEURONS. S. Liljequist* and G. Cebers. Dept. of Clin. Neuroscience, Sect. Drug Dependence Res., Karolinska Institute, Stockholm, Sweden.

α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-induced Ca^{2+} responses, and their modulation by cyclothiazide, were investigated in two functional assays of Ca^{2+} channel activity. AMPA produced a marked increase in cytoplasmic free Ca^{2+} levels $[Ca^{2+}]_i$ in single neocortical neurons, whereas no such effects of AMPA could be observed in intact cerebellar granule neurons. In monolayer cultures of cortical cells, cyclothiazide caused a pronounced enhancement of AMPA-induced stimulation of $^{45}Ca^{2+}$ uptake, whereas similar studies in cerebellar granule neurons revealed only a weak potentiation of AMPA-induced $^{45}Ca^{2+}$ uptake. Higher concentrations of cyclothiazide alone produced a dose-dependent increase of spontaneous $[Ca^{2+}]_i$ oscillations as well as an increase of basal $^{45}Ca^{2+}$ uptake in cortical neurons, whereas no such effects were obtained in cerebellar granule neurons. Our data indicate that AMPA receptors located on cortical and cerebellar granule neurons, respectively, may differ in their permeability to Ca^{2+} and that this difference is markedly potentiated following the application of cyclothiazide.

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337.15

DIFFERENTIAL EFFECT OF PHOSPHATIDYLSERINE ON THE BINDING PROPERTIES OF AMPA RECEPTORS IN BRAIN SECTIONS OF NEONATAL AND ADULT RATS. J. Gagné, C. Giguère, G. Tocco, R. F.Thompson, M. Baudry and G. Massicotte*. Lab. of Neurobiology, Univ. Québec à Trois-Rivières, Canada G9A 5H7.

The effects of phosphatidylserine (PS) on the binding properties of the AMPA (α-amino-3-hydroxy-5-methylisoxazolepropionic acid) and NMDA (N-methyl-D-aspartate) subtypes of glutamate receptors were analyzed by using qualitative as well as quantitative autoradiographic analysis of ^{3}H-AMPA and ^{3}H-6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and ^{3}H-glutamate binding on rat brain tissue sections. Preincubation of brain sections with PS produced an increase in ^{3}H-AMPA binding, while the same treatment had no effect on the binding properties of ^{3}H-CNQX, an antagonist of AMPA receptors. Furthermore, PS-induced increase in ^{3}H-AMPA binding was different among various brain structures, being larger in the molecular layer of the cerebellum and almost absent in the striatum. The effect of PS appeared to be specific for the AMPA subtypes of glutamate receptors as the same treatment did not modify ^{3}H-glutamate binding to the NMDA receptors. Finally, the effect of PS on AMPA receptor properties was markedly reduced in rat brain sections prepared from neonatal rats. The present data are consistent with the hypothesis that an alteration in lipid environment of synaptic membranes may be an important mechanism for regulating AMPA receptor properties, which could produce long-lasting changes in synaptic operation.

337.16

CHARACTERIZATION OF NATIVE AMPA RECEPTORS BY MEASURING RECEPTOR-MEDIATE CALCIUM INFLUX IN CEREBELLAR GRANULE CELLS. F. Li*and T. A. Verdoorn , Dept. Pharmacology, Vanderbilt Univ. Sch. of Med., Nashville, TN 37232

We have tested the ability of glutamate receptor agonists to cause accumulation of $^{45}Ca^{2+}$ in cultured cerebellar granule neurons. Kainate and L-glutamate caused $^{45}Ca^{2+}$ accumulation in a time- and concentration dependent manner (average kainate EC50=85.41±9.87 μM, n=6, glutamate EC50=21.02±1.77 μM, n=15). AMPA by itself evoked little excess accumulation, but in the presence of 50 μM cyclothiazide AMPA induced accumulation that was similar in magnitude to that of kainate (AMPA EC50= 7.65±1.38 μM, n=6). $^{45}Ca^{2+}$ accumulation evoked by 30 μM L-glutamate was blocked completely by D(-)-AP-5 (IC50=6.09 ±1.19 μM, n=3) or CNQX (IC50=1.76±0.325 μM , n=5). Although CNQX completely blocked the accumulation caused by 100 μM kainate or 30 μM AMPA plus 50 μM cyclothiazide (IC50's in two experiments were 2.197 μM for AMPA and 0.417 μM for kainate), D(-)-AP-5 blocked only 75.7 to 60.8% of the accumulation evoked by these two agonists. High concentrations of potassium (50 to 100 mM) were ineffective at causing $^{45}Ca^{2+}$ accumulation in these cells (between 4 to 7 DIV) and the calcium channel antagonists nifedipine and omega-Conotoxin MVIIC did not block agonist-induced accumulation. Electrophysiology experiments showed that the Ca^{2+} permeability of the NMDA receptors in these cells was high (average PCa^{2+}/PNa^{+} was 2.84±0.422, n=6, at 6 DIV) whereas the AMPA receptors had low calcium permeability (PCa^{2+}/PNa^{+} was 0.053±0.0033, n=6, at 6 DIV). Our results suggest that glutamate evoked calcium accumulation has both NMDA and non-NMDA receptor components.

337.17

INTRACELLULAR POLYAMINES MAY DETERMINE RECTIFICATION IN THE AMPA SUBTYPE OF GLUTAMATE RECEPTORS , C. B. McCullum*, R.I. Hume, Dept. of Biology, Univ. of Michigan, Ann Arbor MI.

Glutamate receptors transduce most of the excitatory synaptic information in the mammalian central nervous system. We have studied the mechanism of rectification in the GluR1, GluR2 and GluR3 subunits of the AMPA class glutamate receptor expressed in Xenopus oocytes. When the GluR1 or GluR3 subunits are expressed alone, the whole cell I-V curves inwardly rectify and the permeability to divalent cations such as calcium is high compared to that of monovalent cations. When the GluR1 or GluR3 subunit is co-expressed with the GluR2 subunit, the whole-cell current-voltage relationship becomes linear and the relative permeability to divalent cations is low. Surprisingly, we found that when outside-out patches were pulled from oocytes expressing homomeric GluR1 or GluR3, the inward rectification was lost within 3-5 minutes following patch excision. This finding led us to hypothesize that a substance within the oocyte induces inward rectification and that this substance diffuses away when outside-out patches are excised. In a search for this putative substance, we decided to test the role of intracellular polyamines. This idea was based on work by Lopatin et al. (Nature 372: 366-369) which demonstrated that intracellular polyamines cause inward rectification in some potassium channels. In outside out patches from oocytes expressing GluR1 or GluR3 receptors, we found that 100 μm spermine can block the change from inward rectification to linear current-voltage relationship. However, 100 μm spermine had no effect on the current-voltage relationship of oocytes co-expressing the GluR3 and GluR2 subunits, suggesting a specific effect. Polyamines may mediate rectification physiologically, because oocytes and many neuronal types contain 100-500 μm polyamines cytoplasmically.

337.18

SINGLE-CHANNEL CONDUCTANCES OF RECOMBINANT CALCIUM-PERMEABLE AND -IMPERMEABLE AMPA RECEPTORS. S. K. Kamboj*, G. T. Swanson and S. G. Cull-Candy, Department of Pharmacology, University College London, London WC1E 6BT, UK.

The presence of the RNA-edited GluR2 subunit reduces Ca^{2+}-permeability of AMPA receptors. To characterize channels of known subunit composition, we have examined the single-channel properties of a Ca^{2+}-permeable AMPA receptor (homomeric GluR4) and two Ca^{2+}-impermeable receptors (homomeric GluR2 and the heteromer GluR2/4), expressed in HEK 293 cells. Flip subunit isoforms were used.

The single-channel conductance of GluR4 receptors was apparently agonist-dependent. Kainate (300 μM) elicited a noise increase but no discrete channel openings. The conductance estimated from noise analysis was 2 pS (n=6 outside-out patches). AMPA (10 μM) gave discrete channel openings which were time-course fitted to levels of 7, 17 and 29 pS (n=6). Glutamate also activated multiple conductances from the GluR4 receptor. Homomeric GluR2 gave an estimated conductance of 200 fS (variance analysis of whole-cell currents activated by various agonists; n=5 cells). Heteromeric GluR2/4 receptors had a mean conductance of 2 pS (determined by noise analysis of AMPA and glutamate activated currents; n=6 patches). Time-course fitting of GluR2/4 channel openings (activated by 10 μM AMPA) revealed a main conductance of 4 pS with few larger events (3 patches).

Our data show that in addition to reducing Ca^{2+}-permeability, the presence of the GluR2 subunit in the AMPA receptor complex appears to reduce the single-channel conductance. Furthermore, the agonist-dependence of the GluR4(flip) single-channel conductance is strikingly similar to that of the 'low-conductance' response seen in cerebellar granule cell patches (J. Physiol., 463, 193-226, 1993).

EXCITATORY AMINO ACID RECEPTORS VIII

338.1

Cd^{2+} IONS "IRREVERSIBLY" INHIBIT EAA-ELICITED INOSITOL PHOSPHATE FORMATION IN RAT TERMINALS

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In 8-day-old rat forebrain synaptoneurosomes (Sn), Cd^{2+} ions inhibit inositol phosphate (IP) formation stimulated by metabotropic glutamate receptor (mGLUR) agonists, but not the IP response elicited by muscarinic agonists. Here, the mechanism of Cd^{2+} action was examined. We found that: 1°) Sn pretreatment with 100 μM Cd^{2+} for 1 to 30min at 37°C, followed by extensive washing, block GLU- but not carbachol-induced IP formations. 2°) A heavy metal chelator, TPEN (100 μM), possessing a high affinity for Cd^{2+} and added after Cd^{2+} pretreatment, does not reverse the inhibitory effect of Cd^{2+}. 3°) 100 μM TPEN Sn pretreatment does not prevent the inhibitory effect of later added Cd^{2+} on GLU-evoked IP formation. However, TPEN blocks the Fura-2 fluorescence increase induced by Cd^{2+} influx, which demonstrates the efficacy of TPEN to form a complex with Cd^{2+}. 4°) Ca^{2+} ions does not reverse Cd^{2+} inhibitory effect. 5°) Only Hg^{2+} ions mimic the Cd^{2+} action.

Taken togethe----------r, these data indicate that Cd^{2+} selectively and irreversibly inhibit IP formation induced by mGLUR activation in terminals. Cd^{2+} does not compete with Ca^{2+} and acts extracellularly, likely by forming covalent bonds with -SH free groups of the mGLUR NH_2 extracellular domain, which possesses numerous cysteine residues.

338.2

L-AP4-SENSITIVE METABOTROPIC GLUTAMATE RECEPTORS ACTIVATE THE G PROTEIN-COUPLED INWARDLY RECTIFYING POTASSIUM CHANNEL IN *XENOPUS* OOCYTES. J.A. Saugstad*, T.P. Segerson and G.L. Westbrook, Vollum Institute for Advanced Biomedical Research, OHSU, Portland, OR 97201.

L-AP4-sensitive metabotropic glutamate receptors (mGluRs) are involved in the inhibition of synaptic transmission, presumably by acting as presynaptic autoreceptors. Mechanisms of presynaptic inhibition include the inhibition of calcium currents or the activation of potassium currents. mGluRs inhibit calcium currents in central neurons, however, the activation of G protein-coupled inward rectifying potassium channels (GIRK) by glutamate has only been demonstrated in molluscs and *Aplysia*. We examined whether L-AP4-sensitive mGluRs could activate GIRK in *Xenopus* oocytes. The mGluRs effects were compared to oocytes injected with the D2 dopamine receptor which is known to couple to GIRK in neurons. Co-expression of mGluR7 and GIRK cRNA results in the activation of the potassium channel in response to glutamate (2 mM) or L-AP4 (1 mM). The peak amplitude of the mGluR7 response is smaller than that induced by the D2 receptor in response to dopamine (1 μm). Also, the time to peak amplitude was longer for mGluR7 than the D2 receptor suggesting that a component of mGluR7 coupling to GIRK is rate-limiting. The mGluR7 response and the D2 response both desensitize with repeated agonist application. However, sequential activation of co-expressed D2 and mGluR7 receptors yield maximal GIRK responses and thus demonstrate no cross-desensitization. These results suggest that mGluR7 and D2 receptors activate GIRK in *Xenopus* oocytes via distinct G proteins. However, the coupling efficiency for mGluR7 appears to be lower than for the D2 receptor. Supported by R01DC01783, F32NS09200 and the Klingenstein Foundation.

338.3

MODULATION OF [^{3}H] GLUTAMATE RELEASE FROM RAT CEREBROCORTICAL SYNAPTOSOMES BY METABOTROPIC GLUTAMATE RECEPTOR ACTIVATION. J.S. Bedingfield, P.J. Roberts*, and J.C. Watkins. Department of Pharmacology, School of Medical Sciences, University of Bristol, Bristol, BS8 1TD, UK.

Of the eight known metabotropic glutamate receptors (mGluRs), subtypes 1 and 5 (group 1) are found to couple to phosphoinositide (PI) hydrolysis, while 2 and 3 (group 2) and 4, 6, 7 and 8 (group 3) all negatively couple to cyclic AMP. All groups of mGluR are found to be present both pre and postsynaptically. Here we report the effects of mGluR agonists on presynaptic receptors.

Synaptosomes were prepared from adult rat cerebral cortices, preloaded with [^{3}H] glutamic acid and superfused continuously. Drugs were applied 1 min before and during a 1min depolarisation by 200μM 4-aminopyridine (4AP) (approximate EC_{50} value). Superfusate was collected in 1min fractions (2ml) and counted by liquid scintillation spectrometry.

The non-selective mGluR agonists, (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid, (2S,3S,4S)-α-(carboxycyclopropyl)-glycine and the group 3 selective agonist L(+)-2-amino-4-phosphonobutyric acid caused a dose-dependent inhibition of 4AP-stimulated [^{3}H] glutamate release with IC_{50} values of 32, 1.2, and 3μM respectively. The group 1 selective agonist (RS)-3,5-dihydroxyphenylglycine produced a dose-dependent potentiation of 4AP-stimulated [^{3}H]glutamate release ($EC_{50}\approx$ 1.2μM).

From this, it is evident that activation of the different presynaptic mGluRs can differentially modulate the synaptic release of glutamate. This may prove to be an important control mechanism and its modulation by drugs may be useful in the prevention of the known pathological effects of excessive or insufficient release of glutamate.

338.4

AGONIST SENSITIVITY OF METABOTROPIC GLUTAMATE RECEPTOR-MEDIATED SUPPRESSION OF CALCIUM CURRENT IN RAT NEOCORTICAL NEURONS IS CONSISTENT WITH RECEPTORS OF THE MGLUR1/5 SUBGROUP. R. J. Sayer* Dept. of Physiology, University of Otago, Dunedin, New Zealand.

Calcium currents in rat neocortical pyramidal neurons are suppressed by the metabotropic glutamate receptor (mGluR) agonist (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD). The aim of this study was to gain further information about the mGluR subtype(s) involved, by testing agonists that are putatively selective for subgroups of mGluR. Neurons were acutely isolated from the dorsal frontoparietal neocortex of 10-21 day old rats. Whole-cell recordings of calcium currents were made at 30-34° C, because responses to 1S,3R-ACPD were found to be larger and more reliably evoked than at room temperature.

The calcium current suppression by 200 μM 1S,3R-ACPD was mimicked by 200 μM (RS)-3,5-dihydroxyphenylglycine (DHPG), a probable selective mGluR1/5 subgroup agonist. (2S,3S,4S)-α-(carboxycyclopropyl)-glycine (L-CCG-I) at 1 μM, a concentration active at mGluR2 in other preparations, had no effect. L(+)-2-amino-4-phosphonobutyric acid (L-AP4) (1 mM), an agonist at mGluR4/6/7, did not inhibit the calcium currents. Responses to 50 μM 1S,3R-ACPD were partially and reversibly antagonized by 1 mM (+)-α-methyl-4-carboxyphenylglycine (MCPG). The findings are consistent with the rank order of potency quisqualate>glutamate>*trans*-ACPD found previously for calcium current suppression in these neurons, suggesting involvement of mGluR1 or mGluR5.

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338.5

METABOTROPIC GLUTAMATE RECEPTOR (mGluR)-ACTIVATED INWARD CURRENTS IN BASOLATERAL AMYGDALA NEURONS. N.B. Keele*, V.L. Arvanov and P. Shinnick-Gallagher. Dept. of Pharmacology, Univ. of Texas Medical Branch, Galveston, TX 77555.

Inward currents evoked by mGluR agonists 1S,3R-ACPD (ACPD) and quisqualate (QUIS) were investigated using standard intracellular and whole-cell recordings in brain slice. In D-APV (50μM), CNQX (30μM) and TTX (1μM), the inward current evoked by ACPD (I_{ACPD}, 50 – 200 μM; 31 ± 9 pA; n = 21) is associated with a membrane conductance (G_m) decrease (control: 7.1 ± 0.5 nS; ACPD: 6.6 ± 0.5 nS; $p < 0.001$; n = 21) and reverses polarity at -95 ± 7 mV (n = 14); whereas the QUIS-evoked current (I_{QUIS}, 50 – 100 μM, 73 ± 12 pA; n = 19) occurs without a reversal or significant change in G_m between -110 and -50 mV ($p > 0.05$, n = 19). I_{QUIS} and I_{ACPD} were similar recorded with K^+-filled patch or standard sharp electrodes.

mGluR-activated current(s) not mediated by a K^+ conductance were further characterized with Cs^+-filled electrodes in the presence of external Cs^+ (2 mM) and Ba^{2+} (0.5 mM). Under these conditions, QUIS (10 – 50 μM; n = 10) and ACPD (20 – 200 μM; n = 7) produce inward currents (I_{QUIS} = 48 ± 11; I_{ACPD} = 26 ± 8), also without significant G_m changes between -110 and -50 mV. Removal of external Ca^{2+} greatly reduced (or blocked) I_{QUIS} (n = 5) and I_{ACPD} (n = 4). In low Ca^{2+} (100 μM), Cd^{2+}-containing (200 μM) external solution (to eliminate voltage-activated Ca^{2+} currents), ACPD and QUIS usually produced currents associated with increased G_m between -110 and + 50 mV. The reversal potential of I_{ACPD} was 0 ± 3 mV (n = 3) whereas I_{QUIS} did not reverse between -110 and + 50 mV in 4 of 5 neurons tested. I_{ACPD} and I_{QUIS} persisted in some but not all neurons after reducing external Na^+ by equimolar substitution of Li^+, suggesting that ACPD and QUIS may activate a nonspecific cation conductance, electrogenic Na/Ca exchange, or perhaps both. [Supported by NS24643].

338.6

FUNCTIONAL ANALYSIS OF CHIMERAS CONSTRUCTED FROM METABOTROPIC GLUTAMATE AND CALCIUM RECEPTORS. L.G. Hammerland, K.J. Krapcho, N. Alasti, R. Simin, J.E. Garrett, E.F. Nemeth* and E.H. Fuller. NPS Pharmaceuticals, Inc., Salt Lake City, UT 84108.

The calcium receptor (CaR) is a novel type of G-protein coupled receptor which responds to extracellular cations and shares sequence homology (~25% amino acid identity) only with metabotropic glutamate receptors (mGluRs). In addition to sequence similarities, the CaR and mGluRs each contain a large (~600 amino acid) extracellular domain (ECD), which precedes the putative seven transmembrane spanning domains (7TMDs). To determine whether these two receptors share functional as well as structural characteristics, and to identify domains necessary for ligand binding, two chimeric receptors were constructed in which the large ECDs of the CaR and mGluR1 were interchanged and their functional activity was assessed following expression in *Xenopus* oocytes. The CaR is activated *in vitro* by several different cations including gadolinium, neomycin or calcium, but not by the mGluR1 agonists glutamate or quisqualate. The chimera CaR/mGluR1 (ECD of the CaR and 7TMD of the mGluR1) was responsive to the above cations but not to glutamate or quisqualate. mGluR1/CaR (ECD of the mGluR1 and 7TMD of the CaR) responded to glutamate and quisqualate as well as to cations. mGluR1/CaR activation by glutamate was blocked by the competitive mGluR1 antagonist 4C3H-PG, but gadolinium-evoked responses were not affected. Consistent with previous studies using mGluRs, these data show that ligand binding specificity is primarily determined by the ECD of the mGluR. However, cation binding domains of the CaR exist in both the ECD and 7TMD. Furthermore, functional determinants in the CaR and mGluR appear to have been conserved such that they can compliment each other with regard to cation responsivity.

338.7

A FREQUENCY-DEPENDENT SUPPRESSION OF THE SLOW AHP IN RAT HIPPOCAMPAL CA1 PYRAMIDAL NEURONS IS BLOCKED BY THE METABOTROPIC GLUTAMATE RECEPTOR ANTAGONIST MCPG.

G. Woodhall*, J.E. Chad & H.V.Wheal. Dept. of Physiology and Pharmacology, University of Southampton, Southampton, SO16 7PX, U.K.

A stepped frequency paradigm was designed to generate stimulation of the Schaffer collateral/commissural input to CA1 at frequencies between 0.67 and 100 Hz, representing a frequency progression on a natural log scale. Stimulation was delivered via a bipolar NiCr electrode placed in radiatum and intensity set at three times that required to elicit a spike in neurons recorded from by the whole-cell current clamp technique. After a stimulus train lasting for 1s at the desired frequency, 500ms positive current injections (0.1-0.7 nA) were used to evoke repetitive spike activity and an AHP. Prior to stimulation, the AHP amplitude measured at peak and 200ms after cessation of current injection was 6.88 ± 0.40 and 5.22 ±0 33 mV respectively (mean ± sem, 13 cells). The sAHP at 200ms was depressed significantly following 13.5, 36 and 100 Hz stimulation for 1s, with a maximum depression after 100 Hz such that the sAHP amplitude was 25 .68 ±5.21 % of control. Recovery was complete within 1-2 minutes.

This suppression of the AHP in response to high frequency stimulation was blocked by prior application of 400μM MCPG which appeared to shift to the left the frequency response curve in the manner of a competitive antagonist. In the presence of MCPG no significant attenuation of the AHP was noted at 13.5 and 36 Hz, and a weak response occurred after 100 Hz stimulation (AHP amplitude at 200ms = 75.44 ±9.66% of control).

The suppression of the AHP after 100 Hz stimulation was also elicited in the presence of 20μM CNQX and 20μM APV, which completely abolished AMPA and NMDA receptor mediated responses to stimulation. Taken together, these data suggest that metabotropic glutamate receptors are activated during high-frequency synaptic activity, as reported by a suppression of the slow AHP.

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338.8

t-ACPD ENHANCEMENT OF NMDA RESPONSES IN FROG MOTONEURONS IS MAGNESIUM DEPENDENT. A.M. Holohean*, J.C. Hackman,and R.A. Davidoff. Neurology Service., VAMC and Neurology Dept., Univ. Miami Sch. Med. Miami, FL 33101.

The metabotropic glutamate receptor agonist, *t*-ACPD [(±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid] has been reported to enhance ionotropic glutamate responses. We compared the effects of *t*-ACPD on depolarizations induced by ionotropic glutamate receptor agonists in frog motoneurons bathed in HCO_3^--buffered Ringer's solution with and without Mg^{2+} ions. We used sucrose gap recording techniques from the ventral roots of the isolated, hemisected, frog spinal cord.

ACPD (30 μM, 20 min) in the presence of TTX had no significant effect on the depolarizations of motoneurons evoked by 5 sec applications of NMDA (100 μM), AMPA (10 μM) or kainate (30 μM). Addition of a "physiological" concentration of Mg^{2+} (1.0 mM) to the Ringer reduced NMDA-induced motoneuron depolarizations by 30 to 90%. These reduced NMDA depolarizations were enhanced 193 ± 47% by *t*-ACPD (30 μM). The depolarizations evoked by AMPA and kainate remained unchanged in the presence of *t*-ACPD (100% and 95%, respectively), whether or not Mg^{2+} was present in the medium.

Unexpectantly, depolarization of motoneurons by addition of AMPA or K^+ ions had no effect on the release of the Mg^{2+} blockade of NMDA. Intracellular mechanisms that may be responsible for the unique effect of t-ACPD are currently being investigated. To date, neither caffeine-induced release of Ca^{2+} stores nor phorbol 12-myristate 13-acetate reduces *t*-ACPD activated enhancement of NMDA depolarizations in the presence of Mg^{2+}. (Supported by VAMC MRIS #1769 and 3369 and USPHS #NS17577).

338.9

REDUCTION OF GABA-INDUCED DEPOLARIZATIONS OF AFFERENT TERMINALS BY *t*-ACPD IN THE FROG SPINAL CORD IS cAMP-DEPENDENT. J.C. Hackman*, A.M. Holohean and R.A. Davidoff. Neurology Service, VAMC and Neurology Dept. Univ. of Miami Sch. Med. Miami, FL 33101.

Activation of metabotropic glutamate receptors by the selective agonist *t*-ACPD [(±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid] reduces GABA-induced depolarizations of frog primary afferent terminals. We used the isolated, hemisected, frog spinal cord superfused with HCO_3^--Ringer solution and sucrose gap recording techniques to investigate the interactions between *t*-ACPD and GABA.

In the presence of TTX, *t*-ACPD (30 μM, 15 min) reduced the afferent terminal depolarizations evoked by short applications of GABA (1.0 mM, 10 sec, 29 ± 10%, n = 3) and the $GABA_A$ agonist muscimol (10 μM, 10 s, 25 ± 4%, n = 5). The phosphodiesterase inhibitor IBMX (1 mM, 25 min) diminished the GABA-induced depolarizations and occluded the effects of *t*-ACDP. The adenylate cyclase activators, 8-bromo-cAMP (0.5 to 1.0 mM) and forskolin (10 to 50 μM) also reduced the GABA-induced depolarizations. In contrast, the calcium uptake blocker, thapsigargin (1 to 2 μM), the calcium releasing agent, ryanodine (5 to 10 μM) and the protein kinase inhibitor, staurosporine (1 to 2 μM) did not affect either the GABA response or the ability of *t*-ACPD to reduce GABA responses.

In sum, the *t*-ACPD-induced reduction of GABA responses in the spinal cord appears to result from enhanced adenylate cyclase activity. (Supported by VAMC MRIS #1769 and 3369 and USPHS #NS17577).

338.10

FACILITATORY METABOTROPIC GLUTAMATE RECEPTORS REGULATE EXCITATORY AMINO ACID RELEASE IN RAT DORSAL VAGAL COMPLEX. P.M. Beart*, A.J. Lawrence and N.M. Jones. Dept. Pharmacology, Monash University, Clayton, Vic. 3168, Australia.

Recent *ex vivo* electrophysiological observations have suggested that metabotropic glutamate receptors (mGLURs) modulate the activity of first and second order barosensitive neurones in the rat nucleus of the solitary tract (NTS: Glaum et al., *Neuropharmacol.* 32, 1419, 1993). Here the functional involvement of mGLURs in NTS has been investigated by studying the effects of (±)-1-aminocyclopentane-trans-1,3-dicarboxylate (tACPD) on the release of L-glutamate (GLU) and L-aspartate (ASP) by *in vivo* microdialysis. In urethane anaesthetized (1.5g/ kg i.p.) male WKY rats, a microdialysis probe was inserted unilaterally into the medial NTS and perfused with artificial cerebrospinal fluid (1 μl/min). After 60 min equilibration, 20 min samples were collected from amino acid analysis (Lawrence & Jarrott, *Neurosci.* 58, 585, 1994). Basal levels of GLU and ASP were 8.6 and 3.3 pmol/ 20 μl sample, respectively. tACPD (10-100 μM) produced concentration-dependent increases in release; at 30 μM 190 and 500% increases in the release of GLU and ASP, respectively. Two periods of stimulation (S1 & S2) with tACPD (30 μM) yielded S2/S1 ratios of 0.95 ± 0.14 for GLU and 1.06 ± 0.28 (both n=5) for ASP. Other experiments indicated release was tetrodotoxin (0.1 μM)- and Ca^{2+}-sensitive. Actions of tACPD (30 μM) were attenuated by α-methyl-4-carboxyphenylglycine (200 μM; S2/S1 ratios 0.19 ± 0.04 (n=4) for GLU and 0.17 ± 0.02 (n=4) for ASP) and by 4-carboxyphenylglycine (500 μM; S2/S1 ratios 0.52 ± 0.07 (n=3) for GLU and 0.51 ± 0.06 (n=3) for ASP) (all $P < 0.05$). These data establish the existence of functional mGLURs, which seem to be facilatory homoreceptors, in the NTS.

338.11

ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS POTENTIATES RESPONSES OF SPINAL DORSAL HORN NEURONS. D. Budai* and A. A. Larson. Department of Veterinary PathoBiology, University of Minnesota, Saint Paul, MN 55108.

We have investigated he role of metabotropic glutamate receptors (mGluRs) in the transmission of sensory information from the primary afferent fibers to secondary dorsal horn neurons of the rat spinal cord. Our method utilized single neuron recordings from wide dynamic range dorsal horn neurons responding to both innocuous and noxious mechanical stimuli delivered to the cutaneous receptive field. Activation of mGluRs at these synapses by local iontophoresis of (1*S*,3*R*)-1-amino-cyclopentane-1,3-dicarboxilic acid (1*S*,3*R*-ACPD) resulted in a long-lasting increased response of dorsal horn neurons to ionotropic glutamate receptor agonists (NMDA and kainic acid) applied by iontophoresis. Application of 1S,3R-ACPD also led to a significant increase in responses to innocuous (brush, pressure) rather than in responses to noxious (pinch, squeeze) mechanical stimulation. The excitatory effects of 1*S*,3*R*-ACPD were selectively blocked by the selective mGluR antagonist, (*S*)-4-carboxy-3-hydroxyphenyl-glycine (*S*-4C3H-PG), confirming that such effects were due to mGluRs. Wind-up, the progressive potentiation of C-fiber-evoked responses during a train of stimuli, was increased and decreased by (1*S*,3*R*-ACPD) and *S*-4C3H-PG, respectively. The long-lasting effects of 1*S*,3*R*-ACPD on wind-up strongly support the view that mGluRs in spinal wide dynamic range neurons may play a role in spinal synaptic plasticity, and therefore, may contribute to the central sensitization during increased pain sensation.

338.12

METABOTROPIC GLUTAMATE RECEPTOR mGLUR1 BUT NOT mGLUR7 AGONISTS ALTER EXCITABILITY OF CULTURED PURKINJE NEURONS. J.G. Netzeband*, D.D. Sweeney and D.L. Gruol. Dept. Neuropharmacol., The Scripps Research Institute, La Jolla, CA 92037.

Cerebellar Purkinje neurons express an abundance of metabotropic glutamate receptors (mGluR), particularly mGluR1, but also mGluR7. We have used both electrophysiological and fura-2 microscopic calcium imaging to record the responses of cultured Purkinje neurons (> 16 days in culture) to mGluR agonists. DNQX (50 μM) and bicuculline (30 μM) were present in the bathing media for all experiments. For electrophysiological studies, current clamp recordings were made using the nystatin-patch technique. Rapid microperfusion (1.5 s) of the mGluR1 agonists (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD, 300 μM) or quisqualate (Quis, 5 μM) caused a membrane depolarization that was accompanied by an increase in both simple and complex spike activity. These responses were blocked by the mGluR1 antagonist (+)-α-methyl-4-carboxyphenylglycine (1mM). Inclusion of caffeine (20 mM) in the bathing media attenuated the electrophysiological responses to ACPD and Quis, whereas calcium-free media did not, suggesting that release of calcium from intracellular stores is important for these responses. In calcium imaging studies, ACPD elicited rises in intracellular calcium in both somata and dendrites. In contrast to the effects of mGluR1 agonists, the mGluR7 selective agonists L(+)-2-amino-4-phosphonobutyric acid (L-AP4, 200 μM and 1 mM) and O-phospho-L-serine (L-SOP, 200 μM and 1 mM) did not alter the level of membrane polarization or spike firing, nor did L-AP4 affect intracellular calcium levels. The data suggest that in Purkinje neurons, mGluR-mediated changes in neuronal excitability and increases in calcium levels are mediated primarily through activation of mGluR1. *Supported by ARC 6420 and AA 0756.*

338.13

HETEROLOGOUS EXPRESSION OF A RAT METABOTROPIC GLUTAMATE RECEPTOR (mGluR2) IN HEK293 CELLS: FUNCTIONAL COUPLING TO A STABLY EXPRESSED ω-CONOTOXIN-SENSITIVE CALCIUM CHANNEL. B.A.McCool*+, P.F.Brust#, M.M.Harpold#, and D.M.Lovinger+. Dept. Molec. Physiol. & Biophys., Vanderbilt Univ. Med. School, Nashville, TN 37232+;The Salk Instit. Biotech./Industrial Assoc., Inc., La Jolla, CA 92037#

Metabotropic glutamate receptors (mGluRs) perform a variety of modulatory roles in the central and peripheral nervous systems. The development of receptor subtype-specific agonists has lagged far behind the isolation of receptor cDNAs; and, the coupling of specific receptor subtypes to various neuronal effectors has not been well studied. We have recently demonstrated (Ikeda et al., **Neuron**, in press) that mGluR2, a Group II metabotropic receptor, is capable of coupling to endogenous N-type calcium channels when its cDNA is heterologously expressed in adult rat sympathetic neurons. To better understand the molecular aspects of mGluR2 modulation of voltage-gated calcium channels, we have transiently expressed this receptor in HEK293 cells which stably express the human α_{1B-1}, α_{2b}, β_{1-3} calcium channel subunits (G1A1 cell line). These mGluR2 receptors reversibly inhibit the ω-conotoxin GVIA-sensitive barium current in G1A1 cells as measured by the whole cell patch clamp technique. Furthermore, this inhibition displays a number of characteristics typical of heterotrimeric G protein-mediated modulation: it is voltage dependent, is partially relieved by strongly depolarizing prepulses, and results in slowing of macroscopic current activation kinetics. These mGluR2 receptors also display the appropriate Group II receptor pharmacology, with L-glutamate (EC_{50}~0.5μM), (+/-)-1-aminocyclopentane-trans-1,3-dicarboxylic acid (t-ACPD; EC_{50}~8.4μM), (2S,1'S,2'S)-2-(carboxycyclopropyl) glycine (L-CCG-I), and (2S,1'R,2'R,3'R)-2-(2,3-dicarboxycyclopropyl) glycine (DCG-IV) acting as agonists. The Group III mGluR agonists L-2-amino-4-phosphonobutyric acid (L-AP4; 100μM) and L-serine-O-phosphate (L-SOP; 100μM) are without effect. These results indicate that mGluR2 is capable of functionally coupling to Ca^{++} channels in a recombinant system using an endogenous HEK293 signal transduction pathway. Supported by NS09719 (B.A.M.) and NS30470 (D.M.L.).

338.14

MODULATION OF SYNAPTIC TRANSMISSION IN LOCUS COERULEUS BY METABOTROPIC GLUTAMATE RECEPTORS. G.R. Dubé* and K.C. Marshall. Dept. of Physiology, Univ of Ottawa, Ottawa, On , K1H 8M5.

Metabotropic glutamate receptors (mGluR) have been implicated in positive and/or negative modulation of synaptic transmission in many different systems, though the significance of this modulation is not always well understood. Here, we report on the effects of selective activation of mGluR on synaptic transmission to intracellularly recorded locus coeruleus (LC) neurons in superfused rat brain slice preparations. Excitatory postsynaptic potentials (EPSPs) were evoked in the presence of 10 μM bicuculline and 0.8mM Mg^{2+}. These were mediated mainly through activation of postsynaptic non-NMDA receptors, as EPSP amplitude could be blocked by more than 85% by 20 μM 6-cyano-7-nitroqinoxaline-2,3-dione (CNQX), while 3-((R)-2-carboxypiperazin-4-yl)--propyl-1-phosphonate (CPP; 20 μM), a specific NMDA antagonist, had little effect. Bath application of the selective mGluR agonists L(+)-2-amino-4-phosphonobutyric acid (L-AP4) or (1S,3R)-1-aminocyclopentane-1,3-dicarboxilic acid (1S,3R-ACPD) (0.1-50 μM), caused a depression of EPSPs in a dose dependent fashion to about 50% of control at high doses. However, the potency of the two agonists was different. The IC50 for this effect was 0.9 μM and 7.2 μM for L-AP4 and 1S,3R-ACPD respectively. Focal application of glutamate to cells also caused a depolarization and this was not affected by the activation of mGluR by either agonist, indicating that the modulation of the EPSPs occurs presynaptically. In addition, neither agonist, had any effect on cell input resistance, intrinsic oscillatory activity, or spontaneous firing rate of the LC neurons. Owing to the specificity of L-AP4 and 1S,3R-ACPD for selected mGluR subclasses, we speculate that the receptor is of the mGluR 4, 6, 7 subtypes (highest affinity to L-AP4). LC provides noradrenergic innervation to extensive regions of the brain. Glutamate feedback signalling through presynaptic autoreceptors may, therefore, represent one mechanism by which LC activity, which results in noradrenaline release throughout the CNS, is modulated. Supported by the MRC, Canada and FCAR (Quebec)

338.15

EFFECTS OF LORAZEPAM TOLERANCE AND WITHDRAWAL ON METABOTROPIC GLUTAMATE RECEPTOR FUNCTION. Christian Thomsen*, Peter D. Suzdak and Martin Mortensen. Health Care Discovery, Neuroscience, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Måløv, Denmark.

Mice were exposed to lorazepam (4 mg/kg) or vehicle by continuous infusion using implanted (s.c.) osmotic minipumps which were removed after seven days. Dose-response curves for stimulation of phosphoinositide (PI) -hydrolysis by the selective metabotropic glutamate receptor (mGluR) agonist, 1S,3R)-ACPD were performed with cortical slices from mice treated with lorazepam or vehicle for 7 days and subject to 0, 1, 2, 3, 4 and 7 days of withdrawal. The efficacy of (1S,3R)-ACPD to stimulate PI-hydrolysis was significantly increased at 2 and 3 days of lorazepam withdrawal when compared to comparable responses in control slices. The effect was blocked by the mGluR antagonist, L-2-amino-3-phosphonopropionate (L-AP3). Enhancement of PI-hydrolysis in cortical slices from mice at 2 days of discontinuation from 7 days exposure to lorazepam was also observed with agonists of alpha(1) adrenergic- and of histamine- receptors, but not with agonists of muscarinic- or serotonin- receptors when compared to comparable responses in control slices. I.c.v. infusion of L-AP3 significantly increased pentylenetetrazol-seizure threshold in mice withdrawn for 2 days from 7 days of lorazepam-exposure, but showed no effect in comparable vehicle-exposed mice. These data suggest that PI-coupled mGluRs may be implicated in regulation of GABAergic functionality as observed following withdrawal from prolonged exposure to lorazepam.

338.16

NOVEL GLIAL-NEURONAL COMMUNICATION MEDIATED BY COACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS AND β-ADRENERGIC RECEPTORS. D.G. Winder*, R.W. Gereau, P.S. Ritch and P.J. Conn. Dept.of Pharmacology and Prog. in Neuroscience, Emory Univ. Sch. of Med., Atlanta, GA 30322.

We have previously reported that activation of group II-like mGluRs in hippocampus results in potentiation of cAMP responses. Activation of this mGluR effector system in conjunction with stimulation of β-adrenergic receptors (βARs) in area CA1 results in depression of synaptic transmission. *In situ* hybridization studies suggest that group II mGluRs are not present in neurons in area CA1. Thus, we tested the hypothesis that this mGluR effector system is present in glia in area CA1. If mGluRs that potentiate cAMP responses were present on CA1 pyramidal cells, it would be predicted that activation of this effector system would enhance cAMP-mediated events in these cells. However, we were unable to see such enhancement. Further, preincubation with either of the selective glial toxins fluorocitrate or L-α-aminoadipic acid completely blocked the cAMP responses elicited by activation of mGluRs and βARs, while having no effect on membrane properties or receptor-mediated increases in cAMP in the CA1 pyramidal cells. Finally, preincubation of hippocampal slices with kainate resulted in complete disruption of neuronal transmission, but had no effect on the cAMP response elicited by activation of mGluRs and βARs. In total, these data suggest that mGluRs coupled to potentiation of cAMP responses may be present on glia rather than neurons in area CA1 of hippocampus.

338.17

DISTRIBUTION OF METABOTROPIC GLUTAMATE RECEPTOR 7 mRNA IN ADULT AND DEVELOPING RAT BRAIN. J. M. Kinzie, M. L. Baccei, J. A. Saugstad, E. A. Zimmerman*, G. L. Westbrook and T. P. Segerson. Vollum Institute and Dept. of Neurology, Oregon Health Sciences University, Portland, Oregon, 97201.

The pattern of mRNA expression of metabotropic glutamate receptor subtypes in brain can give clues to subtype-specific functions. mGluR7 is a metabotropic glutamate receptor that is sensitive to the agonist L-2-amino-4-phosphonobutyric acid (L-AP4), a presynaptic inhibitor of neurotransmitter release. To determine the anatomical distribution of mGluR7 mRNA, we performed *in situ* hybridization on rat brain slices using an antisense mGluR7 RNA probe. We found that mGluR7 mRNA is widely distributed in both adult and developing rat brain with expression in neocortex, hippocampus, olfactory bulb, olfactory cortex, thalamus, hypothalamus, spinal cord and superior and inferior colliculi. mGluR7 mRNA is also expressed in most neuronal groups known to respond to L-AP4 including mitral cells of the olfactory bulb, granule cells of the dentate gyrus and neurons of the entorhinal cortex and dorsal root ganglion. The expression of mGluR7 mRNA in neurons sensitive to presynaptic effects of L-AP4 implies that mGluR7 is a candidate for the presynaptic L-AP4 receptor in these neurons. In addition, mGluR7 mRNA is highly expressed in areas involving sensory processing. Most strikingly, neurons at all levels of olfactory circuitry including mitral cells, neurons of the anterior olfactory nucleus and pyramidal neurons of the olfactory tubercle and primary olfactory cortex express high levels of mGluR7 mRNA, suggesting that mGluR7 activation may participate in olfactory information processing. This work was supported by NIH grants MH10314, NS09200, DC01783 and the Klingenstein Foundation.

338.18

DIFFERENTIAL GENE EXPRESSION OF METABOTROPIC GLUTAMATE RECEPTOR SUBTYPES (mGLURs) AFTER KAINIC ACID INDUCED STATUS EPILEPTICUS IN RAT PUPS AND MATURE RATS.
Eleonora Aronica*, Jan A. Gorter, Hong Shen, Linda K. Friedman, Marie C. Paupard, Michael V.L. Bennett, R. Suzanne Zukin. *Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY10461.*

It has been well documented that kainic acid induced seizures lead to cell loss in several brain regions including the hippocampal CA3 region in the adult rat but not in the rat pup. We examined the expression of the metabotropic glutamate receptors (mGluRs) after status epilepticus induced by subcutaneous kainate injection in pups at postnatal day 10 (P10) (2 mg/kg) and adult rats (P40; 10 mg/kg). At 24 hrs mGluR1, mGluR3 and mGluR5 mRNAs were not affected after status epilepticus. mGluR2 mRNA expression however, was strongly downregulated in pups as well as in the adult rats in all brain regions examined (dentate gyrus, neocortex). Since mGluR2 is involved in the activation of potassium channels, decreasing excitability, a downregulation of this receptor could contribute to the enhanced excitability. mGluR4 mRNA was significantly upregulated in the CA3 region of the hippocampus in the rat pup but not in the adult rat. Since mGluR4 receptors are putative presynaptic receptors (inhibiting glutamate release), upregulation of mGluR4 in the CA3 region in the rat pup might protect these neurons against excessive glutamate release from CA3 recurrent collaterals. Failure of such mGluR4 upregulation however, could lead to CA3 cell loss as is observed in adult rats.

338.19

DEVELOPMENTAL SWITCH IN THE EXPRESSION OF mGluR5 AND mGluR1a RECEPTORS IN CULTURED CEREBELLAR NEURONS. A. Copani*[1], G. Casabona[1], V. Bruno[1], T. Knopfel[2], R. Kuhn[2], and F. Nicoletti[3]. [1]*Institute of Pharmacology, University of Catania, and* [3]*Dept. Exp. Med. Biochem Sci. University of Perugia, Italy;* [2]*CIBA-GEIGY, Basel, Switzerland.*

In cultured cerebellar granule cells, stimulation of polyphosphoinositide (PI) hydrolysis by metabotropic glutamate receptor (mGluR) agonists is developmentally regulated and exhibits a transient peak after 4 days *in vitro* (DIV), which is observed when cultures are grown in either 25 or 10 mM K^+ (the latter condition leading to apoptotic death). We have characterized the expression of the various mGluR subtypes coupled to PI hydrolysis using polyclonal antibodies raised against non-conserved regions of the carboxy-terminal domains. Cells were stained with mGluR5 antibodies since the earlier stages of maturation, and, at 4 DIV virtually all viable cells were immunopositive. At 8 DIV, the percentage of mGluR5-positive cells was reduced. Immunostaining was more intense in cultures grown in 10 mM K^+ throughout the cultivation time. In contrast, immunostaining with mGluR1a antibodies increased with age and was maximal at 8 DIV. Interestingly, quisqualate-stimulated PI hydrolysis at 4 DIV (when mGluR5 was prevalent) was insensitive to inhibition by either pertussis toxin or phorbol esters, which instead substantially reduced the action of quisqualate at 8 DIV (when mGluR1a was prevalent). This is in agreement with results obtained in cells transfected with mGluR5 and mGluR1a, respectively.

338.20

DIFFERENTIAL PRESYNAPTIC LOCALIZATION OF METABOTROPIC GLUTAMATE RECEPTOR SUBTYPES, mGluR2/3 and mGluR7, IN THE RAT HIPPOCAMPUS. R. Shigemoto[1,2], E. Wada[1], H. Ohishi[1], M. Takada[1], N. Mizuno*[1] J. D. B. Roberts[2], and P. Somogyi[2].
1. Dept. Morph. Brain Science, Kyoto Univ., Japan, 2. Medical Res. Council, Anatomical Neuropharm. Unit, Oxford Univ., Mansfield Rd. Oxford, U. K.

The release of glutamate from nerve terminals is depressed by agonists for metabotropic glutamate receptors (mGluRs). At least 8 mGluR subtypes have been cloned and they are classified into 3 subgroups based on their pharmacology and transduction mechanisms. Subtypes in the first subgroup (mGluR1 and mGluR5) are largely localized in postsynaptic elements (Baude et al., Neuron 1993. 11, 771; Shigemoto et al. Neuroscience lett., 1993, 163, 53). In the present study, antibodies specific to mGluR2/3 in the second subgroup and mGluR7 in the third subgroup were used for immunocytochemical analysis in the rat hippocampus.

mGluR2/3 immunoreactivity (IR) was prominent in terminal zones of mossy and perforant path fibers, while mGluR7 IR was dense in all dendritic layers. By generating lesions in the entorhinal cortex, granule cell layer, or CA3 pyramidal cell layer, it was revealed that axons constituting the perforant path and mossy fibers have mGluR2/3 and mGluR7 IR, while axons from CA3 pyramidal cells have only mGluR7 IR. Electron microscopic immunogold localisation revealed an exclusively presynaptic distribution of mGluR7 IR in axon terminals that formed type 1 (asymmetrical) synapses. mGluR7 immunoparticles were restricted to the presynaptic membrane specialisation at the synaptic junctions. On the contrary, mGluR2/3 immunoparticles were found on membranes of the preterminal rather than terminal portions of axons, and never at the presynaptic membrane specialisation.

In conclusion, the results demonstrate that there is differential compartmentalised distribution of mGluRs in the plasma membrane of glutamatergic afferents; mGluR7 IR is restricted to the presynaptic grid where the receptor may interact with the vesicle fusion apparatus and/or with calcium channels to participate in dynamic changes in the release of glutamate. The location of mGluR2/3 away from the release site suggest a different effector mechanism for the regulation of glutamate release.

338.21

THROMBIN DOWN-REGULATES mGLUR5 LEVELS AND *TRANS*-ACPD STIMULATION OF PHOSPHOINOSITIDE HYDROLYSIS IN CORTICAL ASTROCYTES. S. Miller[1*], N. Sehati[1], C. Romano[2], and C.W. Cotman[1]. [1]Brain Aging and Dementia Institute, University of California, Irvine, CA 92717. [2]Departments of Ophthalmology and Neurobiology, Washington University Medical School, St. Louis, MO 63110.

Disruptions of glutamate homeostasis have been implicated in several pathological conditions in the CNS. We have thus investigated the effects of regulatory molecules involved in CNS pathology on the expression of metabotropic glutamate receptors. We have recently demonstrated that treatment of cultured astrocytes with EGF, TGF-α, or basic FGF produces a robust increase in the expression of mGluR5 and in the ability of metabotropic glutamate receptor agonists to stimulate phosphoinositide (PI) hydrolysis (*J. Neurosci.*, in press). Treatment with these growth factors also alters astrocyte morphology, producing a stellate shape with numerous branching processes. Thrombin is one of the first regulatory molecules present at sites of CNS trauma or injury, and it has diverse biological actions both through its extracellular proteolytic activity and through direct activation of cell-surface receptors. Since thrombin has been shown to reverse the morphological differentiation of astrocytes, we investigated its effects on mGluR5 levels and agonist-stimulated PI hydrolysis. Treatment of astrocytes with concentrations of thrombin as low as 100 pM inhibited the ability of TGF-α to up-regulate *trans*-ACPD stimulation of PI hydrolysis. Interestingly, thrombin treatment did not affect the stimulation of PI hydrolysis by norepinephrine or carbachol. Western immunoblotting demonstrated that the thrombin treatment also produced a reduction of mGluR5 expression which corresponded with the reduction in PI hydrolysis.

338.22

NAAG ACTIVATES CLONED mGluR3 RECEPTOR. B. Wroblewska*, M.R. Santi, J.H. Neale. Dept. Biology, Georgetown Univ., Washington D.C. 20057

We have shown previously that in cultured cerebellar granule cells N-acetylaspartylglutamate (NAAG) decreases forskolin-stimulated cAMP formation, indicating the activation of metabotropic glutamate receptor(s). The effects of NAAG are as potent as glutamate and trans-ACPD. Inhibition of forskolin-stimulated cAMP formation is pertussis toxin-sensitive, and not due to the stimulation of phosphodiesterase activity. In cerebellar granule cells inhibition of forskolin-stimulated cAMP by NAAG was additive to the inhibition caused by AP4, but not trans-ACPD. These pharmacological data suggested that in cultured cerebellar granule cells NAAG interacts with mGluR2 and/or mGluR3 type of metabotropic glutamate receptor. To evaluate this hypothesis we prepared cell lines stably transfected with mGluR2, mGluR3 or mGluR4 gene. The clones of metabotropic glutamate receptors mGluR2, mGluR3, and mGluR4 (kindly provided by Dr. S. Nakanishi) were excized from pmGR2, pmGR3, and pmGR4, respectively and inserted into the R1 site of pcDNA 1/Amp vector. Stably transfected cell lines were developed in Chinese hamster ovary cells using the calcium phosphate technique, and selection by G-418 sulfate. Selected positive clones were analyzed for cAMP formation in the presence of forskolin, and glutamate, trans-ACPD, AP4, and NAAG. Forskolin-stimulated cAMP responses in the cells transfected with mGluR3 were inhibited by glutamate, trans-ACPD, and NAAG to about 40-50% of maximal stimulation with forskolin alone. Selective agonist of the mGluR4 receptor, AP4, had no inhibitory effect in these cells. In the cells permanently expressing mGluR2 receptor, forskolin-stimulated cAMP formation was inhibited by trans-ACPD, and glutamate (by about 80 %), while NAAG and AP4 had no effect. These results indicate that NAAG selectively activates mGluR3 receptor.

338.23

NAAG ACTS THROUGH METABOTROPIC GLUTAMATE RECEPTORS IN CULTURED GLIAL CELLS. M. R. Santi, B.Wroblewska and J.H. Neale*. Department of Biology, Georgetown University, Washington D.C. 20057.

Recent findings suggest that glial cells can actively participate in neurotransmission. The presence of several voltage- and receptor operated ion channels in glial cell membranes has been shown by both electrophysiological methods and molecular cloning. Glia also express several receptors which are positively linked to adenylate cyclase and PI/Ca $^{2+}$ signal transduction. Our goal was to investigate glial cell responses to glutamate and N-acetylaspartylglutamate (NAAG) through the metabotropic glutamate receptors (mGluRs). Cerebellar glial cell cultures were obtained from 8 days old rats by trypsin digestion. Cells were grown in medium containing D-Valine to reduce fibroblast contamination; at confluence, cultures contained about 80 % astrocytes type I, and 10-15 % astrocytes type II (as characterized by GFAP and A2B5 staining), and no oligodendrocyte progenitor cells. Qualitative RT-PCR assays with specific primers for mGluR subtypes 1a, 2, 3, 4, 5, 6, and 7 subtypes indicates that in cultured glial cells only mRNA for mGluR2 is absent. Treatment of cultured glial cells with forskolin causes a dose-dependent increase in cAMP accumulation. This increase was inhibited, dose-dependently, by mGluR agonists: glutamate, *trans*-ACPD, NAAG, and AP4. The most potent agonists to inhibit forskolin-stimulated cAMP formation in glial cells were glutamate, and NAAG. The effects of NAAG were not mimicked by β-NAAG. Our results suggest that in cultured glial cells, mGluR3 is a predominant subtype within the family of glutamate metabotropic receptors, and may respond physiologically to NAAG.

GABA RECEPTORS II

339.1

LOCALIZATION OF THE HUMAN GABA$_A$ RECEPTOR α4 SUBUNIT GENE TO CHROMOSOME 4 DEFINES AN α2-α4-β1-γ1 GENE CLUSTER. P.J. McLean*, D.H. Farb and S.J. Russek. Laboratory of Molecular Neurobiology, Department of Pharmacology, Boston University School of Medicine, Boston, MA 02118.

Many of the genes encoding GABA$_A$ receptor subunits have been localized to human chromosomes and these mapping experiments have revealed a clustering of loci. We demonstrated previously that the genes encoding subunits of the most abundant isoform of the GABA$_A$ receptor are located in an α1-β2-γ2 cluster on human chromosome 5 (Russek and Farb, (1994), *Genomics*, 23:528-533) and that an ancestral α-β-γ gene cluster probably spawned clusters on chromosomes 4, 5 and 15. We now report the localization of the α4 subunit gene to human chromosome 4 using PCR to identify sequences unique to the human α4 intron-1 in a panel of human-hamster somatic cell hybrid DNAs. Our results demonstrate that the α4 gene is located on chromosome 4p14-q12, a region that also contains the α2, β1 and γ1 genes. The mapping of the α4 gene to an α2-α4-β1-γ1 gene cluster on human chromosome 4, and the fact that human chromosome 5 contains a cluster of α1, α6, β2 and γ2 genes, suggests that duplication of an ancestral α subunit gene occurred prior to duplication of an ancestral gene cluster. Furthermore, these results suggest that there may be a heretofore undiscovered subtype of α subunit on chromosome 15, since this chromosome also contains an α-β-γ gene cluster. The fact that GABA$_A$ receptor subunit genes have remained in clusters indicates that this chromosomal organization is crucial to the coordinate regulation of GABA$_A$ receptor gene expression in the nervous system.

339.2

IDENTIFICATION OF PUTATIVE GABA-A RECEPTOR β2 SPLICE VARIANT IN SINGLE CULTURED MOUSE CEREBELLAR GRANULE CELLS. S.N. Sudweeks[1*] and R.E. Twyman[1,2] Dept. of Pharmacology and Toxicology[1,2], Program in Human Molecular Biology and Genetics[1,2], and Dept. of Neurology[2], University of Utah, SLC, Utah, 84112.

A large number of GABA-A receptor subunits have been cloned and examined experimentally. Subunit heterogeneity in GABA-A receptor content has been shown to cause functional diversity. Additional functional diversity and modulatory potential is supplied by splice variants of certain subunits that contain phosphorylation sites. The γ2 subunit short and long forms are the best characterized examples. Single cell reverse transcription-polymerase chain reaction (RT-PCR) analysis of chronically depolarized (high KCl) mouse cerebellar granule cell neurons in culture for GABA-A receptor subunits revealed the existence of an apparent β2 subunit splice variant (approximately 50 b.p. in length) in the intracellular loop between putative transmembrane regions three and four. This is possibly analogous to the β2 long variant isolated in chick brain by Harvey *et al.* reported in *J. Neurochem.* 62:10-16 that contains a consensus sequence for both protein kinase C and casein kinase I, both of which could be important in the physiological modulation of the receptor. Enzyme restriction digest of the amplified DNA yeilds bands of the expected sizes for the β2 subunit with the inclusion of the inserted sequence. RT-PCR analysis of RNA obtained from whole culture dishes and adult mouse cerebellum did not yeild any bands of the larger size, indicating that the splice variant may be rare and difficult to detect at a level higher than individual neurons. This may be why Harvey *et al.* report not finding the splice variant in either rat or bovine RNA. Such a rare splice variant could be an example of localized or transient control of GABA responsiveness.

339.3

THE RECEPTOR CHANNEL SYSTEM RESPONSE: RAPID "FINGERPRINTING" OF NATIVE AND EXPRESSED GABA-A RECEPTORS. A.M. LAVOIE* & R.E. TWYMAN. Programs in Neuroscience, Human Molecular Biology & Genetics, Departments of Neurology & Pharmacology, Univ. of Utah, Salt Lake City, UT

Exposure of a multichannel patch to a sufficiently brief pulse of agonist produces a characteristic current response ("fingerprint") with receptor specific kinetic rates, which we have termed the *receptor channel system response*. GABA-A receptor system responses were obtained using ultrafast ligand exchange techniques (100μs exchange time) to apply brief pulses (800μs) of GABA (10μM-10mM) to native (fetal mouse cortex) and expressed (acute transfection of $\alpha1\beta1\gamma2$ & $\alpha2\beta1\gamma2$ in HEK293 cells) receptor populations. Outside-out patches contained >10 channels and were voltage clamped at -75mV in symmetrical chloride solutions.

Receptor channel system response kinetics were consistent within a defined ($\alpha1\beta1\gamma2$ or $\alpha2\beta1\gamma2$) receptor set, but varied across undefined (native) receptor populations. There were kinetic similarities within subgroups of native system responses, suggesting some subunit homology. However, none of the native system responses resembled those of expressed receptors. Modeling programs demonstrated that native system responses could not be replicated by receptor populations containing only various proportions of $\alpha1\beta1\gamma2$ & $\alpha2\beta1\gamma2$ containing receptors. These results suggest that native populations of GABA-A receptors are not comprised of either homogeneous or heterogeneous populations of $\alpha_1\beta_1\gamma_2$ or $\alpha_2\beta_1\gamma_2$ containing receptors, but may consistently contain other preferred subunit combinations.

339.4

HYDRODYNAMIC PROPERTIES OF $\alpha1\beta1$ AND $\alpha1\beta1\gamma2$ RECOMBINANT $GABA_A$ RECEPTORS EXPRESSED IN Sf9 CELLS. A.R. Knight*, M. Brown, C. Kostas, C. Hartnett, D. Gallager, J. Tallman, T.V. Ramabhadran. Neurogen Corporation, Branford, CT. 06405.

Expression of $GABA_A$ receptor subunits in Sf9 insect cells using recombinant baculoviruses is a valuable method for investigating the assembly of individual $GABA_A$ receptor subunits into functional ion channels. It has been shown previously that such receptors show the appropriate pharmacological and electrophysiological properties. In the present study, the physical characteristics of $GABA_A$ receptors obtained from Sf9 cells expressing $\alpha1$ and $\beta1$ subunits and cells expressing $\alpha1$, $\beta1$, and $\gamma2$ subunits were compared. Receptors were solubilized in 1% Triton X100. In sucrose density gradients, $\alpha1\beta1$ and $\alpha1\beta1\gamma2$ constructs both sedimented as sharp peaks suggesting constrained assembly into specific oligomeric complexes. The sedimentation coefficients and partial specific volumes for $\alpha1\beta1$ and $\alpha1\beta1\gamma2$ $GABA_A$ receptors were calculated by comparison of sedimentation rates through sucrose density gradients prepared in H_20 and D_2O. GABA receptors from $\alpha1\beta1$ expressing cells (3H-muscimol binding) sedimented more slowly ($S_{20,w}$ = 7.3 S) than GABA receptors from $\alpha1\beta1\gamma2$ expressing cells (3H-Ro 151788 binding)($S_{20,w}$ = 8.2 S). Gel filtration chromatography through a Sephacryl S-300 column was also used to determine the Stokes radii of the receptors. An estimate of the molecular weights of the two constructs is consistent with a 4 ($\alpha1\beta1$) and a 5 ($\alpha1\beta1\gamma2$) subunit construct.

339.5

THE EFFECT OF UNSATURATED FFA ON THE $GABA_A$/BENZODIAZEPINE RECEPTOR IS SUBUNIT SPECIFIC. M.R. Witt, S.E. Westh-Hansen and M. Nielsen*. Dept. of Biochemistry, Research Institute of Biological Psychiatry, St. Hans Hospital, DK-4000 Roskilde, Denmark.

Unsaturated free fatty acids (FFA) strongly enhance the binding of agonist benzodiazepine receptor ligands in the CNS *in-vitro*. To determine the selectivity and specificity of these interactions, recombinant human $GABA_A$/benzodiazepine receptor complexes assembled by different subunit compositions ($\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, $\alpha_5\beta_2\gamma_2$) were expressed using the baculovirus-transfected Sf9 insect cell system. At 10^{-4}M, unsaturated FFA, in particular, arachidonic (20:4) and docosahexaenoic (22:6) strongly stimulated (>200% of control) the binding of ^{3}H-flunitrazepam to the $\alpha_3\beta_2\gamma_2$ receptor combination in whole cell preparations. No effect of unsaturated FFA on ^{3}H-flunitrazepam binding to $\alpha_1\beta_2\gamma_2$ and $\alpha_2\beta_2\gamma_2$ receptor combinations was observed, and only weak effects (30% of control) were observed using the $\alpha_5\beta_2\gamma_2$ receptor combination. The saturated FFAs stearic and palmitic acid were without effect. The hydroxylated unsaturated FFAs ricinoleic and ricinelaidic acid were shown to decrease the binding of ^{3}H-flunitrazepam only if an $\alpha_1\beta_2\gamma_2$ receptor combination was used, while being inactive in all other receptor combinations. The binding of the benzodiazepine antagonist Ro15-1788 was not influenced or weakly enhanced by unsaturated FFA in any receptor combination. Scatchard plot analysis of the stimulation of ^{3}H-flunitrazepam binding to an $\alpha_3\beta_2\gamma_2$ combination shows that the enhancement is due to an increase in binding affinity (decrease of the dissociation constant K_D).

Our data indicate the effect of FFA on agonist benzodiazepine receptor ligands is dependent on the receptor subunit combination used and that the α subunit is essential to mediate these effects. Given the heterogeneity of the $GABA_A$/benzodiazepine receptor subunit distribution in the CNS, the overall effect of FFA on the benzodiazepine receptor can be assumed to vary at both cellular and regional level.

339.6

MODULATION OF RECOMBINANT $GABA_A$ RECEPTORS BY THE GENERAL ANAESTHETIC ETOMIDATE IS SUBUNIT DEPENDENT. C. Hill-Venning*, D. Belelli, A.G. Hope, J.A. Peters and J.J. Lambert. Department of Pharmacology, Ninewells Hospital and Medical School, The University, Dundee DD1 9SY, U.K.

The GABA enhancing and direct 'agonist' effects of the general anaesthetic etomidate have been determined on *Xenopus laevis* oocytes expressing human recombinant $GABA_A$ receptors of different subunit composition. cRNAs coding for human α (1,2,3 or 6) β (1 or 2) γ_{2l} receptor subunits were injected into *Xenopus* oocytes and responses to GABA and etomidate were determined using a two-point voltage-clamp. In all combinations tested, etomidate concentration-dependently enhanced the current response to GABA (EC_{10}), but preferentially augmented currents mediated by receptors containing β_2 rather than β_1 subunits. For example, etomidate EC_{50} values for $\alpha_1\beta_2\gamma_{2l}$ and $\alpha_1\beta_1\gamma_{2l}$ are 1.2±0.1μM (n=4) and 10.8±1.1μM (n=4) respectively and the E_{max} values (expressed as percent of the maximal GABA response) are 127±12% (n=4) (30μM) and 79±2% (n=4) (100μM) respectively. Additionally, the maximal direct currents induced by etomidate were substantially larger for receptors containing a β_2 subunit, *e.g.* 19±2% (300μM) and 4±0.5% (1mM) in $\alpha_1\beta_2\gamma_{2l}$ and $\alpha_1\beta_1\gamma_{2l}$ respectively. The type of α subunit expressed also influenced the modulatory effect of etomidate; the ratio of potency for the $\alpha_x\beta_1\gamma_{2l}$ versus $\alpha_x\beta_2\gamma_{2l}$ constructs was 13.3, 6.9, 8.8, 8.0, where x was α_6, α_2, α_1, α_3 respectively. By contrast, the anaesthetics propofol and 5α-pregnan-3α-ol-20-one displayed similar potencies in β_1 versus β_2 containing receptors both as modulators and agonists. These findings suggest that comparison of the β_1 and β_2 amino acid sequences will be instructive in identifying the molecular determinants of the action of this anaesthetic.

Work supported in part by Organon Teknika and the MRC.

339.7

PROPOFOL AND FLURAZEPAM ACT SYNERGISTICALLY TO POTENTIATE $GABA_A$ RECEPTOR ACTIVATION IN HUMAN RECOMBINANT RECEPTORS. J.N. Reynolds* and R. Maitra. Faculty of Medicine, Memorial University of Newfoundland, St. John's, Nfld., Canada, A1B 3V6.

Propofol (2,6-di-isopropylphenol) is an increasingly popular intravenous general anesthetic which is frequently combined with a benzodiazepine, either to enhance anesthesia or to alleviate anxiety. We have investigated the interaction between propofol and flurazepam on human recombinant $GABA_A$ receptors expressed in *Xenopus* oocytes. Combinations of cRNAs encoding the $\alpha1\beta2\gamma2L$ or the $\alpha2\beta2\gamma2L$ subunits of the $GABA_A$ receptor were injected into *Xenopus* oocytes, and the effects of GABA and modulation of GABA-evoked currents by propofol and benzodiazepines were measured using standard two-electrode voltage-clamp recordings 2-10 days after cRNA injection. Propofol (0.5-20 μM) produced a concentration-dependent increase in membrane currents activated by GABA. When flurazepam (0.5 μM) was combined with propofol, the resulting current in the presence of 3 μM GABA was significantly greater than that predicted from an additive effect. This synergism between propofol and flurazepam was evident over the whole range of concentrations (0.5-20 μM) of propofol examined. There was a strong concentration dependence to this effect; higher concentrations of GABA or flurazepam failed to produce this synergism. In contrast, the cyclopyrrolone derivative, zopiclone, which is classified as a full agonist at the benzodiazepine site of the $GABA_A$ receptor, failed to act either synergistically or additively with propofol. Our studies suggest that the synergism observed between benzodiazepines and propofol *in vivo* can be acounted for by an interaction between these two drugs at the $GABA_A$ receptor. It is also suggested that zopiclone acts at a site which is distinct from the site of binding of classical benzodiazepine agonists, since the combination of zopiclone with propofol results in a negative allosteric interaction.

339.8

MODULATION OF β3 AND α1β3 $GABA_A$ RECEPTORS BY PROPOFOL. P. A. Davies, T. G. Hales and E. F. Kirkness*. Dept. Anesthesiology, UCLA, LA, CA 90025 and Inst. for Genomic Research, 932 Clopper Rd. Gaithersburg, MD 20878.

The general anesthetic propofol modulates neuronal receptors for the inhibitory transmitter γ-aminobutyric acid (GABA). Low concentrations of propofol potentiate responses to GABA and at higher concentrations propofol directly activates $GABA_A$ receptors in the absence of GABA. There are 16 different $GABA_A$ receptor subunits which may combine to form pentameric receptors with different pharmacological properties. Using radioligand binding and patch-clamp techniques we are investigating which subunit(s) are involved in the modulation of the $GABA_A$ receptors by propofol.

Human embryonic kidney (HEK) cells were transfected with rat α1 and β3 cDNAs, alone, or in combination. Both GABA and propofol displaced [^{35}S]TBPS binding from membranes of HEK cells expressing $\alpha1\beta3$ $GABA_A$ receptors. Both GABA (100 μM) and propofol (100 μM) directly activated whole cell currents recorded from 42% of HEK cells tested expressing $\alpha1\beta3$ receptors. Propofol (10 μM) also potentiated GABA (100 μM)-activated currents in these cells. Cells transfected with α1 subunits alone did not bind [^{35}S]TBPS. Likewise, neither GABA nor propofol activated currents when applied to cells expressing only α1 subunits. By contrast, propofol but not GABA displaced [^{35}S]TBPS from the membranes of cells expressing β3 homomers. Propofol evoked currents in 63% of cells transfected with β3 cDNA, but GABA was ineffective as an agonist in the same cells.

Therefore α1 and β3 subunits together form functional $GABA_A$ receptors. GABA-evoked currents mediated by $\alpha1\beta3$ receptors are potentiated by propofol and propofol directly activates this combination. When expressed alone α1 subunits did not form functional $GABA_A$ receptors. By contrast, β3 homomers formed receptors which were activated by propofol, but were insensitive to GABA.

339.9

SUBUNIT SELECTIVITY FOR ANESTHETIC MODULATION OF $GABA_A$ RECEPTORS EXPRESSED IN Sf9 CELLS USING BACULOVIRUS. BX Carlson*, S Srinivasan, RW Olsen, Dept. of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90095.

Anesthetic interactions with various subunit combinations of recombinant $GABA_A$ receptors (GABAR) were compared in the baculovirus Sf9 cell expression system. $GABA_A$ chloride ionophores have potentially a diverse composition of polypeptides, and except for the ρ subunit, all GABAR subunit combinations tested have demonstrated sensitivity to most anesthetic agents. Using receptor autoradiography, however, it has been shown that anesthetics like alphaxalone and pentobarbital modulate GABAR ligand binding differentially throughout the brain (Carlson et al., 1992; Sapp et al., 1992; Bureau and Olsen, 1993; Nguyen et al., 1995). The greatest increases in barbiturate enhancement of benzodiazepine binding occurred in brainstem nuclei. In the presence of intravenous and volatile anesthetics, glucose metabolism and acetylcholine release are also enhanced in similar brainstem nuclei (Herkenham, 1981; Taguchi et al., 1991). What is the subunit composition of GABAR in these brainstem nuclei? *In situ* hybridization and immunohistochemistry have demonstrated that the subunits, α5, β3, and γ1 appear to colocalize in the brainstem. Using the baculovirus expression system, α5, β3, and γ1 subunits were subcloned and expressed into Sf9 insect cells in order to test the hypothesis that GABAR comprised of α5β3γ1 are more sensitive to anesthetic agents than the generic composition of α1β2γ2. Subunit selectivity for intravenous and volatile anesthetics was studied using radioligand binding and electrophysiological assays.

Supported by NIH grant NS28772.

339.10

PHARMACOLOGY OF α5β2 and α5β2γ2 $GABA_A$ RECEPTORS IN Sf9 CELLS. S. Srinivasan*[1], G.M. Lawless[2], A.J. Tobin[2-5] and R.W. Olsen[1,4,5]. Department. of [1]Pharmacology, [2]Physiological Sciences, [3]Neurology, [4]Brain Research Institute and [5]Molecular Biology Institute, University of California, Los Angeles, CA 90095.

We have used a "bacmid" expression system, based on baculovirus, to express large amounts of $GABA_A$ receptors of known subunit compositions. Our initial experiments have compared the pharmacology of $GABA_A$ receptors that contain the α5 and β2 subunits with those containing the α5, β2, and γ2 subunits. Membranes from these cells were tested for their ability to bind [^{3}H]muscimol and [^{3}H]flunitrazepam, and for the relative affinities of the receptors for other benzodiazepines, including diazepam, flurazepam, clonazepam and zolpidem. We have also compared ligand binding of receptors containing α5β2 and α5β2γ2 with that of receptors containing α1β2 and α1β2γ2 respectively.

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339.11

A NOVEL $GABA_A$ RECEPTOR SUBUNIT THAT IS EXPRESSED IN REPRODUCTIVE TISSUES AFFECTS SENSITIVITY TO STEROIDS. E.F. Kirkness, E. Hedblom and C.M. Fraser* The Institute for Genomic Research, 932 Clopper Road, Gaithersburg, MD 20878.

The cloning of a novel member of the $GABA_A$ receptor subunit family has revealed a new subunit class, termed π. The human π subunit mRNA is expressed at low levels within specific brain regions but is relatively abundant in those tissues of the reproductive system that have been reported to express $GABA_A$ receptors.

The human π gene is 30 kb in length and exhibits an intron/exon structure that is identical to known $GABA_A$ receptor subunit genes within its protein-coding sequence. Unusually, the π gene also contains a 5'-untranslated exon. The π gene has been localized to chromosome 5q33-34 by FISH mapping. The $GABA_A$ receptor α1, α6, β2 and γ2 subunit genes have also been mapped to this region. However, the π gene is not contained within the YAC clone yGABRγ2, and its physical proximity to the other 4 subunits is unknown at present.

When expressed alone in HeLa or 293 cells, π subunits do not form homomeric binding sites for known $GABA_A$ receptor ligands. In contrast, β3 subunits will form homomeric TBPS-binding sites that are sensitive to barbiturates, propofol and pregnanolone. Co-transfection of β3 and π subunits results in expression of TBPS-binding sites that are distinct from either π or β3 homomers. In particular, the π/β3 receptor is much less sensitive to the steroid pregnanolone. It is therefore likely that the π subunit plays a role in the steroid-sensitivity of $GABA_A$ receptors within the reproductive system.

339.12

A NOVEL GABA RECEPTOR IS EXPRESSED BY LOBSTER OLFACTORY INTERNEURONS. M. Wachowiak*, A. Boettcher, A.B. Zhainazarov, S. Elenes, and B.W. Ache. Whitney Lab and Dept. of Neuroscience, Univ. of Florida, St. Augustine, FL 32086.

GABA is an important central neurotransmitter in many animals, yet GABA receptors in crustaceans remain poorly characterized. In the spiny lobster *Panulirus argus*, GABA applied to the somata of olfactory interneurons elicits a current which reverses at E_{Cl}, has a half-maximal GABA response at approximately 30 μM, and an agonist profile of muscimol > GABA > CACA (a $GABA_C$ agonist), with no response to baclofen. The receptor is potently blocked by picrotoxin, but not by the noncompetitive antagonist TBPS or the $GABA_A$ antagonists bicucculine and hydrastine. The receptor is also insensitive to the $GABA_C$ antagonists I-4AA, 3-APA and THIP, with I-4AA and 3-APA actually acting as weak agonists. The receptor is also insensitive to strychnine, a glycine receptor antagonist, and cimetidine, which blocks a histamine-activated chloride current in these neurons. In outside-out patch recordings from these neurons in culture, GABA directly gates a 44 pS channel with a current reversal at E_{Cl}, consistent with the pharmacological evidence that the receptor is an ionotropic chloride channel. This receptor is similar if not identical to one reported by Jackel et al. (J. Exp. Biol. 191:167-193) on cultured CNS neurons from the clawed lobster *Homarus gammarus*. Our data extend their findings to show that the lobster receptor is pharmacologically distinct from vertebrate $GABA_C$ receptors as well as from other known GABA receptors. Its presence in two functionally diverse systems in different crustacean species underscores the importance of this novel receptor type in mediating the effects of GABA in the crustacean CNS.

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339.13

PENTOBARBITAL INDUCES TERNARY EFFECTS ON RECOMBINANT $GABA_A$ RECEPTORS. K.J. Gingrich, W.A. Roberts*, P.M. Burkat., R. Wissler, Departments of Anesthesiology and Physiology, University of Rochester, School of Medicine, Rochester, N.Y. 14642.

Pentobarbital (PB) directly activates $GABA_A$ receptors (GABAR) but paradoxically enhances current magnitude during washout (Akaike, 1985). To elucidate the gating mechanisms underlying this complex function, we studied Cl^- currents during the rapid application (I_{BARB}) and termination (I_{TAIL}) of PB pulses (50-5000μM, 0.10-30s) in single HEK-293 cells expressing rat brain α1β2γ2S GABAR subunits. The peak I_{BARB} concentration response relationship was bell-shaped (peak=500μM). Increasing PB concentrations enhanced I_{BARB} desensitization. The termination of high concentration PB (>750μM) pulses triggered a rapid increase in current causing enhancement of peak I_{TAIL} before decay. I_{TAIL} enhancement showed a sigmoidal relationship with PB concentration (Hill coefficient=2, EC50=740μM). I_{TAIL} relaxation kinetics manifested a complex dependence on PB concentration (10s pulses, mean±SEM): fast monoexponential decay (time constant (τ)=224±18ms, 100μM PB) at low PB concentrations (<250μM), biexponential decay (τ_{FAST} = 96±11ms, τ_{SLOW} = 570 ±39ms, percentage fast component = 49±5.5, 750μM PB) at higher concentrations (250-750μM), and a near-plateau period (duration-T_p) followed by slow monoexponential decay (T_p=646±20ms, τ=828±77ms, 1.25mM PB) at high PB concentrations (>750μM). T_p also increased monoexponentially with pulse duration (τ=4.4s, plateau=560ms, 3mM). The results demonstrate that PB modulates the enhancement and relaxation kinetics of I_{TAIL}, as well as induces receptor activation. These findings are consistent with PB inhibition accomplished by binding to low affinity sites that result in rapid and slow transitions to non-conducting states.

339.14

STABLE HIGH EXPRESSION OF HUMAN GAMMA-AMINOBUTYRIC $ACID_A$ RECEPTORS COMPOSED OF ALPHA AND BETA SUBUNITS. J.-S. Chen, J.A. Drewe, A.A. Reyes # and N.C. Lan*, CoCensys Inc., 213 Technology Drive, Irvine, CA 92718.
Current address: Bio-Rad Laboratories, Inc., Life Science Group, Hercules, CA

Multiple classes of pharmacological agents including benzodiazepines, "cage convulsants" like *t*-butylbicyclophosphorothionate (TBPS), barbiturates and neuroactive steroids allosterically modulate the γ-aminobutyric $acid_A$ receptor-chloride ionophore complex (GRC). The function of benzodiazepines requires a GRC comprised of α, β and γ subunits, while TBPS, barbiturates and neuroactive steroids will allosterically modulate GRCs comprised of α and β subunits. Binary αβ complexes are still hypothesized to be expressed in the mammalian brain particularly during development and could contribute to the pharmacological action of neuroactive steroids and barbiturates. In order to examine binary αβ complexes we report here the establishment of stable cell lines that express high levels of human $GABA_A$ receptors comprised of α1β1, α2β1 and α3β1 subunit combinations. The apparent potencies for allosteric modulation of [^{35}S]TBPS for most naturally occurring neuroactive steroids for the binary subunit combinations was similar to that of the γ-containing subunit combinations. Also discussed is the usefulness of these cell lines for the biophysical analysis of the $GABA_A$ receptor stoichiometry.

339.15

CLONING AND CHARACTERIZATION OF THE HUMAN $GABA_A$ RECEPTOR α4 SUBUNIT: IDENTIFICATION OF A UNIQUE DIAZEPAM-INSENSITIVE BINDING SITE. W. Yang*, J.A. Drewe and N.C. Lan. CoCensys Inc., 213 Technology Drive, Irvine, CA 92718

The heterogeneity of diazepam-insensitive (DI) benzodiazepine binding sites are found, spatially distributed between cerebral cortical and cerebellar regions. Coexpression of α6 with β2 and γ2L subunits creates a pharmacologically similar benzodiazepine receptor to the DI site observed in cerebellum, however, there is no evidence regarding the possible subunit combination forming the DI site in cerebral tissues. Here we report the cloning of the human α4 cDNA and the pharmacology derived from coexpression of this α4 subunit with β2 and γ2L subunits. This recombinant receptor complex showed a high affinity for the previously described benzodiazepine partial agonist bretazenil, the pyrazoloquinoline compounds CGS-9895 and CGS-9896, as well as the inverse agonists DMCM and Ro15-4513 as determined by [^{3}H]Ro15-4513 binding. However, it is insensitive to the benzodiazepine type I selective compounds CL 218,872 and zolpidem as well as the benzodiazepine full agonists diazepam, halazolam and midazolam. In addition, the benzodiazepine receptor ligands DMCM, β-CCE, β-CCM, FG-7142, CGS-9895 and CGS-9896 showed 7 to 10 times higher affinity for α4β2γ2L than for α6β2γ2L. The pharmacology of the α4β2γ2L receptor complex appears to resemble those of the DI site found in the cerebral cortex. Our study thus suggests that this subpopulation of DI $GABA_A$ receptors may be composed of α4β2γ2L subunits.

339.16

FUNCTIONAL AND PHARMACOLOGICAL CHARACTERIZATION OF THE HUMAN $GABA_A$ RECEPTOR α4 SUBUNIT EXPRESSED IN *XENOPUS* OOCYTES. E. R. Whittemore*, W. Yang, J. A. Drewe, and R. M. Woodward. CoCensys, Inc., 213 Technology Drive., Irvine, California 92718.

The human $GABA_A$ receptor α4 subunit has recently been cloned and characterized pharmacologically using binding techniques [Yang *et al.* see adjacent abstract]. These studies suggest that the α4 subunit forms a novel diazepam-insensitive benzodiazepine binding site. To further investigate the functional pharmacology of the α4 subunit we expressed α4β2γ2L subunit combinations in *Xenopus* oocytes, comparing the properties of these receptors with α1β2γ2L and with various binary subunit combinations (e.g. α4β2, α4γ2L, and β2γ2L). Maximal GABA responses were generally smaller for α4β2γ2L, as compared to α1β2γ2L, while apparent GABA affinity was higher for α4β2γ2L. Modulatory effects of benzodiazepine site ligands and other $GABA_A$ receptor modulators were assayed on currents that were ~10% of the maximal GABA response. In agreement with the binding studies, diazepam (0.01-1 μM), normally classed as a full agonist at benzodiazepine sites, had no affect on α4β2γ2L, whereas it increased α1β2γ2L responses up to ~100 %. Ro15-4513 (1 μM), a benzodiazepine partial inverse agonist, potentiated α4β2γ2L by ~60 %, but inhibited α1β2γ2L by ~10 %. Flumazenil (1 μM), a benzodiazepine antagonist, potentiated α4β2γ2L by ~40 % and did not modulate α1β2γ2L. The benzodiazepine ligands bretazenil, CGS-9895, and DMCM had qualitatively similar effects at both subtypes of receptor. Pentobarbital, loreclezole, mefanamic acid and 3α-hydroxy-5α-pregnan-20-one all induced potentiation of α4β2γ2L responses, similar to that seen with α1β2γ2L. Our results suggest that the α4 subunit selectively confers novel benzodiazepine site pharmacology on $GABA_A$ receptors.

339.17

NOVEL PHARMACOLOGY OF THE $GABA_A$ RECEPTOR α5 SUBUNIT EXPRESSED IN XENOPUS OOCYTES STUDIED WITH THE TWO ELECTRODE VOLTAGE-CLAMP TECHNIQUE. D.A. Gurley*, V. Stirling and G. White. Neurogen Corporation, Branford, CT 06405.

The pharmacology of zolpidem at $GABA_A$ receptors ($GABA_AR$s) expressed in HEK cells and Xenopus oocytes is consistent for $GABA_AR$s containing α1, α2 and α3 subunits. While zolpidem does not bind to $GABA_AR$s containing the α5 subunit in HEK cells, we report that zolpidem potentiates GABA current by 194±24% (EC_{50}= 227±38nM, Hill slope= 0.9±0.1, n=7) in oocytes injected with cRNA coding for α5β1γ2 subunits. We also observed 10-30% potentiation to zolpidem (500nM-1000nM) in most cells injected with α5β2γ2 and α5β3γ2. Zolpidem potentiation was blocked by flumazenil in all subunit combinations.

Zolpidem also potentiated GABA current in oocytes expressing β1γ2 by 211±24% (EC_{50}=247±47nM, Hill slope= 1.0±0.1, n=10), thus a positive control was required to demonstrate that the a5 subunit was incorporated into the $GABA_AR$s that were potentiated by zolpidem in oocytes injected with cRNA coding for α5, β1, and γ2 subunits. The α5 subunit was mutated within the M2 region to abolish sensitivity to picrotoxin (PTX) as described previously (Gurley et al., 1995). Expression of α5(T295F/T301A)β1γ2 $GABA_AR$s yielded GABA-gated current that was not blocked by 100μM PTX. Wild-type β1γ2 $GABA_AR$s were blocked 99±1% by 100μM PTX. Zolpidem sensitivity of α5(T295F/T301A)β1γ2 was determined in the presence of 100μM PTX to block any existing β1γ2 $GABA_AR$s in the receptor population. This α5(T295F/T301A)β1γ2 PTX-resistant current was potentiated by zolpidem 276±30% (EC_{50}= 146±14nM, Hill slope= 1.1, n=6). Potentiation of α5(T295F/T301A)β2γ2 and α5(T295F/T301A)β3γ2 to zolpidem in the presence of 100μM PTX was not different from the potentiation observed for the wild-type $GABA_AR$s.

These results indicate that some pharmacological properties of α5-containing $GABA_AR$s differ between expression systems in vitro. Similarly, α5-containing $GABA_AR$s with identical subunit composition may differ pharmacologically between cell types in vivo.

339.18

CHARACTERIZATION OF THE MURINE $GABA_A$ RECEPTOR α6-SUBUNIT GENE. T.A. Glencorse*, D. Livingston, H.H. Xue and R.W. Davies. Robertson Laboratory of Biotechnology, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G11 6NU, Scotland, U.K.

γ-Aminobutyric acid (GABA), the major inhibitory neurotransmitter in the vertebrate central nervous system, primarily mediates its actions via the pentameric $GABA_A$ receptor. To date, 15 genes encoding $GABA_A$ receptor subunits have been identified, each of which exhibit distinct developmental-, regional- and cell-specific expression patterns. Further, chronic treatment with therapeutic agents such as the benzodiazepines (eg., diazepam and lorazepam which are used in the treatment of anxiety) and with ethanol, modulates the expression and function of $GABA_A$ receptors. The molecular mechanisms by which $GABA_A$ receptor expression is regulated in normal and pathological states are not known. We are currently investigating the transcriptional regulation of the $GABA_A$ receptor α6-subunit gene since the expression of this gene is tightly regulated. It is expressed solely in cerebellar granule cells, is developmentally regulated and is altered in response to chronic benzodiazepine treatment. Using a PCR probe from the 5' end of the published α6-subunit cDNA sequence we have isolated several genomic clones from mouse 129 genomic library (λ2001). Three of these clones, which were found to overlap in the region of the translational start site, were further characterized by restriction mapping, DNA sequence analysis and localization of the transcriptional start site(s). A series of $GABA_A$ receptor α6-subunit gene promoter deletion constructs are presently under investigation in cultured primary cerebellar granule cells.

339.19

PRIMATE DERIVED $GABA_A$ α1, α2, α3, or α5, SUBUNITS IN COMBINATION WITH EITHER β1γ2L, β2γ2L, OR β3γ2L SUBUNITS FORM TWELVE PHARMACOLOGICALLY DISTINCT RECEPTOR SUBTYPES IN XENOPUS OOCYTES. G. White* and D. A. Gurley. Neurogen Corp. 35 NE Industrial Rd, Branford, CT 06405.

α subunits of the $GABA_A$ receptor complex ($GABA_AR$) are known to influence $GABA_AR$ pharmacology. Little information exists on the influence of β subunits and on the interaction between α and β subunits for influencing pharmacological properties of the $GABA_AR$. Using the two electrode voltage-clamp technique, the effects of α and β subunits on pharmacological properties of the $GABA_AR$ were evaluated in Xenopus oocytes injected with cRNA coding for the γ2L subunit plus one α and one β subunit subtype of the $GABA_AR$. For each subunit combination, we evaluated a concentration response curve to GABA. For GABA evoked current at each subunit combination, we evaluated a concentration effect curve for Zn and a number of benzodiazepine receptor ligands. A two-way ANOVA was carried out on EC50s of GABA. A two-way ANOVA was carried out on inhibition by Zn and a separate two-way ANOVA was carried out on the effects of each benzodiazepine site ligand. In every ANOVA, the interaction effect between α and β subunits was significant ($p<0.03$). In addition, no pair of subunit combinations shared the same pharmacological profile across the different drug treatment groups. Thus, each subunit combination exhibited a unique and identifiable pharmacological profile. For some benzodiazepine site ligands, effects at α1- and α3-containing $GABA_AR$s were not significantly different, whereas effects at α1- and α3-containing $GABA_AR$s were different from effects at α2 containing $GABA_AR$s. In other comparisons, α1- and α2-containing $GABA_AR$s were not significantly different, whereas effects at α1- and α2-containing $GABA_AR$s were different from α3 containing $GABA_AR$s. These findings indicate that Type I and II terminology may no longer be appropriate for benzodiazepine receptors. More importantly, the finding that each subunit combination has a unique pharmacological profile suggests that each subunit combination may be selectively targeted by therapeutic agents and investigational tools.

339.20

GAMMA SUBUNIT INFLUENCES THE MECHANISM OF ZINC INHIBITION IN RECOMBINANT $GABA_A$ RECEPTORS. P.M. Burkat, W.A. Roberts, K.J. Gingrich*, Departments of Anesthesiology and Physiology, University of Rochester, School of Medicine, Rochester, N.Y. 14642.

The inclusion of the γ subunit class in recombinant $GABA_A$ receptors (GABAR) changes the apparent mechanism of Zn^{2+} inhibition from a non-competitive (Draguhn et al, 1990) to a competitive mechanism (Chang et al, 1995). Yet, the details of Zn^{2+} interaction with αβ(γ) GABARs remain unclear. To investigate the nature of Zn^{2+} inhibition and its dependence on the γ subunit, we performed whole-cell patch clamp on single HEK-293 cells transfected with cDNAs encoding rat brain α1β2 or α1β2γ2S GABA receptor subunits. Cl^- currents (I_{GABA}) triggered by rapidly applied GABA pulses (1-5000μM, 2-10s) were examined in the presence of Zn^{2+} (0.5-5000μM). Zn^{2+} inhibited I_{GABA} (5uM GABA) of both receptors in sigmoidal fashion (α1β2: Hill coefficient-0.6, EC50-1.4μM; α1β2γ2S: Hill coefficient-1.01, EC50-177μM) consistent with previous reports. Zn^{2+} (10μM) suppressed peak I_{GABA}s of α1β2 independent of GABA concentration and without appreciable changes in time-course. However, Zn^{2+} (200μM) reduced peak I_{GABA}s of α1β2γ2S that was relieved by increasing GABA concentration and nearly abolished at high concentrations (>1000μM), as reported for *Xenopus*. Furthermore, Zn^{2+} (200μM) accelerated markedly α1β2γ2S I_{GABA} (1mM) desensitization kinetics (biexponential function fits (mean± SEM): fast time constant - 281±64ms to 95±24ms, slow time constant - 2480±634ms to 1340±149ms, and percentage fast component - 33±5 to 47±7). These results suggest that the nature of the Zn^{2+} inhibition of GABARs is influenced by the presence of the γ subunit class. When present (α1β2γ2S), Zn^{2+} suppresses GABARs through dual mechanisms of competitive inhibition of GABA at activating sites and accelerated desensitization.

339.21

STUDY OF THE BENZODIAZEPINE SITE ON THE $\alpha6\beta2\gamma2$ $GABA_A$ RECEPTOR SUBTYPE VIA SITE-DIRECTED MUTAGENESIS. J.F.Pregenzer, G.L.Alberts, W.B.Im* and J.A.Binder. CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

The $\alpha6\beta2\gamma2$ subtype of cloned $GABA_A$ receptors is unique among similar subtypes made of different α isoforms in its inability to interact with classical benzodiazepines, its agonistic response to Ro 15-1788, and its low half-maximal concentration (EC_{50}) for GABA to induce Cl^- currents. A point mutation of arginine 100 of $\alpha6$ to histidine (the corresponding residue in $\alpha1$) enables the subtype to interact with diazepam (Wieland *et al.*, J. Biol. Chem. 267:1426, 1992). Here we generated $\alpha6$ mutants where several nonconservatively substituted amino acid residues near R100 were mutated to the corresponding $\alpha1$ residues (H121, N122, V142, N143, and the segment from P161 to L187). Studies with the mutants indicate that the agonistic activity of Ro 15-1788 disappeared only with mutation of all the residues cited above, and that the EC_{50} for GABA to induce Cl currents was variable among the mutants, ranging from 0.1 to 1.2 μM (although far from that for the $\alpha1\beta2\gamma2$, about 10 μM). It appears that the N-terminal region of the α subunit includes functional and binding pharmacophores for the benzodiazepine site and influences low affinity GABA sites, allosterically.

339.22

MAPPING THE PICROTOXIN BINDING SITE IN THE GABA-A RECEPTOR CHANNEL USING CYSTEINE MUTAGENESIS. Myles H. Akabas * and Ming Xu. Center for Molecular Recognition, Columbia University, New York, NY 10032.

We identified the residues from the entire M2 membrane spanning segment of the rat $GABA_A$ receptor $\alpha1$ subunit which line the ion channel using the scanning cysteine accessibility method (JBC 268:21505-21508). The cysteine-substitution mutant $\alpha1$ subunits were expressed with wild type $\beta1$ & $\gamma2$ subunits in Xenopus oocytes. Irreversible alteration of GABA-induced currents following reaction with water soluble, charged sulfhydryl-specific reagents indicates that the residues N275, S272, I271, T268, T265, L264, T262, T261 and V257 are exposed in the channel. Picrotoxin and cyclodiene insecticides are non-competitive inhibitors of GABA-induced currents. When picrotoxin is coapplied with the sulfhydryl reagents in the presence of GABA it protects V257C from modification. Although picrotoxin blocks conduction in the T261C mutant, it does not protect T261C from modification. In an α helix, T261 would be 6 Å closer to the extracellular end of the channel than V257. This suggests that picrotoxin binds in the channel lumen at the level of V257. Supported by NIH NS30808, AHA Grant-in-Aid, NYHA Estab. Sci. Award, Klingenstein Award in Neuroscience.

339.23

ACTIONS OF ALLOSTERIC MODULATORS ON EXPRESSED HOMO-OLIGOMERIC *DROSOPHILA* GABA RECEPTORS: INSIGHTS INTO STRUCTURE OF NATIVE RECEPTORS. A. M. Hosie and D. B. Sattelle.* Babraham Institute Laboratory of Molecular Signalling, Department of Zoology, University of Cambridge, Downing St., Cambridge CB2 3EJ, U. K.

The activity of insect GABA receptors, like that of vertebrate $GABA_A$ receptors, is subject to allosteric modulation. Under two-electrode voltage clamp, we have tested a range of candidate modulators on homo-oligomeric GABA-gated chloride-channels formed by the expression, in *Xenopus* oocytes, of cRNA encoding the *Drosophila* GABA receptor subunit, RDL. The potency and effects of allosteric modulators on RDL homo-oligomers and native insect GABA receptors were strikingly similar for those compounds that do not show subunit specificity in vertebrate $GABA_A$ receptors (i.e. barbiturates, pregnane steroids and picrotoxin binding site ligands). In contrast to *in situ* insect receptors, 1000-fold higher concentrations of 4'-chloro-diazepam (Ro5-4864) were required to enhance the GABA response of RDL homo-oligomers whereas flunitrazepam was without effect. In vertebrates, the co-expression of specific subunits is a prerequisite for native benzodiazepine pharmacology. Thus, the differences between the pharmacology of RDL homo-oligomers and *in situ* insect receptors may reflect the hetero-oligomeric nature of native insect GABA receptors.

339.24

CO-ASSEMBLY OF TWO DIVERGENT INSECT GABA RECEPTOR SUBUNITS

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In comparison with the large number and variety of vertebrate $GABA_A$ receptors, few insect GABA receptosr or 'GABA receptor like' subunits have been cloned to date. We have investigated the coexpression of two *Drosophila* GABA receptor subunits, *Resistance to dieldrin* or *Rdl* and a homolog of the vertebrate $GABA_A$ receptor β subunit. Unlike vertebrate $GABA_A$ receptors *Rdl* forms highly functional picrotoxin (PTX) sensitive but bicuculline (BIC) insensitive homomeric channels, and cannot be readily classified within any of the known $GABA_A$ receptor subtypes. In contrast to *Rdl*, successful functional expression of the *Drosophila* β subunit homolog has not been reported. Here we report that co-infection of cells with recombinant baculoviruses containing both subunits induces expression of GABA receptors having distinct pharmacological and kinetic properties. Whole cell patch clamp recordings reveal two separate populations, one highly sensitive to PTX but BIC insensitive (*Rdl* homomultimers) and the other PTX insensitive and BIC sensitive (*Rdl*+β heteromultimers). Putative *Rdl*+β channels also show very different channel properties with slow activation, rapid bursting, prolonged open time and a shorter closed time. These studies not only localize sensitivity to PTX and BIC to two distinct GABA receptor subunits but also demonstrate an unprecedented assembly of two highly divergent GABA receptors subunits. Further, the difference in channel conductance between *in vivo* and recombinant channels predicts the existence of a novel uncharacterized GABA receptor subunit in *Drosophila*.

339.25

Assembly and cell surface expression of functional γ-aminobutyric acid type A receptors. C.N.Connolly*[2], B.J. Krishek[1], B.J. McDonald[2], T.G. Smart[1], and S.J. Moss[2]. 1. Department of Pharmacology, The School of Pharmacy, London WC1N 1AX. 2. MRC LMCB, U.C.L., London WC1E 6BT.

The ability of differing subunit combinations of $GABA_A$ receptors, produced from $\alpha1$, $\beta2$ and $\gamma2L$ subunits, to form functional cell surface receptors was analysed in both A293 cells and *Xenopus* oocytes. Receptor assembly occurred within the endoplasmic reticulum (ER) where the chaperone molecules Heavy chain binding protein (BiP) and calnexin participated. Assembly was unaffected by glycosylation inhibitors and could occur rapidly between immature subunits. Despite all three subunits possessing the ability to oligomerise with each other, only $\alpha1\beta$ 2 and $\alpha1\beta2\gamma2L$ subunit combinations could produce functional surface expression. Single subunits and the $\alpha1\gamma2L$ and $\beta2\gamma2L$ combinations were retained within the ER. These results suggest that receptor assembly occurs by defined pathways, which may serve to limit the diversity of $GABA_A$ receptors that are expressed on the surface of neurones.

340.1

CHARACTERIZATION OF PD 152255, A NOVEL DIMERIC BENZIMIDAZOLE DOPAMINE D_3 ANTAGONIST. A.E. Corbin*, T.A. Pugsley, H.C. Akunne, S.Z. Whetzel, R.G. MacKenzie, L.M. Georgic, J.L. Wright, L.D. Wise, T.G. Heffner. Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105.

PD 152255 (*E*-1,1'-(2-butene-1,4-diyl)bis[2-[4-[3-(1-piperidinyl)propoxy]-phenyl]-1H-benzimidazole]) is a novel agent with high affinity for dopamine (DA) D_3 receptors. PD 152255 displaced the DA antagonist [^{3}H]-spiperone with a K_i value of 10 nM from human DA D_3 receptors expressed in CHO K1 cells. PD 152255 had low affinity for human DA D_2 receptors expressed in CHO K1 cells, whether labelled with [^{3}H]-spiperone (K_i: 516 nM) or [^{3}H]-N-0437 (K_i: 271 nM) and lacked affinity for DA D_4 and DA D_1 receptors (K_i >3 μM). PD 152255 exhibited effects consistent with DA D_3 receptor antagonist activity in vitro: it did not cause DA agonist-like stimulation of mitogenesis in D_3-transfected CHO p-5 cells and it antagonized the stimulation of mitogenesis produced by the DA agonist quinpirole in this assay (IC_{50}: 19 nM), in line with its D_3 receptor binding affinity. PD 152255 produced dose-related inhibition of spontaneous locomotor activity in mice (ED_{50}: 7 mg/kg IP; maximal effect: 70%). Unlike classical DA antagonists, PD 152255 produced minimal effects on brain DA synthesis and spontaneous or amphetamine-stimulated locomotor activity in rats. However, PD 152255 increased locomotion in rats showing low basal activity after a period of habituation to the test chambers, a profile also seen with other D_3 antagonist-like compounds, including U-99194, (+)-AJ-76 and (+)-UH-232. These results suggest that PD 152255 is a DA D_3 antagonist that does not produce behavioral effects characteristic of classical DA antagonists.

340.2

PD 156586, A NOVEL DOPAMINE (DA) D3 PREFERRING PARTIAL AGONIST: BIOCHEMICAL AND LOCOMOTOR EFFECTS IN RODENTS. T. A. Pugsley*, H.C. Akunne, S. Z Whetzel, A. E. Corbin, L. M. Georgic, Y. H. Shih, R. G. MacKenzie, J. L. Wright, L. D. Wise, T. G. Heffner. Departments of Therapeutics and Chemistry, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, 48105.

The present study examines some aspects of the in vitro and in vivo pharmacology of PD 156586 (3-[4-(1H-benzimidazol-2-yl)-phenoxy]-propyl}-propyl-(2-thiophen-2-yl-ethyl)amine). In transfected Chinese hamster ovary cells (CHO K1) using [^{3}H]spiperone as the ligand, PD 156586 had Ki values of 8 nM, 118 nM, and 584 nM for human D3, D2L and D4.2, respectively. PD 156586 stimulated [^{3}H]thymidine uptake in CHO p-5 cells transfected with either D3 (EC50 = 1.1 nM) or D2 receptors (EC50 = 138 nM) with intrinsic activities suggestive of partial agonist action at both sites. PD 156586 (3-30 mg/kg, s.c.) produced a mild behavioral stimulation in habituated rats, without an effect on locomotor activity in nonhabituated rats or mice, effects like those of (+)AJ76 and (+)UH23. PD 156586 partially antagonized apomorphine-stimulated locomotor activity in rats. Like classical DA antagonists, PD 156586 (5-30 mg/kg, i.p.) increased rat brain DA synthesis. PD 156586 (5 mg/kg, i.p.) reversed the decrease in rat striatal DA synthesis induced by the D3 preferring agonist PD 128907. By itself PD 156586 had no effect on DA synthesis in gamma-butyrolactone pretreated rats indicating a lack of DA autoreceptor agonist action. These results suggest that PD 156586 is acting as an antagonist at DA synthesis-regulating autoreceptors. It is tempting to suggest that the in vivo actions of PD 156586 are due in part to a D3 receptor interaction. However, the influence of the DA D3 receptor preference of such compounds on their pharmacological profile is still not fully understood. Compounds such as PD 156586 may help to further our understanding of the function of D3 receptors.

340.3

DIFFERENTIAL SENSITIVITY OF [^{3}H]7-OH-DPAT-LABELED BINDING SITES IN RAT BRAIN TO INACTIVATION BY EEDQ. Beth Levant*. Department of Pharmacology, University of Kansas Medical Center, Kansas City, KS 66160-7417

The alkylating agent EEDQ irreversibly inactivates a variety of neuroreceptors including the classical dopamine receptor subtypes, D_1 and D_2. [^{3}H]7-OH-DPAT is a selective ligand for the novel D_3 dopamine receptor (Ki = 1.4 nM). The effects of EEDQ on [^{3}H]7-OH-DPAT labeled receptors were assessed in ventral striatal (n. accumbens and olf. tubercle) membranes of adult, male Sprague-Dawley rats according to the methods of Levesque et al. (PNAS, 89:8155, 1992). [^{3}H]Spiperone binding in striatal membranes was also determined as a positive control. *In vitro*, EEDQ inactivated both [^{3}H]7 OH-DPAT- and [^{3}H]spiperone-labeled receptors with IC_{50} values of 13 µM and 19 µM, respectively. *In vivo*, [^{3}H]spiperone binding was rapidly inhibited in a dose-dependent manner. In contrast, [^{3}H]7-OH-DPAT binding was not significantly altered by EEDQ at any dose (3 - 10 mg/kg, sc), or at any time point (2 - 24 h) examined. Depletion of endogenous catecholamines with α-methyltyrosine (300 mg/kg, ip) and reserpine (5 mg/kg, ip), revealed a second, higher affinity [^{3}H]7-OH-DPAT binding site (Ki = 0.15 nM). EEDQ treatment in catecholamine depleted animals decreased [^{3}H]7-OH-DPAT binding by 33% relative to controls and yielded a single population of sites (Ki = 1.3 nM). This suggests that [^{3}H]7-OH-DPAT labels two populations of binding sites in rat brain which differ in their susceptibility to modification by EEDQ. These sites may represent high and low affinity states of the D_3 receptor. *Supported by the Pharmaceutical Research and Manufacturers Association and NIMH MH 52839.*

340.4

NEUROCHEMICAL INVESTIGATIONS OF THE ROLE OF DOPAMINE D-3 RECEPTORS IN COCAINE SELF-ADMINISTRATION. E. Weiss*, L.H. Parsons, S.B. Caine, J.C. Schwartz, P. Sokoloff, and G.F. Koob. Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

The present study was designed to test the hypothesis that the D-3 receptor modulates cocaine self-administration via a post-synaptic mechanism. The selective D-3 agonist quinelorane attenuated cocaine self-administrtion at a 16-fold lower dose than 7-OH-DPAT. The addition of quinelorane (0.25 µg/infusion) to the self-administered cocaine solution (0.25 mg/inf) reduced cocaine intake by 50% (from 2.4±0.1 to 1.2±0.1 infusions per ten minutes), while also decreasing nucleus accumbens (NAC) dialysate DA levels from 366±63% of baseline during cocaine self-administration to a stable level of 187±25% of baseline. When the drug solution was switched to quinelorane alone (0.25 µg/inf) the number of self-administered infusions increased to an average of 5.3±0.2 infusions per ten minutes. During this time dialysate DA levels decreased to a stable level of 71±8% of baseline. Combined with the 7-OH-DPAT findings referred to above, these results suggest that D-3 receptor agonists modify cocaine reinforcement via a post-synaptic mechanism. To test the regional specificity of 7-OH-DPAT, this agonist was locally applied to the NAC and ipsilateral striatum of the same animal (n = 5) via reverse dialysis. DA efflux in the NAC was dose-dependently decreased by 7-OH-DPAT (30, 100 nM) while there was no effect of this agonist on DA efflux in the striatum. These results suggest that this agonist selectively modifies mesolimbic DA neurotransmission, and at low concentrations may selectively activate D-3 receptors. Finally, the the D-3 receptor antagonist nafadotride dose-dependently (0.1 - 0.3 mg/kg s.c.) increased both locomotor activity and NAC dialysate DA (133±13% and 160±22% of baseline, per dose). These findings suggest that at low doses D-3 antagonists induce locomotor activity via an increase in DA efflux produced by a presynaptic action. These results suggest that the D-3 receptor has both a presynaptic and postsynaptic functional action similar to dopamine D-2 receptors except that they appear restricted to the mesolimbic system.

340.5

S-(-)-HA-966, A γ-HYDROXYBUTYRATE-LIKE AGONIST, BLOCKS BEHAVIORAL AND MESOACCUMBAL, IN ADDITION TO MESOCORTICAL, DOPAMINERGIC STRESS ACTIVATION. B.A. Morrow*, E.J.K. Lee, J.D. Elsworth, H.E. Nye and R.H. Roth. Depts. of Pharmacology and Psychiatry, Yale Univ. School of Med., New Haven, CT 06520

Recently, we demonstrated that R-(+)-HA-966, a glycine/NMDA antagonist, selectively attenuated stress-induced increases in dopamine (DA) metabolism in the medial prefrontal cortex (mPFC), but not the nucleus accumbens (NAS). S-(-)-HA-966, however, has been noted to have actions similar to γ-hydroxybutyrate (Singh et al. *Proc. Natl. Acad. Sci.* 87:347-3451, 1989). We tested the effect of S-(-)-HA-966 on restraint and conditioned fear stressor-induced monoamine metabolism and fear-associated behaviors--immobilization and defecation. S-(-)-HA-966, 5 mg/kg i.p., prevented the stress-induced increase in mesocortical DA, but not serotonin, metabolism, similar to the R-(+) enantiomer, but at 1/3 the dose. Additionally, S-(-)-HA-966 blunted condition fear-induced immobility and increased defecation. In contrast to these similar effects of both enantiomers, S-(-)-HA-966 had unique actions on mesoaccumbal DA activity. Interestingly, conditioned fear elevated DA metabolism in both the core and shell subdivisions of the NAS. The S-(-) enantiomer blocked the stress-induced increase in DA metabolism in the whole NAS and in both the core and shell subdivisions, by restraint and conditioned stressors, respectively. These studies indicate differences with regards to the stress-activation of the mesoaccumbal DA projections between the R-(+) and S-(-) enantiomers. The mechanism of the action of S-(-)-HA-966 is not clear, however a GABA-B linked mechanism has been proposed. Initial studies, however, do not indicate that S-(-)-HA-966 displaces ^{3}H-baclofen binding. Additional studies will further examine the possibility of a GABAergic mechanism for S-(-)-HA-966, as well as the effects of both enantiomers on post-synaptic activity utilizing the immediate-early gene, fos, as a marker. Supported in part by MH-14092.

340.6

D-2 AND D-3 RECEPTORS MODULATE LEARNING AND MEMORY PROCESSES IN THE RAT. S. Sigala*, C. Missale, M. Frassine, P.F. Spano. Div. Pharmacol., Dept. Biomed. Sci. & Biotech., Brescia Univ. Med. Sch., 25123 Brescia, Italy.

Molecular cloning techniques revealed the existence of different dopamine (DA) receptors. Currently, six receptors have been cloned and grouped into two original families, D-1 and D-2. However, the paucity of highly selective ligands for each of these receptor subtypes makes functional studies difficult. Recently, a new D-3 antagonist compound called nafadotride has been developed.

It is well known that there is a DAergic control of septohippocampal cholinergic system involved in learning and memory; in particular DA has a permissive role on hippocampal acetylcholine release, mediated by receptors belonging to the D-2 DA family.

In the present study, using the model of transient memory disruption induced by scopolamine in the passive avoidance behavioural test, pharmacological characterization confirm that the DAergic modulation of septohippocampal system is due to D-2 DA receptor family; in particular, two members of this family are involved, namely D-2 and D-3 receptors. Interestingly, they shown an opposite effect on antagonizing scopolamine-induced memory impairment; in fact the amnesic effect elicited by scopolamine is blocked by the D-2 DA receptor stimulation and this effect is potentiated by the contemporary block of D-3 DA receptor activity.

Our data firstly demonstrated a functional role of D-3 receptors located in limbic areas; this could have important pharmacological and therapeutical implications and it could give input for the development of new selective drugs, which would help to well define the localization and the role of the various receptor subtypes.

This work was supported by the European Commission, contract Biomed I BMH1 - CT92 - 1086.

340.7

U-95666A, A DOPAMINE D2 AGONIST: BEHAVIORAL STUDIES IN RATS. P.J.K.D. Schreur* and N.F.Nichols. CNS Research, The Upjohn Company, Kalamazoo, MI 49001

U-95666A is a dopamine (DA) agonist which binds to D2 receptors but not D1, D3, or D4 (M.W.Smith et al., this meeting). We examined the activity of U-95666A in turning and locomotor assays using Omnitech Roto-Scan and Digiscan equipment. In rats with unilateral substantia nigra (SN) lesions, 1-3 mg/kg s.c. U-95666A caused contralateral turning in the manner of direct-acting postsynaptic dopamine agonists, but with a delayed time to maximum turning. The response was long-lasting, with a plateau of 2-2.5 hrs, and ending at about 5 hrs. Haloperidol, which had no effect on rotation by itself, completely antagonized the turning induced by U-95666A. When given daily for 2 weeks, U-95666A-induced turning showed a profound sensitization which remained high for weeks or months afterward. These withdrawn, sensitized rats respond with cross-sensitivity to the D1 agonist SKF 38393 (which did not cause sensitization by itself), but without cross-sensitivity to the D2/D3/D4 agonist quinpirole (which did cause sensitization by itself).

In intact, habituated rats, U-95666A stimulated locomotor activity at 3 mg/kg s.c. at 50-120 mins after injection (1 mg/kg was not active). Given U-95666A 1 mg/kg daily for 2 weeks. rats did not have increased activity in either their baseline activity or their response to an acute dose of 1 mg/kg s.c. Hence, no sensitization was seen here, in contrast to that seen in rats with SN lesions.

340.8

MONOAMINERGIC RECEPTOR PROFILES OF IMIDAZOQUINOLINONES: AGONISTS POTENTIALLY USEFUL FOR THE TREATMENT OF PARKINSON'S DISEASE. M.W. Smith, M.J. Bienkowski, W.H. Darlington, D.M. Dinh, D.W. Harris, R.M. Huff, M.E. Lajiness, C.F. Lawson, R.B. McCall*, J.C. McGuire, M.W. Moon, and S.K. Schlachter. Upjohn Laboratories, The Upjohn Company, Kalamazoo, MI 49001.

Parkinson's Disease (PD) results from the progressive degeneration of a small population of dopamine producing brain cells. Although the traditional symptomatic treatment for the disease has been with the dopamine precursor L-DOPA, direct-acting dopamine agonists have been increasingly used to supplement or replace L-DOPA therapy. The imidazoquinolines and imidazoquinolinones are a series of mixed serotonergic/dopaminergic agonists under development at The Upjohn Company. Members of this series have a variety of interesting effects in biochemical, neurochemical and behavioral assays. We have evaluated the *in vitro* binding and functional intrinsic activities of selected members of this series for mammalian monoaminergic receptors expressed in cultured cell lines. Members of the series were found to have generally low affinity for D1-, D3- and D4-dopamine, 5-HT2 and 5-HT1Dα sites. Some had high to moderate affinity for and were found to activate D2-dopamine, 5-HT1A and/or 5-HT1Dβ receptors. The imidazoquinolinone U-95666A, in particular, had potent *in vitro* D2-dopamine activity, exceptionally good selectivity within the dopamine receptor subtypes, and modest affinity for 5-HT1A and 5-HT1Dβ sites. The structure-activity relationships within this series will be discussed and compared with the binding properties of a variety of standard agonist compounds either currently used or under development for the treatment of PD.

340.9

PRESYNAPTIC AND POSTSYNAPTIC PHARMACOLOGY OF U-95666A, A DOPAMINE AGONIST SELECTIVE FOR THE D_2 RECEPTOR SUBTYPE: INCREASE IN POSTSYNAPTIC RESPONSE IN PARKINSON'S DISEASE (PD) MODEL. M.Camacho-Ochoa*, W.E.Hoffmann, M.W.Moon, L.M.Figur, A.H. Tang, C.S. Himes, N.F.Nichols, and M.F.Piercey, The Upjohn Company, Kalamazoo, MI 49001

In receptor binding assays using cloned receptors in CHO cells, U-95666A bound with high affinity to dopamine (DA) D_2 receptors; it also bound with moderate affinity to 5-HT_{1A} and 5-$HT_{1Dβ}$ receptors (Smith *et al.*, this meeting). Large differences in affinity for the high and low affinity forms of the D_2 receptor are consistent with functional data demonstrating high intrinsic activity for U-95666A (Smith *et al.*, this meeting). U-95666A depressed locomotor activity in rats with low doses and stimulated it with a high (10 mg/kg) dose; stereotypy was minimal with all doses but was prominent when U-95666A was combined with SKF 38393. In electrophysiology tests, U-95666A depressed firing rates of DA neurons in substantia nigra pars compacta (SNPC) by a haloperidol-sensitive mechanism, but it did not alter firing rates of 5-HT neurons in dorsal raphe. In normal rats, U-95666A did not alter neuronal firing rates in substantia nigra pars reticulata (SNPR), an area sensitive to striatal control. However, in animals where SNPC DA neurons were destroyed by 6-OHDA lesions, U-95666A depressed SNPR neuron firing rates with potencies similar to those depressing SNPC neuron firing. It is concluded that, in normal animals, presynaptic D_2 receptors are more responsive than postsynaptic D_2 receptors; however, in denervated animals, postsynaptic D_2 sensitivity is equal to that observed for presynaptic D_2 receptors in normal animals. These data suggest a potential utility for a selective D_2 agonist such as U-95666A in treatment of PD.

340.10

EFFECTS OF THE PUTATIVE ANTI-PARKINSON COMPOUND U-95666A ON BRAIN DOPAMINE ON SEROTONIN NEUROCHEMISTRY. K.A.Svensson*, M.P.Stone, J.E.Myers, S.Koch and N.F. Nichols, The Upjohn Company, Kalamazoo, MI 49001

The dopamine D_2 receptor agonist U-95666A is an imidazoquinolinone analog that induces locomotor stimulation and stereotypies in habituated and reserpine pretreated rats, and strong turning behavior in the 6-OHDA lesioned rat. No signs of the 5-HT behavioral syndrome were noted. U-95666A binds to D_2 receptors with high affinity, and with moderate affinity to 5-$HT_{1Dβ}$ and 5-HT_{1A} receptors (cf. Smith *et al.* and Schreur *et al.*, this meeting). In neurochemical studies in the rat, U-95666 (1-10 mg/kg s.c.) reduced limbic and striatal DOPA and 5-HTP accumulation with a similar potency and a high degree of intrinsic efficacy. Also, the steady-state levels of dopamine and 5-HT were increased, while the metabolites DOPAC, HVA and 5-HIAA were reduced, thus indicating reduced dopamine and serotonin turnover. These data suggest that U-95666 acts as an agonist at both dopamine and serotonin synthesis/release regulating autoreceptors. The inhibitory effects on striatal dopamine release were confirmed in a microdialysis study in freely moving rats. The inhibition of DA turnover may add "neuroprotective properties" that may impact the progression of Parkinson's disease.

340.11

ANTI-AGGRESSION EFFECT OF THE D2 AGONIST U-95667E: AN ANALOG OF U-95666A N.F. Nichols*, P.J.K.D. Schreur. CNS Research, The Upjohn Co., Kalamazoo, MI 49001

The shock-induced aggression test in mice picks up activity of both benzodiazepine- and serotonin-related anxiolytic compounds. U-95667E, a compound with both dopamine (DA) and serotonin (5-HT) agonist activity, is highly efficacious in this test. Stress increases the synthesis and release of DA, and increases DA metabolites in the prefrontal cortex of rats, and this can be modulated by benzodiazepine (BZD) receptors. There is also a close link between this BZD modulation and stress-induced behavior. However, it is unclear to what extent DA "drives" stress-induced behaviors. Pretreatment with α-methyl-para-tyrosine (α-MPT) alone gave only weak activity at best, but facilitated anti-aggression effects of 8-OH-DPAT (DPAT), diazepam (DIAZ) and U-95667E. Neither apomorphine (APO) nor haloperidol (HALO) alone altered latency to fighting in this assay, but APO antagonized and HALO potentiated all of the following: DPAT, DIAZ and U-95667E. Flumazenil attenuated DIAZ latencies, but had no effect on DPAT or U-95667E latencies. In conclusion, this testing shows U-95667E anti-aggression activity to be independent of BZD. This leaves the DA agonist property of U-95667E linked to the anti-aggression effect. Since APO alone was inactive, and in fact antagonized the anti-aggression effects of DPAT, DIAZ and U-95667E, it would seem paradoxical for the DA agonist activity of U-95667E to be responsible for increasing latency to fighting. Peripheral effects, regional specificity or effects on other transmitters may be involved.

340.12

UP-REGULATION OF RAT D_3 DOPAMINE RECEPTOR mRNA BY NEUROLEPTICS. W. Wang, K.-H. Hahn, J. F. Bishop, D.-Q. Gao, P. A. Jose*, M. M. Mouradian. Genetic Pharmacology Unit, Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD, 20892 and Department of Pediatrics, Georgetown University Medical Center, Washington, D.C. 20007.

The predominant localization of the D_3 dopamine receptor in the limbic part of the striatum complex, including the olfactory tubercle-island of Calleja complex, the bed nucleus of stria terminals and nucleus accumbens suggests that this receptor could be an important mediator of the antipsychotic action of neuroleptics. In this study, the effects of four neuroleptics on the expression of D_3 dopamine receptor gene after 14 days treatment were investigated in different regions of the rat brain using a sensitive quantitative polymerase chain reaction (Q-PCR) assay. The levels of D_3 dopamine receptor mRNA increased in olfactory tubercle following 0.5mg/kg/day haloperidol (40%), 0.25mg/kg/day pimozide (56%) and 40mg/kg/day sulpiride (63%) administration (ANOVA $p<0.002$). In nucleus accumbens, D_3 mRNA levels increased after haloperidol (50%) and sulpiride (50%) (ANOVA $p<0.05$). D_3 message levels in the motor striatum did not change with any antagonist tested. The atypical neuroleptic clozapine (20mg/kg/day) did not affect D_3 expression in any brain region examined. These findings suggest that the extrapyramidal side effects of chronic antipsychotic therapy are not due to D_3 receptor up-regulation in the motor striatum and thus neuroleptics with selective D_3 antagonism may be free of these complications.

340.13

D2 DOPAMINE RECEPTOR ACTIVATION INDUCES FOS IN THE RAT STRIATUM. A.E. Pollack* and J.S. Fink, Molecular Neurobiology Laboratory, Massachusetts General Hospital and Department of Neurology, Harvard Medical School, Boston, MA 02114.

Stimulation of D1 dopamine (DA), but not D2 DA receptors is associated with c-Fos activation in the striatum. In this study we tested whether repeated treatment of 6-hydroxydopamine (6-OHDA) lesioned rats with the mixed D1-D2 DA agonist apomorphine (APO) could alter the responsiveness of striatal neurons upon challenge with the D2 DA agonist quinpirole (QUIN). Three weeks following unilateral injections of the 6-OHDA in the medial forebrain bundle rats were treated with APO 3x over 10 days. One week later when these 3xAPO rats were treated with QUIN (0.25mg/kg) there was a significant increase in contralateral rotation (554 ± 70) and ipsilateral Fos-like immunoreactivity (Fos-LI) (835 ± 69) in the dorsal striatum after 2 hours as compared to 3xAPO control rats (H_2O-injected) (rotation: 5 ± 2; Fos-LI: 91 ± 7). The effect of QUIN on contralateral rotation and ipsilateral striatal Fos-LI was dose-dependent and could be blocked by the D2 DA antagonist eticlopride (0.5mg/kg), but not by the D1 DA antagonist SCH 23390 (2 or 0.5 mg/kg). Fos immunohistochemistry combined with colloidal gold retrograde tract-tracing showed that 78 ± 1% of cells expressing Fos-LI following administration of QUIN were gold-labeled striatoentopeduncular neurons. Lastly, 6-OHDA lesioned rats pretreated with H_2O (No APO) showed little contralateral rotation (20 ± 8) or ipsilateral striatal Fos-LI (223 ± 40) following administration of QUIN (0.25mg/kg), while 1xAPO rats treated with QUIN had an intermediate amount of rotation (314 ± 52) and striatal Fos-LI (489 ± 98) compared to 3xAPO rats treated with QUIN. These data suggest that pretreatment of 6-OHDA lesioned rats with APO alters the responsiveness of striatal neurons to a D2 DA agonist.

340.14

c-*fos* AND NEUROTENSIN mRNA INDUCTION IN THE RAT MEDIAL PREFRONTAL CORTEX AND NUCLEUS ACCUMBENS BY ATYPICAL ANTIPSYCHOTICS: ROLE OF D3 DOPAMINE RECEPTORS. L.M.Figur*, D.L.Evans and K.M.Merchant, CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001

Previous studies have shown that administration of some atypical antipsychotic agents (but not typical ones) increases c-*fos* gene expression in the rat medial prefrontal cortex. Characterization of the effects of a dopamine receptor antagonist, U-99194A, with 20-fold preference for D3 versus D2 receptors, has aided in investigating the role of D3 receptors in this phenomenon. Acute administration of U-99194A at 25, 50 or 100 µmol/kg (i.p). produced large increases in c-*fos* mRNA levels in the medial prefrontal cortical regions. Unlike the effects of clozapine, the response to U-99194A was not restricted largely to the deep cortical layers. The greatest response was observed in the anterior dorsal cingulate cortex, followed by the prelimbic, infralimbic (IL), and the lateral orbital (Orb) cortices. Interestingly, the highest dose of 100 µmol/kg produced lower responses in the IL and Orb cortices as compared to those induced by 50 µmol/kg. These data raise the question whether blockade of D2 receptors may prevent c-*fos* gene induction in the IL and Orb cortex. In the nucleus accumbens-shell where several clinically efficacious antipsychotics (typical and atypical) induce neurotensin (NT) gene expression, U-99194A did not produce statistically significant alterations at any dose tested. However, at the two higher doses, it induced NT mRNA in the accumbal shell and dorsolateral striatum in 2 out of 5 animals. It is likely that the effects of the higher doses are mediated by D2 receptors. These data show that D3 receptors may regulate the activity of prefrontal cortical neurons, but not accumbal systems.

340.15

D1 AND D2 DOPAMINERGIC AGONISTS INCREASE FOS IMMUNOREACTIVITY IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS AND CENTRAL NUCLEUS OF THE AMYGDALA IN RAT. M.J. Eaton*, K.E. Moore and K.J. Lookingland. Dept. of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824.

Populations of corticotropin-releasing hormone (CRH)-containing neurons which play an important role during stress are found in the paraventricular nucleus of the hypothalamus (PVN) and the central nucleus of the amygdala (cAMY). Dopaminergic (DA) fibers terminate in close association with CRH-containing neurons in both PVN and cAMY suggesting that CRH-synthesizing neurons could be regulated by DA receptors. The purpose of the present study was to determine the effects of selective D1 and D2 DA agonists on FOS immunoreactivity in neurons of PVN and cAMY (some of which contain CRH). Transverse sections through the middle of PVN or cAMY were chosen macroscopically and FOS-positive cells within each nucleus were counted. Ninety min after systemic injection of either the D1 agonist SKF 38393 (20 mg/kg; i.p.) or its 0.1% ascorbic acid vehicle (5 ml/kg; i.p.), the number of FOS-positive cells increased from 69.6 ± 12.7 to 145.4 ± 29.3 in PVN and from 18.8 ± 3.7 to 146.2 ± 14.9 in cAMY. Similarly, 90 min after systemic injection of either the D2 agonist quinelorane (100 µg/kg; i.p.) or its 0.9% saline vehicle (1 ml/kg; i.p.), the number of FOS-immunoreactive cells increased from 52.2 ± 7.5 to 141.4 ± 11.0 in PVN and from 30.6 ± 4.1 to 123.8 ± 29.3 in cAMY. The results of this study reveal that activation of either D1 or D2 receptors stimulates neurons in both PVN and cAMY. (Supported by NS 15911)

340.16

ACUTE ADMINISTRATION OF A D_2 DOPAMINERGIC RECEPTOR AGONIST DECREASES FOS IMMUNOREACTIVITY IN THE PERIVENTRICULAR NUCLEUS, MEDIAL PREOPTIC AREA AND HORIZONTAL LIMB OF THE DIAGONAL BAND OF BROCA IN MALE RATS. S. Cheung*, M.J. Eaton, K.E. Moore and K.J. Lookingland, Dept. of Pharmacology/Toxicology, Michigan State University, East Lansing, MI 48824.

Dopaminergic (DA) agonists increase gonadotropin-releasing hormone (GnRH) mRNA levels in the brain of male rats. In the rat brain, GnRH containing cells are diffusely distributed within several nuclear groups, including periventricular nucleus (PeVN), medial preoptic area (MPOA), and the horizontal limb of the diagonal band of Broca (HDB). The purpose of the present study was to determine the effects of selective D_1 and D_2 agonists on FOS immunoreactivity in GnRH neurons in these regions. Transverse sections through the middle of the PeVN, MPOA and HDB were chosen macroscopically and FOS-positive cells within each nucleus were counted bilaterally. GnRH positive neurons were identified by immunohistochemistry, but no FOS-positive GnRH neurons were found in any of these brain regions. Ninety min after systemic injection of either the D_1 agonist SKF 38393 (20 mg/kg; i.p.) or its 0.1% ascorbic acid vehicle (5 ml/kg; i.p.), there was no difference in the number of FOS-positive cells between control and treatment groups. However, 90 min after systemic injection of either the D_2 agonist quinelorane (100 µg/kg; i.p.) or its 0.9% saline vehicle (1 ml/kg; i.p.), the number of FOS-immunoreactive cells decreased from 25.4 ± 0.98 to 14.4 ± 2.23 in the PeVN, from 193 ± 11.91 to 119.2 ± 20.3 in the MPOA and from 39.4 ± 5.12 to 11.2 ± 2.96 in the HDB. The results of this study reveal that activation of D_2 receptors inhibits FOS expression in unidentified, non-GnRH neurons in the PeVN, MPOA and HDB. (Supported by NS15911)

340.17

ACUTE AND PROLONGED ANTIPSYCHOTIC TREATMENTS LEAD TO DIFFERENT PATTERNS OF IEG EXPRESSION IN THE CNS. P. Rogue*, T. Leveillard, A. N. Malviya. UPR416-CNRS, Center Neurochem., 5 rue Blaise Pascal, Strasbourg 67084 France.

Using gel-shift assays, we studied the effect of antipsychotics on AP-1 binding activity in the CNS. A single injection of haloperidol (2 mg/kg I.P.), which induces c-*fos* and *jun* B mRNA in the rat striatum, similarly produces a transient increase in AP-1 binding activity in both the dorsal and the ventral striatum. After prolonged haloperidol administration (2 mg/kg for 15 days), both c-*fos* and *jun* B induction were significantly dessensitized, though not completely abolished. However, despite this down-regulation, AP-1 binding activity remained elevated at levels comparable to those seen after acute haloperidol administration. This increase persisted for at least 4 days following the last administration of haloperidol. Similar results were found in the dorsal and in the ventral striatum. Thus the composition of the AP-1 transcription factor complex appears modified upon prolonged neuroleptic administration. The effect of clozapine will be presented. Supershift assays were also conducted using various antibodies.

340.18

DECREASE IN TYROSINE HYDROXYLASE mRNA EXPRESSION IN THE CAROTID BODY DURING DEVELOPMENT: POSSIBLE REGULATION BY D2-DOPAMINE AUTORECEPTORS. E.B. Gauda[1], C.R. Gerfen[2]*. [1]Johns Hopkins Medical Institutions, Baltimore, MD 21287 and [2]Laboratory of Systems Neuroscience, NIMH, Bethesda, MD 20892.

Type 1 cells in the carotid body synthesize dopamine and express D2-dopamine receptors. Binding of dopamine to D2 receptors decreases cAMP which has been shown to alter gene transcription of tyrosine hydroxylase (TH), the rate limiting enzyme for dopamine synthesis. Furthermore, D2 receptor antagonists increase TH mRNA expression in nigrostriatal neurons in the brain. We have shown that in the newborn rat, TH mRNA expression in the carotid body at birth is significantly greater than it is at postnatal days 2 and 14. In this study, we examined the relationship between TH and D2 mRNA expression in the carotid body in animals at postnatal days 0 and 14. Quantitative, double-label *in situ* hybridization using ^{35}S-UTP and digoxigenin labeled ribonucleotide probes enabled the comparison of TH and D2 mRNAs in the same cells in the carotid body of both age groups. At postnatal day 0, TH mRNA expression was greater ($P<0.002$) while D2 mRNA was less ($P<0.001$) than TH and D2 mRNA expression, respectively, in animals at postnatal day 14. Thus, TH mRNA expression is inversely correlated with D2 mRNA expression in the carotid body at two time points during development. Experiments to determine the mechanism of this correlation are being performed. [1]Supported by RWJ Foundation.

340.19

CHRONIC NEUROLEPTICS DO NOT ALTER G PROTEIN EXPRESSION IN RAT BRAIN. E. Meller,* K. Bohmaker and A.J. Friedhoff. Dept. of Psychiatry, NYU Medical Center, New York, NY 10016.

Chronic treatment with a number of psychotropic drugs (morphine, cocaine, lithium, antidepressants) alters expression of various G proteins in discrete regions of rat brain. The present study assessed the effects of chronic treatment with several dopamine receptor antagonists on expression of G proteins. Groups of rats were treated for 3 weeks with vehicle, haloperidol (0.5 mg/kg), SCH 23390 (0.2 mg/kg) and clozapine (20 mg/kg). Animals were sacrificed 3 hr after the final treatment and micropunches of tissue were obtained from 6 brain regions (striatum, nucleus accumbens, frontal cortex, hippocampus, substantia nigra and ventral tegmental area). Membrane fractions were denatured by standard techniques and G proteins were separated by SDS-PAGE on 28 cm long gels, followed by Western blotting and detection of G protein α subunits with specific antibodies using the enhanced chemiluminescence technique. This method allowed the clear separation and effortless densitometric analysis of the 3 main pertussis toxin-sensitive G proteins present in rat brain ($G_{i\alpha1}$, $G_{i\alpha2}$ and $G_{o\alpha}$) in a single lane using an appropriately diluted mixture of two antibodies (α_1/α_2, Calbiochem; α_o, Upstate Biotechnology, Inc.).

Despite reports that other psychotropic drugs alter expression of specific G proteins in discrete brain regions, none of the D1 or D2 receptor antagonists modified the expression of these G protein α subunits in the 6 brain regions examined. Supported in part by PHS grants NS 23618 and MH 08618.

340.20

G-PROTEIN EXPRESSION IN 6-HYDROXYDOPAMINE LESIONED RATS AND 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE TREATED MICE. E.R. Marcotte,* A. Chugh, C. Barlas, and R.K. Mishra. Depts. of Psychiatry and Biomedical Sciences, McMaster University, Hamilton, Ontario, L8N 3Z5, Canada.

Parkinson's disease (PD) is a progressive neurodegenerative condition characterized by the loss of dopaminergic neurons in the substantia nigra, with the subsequent depletion of striatal dopamine levels. In the 6-hydroxydopamine (6-OHDA) lesioned rat model of PD, striatal dopamine receptors exhibit behavioural and pharmacological supersensitivity to both D_1 and D_2 receptor agonists, despite significant upregulation of only D_2 receptors. Here we report the persistent elevation of striatal stimulatory G-proteins Gs and Golf, but not Gi or Go, as measured by immunoblotting. These results suggest that D_1 receptor supersensitivity may be maintained by stimulatory G-proteins in the absence of D_1 receptor upregulation. To examine the relative contributions of Gs and Golf in post-synaptic supersensitivity, antisense oligonucleotides have been prepared for in vivo administration in 6-OHDA lesioned rats. G-protein levels have also been examined in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated C57 BL/6 mice (30mg/kg/day for 10 days). Although these animals show >90% striatal dopamine depletion, dopamine receptor upregulation has only rarely been reported. We have found no significant change in Gs, Gi, or Go levels 10 days post-lesion. However, preliminary data suggests that Golf levels may be reduced by MPTP treatment, in contrast to 6-OHDA lesions. In any case, these results clearly indicate that G-proteins are primarily involved in the expression of post-synaptic dopamine receptor supersensitivity (supported by PFD Canada).

CATECHOLAMINE RECEPTORS: IN VIVO DRUG EFFECTS II

341.1

EFFECTS OF HALOPERIDOL TREATMENT ON DOPAMINE D2 AND D3 RECEPTOR DENSITIES IN RAT BRAIN. M.-P. Kung*, D. Frederick, J. Vessotskie and H. F. Kung. Departments of Radiology and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

Up-regulation of dopamine D2-like receptors following neuroleptic treatment (i.e., haloperidol) has been reported previously, and may produce extrapyramidal side effects. Lack of selective ligands, however, has hampered the study of differential changes of various D2-like subtypes. Recently, a novel radioiodinated 5-OH-DPAT analog, S(-)5-OH-PIPAT, displaying high affinities for dopamine D3 and the high affinity state of D2 (D2H) receptors, was reported (Chumpradit et al., J. Med. Chem. 37:4245-4250, 1994). Regional changes of dopamine D2 (high and low affinity states) and D3 receptor densities in rat brain after chronic treatment with haloperidol (1.5 mg/kg/day, i.p., for 18 days) were re-examined using homogenate binding and quantitative receptor autoradiography. The binding affinities (K_d) with both [I-125]S(-)5-OH-PIPAT (measuring D2 and D2H) and [I-125]NCQ298 (measuring total D2 and D3 binding sites) were similar between control and treated rats. A prominent increase of specific binding of [I-125]S(-)5-OH-PIPAT (40-80%) in regions of striatum, nucleus accumbus and olfactory tubercle was observed for haloperidol-treated rats as opposed to a moderate increase shown in [I-125]NCQ298 binding (10-30%). Autoradiographic analysis of brain sections revealed that there was no significant difference in the densities in cerebellar lobules 9 and 10 (pure D3) when labeled with either [I-125]NCQ298 or [I-125]S(-)5-OH-PIPAT. These data suggested that dopamine D2 and D3 receptors may be regulated differently by the neuroleptic treatment. It appeared that the high affinity state (agonist binding) of D2 receptors was up-regulated, while D3 receptors remained relatively constant. (supported by MH-51880)

341.2

CHARACTERIZATION OF DOPAMINE D3 RECEPTORS IN A NATIVE STATE USING QUANTITATIVE AUTORADIOGRAPHY. J. Vessotskie*, M.-P. Kung, S. Chumpradit and H. F. Kung. Departments of Pharmacology and Radiology, University of Pennsylvania, Philadelphia, PA 19104.

Recently, a radioiodinated analog of R(+)7-OH-PIPAT, S(-)-5-OH-PIPAT (5-hydroxy-2-(N-n-propyl-N-3'-iodo-2'-propenyl)aminotetralin), was shown to bind with similar high affinities (K_d = 0.2-0.4 nM) to dopamine (DA) D2-like receptors expressed in cell lines (Vessotskie, Neurosci. Abs. 524, 1994). The presence of $MgCl_2$ (2 mM) is necessary for binding of [I-125]S(-)-5-OH-PIPAT to high affinity states of DA D2 and D4 receptors expressed in HEK293 and CHO cells, but 100 mM Gpp(NH)p greatly reduces binding by converting the receptors to low affinity states. However, increasing concentrations of Gpp(NH)p or $MgCl_2$ did not influence binding of [I-125]S(-)-5-OH-PIPAT to DA D3 receptors expressed in HEK293 cells. The results of experiments performed to compare D3 receptor density (expressed in HEK293 and Sf9 cells) revealed that putative agonist [I-125]S(-)-5-OH-PIPAT labeled 27% and 39% less D3 sites, respectively, than antagonist [I-125]NCQ298. To further investigate the effects of $MgCl_2$ and Gpp(NH)p on the D3 receptor binding of these iodinated ligands and the difference in the binding sites labeled with the two ligands, experiments were carried out in the native state. Molecular layers 9 and 10 of the rat cerebellum, containing high D3 densities with minimal contamination of D2 or D4 receptors (based on mRNA expression; Bouthenet et al., Brain Res. 564:203, 1991; Van Tol et al., Nature 350:610, 1991), were chosen for the studies. Preliminary results using quantitative autoradiography are consistent with cell line findings. [I-125]S(-)-5-OH-PIPAT appears to label fewer sites in molecular layers 9 and 10 than [I-125]NCQ298, and the presence of $MgCl_2$ or Gpp(NH)p does not influence this binding. These results suggest that DA D3 receptors in native tissue bind to agonist and antagonists differently and also that binding to D3 receptors is GTP insensitive. (supported by MH-51880)

341.3

EFFECT OF 6-OHDA LESION ON THE DENSITIES OF D1, D2, D3, AND D4 DOPAMINE RECEPTORS. G.D. Stanwood*, I. Lucki, and P. McGonigle, Institute of Neurological Sciences and Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

Adult male Sprague-Dawley rats received a unilateral infusion of the neurotoxin 6-hydroxydopamine (6-OHDA) in the medial forebrain bundle to produce a selective lesion of the ascending dopaminergic pathways and were sacrificed 7 or 28 days after lesion (n=6-8/group). The extent of the lesion was determined by measurement of DA uptake sites using ^{3}H-WIN 35,428 (mean loss = 95.3±1.2%). Consistent with previous reports, D1 receptors labeled with ^{3}H-SCH 23390 were unchanged both at 7 and 28 days post-lesion in the striatum, nucleus accumbens (NA), and substantia nigra (SN). Additionally, a 20% increase was observed in the binding of ^{3}H-spiperone (which has high affinity for D2, D3, and D4 receptors) in the striatum of animals sacrificed 28 days after lesion. However, no corresponding change was observed in the binding of ^{125}I-NCQ 298 under conditions which limited its binding only to D2 receptors. As there was no detectable labeling of D3 receptors with ^{125}I-7-OH-PIPAT in this region, the increase in striatal ^{3}H-spiperone binding appears to represent an upregulation of D4 receptors. This upregulation may mediate the electrophysiological and behavioral supersensitivity which has been observed following 6-OHDA lesion. D3 receptors, labeled with ^{125}I-7-OH-PIPAT, were significantly reduced in the NA and SN both at 7 days (-35% and -45%, respectively) and 28 days (-50% and -52%, respectively) following 6-OHDA lesion. These data, in conjunction with a previous report from this laboratory (Chen, et al., Neurosci Abs 555.2, 1994) support a dual pre- and post-synaptic localization of the D3 receptor in the NA and SN. (Supported by NS-18591, GM-34781 and MH-51880.)

341.4

STREPTOZOTOCIN-INDUCED DIABETIC RAT IS RESISTANT TO CHRONIC EFFECTS OF HALOPERIDOL ON DOPAMINE-D2 RECEPTORS. T. Sumiyoshi*, J. Ichikawa, H.A. Johnston and H.Y. Meltzer, Department of Psychiatry, Case Western Reserve University, Cleveland, OH 44106.

The incidence of neuroleptic-induced tardive dyskinesia in patients with schizophrenia is increased in patients who are co-morbid for diabetes mellitus (DM). The present study was performed to determine the effect of chronic haloperidol (HPD) treatment on dopamine-D_2 receptor number and vacuous chewing movements (VCMs) in the streptozotocin (STZ)-induced DM rat. Nine weeks after STZ (65 mg/kg i.v.) treatment, male Sprague-Dawley rats with DM showed an increase in the number of VCMs (mean ± S.E.M.) counted for 90 min following an injection of (-)apomorphine (250 µg/kg s.c.), as compared to controls (55.1 ± 8.4 vs 13.5 ± 3.9). There was no significant change in the density of [^{3}H]spiperone binding sites in the striatum, representing D_2 (and partially, D_3+D_4) receptors, in DM rats (26.3 ± 1.3 vs 28.2 ± 1.1 pmol/g tissue in controls). Four weeks of depot HPD treatment (4 mg/kg/wk, i.m.) followed by a one-week withdrawal period did not affect either the density of D_2 receptors (28.4 ± 1.6 pmol/g tissue) or VCMs (47.0 ± 5.8) in DM rats. In non-DM rats, HPD treatment caused D_2 receptor up-regulation (37.0 ± 2.3 pmol/g tissue) and an increase in VCMs (105.1 ± 6.0). These results indicate increased abnormal oral movements and the lack of effect of HPD on post-synaptic D_2 receptors in the STZ-induced DM rat.

341.5

CRITICAL EVALUATION OF [^{3}H]SPIPERONE, [^{3}H]RACLOPRIDE, [^{3}H]YM-09151-2 AND [^{125}I]NCQ-298 BINDING TO DOPAMINE D_2 RECEPTORS. Å. Malmberg[a,c], E. Jerning[a], D.M. Jackson[b*] and N. Mohell[a]. Departments of Molecular Pharmacology[a] and Behavioural and Biochemical Pharmacology[b], Preclinical R & D, Astra Arcus AB, S-151 85 Södertälje and Department of Organic Pharmaceutical Chemistry[c], Uppsala Biomedical Center, Uppsala University, S-751 23 Uppsala, Sweden

Neuroleptics from several chemical classes have been used as radioligands to selectively label dopamine D_2 receptors in various parts of the brain. There are, however, several inconsistencies in the literature as regards the characteristics of benzamide and butyrophenone binding to D_2-like receptors. We have previously suggested that the discrepancies in B_{max} as well as K_d and K_i values may be related to methodological difficulties associated with the use of very high-affinity radioligands (e.g. ligand depletion and failure to achieve equilibrium). The present study was designed to reinvestigate the characteristics of [^{3}H]spiperone, [^{3}H]YM-09151-2, [^{125}I]NCQ-298 and [^{3}H]raclopride binding to cloned human D_{2A} and rat striatal D_2 receptors using optimized experimental assay conditions. We found that the K_d values of [^{3}H]spiperone, [^{125}I]NCQ-298 and [^{3}H]YM-09151-2 were about 20 pM and that of [^{3}H]raclopride about 1 nM. There were no significant differences between the B_{max} values determined with the various radioligands. The cross-competition studies showed that the K_i values of spiperone and NCQ-298 for D_2 receptors labeled with [^{3}H]spiperone or [^{125}I]NCQ-298 were in good agreement with the corresponding K_d values. Furthermore, the binding of both radioligands was fully displaceable by spiperone and NCQ-298. We conclude that, when studied under correct experimental conditions, all four radioligands label an identical receptor population.

341.6

ENDOGENOUSLY RELEASED DOPAMINE INHIBITS THE BINDING OF DOPAMINERGIC PET AND SPECT RADIOLIGANDS IN SUPERFUSED RAT STRIATAL SLICES. Andrew N. Gifford*, S. John Gatley and Charles R. Ashby, Jr.. Medical Department, Brookhaven National Laboratory, NY 11973.

Pharmacologically induced changes in synaptic cleft levels of dopamine (DA) have been found, in some cases, to affect the in vivo binding of dopaminergic radioligands. In the present study we used a superfused brain slice preparation to examine the effect of synaptically released dopamine on the binding of some commonly used PET and SPECT radioligands under more controlled conditions than those present in vivo. The release of DA was evoked by electrical stimulation of the slices and the sensitivity of binding of the D_1 receptor ligand, [^{3}H]SCH 23390, the D_2 receptor ligands [^{3}H]raclopride and [^{123}I]epidepride, and the DA uptake transporter ligands, [^{3}H]WIN 35,428 and [^{123}I]RTI-55, to the frequency of stimulation examined. Most affected by stimulation was the specific binding of [^{3}H]SCH 23390, which was fully inhibited at 2.5 Hz. This was followed by [^{3}H]raclopride and [^{123}I]epidepride, respectively, the binding of the latter showing only a 50% reduction at the highest frequency of 10 Hz. [^{3}H]WIN 35,428 and [^{123}I]RTI-55 binding was unaffected by stimulation. Examination of the effects of dopamine depletion on the inhibition of [^{3}H]raclopride binding by electrical stimulation revealed that the effects of stimulation could be prevented by reserpine treatment of the rat, when combined with inclusion of the dopamine synthesis inhibitor, α-methyl-p-tyrosine, in the superfusion medium. We conclude that, at least in brain slices, the binding of D_1 and D_2 receptor ligands, but not that of DA uptake transporter ligands, is readily inhibited by DA released into the synaptic cleft.

341.7

EFFECTS OF THE SELECTIVE DOPAMINE D_2 AGONIST U91356A ON REGIONAL CEREBRAL BLOOD FLOW. M. Gado, K. J. Black, J. S. Perlmutter, M.E. Raichle.* Departments of Radiology, Psychiatry and Neurology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Based on known basal ganglia anatomy, and the results of our previous work with the dopamine D_2-like agonist quinpirole, we hypothesized that blood flow in the globus pallidus (GP) would fall after administration of the more specific D_2 agonist U91356a. We measured quantitative regional cerebral blood flow using $H_2{}^{15}O$ and positron emission tomography in 6 baboons before and after intravenous administration of U91356a. We then performed automated registration of the PET blood flow images with anatomic magnetic resonance images of the same animal using the method of Woods et al. (**J Comput Assist Tomogr 17**:536-546, 1993). In this manner, we could precisely localize blood flow responses to predefined anatomic areas. At high doses of U91356a (0.2 mg/kg), global blood flow declined. However, at intermediate doses (0.02-0.1 mg/kg), there was a significant regional decline in GP blood flow (mean $\Delta rCBF = -4.79\%$, $p = 0.05$) when averaged across all 6 animals. The response was bilateral in normals and was present in five of six animals. The decline in pallidal flow was completely blocked by prior intravenous administration of the dopamine D_2 antagonist eticlopride, but was unaffected by prior intravenous administration of the D_1 antagonist SCH 23390. Two animals with unilateral MPTP lesions had the usual decline in globus pallidus blood flow in the normal hemisphere but not in the lesioned hemisphere. These preliminary results strongly suggest that this technique is an effective *in vivo* test of the function of dopamine D_2-receptor-bearing neurons. This "pharmacological activation" technique complements receptor binding studies, and may prove to be a more sensitive probe for investigation of neuropsychiatric diseases in which a functional abnormality of dopamine D_2 neuronal circuits is suspected. (We thank the Upjohn Company for supply of U91356a).

341.8

BIPHASIC EFFECT OF DOPAMINE ON HYPOTHALAMIC ANF-PRODUCING NEURONS: INTERACTION OF D_1 AND D_2 RECEPTORS

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Previously we have reported that ANF neurons of rat hypothalami are dopamine sensitive and shown that the catecholamine exerts a direct stimulatory or inhibitory effect on the neurons acting on D_1 or D_2 receptors respectively, through the modulation of cAMP dependent pathway. Employing well characterised radioimmunoassay and colorimetric Northern blot analysis with synthetic oligonucleotide probes complementary to pro-ANF mRNA, we report here the effect of dopamine (DA), a physiological ligand for both D_1 and D_2 receptors, on ANF neurons in long term primary cultures of neonatal rat hypothalamic cells. At low concentrations (10^{-8} to 10^{-7} M), DA was found to suppress pro-ANF mRNA expression as well as immunoreactive (ir) ANF release, whereas at high doses (10^{-6} to 10^{-5} M), the amine acts by stimulating both the production and the secretion of irANF. The inhibitory effect of DA was abolished by sulpiride, a D_2 antagonist and the stimulatory effect was suppressed by SCH-23390, a D_1 antagonist. Furthermore, the augmenting effect of DA was mimicked by quinpirole, a D_2 receptor agonist, coincubated with 10^{-5} M of SKF-38393, a D_1 receptor agonist. This synergistic effect of SKF-38393 and quinpirole was reproduced by concurrent treatment of quinpirole with forskolin or cholera toxin, but not with cAMP. The stimulatory effects of SKF-38393 and quinpirole as well as that of DA alone were suppressed in cultures incubated with antisense oligonucleotide probes complementary to type 2 adenylyl cyclase (ACII) mRNA. We thus conclude that in rat hypothalamic cultures, DA produces a biphasic effect on the function of ANF neurons. The inhibition of DA is mediated through D_2 receptors whereas the stimulatory effect involves a complex interaction between D_1 and D_2 receptors, partly through modulating the biological activity of the adenylyl cyclase-cAMP system.

341.9

PHARMACOLOGICAL CHARACTERIZATION OF MAO INHIBITOR INTERACTIONS WITH [^{3}H]QUINPIROLE BINDING IN RAT BRAIN. K.A. Morgan*, J.D. Moehlenkamp, N.L. Leonard, C.C. Cheng, and B. Levant. Department of Pharmacology, University of Kansas Medical Center, Kansas City, KS 66160

[^{3}H]Quinpirole is an ergoline dopamine agonist with 1.2 nM affinity for the D_2 and D_3 dopamine receptor subtypes. MAO inhibitors (MAOI) inhibit the binding of [^{3}H]quinpirole, but not [^{3}H]spiperone or [^{3}H]propylnorapomorphine, in rat striatal membranes by a competitive mechanism that does not appear to involve the enzymatic activity of MAO. To further characterize this observation, studies were undertaken to determine the activity profile of MAOI-displaceable [^{3}H]quinpirole binding. Binding assays were performed in striatal membranes from adult, male Sprague-Dawley rats. Clorgyline, Ro 41-1049, pargyline, and phenelzine exhibited high affinity in competition for [^{3}H]quinpirole binding (Ki = 21, 33, 85 and 100 nM, respectively) and greater than 1,000-fold selectivity between [^{3}H]quinpirole and [^{3}H]spiperone binding. Other MAOI's inhibited [^{3}H]quinpirole binding with the following rank order of potency: (-)deprenyl ≥ tranylcypromine > (+)deprenyl > isocarboxazid > Ro 16-6493 ≥ nialamide > iproniazid ≥ moclobemide > semicarbazid. The affinities of additional, structurally-related compounds were also evaluated. In contrast, classical dopaminergic compounds, such as haloperidol and (+)butaclamol, exhibited high affinity in competition with both ligands. These studies should aid in the elucidation of the specific mechanism by which these compounds inhibit [^{3}H]quinpirole binding.
Supported by NARSAD.

341.10

MONAMINE OXIDASE INHIBITOR INTERACTIONS WITH [^{3}H]QUINPIROLE BINDING IN RAT BRAIN AND PERIPHERAL ORGANS. J.D. Moehlenkamp* and B. Levant. Dept. of Pharmacology, University of Kansas Medical Center, Kansas City, KS 66160

Quinpirole, a dopamine receptor agonist, has high affinity for the D_2 and D_3 receptor subtypes. We have shown that monoamine oxidase inhibitors (MAOI) competitively inhibit [^{3}H]quinpirole binding in rat striatal membranes by a mechanism that does not appear to involve the enzymatic activity of MAO. Ro 41-1049 is a MAOI that inhibits [^{3}H]quinpirole binding with a K_i of 34 ± 6 nM, but has low potency in competion with other D_2 ligands, such as [^{3}H]spiperone or [^{3}H]NPA. Additional studies were performed to further characterize the interaction of Ro 41-1049 at [^{3}H]quinpirole labeled sites. Assays were performed using Sprague-Dawley rats as described by Levant, et al (EJP 246:171, 1993). In striatal membranes, Ro41-1049 inhibited [^{3}H]quinpirole binding at a variety of incubation temperatures, (4°, 23°, 30°, 37°C), assay tissue concentrations, (5, 10, 20 mg/ml) and time points (2 -240 min). Ro41-1049 displaceable [^{3}H]quinpirole binding was observed in hippocampus, hypothalamus, cerebellum, cortex, substantia nigra, nucleus accumbens, amygdala in addition to the striatum. In the periphery, Ro 41-1049 displaceable [^{3}H]quinpirole binding was observed in the liver. These data suggest that MAOI may interact by a novel binding site which is labeled by [^{3}H]quinpirole or which modulates [^{3}H]quinpirole binding to D_2-like receptors. *Suppported by NARSAD.*

341.11

DOPAMINE AND SEROTONIN RECEPTOR OCCUPANCY BY NOVEL ANTIPSYCHOTIC DRUG, SM-9018 AND ITS METABOLITE, *IN VIVO*.

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In vivo occupancy of dopamine D_1, D_2 and serotonin $(5\text{-HT})_2$ receptors by novel antipsychotic drug, SM-9018 and its metabolite (M-1) was measured using N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an irreversible antagonist at these receptor sites. EEDQ was administered i.p. an hour after pretreatment with SM-9018 or M-1. Twenty-four hours later, male Wistar rats (130-160g) were decapitated and the brains were removed. Receptor occupancy was estimated by measuring the receptor number that had escaped EEDQ-induced reduction.

SM-9018 (1mg/kg i.p.) was nearly equipotent in occupying D_2 (76.7%) and 5-HT_2 receptors (95.5%), whereas it had no effect on D_1 receptor. The metabolite of SM-9018, M-1 (1mg/kg i.p.), however, was more potent in occupying 5-HT_2 (70.7%) than D_2 receptor (21.2%). We previously reported atypical antipsychotic drugs such as clozapine were characterized by high occupancy of 5-HT_2 receptors with low or minimum occupancy of D_2 receptors *in vivo*. The present study suggests that clozapine-like atypical properties of SM-9018 may be, at least in part, due to the pharmacological actions of M-1.

341.12

A TRIFLATE SUBSTITUTED ISO-CLOZAPINE ANALOGUE: A NEW, POTENT, ATYPICAL NEUROLEPTIC.

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Clozapine is the most effective neuroleptic today, and it has inspired the search for other, equally efficient antipsychotic drugs with even lower propensity of side-effects. One approach is to change the substition pattern on the 5H-dibenzo[b,e][1,4]diazepine skeleton. In this study, we report on the pharmacological effects of 8- and 2-trifluorosulfonyloxy analogues **1** and **2** of clozapine and iso-clozapine, respectively. The compounds were tested for their effects on dopamine release *in vivo* in dorsal and ventral striatum; their propensity to induce catalepsy; their effects on locomotion in a novel environment and inhibition of locomotion after administration of 1 mg/kg apomorphine. Both clozapine and **2** were characterized as atypical neuroleptics in this test system. Compound **2**, however, proved to be acting on dopamine output at doses that were 100 times lower than for clozapine (0.1 µmol/kg vs. 10 µmol/kg). This difference cannot be explained from the binding profiles of clozapine and **2**. Consequently, compound **2** may have an efficacy advantage over clozapine, potentially leading to a lower clinical dose necessary, which may prove to be important for avoiding side-effects, including blood dyscrasias.

341.13

NEUROCHEMICAL EFFECTS OF INTRA-STRIATAL ADMINISTRATION OF ADTN.

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We have shown previously that an injection of ADTN, a potent and long acting mixed dopamine agonist, results, 2 hours later, in a prominant motor hyperactivity which is accompanied by enduring changes in dopamine, serotonin, and their metabolites in several brain regions (Neuropsychopharmacology 9:147, 1993) . In order to examine the specificity of these effects to the nucleus accumbens, the neurochemical effects of intra-striatal administration of ADTN were examined .The effects of bilateral administration of ADTN (12.5ug) on concentrations of dopamine (DA), its metabolites DOPAC and HVA, serotonin (5-HT), and its metabolite 5-HIAA were examined in several regions , including substantia nigra (SN), ventral tegmentum(VTA),prefrontal cortex (PFC), nucleus accumbens (NA), septum (SEP), globus pallidus (GP), amygdala (AM), and the striatum (ST) itself. Neurochemical changes were assessed at 120 minutes following ADTN injections. Concentrations of DA were significantly increased in the ST, NA, and SEP and significantly decreased in the PFC, GP, and AM. DOPAC concentrations were significantly increased in the SEP and significantly decreased in the GP.Except for the SEP, ADTN induced prominant and significant increases in HVA concentrations in all regions. For example, concentrations of HVA in the PFC were 240 times higher than control levels. 5-HT was significantly decreased in both the SEP and AM and concentrations of its metabolite 5-HIAA also showed significant decreases in the ST, SEP, and GP.

These results indicate that, similarly to what was observed in the NA , a strong DA stimulation in the ST induces pervasive and enduring neurochemical changes. However, the nature of the changes were different between the two regions. These differences may underlie the different behavioral effects induced by DA stimulation in each of these regions. Supported by the MRCC.

341.14

UNUSUAL PROFILE OF ^{3}H-CLOZAPINE BINDING IN CALF BRAIN.

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Clozapine has become an important alternate drug of choice in the treatment of schizophrenia. We have previously questioned its apparent lack of regulation on the dopamine D_2 receptors and demonstrated its potent action in reducing serotonin S_2 receptor numbers (Lee and Tang, Psychiatry Res. 12(4):277 1984). Loxapine and amoxapine also showed these same effects as clozapine. To evaluate further the mechanism of action of clozapine, we examined the binding of ^{3}H-clozapine to calf frontal cortex and striatal tissues. In view of the recent report showing that ^{3}H-clozapine is not suitable for dopamine D_4 labeling, we also examined the effect of assay conditions on the binding profile. A variety of drugs was used to compete against ^{3}H-clozapine in binding to calf frontal or striatal membranes in either Hepes (Flamez et al., Neuroscience Letters 175:17 1994) or D_4 (Van Tol et al., Nature 358:149, 1992) buffer. The profile of ^{3}H-clozapine binding under the two different buffer/assay conditions was very similar. Essentially, under our conditions, there was no nanomolar displacement of ^{3}H -clozapine binding by dopamine, sulpiride, or spiperone. Atropine, however, potently displaced ^{3}H -clozapine and clearly showed a biphasic competition curve. At micromolar concentrations, spiperone, mianserin, loxapine and clozapine showed the ability to displace ^{3}H-clozapine from a low affinity site. Changing the assay conditions did not affect the binding profile to a significant degree. The in vitro binding profile of ^{3}H -clozapine again raises questions about its clinical sites of action.

341.15

U-99194A, A D_3 RECEPTOR ANTAGONIST, SELECTIVELY ABOLISHES AMPHETAMINE'S NON-STRIATAL INCREASES IN BRAIN ENERGY METABOLISM: IMPLICATIONS FOR ANTIPSYCHOTIC THERAPY.

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The dopamine (DA) D_3 receptor is a member of the D2 receptor subfamily that, based on mRNA distribution, is speculated to be preferentially distributed in limbic rather than striatal pathways (Bouthenet *et al.*, Br Res 564:203, 1991). We now describe the regional distribution of the functional effects of U-99194A, a D_3 receptor antagonist. Receptor binding experiments demonstrated a K_i of 46 nM for displacing ^{3}[H]spiperone from cloned D_3 receptors and a K_i of 1393 nM for displacing ^{3}[H]U-86170E from cloned D_2 receptors. In 2-deoxyglucose (2-DG) autoradiography studies (Sokoloff *et al.*, J Neurochem 28:897, 1977), amphetamine (AMP, 1.0 mg/kg i.v.) increased brain energy metabolism in 21 of 65 brain regions, including several regions of cerebral cortex (anterior cingulate, sensorimotor, parietal, auditory) and basal ganglia (caudate, globus pallidus). Alone, U-99194A (10 mg/kg i.p.) had no effect on regional brain energy metabolism. However, U-99194A antagonized AMP's stimulant effects in 10 regions, including all cortical areas where AMP induced significant effects. However, AMP effects in basal ganglia areas were not significantly altered. It is concluded that D_3 antagonist activity could lead to selective antagonism of mesocorticolimbic as opposed to nigrostriatal DA function. Such actions hold promise for treatment of schizophrenia without production of extrapyramidal symptoms (EPS). Consistent with this prediction, U-99194A was not active in producing catalepsy in rats, an assay predictive of EPS.

341.16

Differential regional potencies of D_3-preferring agonists at synthesis modulating dopamine autoreceptors *in vitro*:

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We have recently reported that 7-OH DPAT, a dopamine (DA) D_3-preferring ligand was more potent in the olfactory tubercles (OT) vs. either the striatum (STR) or n. accumbens (NAC) using the GBL model *in vivo* (Aretha et al., JPET, 1995). To further examine the role of D_3 receptors in the autoregulation of DA synthesis, the potencies of D_3-preferring agonists were determined *in vitro*. K^+ (25 mM) increased DOPA accumulation by approximately 50% in STR slices and 110% in OT slices, an effect that was inhibited by the D_3-preferring agonists PD128907 and 7-OH DPAT. 7-OH DPAT completely blocked the K^+-induced increase in both regions with ED-50's of 144 ± 2 and 400 ± 1 nM in OT and STR slices, respectively. PD128907 also significantly inhibited the K^+-stimulated increase in the two regions although with less efficacy and greater potency than 7-OH DPAT (ED-50 = 35 ± 2 and 162 ± 2 in OT and STR slices, respectively). The greater potency of 7-OH DPAT and PD128907 observed in the OT may be related to the greater density of D_3 receptors in the OT. The adenylyl cyclase activator, forskolin (10 µM), increased DOPA accumulation by 210% in OT slices, and 140% in STR slices. While PD128907 (10 µM) significantly inhibited forskolin-stimulated DA synthesis in STR slices, PD128907 did not affect forskolin-stimulated synthesis in OT slices, consistent with the lack of coupling of D_3 receptors to adenylyl cyclase activity. Together, these data support the probable role of D_3 receptors as DA autoreceptors. Support: NIDA 04120 and Joe Young Sr. Research Fund.

341.17

PHARMACOLOGY OF DOPAMINE D_3 RECEPTORS IN THE RAT CAUDATE-PUTAMEN. M. Hillefors-Berglund* and G. von Euler. Dept. of Neuroscience, Karolinska Institutet, S-17177 Stockholm, Sweden.

We have analyzed the binding properties of [^{3}H]*R*(+)-7-OH-DPAT binding in freshly prepared membrane preparations from the caudate-putamen of the male rat. Both the association rate and the dissociation rate could be resolved into two components. Also, saturation curves of specific [^{3}H]*R*(+)-7-OH-DPAT binding could be resolved into two binding sites. The $B_{max(H)}+B_{max(L)}$ value of [^{3}H]*R*(+)-7-OH-DPAT binding of about 600 fmol/mg protein was similar to the B_{max} value of [^{3}H]*N*-propylnorapomorphine, and 3-4 times higher than [^{3}H]*R*(+)-7-OH-DPAT binding in the subcortical limbic area. The $K_{D(H)}$ value of 0.7 nM in the caudate-putamen were slightly higher than that in the subcortical limbic area, whereas the $K_{D(L)}$ value of 9.6 nM was similar. Coincubation with GTP (100 μM) reduced the total B_{max} value by about 40 % in the caudate-putamen, and by 20 % in the subcortical limbic region. The binding of [^{3}H]*R*(+)-7-OH-DPAT could be resolved into four specific binding sites (R_1-R_4) and one nonspecific binding site. The major component was made up by R_1 and R_2. The rank order of potencies for various agonists and antagonists were R(-)-NPA > dopamine > PD 128907 ≈ quinpirole > bromocriptine and haloperidol > raclopride > remoxipride > clozapine, respectively, at R_1, and PD 128907 > R(-)-NPA > dopamine > quinpirole > bromocriptine and haloperidol > raclopride ≈ clozapine > remoxipride, respectively, at R_2. These results indicate that D_3 receptors are present at high concentrations in the caudate-putamen, and are coupled to endogenous G proteins.

341.18

ARE THE PUTATIVE D3 ANTAGONISTS (+)AJ 76 AND U99194A AGONISTS AT A DOPAMINE RECEPTOR SUBTYPE? A. Ekman, S.R. Haadsma-Svensson, I.A. Engel*, and E. Eriksson. Department of Physiology and Pharmacology, Division of Pharmacology, Göteborg University, Medicinaregatan 7, S-413 90 Göteborg, Sweden.

(+)AJ 76 has been characterized as a dopamine (DA) antagonist preferentially blocking DA D3 receptors and DA D2 autoreceptors. The present study shows that (+)AJ 76 displaces [^{3}H]spiperone *in vitro* in the limbic region of male rat brain in a biphasic manner with one binding site - comprising about 15% of the total amount of receptors - showing an unexpectedly high affinity (K_H 1.4±0.4 nM, K_L 319±39 nM). In the presence of guanosine triphosphate (GTP), the high affinity site was shifted to the right whereas the low affinity site was unaffected; thus, the displacement curve became monophasic. The data indicate that (+)AJ 76 acts as an agonist at the high affinity site and as an antagonist at the low affinity site. The putative preferential D3 receptor antagonist U99194A also displaced [^{3}H]spiperone in limbic regions of rat brain in a similar biphasic manner (K_H 55±21 nM, K_L 2511±209 nM); again the high affinity site comprised 15% of the receptor population and was shifted to the right in the presence of GTP. For both substances, it was more difficult to detect a high affinity site in striatal tissue than in limbic regions. The possibility that some of the behavioral effects observed after administration of (+)AJ 76 and U99194A to rodents are due to the activation of a subtype of DA receptors (D3?) should be taken into consideration.

341.19

3H-EMONAPRIDE BINDS WITH HIGH AFFINITY TO SIGMA SITES IN THE GUINEA PIG BRAIN. T.F. Seeger*, C.G. Johnson and S. Zorn. Department of Neuroscience, Pfizer Inc., Central Research Division, Groton, CT 06340.

[3H]-emonapride (YM09151-2), binds with high affinity to D2, D3, and D4 dopamine receptors, while [3H]-raclopride binds with high affinity to D2 and D3, and with low affinity to D4 receptors. Recent studies in human postmortem brain tissue have subtracted 3H-raclopride binding from that found with 3H-emonapride, and assumed that the difference reflected D4 receptor density. These results suggested an increase in D4 receptor density in schizophrenic vs. normal brain. These conclusions presume minimal interaction with other receptor types. We found that emonapride appears to bind to the guinea pig sigma binding site with nM affinity, while raclopride binds with μM affinity. Autoradiographic distribution of 3H-emonapride binding in guinea pig cerebellum and frontal cortex matches that found with the selective sigma ligand, 3H-DTG, and is displaced by (+)-pentazocine, a high affinity sigma ligand. Saturation analysis with 3H-emonapride in guinea pig cerebellum and frontal cortex homogenates fits a one site model with Kd's between 0.65-0.9nM and Bmax between 350-600 fmol/mg protein. DTG, 3PPP, (+)pentazocine, and (-)butaclamol potently displace 3H-emonapride binding, although these compounds have low affinity for both D2 and D4 dopamine receptors (IC50's > 2μM). 3H-emonapride binding is displaced more potently by (-)butaclamol than by (+)butaclamol while the reverse is true for all D2-like receptors. Since human brain has moderate levels of sigma binding, if 3H-emonapride is used as a D4 label then an appropriate sigma masking compound should be included in the assay.

341.20

MODULATION OF DOPAMINE RECEPTOR SENSITIVITY BY L-PROLYL-L-LEUCYL-GLYCINAMIDE (PLG) ANALOGUES: NEUROCHEMICAL AND BEHAVIOURAL STUDIES. P.W. Bauer[1], R.L. Johnson[1], A. Chugh[2], E. Marcotte[2], C. Barlas[2], and E.W. Werstiuk[2]*. [1]Dept. of Medicinal Chem., Univ. of Minnesota, Minneapolis, MN, USA [2]Dept. of Biomed. Sci., McMaster Univ., Hamilton, Ont., Canada.

In order to establish the mechanism of action of the endogenous peptide, PLG, a series of analogues were designed, the most potent being 2-oxo-3-(R)-[(2(S)-pyrrolidinylcarbonyl)amino)]-1-pyrrolidineacetamide. The modulatory effect of several of these compounds was studied in three models of dopamine receptor sensitivity: (a) in mice treated with MPTP, which induces a depletion of dopamine, (b) in radioligand binding assays, to determine the prevention of GTP-induced conversion of dopamine D_2 receptors from the high-affinity to the low-affinity state, and (c) in 6-hydroxydopamine-lesioned rats, to observe the potentiation of rotational behaviour, induced by apomorphine. In all of these paradigms, PLG, as well as the potent analogues, displayed a modulatory effect on dopamine receptor sensitivity. These compounds were able to prevent the MPTP-induced depletion of dopamine, at concentrations as low as 1-10 μg/kg and were also effective at extremely low concentrations in the other two paradigms. Furthermore, these analogues were 100-1000 times more potent than PLG itself. These results suggest, that these analogues also interact at the same site as PLG and indicate their potential therapeutic use in the management of drug-induced tardive dyskinesia and Parkinsonism. (Supported by NIH-USA, OMHF).

SEROTONIN: REGULATION

342.1

THE 5-HT1A AGONIST 8-OH-DPAT MODULATES EXTRACELLULAR DOPAMINE AND SEROTONIN IN THE MPOA. D. S. Lorrain, L. Matuszewich, R. Trujillo and E. M. Hull*Dept. Psychology, State Univ. of New York at Buffalo, Buffalo, NY 14260 USA

Serotonin (5-HT) in the medial preoptic area (MPOA) inhibits, while MPOA dopamine (DA) facilitates, male rat sexual behavior (Verma et al., 1988; Hull, et al., 1986). In spite of the generally inhibitory effects of 5-HT, stimulation of the 5-HT1A receptor subtype enhances copulation (Ahlenius & Larsson, 1991; Fernandez-Guasti et al., 1992). The present experiments investigated effects of intracranial and systemic 5-HT1A agonists on extracellular levels of DA and 5-HT.

Five male rats received 8-OH-DPAT (250, 500, and 1000 μM) via a microdialysis probe in the MPOA for 18 min (flow rate, .4 μL/min). Two 6-min samples were collected during drug infusion and assayed for 5-HT and DA by HPLC-EC. The 5-HT1A agonist significantly increased extracellular levels of both DA and 5-HT. In different animals (n=7), a systemic injection of 8-OH-DPAT (.4 mg/kg i.p.) significantly increased DA metabolites DOPAC and HVA. Both the .4 mg/kg and a lower dose (.2 mg/kg, i.p., n=5) significantly decreased 5-HT levels. Thus, both central and systemic injections of 8-OH-DPAT modulate MPOA DA and 5-HT activity.

The decrease in 5-HT following systemic 8-OH-DPAT may be due to stimulation of autoreceptors in the raphe nuclei and may contribute to the enhancement of male sexual behavior. The facilitation of MPOA DA activity by both central and systemic administration of 8-OH-DPAT may also enhance the copulatory ability of the males. However, the mechanism of this interaction remains unknown. The increase of 5-HT in the MPOA during MPOA administration of 8-OH-DPAT is more difficult to explain.
Supported by NIH grant MH-40826 to EMH.

342.2

FUNCTIONAL EVALUATION OF SEROTONINERGIC PLASTICITY AFTER NEONATAL DOPAMINERGIC DENERVATION. M.A. Alonso-Vanegas*, A.F. Sadikot, A. Jevric-Causevic, A. Olivier, M. Diksic. Cone Laboratory for Neurosurgical Research, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada, H3A 2B4.

Under normal circumstances, serotonin (5-HT) neurons innervate both the substantia nigra (SN) and the neostriatum, though the density of striatal 5-HT innervation is lower than that of dopamine (DA). Previous studies have shown that DA denervation results in a marked increase in the density of 5-HT immunoreactive fibers and synapses throughout the neostriatum. We examine the functional response of the striatal 5-HT system using ^{14}C-labeled alpha-methyl-L-tryptophan, a synthetic analogue of L-tryptophan, to study 5-HT synthesis. Neonatal rat pups (P3) underwent catecholamine denervation using 6-hydroxydopamine injected bilaterally into the striatum. Control rats received similar injections of vehicle only. At 30 days after denervation rats were processed for ^{14}C-labeled α-methyl-L-tryptophan (α-MTrp) radioautography as previously described (Diksic et al., 1990). Our data suggests that the rate of 5-HT synthesis increases by 50% in the striatum in lesioned animals as compared to vehicle-injected controls. These results suggest that 30 days after DA denervation, 5-HT sprouting correlates with an increase in 5-HT synthesis rate. (Supported by MRC, FRSQ, AANS, NIH)

342.3

DOPAMINERGIC FACILITATION OF SEROTONIN RELEASE FROM THE RAT HIPPOCAMPUS : *IN VIVO* MICRODIALYSIS STUDY.

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The present study was undertaken to elucidate the functional regulation of dopaminergic receptors on serotonin (5-HT) release from the rat hippocampus as measured by *in vivo* microdialysis coupled with HPLC-ECD. Dialysate 5-HT concentrations were reduced by co-perfusion of TTX (10 μM) and were increased by both local (10 μM) and systemic (10 and 20 mg/kg, i.p.) administration of fluoxetine. These results indicate that the spontaneous 5-HT levels can be used as indices of neuronal origin from the serotonergic nerve terminals. Non selective dopamine (DA) receptor agonist, apomorphine (1, 10 and 100 μM) co-perfused during 40 min produced increases in the 5-HT release in a concentration-dependent manner. Apomorphine-induced (100 μM) increases in 5-HT release were abolished by pretreatment with D2 receptor antagonist, S(-)-Sulpiride (1 and 10 μM), but not by pretreatment with D1 receptor antagonist, R(+)-SCH-23390 (1 μM). S(-)-Sulpiride by itself did not alter the 5-HT levels. The 5-HT release was also elevated by co-perfused D2 receptor agonist, (±)-PPHT (1, 10 and 100 μM) in a concentration-dependent manner. (±)-PPTH-induced (100 μM) increases in 5-HT release were inhibited by 10 μM of S(-)-Sulpiride. The inhibitory effect of S(-)-Sulpiride was augumented by pretreatment with alpha2-adrenoceptor antagonist, idazoxn (5mg/kg, i.p.). In 6-OHDA (200 μg/rat, i.c.v.) treated rats, (±)-PPHT (100 μM) failed to increase 5-HT release. These findings suggest the possibility that the functional regulation of 5-HT release via D2 receptors exists in the rat hippocampus, which may be indirectly mediated by noradrenergic neurons.

342.4

DECREASED SYNAPTIC DENSITY IN DENTATE MOLECULAR LAYER AFTER 5-HT DEPLETION OR 5-HT_{1a} ANTAGONIST TREATMENT IN ADULT RATS.

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Evidence for synapse stabilization by 5-HT in adult rats has appeared in reports of synapse loss in neocortex (J. Neurobiol. 24:687, 1993) and a decrease in synaptophysin staining in area dentata (Soc. Neurosci. Abstr. 20:290, 1994) after 5-HT depletion. The present EM study evaluated changes in synaptic density in the molecular layer of area dentata of adult rats (180-200g) after 5-HT depletion (PCA, 15mg/kg, 2 injections 24h apart), NE depletion (DSP-4, 50mg/kg) and treatment with NAN-190 (3.5mg/kg daily for 7d). Rats were studied 7 days after the start of treatment. Controls received vehicle injections. Synaptic densities were counted in electron photomicrographs of a strip of molecular layer related to the middle of the suprapyramidal blade extending from the hippocampal fissure to the granule cell layer . PCA and NAN-190 rats had significant reductions in molecular layer synaptic densities. Synapses were within normal range in DSP-4 rats. These data indicate that 5-HT may stabilize synapses via 5-HT_{1a} stimulation.

342.5

COMPARATIVE *IN VIVO AND IN VITRO* STUDIES OF STRIATAL C-FOS EXPRESSION AFTER STIMULATION OF 5-HT1A and 5-HT2A/2C RECEPTORS.

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We have previously demonstrated that *in vivo* concomitant stimulation of 5-HT1A and 5-HT2A/2C has a powerful synergistic effects on striatal (STR) and accumbal *c-fos* expression. Expression of Fos-like immunoreactivity (Fos-LI) was restricted to dorsomedial and ventromedial parts of the STR. No Fos-LI was found in the lateral part of the STR. This topographical expression could be the result of intrinsic striatal factors or the result of a differential modulation by afferences. To investigate this latter possibility, we chose to compare c-*fos* expression induced by 5-HT agonists *in vivo* and in a brain slice preparation which correspond to a deafferented system. For *in vivo* studies, rats were injected with 5-HT agonists and sacrificed 2 hrs later. Groups were: a) 8-OHDPAT (2.5 mg/kg i.p), b) DOI (2.5 mg/kg i.p.), and c) 8-OHDPAT +DOI. For *in vitro* studies, coronal striatal slices (400 μm) were prepared from rats weighing 150-175 g. Slices were transferred into a chamber and perfused with oxygenated Ringer solution (95% O2-5% CO2) at room temperature for 1 hour. After, the chamber was heated slowly from room temperature to 34°C. Slices were then perfused with Ringer containing 5-HT drugs for an additional 2 hrs. Groups were: a) 8-OH-DPAT (10 μM), b) DOI (10 μM) and c) 8-OH-DPAT+DOI. After removal from the chamber, slices were fixed in 2% paraformaldehyde and equilibrated in 30% sucrose. Sections were cut at 40 μm on a cryostat and processed for Fos-LI. *In vivo*, only the combination of 8-OHDPAT + DOI induced strong expression of Fos-LI and the expression was restricted to the medial part of the STR. *In vitro*, Fos expression was induced by each drug treatment and was uniformly distributed over the entire extent of the STR. These results strongly suggest that striatal afferences play an important role on Fos expression induced *in vivo* by 5-HT agonists. (Supported by NSERC and FRSQ)

342.6

mCPP INDUCED INCREASE OF SEROTONIN AND DOPAMINE RELEASE: ANALYSIS OF THE MECHANISM OF ACTION.

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m-Chloro-phenylpiperazine (mCPP) has recently gained marked attention as a putative probe of serotonergic function in clinical psychiatric research. The administration of mCPP has been shown to elicit, and/or aggravate, anxiety attacks in patients with panic disorder, obsessions in patient with obsessive compulsive disorder, elation in alcoholics and cocaine addicts, and positive symptoms in schizophrenic patients. The effects of mCPP have generally been attributed to direct interactions with various subtypes of serotonin receptors although recent findings suggest that mCPP also releases serotonin.

In order to further elucidate the mechanism of action of mCPP, the effects of the drug on serotonin release in the hippocampus and on dopamine release in the striatum and nucleus accumbens were investigated by in vivo microdialysis in awake rats.

Intravenous administration of mCPP (0.25 or 2.5 mg/kg) induced a marked and dose-dependent increase in extracellular concentrations of serotonin. The highest dose of mCPP increased the serotonin levels by more than 1000%. The effects of the drug on extracellular dopamine concentrations were less pronounced; mCPP (2.5 mg/kg) increased the dopamine concentrations by 50% in the nucleus accumbens and only slightly in the striatum. The increase of serotonin concentration was antagonised by pretreatment with the serotonin reuptake inhibitor citalopram (10 mg/kg) but was unaffected by local administration of the sodium blocker tetrodotoxin (TTX), whereas the increase in dopamine levels were completely abolished by TTX.

The findings give support to the notion that mCPP releases serotonin by reversing the serotonin transporter. The effects of mCPP on dopamine release can, however, not be explained by a reversal of the dopamine transporter, but can tentatively be a secondary effect due the release of serotonin. The possibility that the clinical effects of mCPP, at least partly, are due to the release of endogenous serotonin and dopamine should be taken into consideration.

342.7

POTENTIATION OF THE FLUOXETINE-DEPENDENT ELEVATION OF EXTRACELLULAR 5-HT LEVELS IN HYPOTHALAMUS BY PINDOLOL, AN ANTAGONIST AT SOMATODENDRITIC 5-HT_{1A} AUTORECEPTORS, IN CONSCIOUS RATS.

L. J. Dreshfield*, E. A. Engleman, K. W. Perry, and D. T. Wong. CNS Division, Eli Lilly and Company, Indianapolis, IN 46285.

Antagonists at the somatodendritic 5-HT_{1A} autoreceptor have been shown to facilitate elevation of extracellular 5-HT levels upon cocomittant inhibition of 5-HT uptake in vivo (Hjorth, S., 1993; Artigas, F., et al., 1994). In the present study, we elaborate on the ability of (-)pindolol to enhance the increase of 5-HT after fluoxetine administration (Wong, D. T., et al., 1994). Rats were implanted with microdialysis probes under chloral hydrate/pentobarbital anesthesia. After a 48-hour recovery period, artificial CSF was perfused through the probe. The output dialysate was passed on to an analytical column for HPLC electrochemical detection of monoamines and their metabolites. Fluoxetine alone (10 mg/kg i.p.) caused 5-HT levels to increase 200% over basal levels. This increase was stable and long lasting. Administration of (-)pindolol (1, 3, and 5 mg/kg s.c.) following fluoxetine led to significant increases in 5-HT levels. Coadministration of fluoxetine (10 mg/kg i.p.) and (-)pindolol (5 mg/kg s.c.) led to an increase similar to that shown by sequential administration (270% over basal). An additional dose of (-)pindolol (5 mg/kg s.c.) caused a further increase in 5-HT levels of 400% over basal levels. These results suggest that coadministration of (-)pindolol with fluoxetine can potentiate fluoxetine-dependent elevations in rat hypothalamic 5-HT.

342.8

DIFFERENTIAL EFFECTS OF GLUTAMATE RECEPTOR AGONISTS ON SEROTONIN IN RAT MIDBRAIN RAPHE AND FOREBRAIN SITES.

R. Tao*, Z. Ma, S.B. Auerbach. Nelson Biol. Lab., Rutgers Univ., Piscataway, NJ 08855.

To investigate the regulation of serotonin (5-HT) release by excitatory amino acids, the effects of NMDA, kainic acid and AMPA in rat midbrain raphe and forebrain sites were examined by microdialysis. In dorsal raphe nucleus (DRN), all three compounds induced significant increases in extracellular 5-HT in a dose-dependent manner. Relative potency was kainate > NMDA > AMPA. NMDA (300 μM) produced a four fold increase in DRN 5-HT. Competitive (AP-5 and CPP) and non competitive (MK-801 and PCP) NMDA antagonists blocked the effect of NMDA. Significant enhancement of 5-HT was also seen in median raphe nucleus (MRN), but the efficacy of NMDA was lower as compared to DRN. In the MRN, NMDA (1000 μM) produced less than a two fold increase in 5-HT.

Infusion of NMDA into the DRN and MRN elicited an increase in 5-HT in the n. accumbens and hippocampus, respectively. In contrast, extracellular 5-HT was slightly decreased in forebrain sites when NMDA was locally applied in the n. accumbens and hippocampus. These data suggest that NMDA, kainate and AMPA act on excitatory amino acid receptors in the midbrain raphe to enhance 5-HT neurotransmission in the forebrain. Supported by NIMH grant #51080

342.9

DIFFERENTIAL INHIBITORY INFLUENCE OF $GABA_A$ AND $GABA_B$ RECEPTORS ON SEROTONIN IN THE RAPHE AND N. ACCUMBENS OF RATS. S.B. Auerbach*, Z. Ma, R. Tao. Nelson Biol. Lab., Rutgers Univ., Piscataway, NJ 08855.

Microdialysis was used to assess the contribution of GABA receptors to regulation of serotonin (5-HT) release in the dorsal raphe nucleus (DRN) and a forebrain project site, nucleus accumbens (NAC). DRN 5-HT was dose-dependently decreased during infusion of the $GABA_A$ receptor agonist muscimol into the DRN. Extracellular 5-HT in the NAC was dose-dependently decreased by the $GABA_B$ receptor agonist baclofen, but not by muscimol, infusion into the NAC.

Infusion of the $GABA_A$ receptor antagonists bicuculline and picrotoxin into the DRN produced dose-dependent increases in extracellular 5-HT in DRN and NAC. In contrast, infusion of the $GABA_A$ receptor antagonists into the NAC had no effect on 5-HT. Infusion of the $GABA_B$ receptor antagonists phaclofen and 2-hydroxysaclofen into the DRN had no effect on 5-HT in the DRN and NAC. These results suggest that $GABA_A$ receptors play a selective role in the DRN to tonically inhibit 5-HT neuronal activity under our experimental conditions. In contrast stimulation of $GABA_B$ receptors may selectively inhibit 5-HT release in forebrain projection sites of the DRN. Supported by NIMH grant #51080

342.10

CITALOPRAM TREATMENT AND HYPOTHALAMIC 5-HT LEVELS AS MEASURED BY IN VIVO MICRODIALYSIS. C. Moret* and M. Briley. Centre de Recherche Pierre Fabre , 17 Ave. J. Moulin, 81100 Castres, France.

Chronic administration with the selective serotonin reuptake inhibitor, citalopram, has been reported to down-regulate serotonergic terminal autoreceptors as measured *in vitro* (Moret & Briley, 1990 *Eur. J. Pharmacol.* 180, 351-356). The present study compared the effects of acute and chronic administration of citalopram on extracellular levels of 5-HT and their terminal autoreceptor modulation in the hypothalamus of freely moving rats by *in vivo* microdialysis. Rats were administered citalopram as in the previous study (50 mg/kg in the diet for 2 days or 21 days). On the last day of treatment or 24 h after change to a non-drug containing diet (washout), extracellular levels of 5-HT were measured before and after the addition (in the perfusion medium) of the non-selective serotonergic autoreceptor antagonists, methiothepin (100 μM) or 1-(1-naphthyl)piperazine (NP) (10 μM). When studied without washout, extracellular levels of 5-HT were increased by both acute and chronic citalopram. In rats treated chronically, however, extracellular 5-HT levels were 43% greater than in those treated acutely. Extracellular levels of 5-HT did not differ between control and citalopram-treated rats when measured after 24 h washout. The enhancing effect of methiothepin or NP, administered through the microdialysis probe, after 24 h washout was similar in both control and chronically treated groups. These results suggest that chronic administration of citalopram does not lead to desensitization of the terminal autoreceptor as measured *in vivo* in contrast to that shown previously *in vitro*. The higher levels of extracellular 5-HT following chronic rather than acute administration with citalopram under conditions where the drug is still present (no washout) suggest, however, that certain adaptive changes do take place in the regulation of serotonergic neurotransmission, presumably at sites other than the terminal autoreceptor.

342.11

SEROTONIN FUNCTION DURING WITHDRAWAL FROM A SENSITIZING REGIMEN OF COCAINE. R.L. Windh*, N. Parekh, B.E. Chadwick and K.A. Cunningham. Dept. Pharmacol., Univ. Texas Med. Branch, Galveston, TX 77555.

Rats sensitize to the behavioral effects of cocaine (COC) with repeated exposure. The mechanisms of this behavioral sensitization may be relevant to psychoses and affective disturbances associated with repeated cocaine use and withdrawal in humans. This laboratory has previously reported enhanced sensitivity of dorsal raphe (DR) serotonin (5-HT) neurons to COC, 8-OH-DPAT (DPAT) and fluoxetine 24 hrs following COC sensitization, as well as decreased numbers and firing rates of DR 5-HT cells (Cunningham *et al*, Synapse 11: 112, 1992). The present studies investigate changes in 5-HT neuron and 5-HT_{1A} receptor function 72 hrs following a sensitizing COC regimen. Male Sprague-Dawley rats (225-350 g) were injected with COC (15 mg/kg, ip, bid) or saline (SAL) for 7 days to induce sensitization. Using single unit extracellular recording techniques, a significant decrease in the number of DR 5-HT cells/electrode pass was observed (sal: 11.8±1.3, coc: 7.8±1.3; n=26), although cell firing rates were not altered (sal: 12.5±1.3, coc: 11.1±0.9; n=26). In contrast to data at 24 hrs, sensitivity of DR 5-HT cells to COC at 72 hrs withdrawal was significantly *decreased* in COC-treated compared to SAL-treated rats. In each of the 1st 3 min following injection of 0.5 mg/kg COC (iv), firing was reduced to 27±6, 9±6 and 14±10% of basal rate in SAL-treated rats (n=4), and 50±10, 32±12 and 48±13% of basal rate in COC-treated rats (n=6). As cocaine inhibits DR 5-HT neurons at least partially via indirect 5-HT_{1A} receptor stimulation, 5-HT_{1A} receptor sensitivity was compared in COC- and SAL-treated rats. No differences in the hypothermia (sal: -1.7±0.2, coc: -1.3±0.1; n=12) induced by 0.12 mg/kg DPAT (s.c.) or the behavioral syndrome (flat body posture, forepaw treading, lower lip retraction and forward locomotion) induced by systemic or intra-raphe DPAT (0.12 mg/kg, s.c, n=12 and 7.5 μg/0.5 μL, n=20) were observed in SAL- and COC-treated rats. With results from the 24 hrs withdrawal time point, these data indicate that the activity and sensitivity of DR 5-HT neurons in sensitized rats are in rapid flux during withdrawal from cocaine. Supported by DA 06511.

342.12

SEROTONERGIC REGULATION OF RAT AMYGDALA ELECTROPHYSIOLOGY: EFFECT OF CHRONIC FENFLURAMINE ON SPONTANEOUS FIRING RATES AND RESPONSE TO COCAINE. E.J. Mah* and K.A. Cunningham, Dept. of Pharmacology, University of Texas Medical Branch, Galveston, TX 77555-1031.

Investigating the role of serotonin (5-HT) as a neurotransmitter in the amygdala could shed light on the etiology of several psychiatric disorders. The present study employs single-unit extracellular recording techniques to test the effect of chronic fenfluramine (FEN), a 5-HT releaser and neurotoxin, on spontaneous firing rates of amygdala (AMY) neurons and their response to bolus doses of intravenous (i.v.) cocaine (COC). Male Sprague Dawley rats (220-300g) were treated subcutaneously with either FEN (12 mg/kg) or saline (SAL; 1 ml/kg) BID for 4 days. Rats were prepared for recording either 36 hr or 2 wks after the final injection. Withdrawal time did not affect spontaneous firing rates within treatment groups, thus both time points were pooled for all analyses. Cells from FEN-treated animals exhibited higher mean firing rates as compared to cells from SAL-treated animals (4.39 ± 0.70/10 sec vs. 1.39±0.14/10sec, respectively; Student's *t*-test, $p<0.05$). FEN treatment also altered the response of AMY neurons to COC. In FEN-treated rats, firing rates were either increased (4/10 cells) or decreased (6/10) following COC (1 mg/kg) whereas in the SAL group, firing rates were either inhibited (7/11) or unaffected (4/11; Fisher's Exact test, $p<0.05$). After a 4 mg/kg dose of COC, the primary neuronal response was inhibitory, but the time course of the inhibition was of shorter duration in FEN animals (ANOVA, $F_{(3,42)}=4.03$, $p<0.05$). The increased firing rates and shift in responsiveness to COC in FEN-treated animals may reflect disinhibition of AMY cells due to removal of 5-HT input. These data suggest a tonic inhibitory role for 5-HT in regulation of AMY neuronal firing and 5-HT involvement in the observed effects of COC on AMY neurons.

Supported by DA 05708 and DA 06511.

342.13

MODIFICATION OF 5-HT RESPONSES IN RAT DORSOLATERAL SEPTAL NUCLEUS (DLSN) NEURONS BY ACUTE AND CHRONIC COCAINE. **D. Simms* and J.P. Gallagher. Dept. of Pharm. and Tox., Univ. of Texas Med. Br., Galveston, TX 77555.**

This lab has previously demonstrated that DLSN neurons can be hyperpolarized by 5-HT via activation of a postsynaptic 5-HT1A receptor (Joëls et al., 1987) that is PTX sensitive. Superfusion with a high concentration of 5-HT can, in addition to a brief hyperpolarization, induce a long-lasting depolarization which is antagonized by ketanserin (Van den Hooff & Galvan, 1992), and unmasked by PTX pretreatment. We used standard intracellular electrophysiological recording techniques to determine whether chronic cocaine administration would: 1) alter the sensitivity of the septal brain slice to exogenous 5-HT application and 2) modify the interaction of 5-HT with cocaine *in vitro*.

Standard intracellular current-clamp recordings were made from neurons in rat brain slices which contained the DLSN obtained from drug naive (DN) rats or rats administered cocaine (15 mg/kg, IP, 2x daily) for periods of 7 (CC7) or 14 (CC14) days. In addition, some of these rats also received intraventricular PTX injections 2-3 days prior to experimentation. In comparison to DN and CC7, CC14 slices showed an increased sensitivity to 5-HT as revealed by a 1 to 2-fold leftward shift in 5-HT EC50 values. Most notable was the shift at the base of the CC14 5-HT dose-response curve. In addition, in PTX-CC14 slices, 5-HT could hyperpolarize the cell membrane while 8-OHDPAT and baclofen failed to do so. We also observed that cocaine (3 μM) in CC14 slices could not significantly potentiate and prolong 5-HT hyperpolarizations. We conclude that in the CC14 septal slice: 1) a 5-HT transporter is downregulated 2) a 5-HT1A receptor is upregulated 3) a 5-HT2 receptor is downregulated and 4) a 5-HT receptor not coupled to a PTX sensitive G-protein is expressed. Supported by DA-07190.

342.14

MORPHINE ACTIVATES THE SEROTONERGIC NEURONS OF THE DORSAL BUT NOT THE MEDIAN RAPHE NUCLEUS OF THE RAT. K.C. Corley*, T.H. Phan and M.C. Boadle-Biber. Physiology Dept, Medical College of Virginia, Virginia Commonwealth Univ., Richmond, VA 23298.

Morphine activates the ascending 5-HT neurons that arise from the midbrain as shown by the increase in the *ex vivo* activity of the rate-limiting enzyme in serotonin (5-HT) synthesis, tryptophan hydroxylase, as well as by the increase in the level of the 5-HT metabolite, 5-hydroxyindoleacetic acid (*Eur. J. Pharmacol.* 139 (1987) 193). In this study, we examined whether morphine activates 5-HT neurons in the median (MRN) and dorsal raphe nuclei, (DRN), or both nuclei, by measuring 5-hydroxytryptophan (5-HTP) accumulation after inhibition of aromatic amino acid decarboxylase (AADC), as an index of *in vivo* tryptophan hydroxylase activity. Male Sprague Dawley rats were injected with morphine (10mg/kg s.c.) or saline, followed 10 min later by the AADC inhibitor, NSD 1015 (m-hydroxybenzylhydrazine, 100 mg/kg i.p.). Thirty min later, rats were killed by decapitation, the DRN and MRN removed by 2 mm punches of a 3 mm coronal section of midbrain (*J. Neurochem.* 64 (1995) S35A) and stored at -70°C until analysed by HPLC-EC for 5-HTP content. Mean levels of 5-HTP, in ng/mg protein ± S.E.M., increased 85 % in the DRN from 8.4 ± 0.2 to 15.5 ± 0.8 ($N=5$, $P < 0.001$) in response to morphine, but were unchanged in the MRN. These results contrast with those obtained with sound stress in which 5-HTP levels increase in MRN but not DRN (*J. Neurochem.* 64 (1995) S35A). This differential responsiveness of DRN and MRN 5-HT neurons suggests the presence of distinct subpopulations of 5-HT neurons, within these nuclei, that can also be differentiated from 5-HT neurons that disharge in patterns related to the alertness state of the animal. Supported by grant NS14090 to MCBB.

342.15

DETECTION OF MDMA NEUROTOXICITY IN VIVO. PET STUDIES IN THE LIVING BABOON BRAIN. U. Scheffel*, Z. Szabo, W. B. Mathews, P. A. Finley, R. F. Dannals, H. T. Ravert, K. Szabo, G. A. Ricaurte. The Johns Hopkins Medical Institutions, Baltimore, Maryland 21205.

In vivo detection of damage to serotonergic (5-HT) neurons with imaging techniques has not been possible so far because of the lack of a suitable imaging ligand. Recently we have developed [^{11}C]labeled McN 5652 ([^{11}C]McN) for in vivo visualization of 5-HT reuptake sites using positron emission tomography (PET). In the present study we evaluated [^{11}C]McN /PET imaging as a method to quantify 5-HT neurotoxicity in the baboon brain.

A 32.7 kg male baboon was anesthetized and received PET scans 3x before and 3x after treatment with MDMA (8 doses of 5mg/kg, injected s.c. twice a day for 4 days). In each study, 25-32 mCi each of [^{11}C](-)McN 5652 (the inactive enantiomer), [^{11}C](+)McN 5652 (the active enantiomer known to bind to 5-HT transporters) and [^{11}C]RTI-55, a cocaine analog which binds to both 5-HT and DA transporters were injected, followed by dynamic PET acquisition over 115 min. Time activity curves were calculated for 13 brain regions.

Comparing the mean radioactivity concentrations (nCi/cc/mCi I.D. between 75-115 min p.i.) before and after MDMA treatment, significant decreases were observed with [^{11}C](+), but not with (-) McN or [^{11}C]RTI-55. Mean reductions in specific [^{11}C](+)McN binding (difference in regional [^{11}C](+) and (-)McN radioactivity) ranged from 44% in pons, 51% in hypothalamus, 70% in striatum to 74-89% in frontal- and occipital cortex. The results indicate that [^{11}C](+)McN 5652 and PET can be used for in vivo measurement of 5-HT neuronal damage by repeated doses of MDMA.

342.16

AGE DEPENDENT SENSITIVITY OF RATS TO THE LONG-TERM EFFECTS OF THE SEROTONERGIC NEUROTOXICANT (±)3,4-METHYLENEDIOXY-METHAMPHETAMINE (MDMA) CORRELATES WITH THE MAGNITUDE OF MDMA-INDUCED HYPERTHERMIA. H.W. Broening*, J.F. Bowyer, and W. Slikker, Jr. Interdisciplinary Toxicology Program, Univ. of Ark. for Med. Sci., Little Rock, AR, and Division of Neurotoxicology, FDA, Nat'l. Center for Tox. Research, Jefferson, AR.

The effects of developmental age on MDMA-induced reductions in 5-hydroxytryptamine (5-HT) content and 5-HT reuptake sites were investigated in conjunction with the effects of developmental age on MDMA-induced thermoregulatory responses. MDMA was administered to rats at postnatal days (PND) 10, 40 and 70 in a range of ambient temperature environments (10°C, 25°C and 33°C). Animals were monitored for alterations in body temperature and sacrificed 1 week after MDMA administration. MDMA administration at PND 10 did not result in persistent reductions in 5-HT content or 5-HT reuptake sites in frontal cortex, nor could a hyperthermic response be elicited. In contrast, MDMA administration at PND 40 and PND 70 resulted in a hypothermic response in cold environments (10°C) and a hyperthermic response in warm environments (≥ 25°C). Increases in developmental age enhanced the hyperthermic response to MDMA. When hypothermia was observed after MDMA (10°C environment), long-term reductions in 5-HT content and 5-HT reuptake sites were significantly attenuated or abolished. Conversely, when a hyperthermic response was observed (25°C and 33°C environments), long-term MDMA-induced reductions in 5-HT content and 5-HT reuptake sites were significantly enhanced. Thus, hyperthermia was predictive, in part, of the degree to which 5-HT content and 5-HT reuptake sites were diminished after MDMA administration. These experiments demonstrate a role for hyperthermi in the expression of serotonergic neurotoxicity after MDMA administration.

342.17

ACUTE DEXFENFLURAMINE AND CORTICAL GLUTAMATE AND ASPARTATE CONCENTRATIONS: IN VIVO MICRODIALYSIS COUPLED WITH CAPILLARY ELECTROPHORESIS AND LASER-INDUCED FLUORESCENCE DETECTION (LIFD). C. Rocher[1], L. Bert[2], F. Robert[2], J-H. Trouvin[1], B. Renaud[2], C. Jacquot*[1] and A.M. Gardier[1]. [1]Dept. Neuropharmacol. JE 92-372, Fac. Pharmacie, Univ. Paris-Sud, F92296-Chatenay-Malabry. [2]Lab. Neuropharmacol., Univ. Claude Bernard, CNRS UMR 105, 69373 Lyon 08, France

The indirect serotoninergic agonist dexfenfluramine (d-fen) releases serotonin (5-HT) and blocks its reuptake. The reason high d-fen doses induce a persistent reduction of brain 5-HT remains unclear. Since 5-HT has been shown to play a modulatory role on glutamate receptor subtypes [Mennini and Miari, *Life Sci.*, (1991) 49: 283], we investigated the effects of an acute d-fen injection on extracellular concentrations of 5-HT, 5-HIAA, Excitatory (glutamate GLU, aspartate ASP) and Inhibitory (glycine GLY, γ-aminobutyric acid GABA, taurine TAU) Amino Acids as measured by *in vivo* microdialysis in the frontal cortex (FC) of awake, freely-moving rats. Forty µl samples collected for a 190 min period after a single d-fen injection (0.5, 1.3, 5 et 10 mg/kg i.p.) were used as follows 1) 20 µl for HPLC with amperometric detection (BAS) for 5-HT and 5-HIAA measurements, 2) 10 µl for capillary electrophoresis with laser-induced fluorescence detection (LIFD, IRIS 2000, Europhor Inst.) for GLU and ASP measurements according to a modification by Robert et al. [*Anal.Chem.*, in press] of a previously described method, 3) 10 µl for HPLC with coulometric detection (ESA) for glutamine GLN, TAU, GLY and GABA measurements. Dialysate 5-HT increased in a dose-dependent manner in FC (max. increase, % of baseline±SEM: 127±17, 219±18, 410±126 and 1082±265 for 0.5, 1.3, 5 et 10 mg/kg, respectively). By contrast, no change in extracellular levels of any Amino Acid was found after acute injection of any d-fen dose, except significant increases in ASP and GABA at 5 mg/kg (309±64% and 290±78%, respectively). Indeed, only a very high d-fen dose (2.4 mM locally applied through the probe in FC), increasing dialysate 5-HT by 1804±336%, was able to increase extracellular GLU (218±34%, $P<0.01$). Thus, no major changes in extracellular transmitters' levels, except those found for 5-HT, occur after acute peripheral injection of a wide range of d-fen doses.

342.18

EFFECTS OF N-METHYLATION ON THE NEUROTOXIC AND BEHAVIORAL EFFECTS OF SELECTED RING-SUBSTITUTED AMPHETAMINES. J. A. Wlos, J. Yuan, G. Hatzidimitriou, N. Castagnoli, J. L. Katz, and G. A. Ricaurte.* Dept. of Neurology, Johns Hopkins Sch. Med. and NIDA, DIR., Baltimore, MD 21224.

N-methylation of the amphetamine derivative methamphetamine (MA) to dimethylamphetamine (DMA) eliminates its neurotoxicity toward brain serotonin (5-HT) neurons, but also diminishes its behavioral potency tenfold. The present study sought to determine: 1) Whether rats given DMA at behaviorally equipotent doses to those of MA show evidence of 5-HT neurotoxicity; and, if not, 2) Whether N-methylation can be used as a strategy to prevent 5-HT neurotoxicity induced by (+)3,4-methylenedioxymethamphetamine (MDMA) or fenfluramine (FEN). Rats were treated with MA, MDMA, FEN, or their respective N-methylated analogues, DMA, MDDMA, and N-Me-FEN. MA (10 mg/kg) and DMA (100 mg/kg) were administered s.c. every 6 hrs X 5, a regimen that permitted use of behaviorally equipotent doses. Other drugs were given s.c. twice daily for 4 days, at doses ranging from 0.1 mg/kg to 40 mg/kg, depending on the drug and paradigm. Behavioral (n=6) and anorectic (n=6) effects were assessed using a drug discrimination paradigm and by measuring daily food intake and body weight. Neurotoxic (n=6) effects were assessed by measuring regional brain 5-HT and 5-HIAA two weeks after the last drug dose. DMA, given at behaviorally equipotent doses to MA, did not lead to a long-term depletion of serotonergic markers. N-methylation of both MDMA and FEN diminished their behavioral effects without preventing dose-related loss of 5-HT and 5-HIAA. These findings indicate that while effective in dissociating behavioral and neurotoxic effects of MA, N-methylation is not an effective strategy for selectively attenuating 5-HT neurotoxicity secondary to MDMA or FEN.

342.19

Anorectic and Neurotoxic Effects of Fenfluramine: Distinct and Separable Phenomena U.D. McCann*, J. Yuan, and G.A. Ricaurte. Dept. of Neurology, Johns Hopkins Bayview Medical Center, Baltimore, MD 21224

Fenfluramine, an amphetamine analog and clinically prescribed anorectic drug, is a serotonin (5-HT) neurotoxin in experimental animals. Fluoxetine, a selective 5-HT reuptake inhibitor, has been shown to prevent fenfluramine-induced 5-HT neurotoxicity, as well as that induced by MDMA, a related toxic amphetamine derivative. Reports from humans who have taken MDMA in combination with fluoxetine indicate that fluoxetine does not significantly interfere with MDMA's unique psychoactive effects. The present studies sought to determine whether fluoxetine, given at doses that prevent fenfluramine-induced 5-HT neurotoxicity, interferes with fenfluramine's anorectic properties. Thirty four SD rats were randomized to one of four treatments: 1) Fenfluramine 5 mg/kg po BID X 6 days; 2) Fluoxetine 5 mg/kg po BID X 6 days; 3) Fenfluramine plus fluoxetine, both at 5 mg/kg po BID X 6 days; or 4) Vehicle po BID X 6 days. Treatment effects on food intake and body weight were evaluated, along with effects on regional brain concentrations of 5-HT, 5-HIAA, paroxetine-labelled 5-HT reuptake sites, and 5-HT immunoreactive (5-HT-IR) axons. Fenfluramine alone or fenfluramine plus fluoxetine led to similar, significant decreases in food intake and body weight that were not seen in animals in the other treatment groups. Fenfluramine alone led to significant decreases in regional brain 5-HT, 5-HIAA, paroxetine-labelled 5-HT reuptake sites and 5-HT-IR axon density two weeks after drug treatment, while animals treated with fluoxetine plus fenfluramine did not exhibit changes in any serotonergic measure. These data indicate that the anorectic and neurotoxic effects of fenfluramine are distinct and separable.

343.1

MATURATION OF CEREBRAL CORTICAL NEURONS *IN VITRO* IS MODULATED BY SEROTONIN RECEPTOR SUBTYPES. E. Köstner and J.P. Hornung*, Institute of Anatomy, University of Lausanne, 1005 Lausanne, Switzerland.

The mammalian cerebral cortex is innervated by serotonergic axons early during fetal development, while cortical neurons are still undifferentiated and migrating from germinal zone. Serotonin transmission in cerebral cortex could affected pre- or postsynaptic neuronal and glial cells via distinct receptor subtypes. Several lines of evidence suggest a morphogenetic function for the early serotonergic transmission. We have studied this issue in an organotypic culture system. Slices of neonatal rat cortex were maintained two weeks on a millipore filter at the air-medium interface, using medium completed with normal horse serum (NHS) or dialyzed - serotonin-free - serum (DHS). The morphology of selected populations of cortical neurons was assessed by immunocytochemistry. In presence of NHS, calretinin-immunoreactive (CR-IR) interneurons developed a complex dendritic arborization. Cultivated with DHS, CR-IR cells reduced drastically their dendritic branches, without change in their population size. On the other hand, slices treated with DHS medium with 5 or 10 µM serotonin (5-HT) showed matured CR-IR neurons with a complex dendritic morphology. In DHS, the 5-HT1a agonist (8-OHDPAT) was ineffective, while 5-HT2 agonist (α-methyl-5-HT) mimicked the effect of 5-HT on the dendritic morphology of CR-IR neurons. Likewise, with 5µM 5-HT in DHS, 5-HT1a antagonist (NAN-190) allowed the differentiation of CR-IR interneurons, whereas 5-HT2 antagonist (ritanserin) prevented it. The overall dendritic labeling for the microtubule-associated protein MAP2 was insensitive to these treatments. The present results suggest a morphogenetic effect of serotonin on cortical neurons mediated by one class of postsynaptic serotonergic receptor. Support: SNF 31-30-852.94

343.2

NEONATAL 5-HT DEPLETION REDUCES DENTATE GRANULE CELL SPINE DENSITY. W. Yan, C.C. Wilson, W.M. Panneton* and J.H. Haring. Dept. of Anatomy and Neurobiology, St. Louis Univ. Sch. Med., St. Louis, MO 63104.

We have been studying the role of 5-HT in dentate granule cell development. Pups were treated on PND 3 with either PCA (15mg/kg, 2 injections SC 24h apart) or 5,7-DHT (50µg in 5µl saline, IC) to produce a transient (Dev. Br. Res. 83:142, 1994) or permanent (Br. Res. 310:67, 1984) depletion of CNS 5-HT, respectively. Controls received vehicle injections. On PND14, 21, 60 and 120, hippocampal slices were prepared from male rats and granule cells were filled with neurobiotin. Filled cells were analyzed for changes in total dendritic length, segment number, segment length and spine density. Dendritic length, segment number and segment length showed some effects of treatment but these were rarely significant. Only spine density was significantly altered by 5-HT depletion. PCA treatment produced around 28% reduction in spines at all ages. 5,7-DHT resulted in a 40% spine decrease by P14 that reached 46% by P21. These results suggest that 5-HT can affect spine density development in a concentration dependent way since 5-HT depletion by IC 5,7-DHT is greater than by PCA.

343.3

POSTNATAL DEFICIENCY OF DOPAMINE BUT NOT SEROTONIN AFFECTS THE DEVELOPMENT OF STRIATAL ENKEPHALIN AND TACHYKININ SYSTEMS. **S. P. Sivam.* Dept. Pharmacol. & Toxicol., Northwest Center for Medical Education, Indiana University School of Medicine, 3400 Broadway, Gary IN 46408**

Previous studies have shown that neonatal dopaminergic lesion augments striatal enkephalin and decreases tachykinin biosynthesis. The present study examined whether a lack of dopamine (DA) and/or 5-hydroxytryptamine (5HT) during early postnatal period will modify the development of enkephalin and tachykinin neurons. The neurotoxins 6-Hydroxydopamine (6OHDA) and 5,7-dihydroxytryptamine (DHT) were used to lesion dopaminergic and serotonergic neurons on the third and fifth day after birth, respectively. Four groups of rats were used for study: control, 6OHDA-lesioned, DHT-lesioned and 6OHDA/DHT-lesioned. The animals were sacrificed at sixty days of age and the striatal tissues were used for neurochemical determinations. The levels of amines (DA and 5HT) and acid metabolites (DOPAC and 5HIAA) were determined by HPLC. The levels of peptides (Met^5-enkephalin, ME; substance P, SP and neurokinin A, NKA) and precursor mRNAs [preproenkephalin, PPE; preprotachykinin, PPT) were determined by radioimmunoassays and Northern blot analyses, respectively. The neonatal 6OHDA lesion depleted DA, DOPAC and increased 5HT, 5HIAA; increased ME and PPE levels; decreased SP, NKA and PPT levels. The neonatal DHT lesion depleted 5HT and 5HIAA and did not affect any other parameter studied. The combined 6OHDA/DHT lesions produced only the changes associated with the 6OHDA or DHT lesion alone. The results suggest that the availability of DA but not 5HT during early postnatal period is essential for the normal development of striatal enkephalin and tachykinin neurons. (Supported by USPHS grant NS 26063).

343.4

PUTATIVE ROLE OF SEROTONIN IN THE MATURATION OF AMPHIBIAN OOCYTES.
L.A. Nikitina[1], G.A. Buznikov[2] and J.M. Lauder[3]. [1]Group of Gametogenesis, [2]Lab. of Embryophysiology, N.K. Koltzov Inst. of Developmental Biology, Moscow 117808, Russia, [3]Dept. of Cell Biology and Anatomy, Lab. for Cell Biology, Univ. of N. Carolina Sch. of Med., Chapel Hill, NC 27599-7090.

Serotonin (5-HT) added to the medium or injected intracellularly inhibits or prevents the maturation of intact and denuded full-grown oocytes of Rana temporaria, Bufo bufo, B. viridis and Xenopus laevis. Some 5-HT antagonists reinitiate the meiosis, i.e. have a progesterone-like effect. 5-HT (both the neuronal and synthesized in follicles) probably maintains the meiosis block in the oocytes up to start of breeding season and modulates the progesterone action during maturation.

343.5

5-HT$_{1a}$ RECEPTORS MEDIATE THE EFFECTS OF 5-HT ON DEVELOPING DENTATE GRANULE CELLS. J.H. Haring*, W. Yan and C.C. Wilson. Dept. of Anatomy and Neurobiology, St. Louis Univ. Sch. Med., St. Louis, MO 63104.

High 5-HT$_{1a}$ receptor densities are present in the neonatal area dentata and neonatal 5-HT depletion results in decreased numbers of dendritic spines on dentate granule cells. 5-HT$_{1a}$ control of spine numbers was tested by treating neonatal rats with PCA (15mg/kg, SC) on PND3 and 4 followed by daily buspirone (agonist) treatment (1mg/kg) to PND14. A second group received daily NAN-190 (antagonist) injections (3.5mg/kg) from PND3 to PND14. Slices were prepared from male rats on PND14, 21 and 60. Granule cells were filled and analyzed. In contrast to the reduced spine denities observed after neonatal PCA treatment, buspirone-treated rats had normal numbers of dendritic spines at all ages studied. NAN-190 treatment resulted in dendritic spine densities similar to those observed after 5-HT depletion by neonatal PCA treatment. The development of normal granule cell spine densities in 5-HT-depleted rats receiving 5-HT$_{1a}$ agonist injections and the replication of 5-HT depletion effects by 5-HT$_{1a}$ antagonist treatment of normal rats indicate a role for 5-HT$_{1a}$ receptors in granule cell development.

343.6

PRENATAL EXPOSURE TO THE ANTIDEPRESSANT FLUOXETINE (PROZAC™) PRODUCES SITE-SPECIFIC ALTERATIONS IN BRAIN SEROTONIN (5-HT) RECEPTORS: AUTORADIOGRAPHIC STUDIES IN RAT OFFSPRING. **T.M. Cabrera*, F. Garcia, L.D. Van de Kar, W. Pinto and G. Battaglia. Department of Pharmacology, Loyola University of Chicago, Stritch School of Medicine, Maywood, IL 60153.**

We have observed altered neuroendocrine responses to the 5-HT$_{1A}$ agonist 8-OH-DPAT and to the 5-HT$_{2A/2C}$ agonist DOI (JPET 269(2):637-645, 1994) in male progeny following prenatal exposure to fluoxetine. The current studies determined the consequences of prenatal fluoxetine exposure on the regional density of central 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors in prepubescent and adult male progeny. Pregnant rats were administered saline or fluoxetine (10 mg/kg, sc) daily from gestational day 13 through 20. Alterations in serotonin receptor density were determined in 5-HT cell body regions as well as in brain regions receiving serotonergic innervation. The density of 5-HT$_{1A}$ receptors was not altered in 5-HT cell body regions (dorsal and median raphe) in either prepubescent or adult offspring. In contrast, regional changes in 5-HT$_{1A}$ receptors were observed in 5-HT target areas. In some of these regions, 5-HT$_{1A}$ receptors were significantly elevated (e.g. entorhinal cortex), whereas in other brain regions, 5-HT$_{1A}$ receptors were significantly reduced (e.g. ventromedial hypothalamus). The nature of the changes were dependent on the age of the offspring. Autoradiographic analysis of 5-HT$_{2A}$ receptors is currently in progress and the results will be presented at the meeting. In conclusion, prenatal exposure to fluoxetine alters the density of 5-HT receptor subtypes in discrete brain regions in both prepubescent and adult progeny. (Supported in part by NSF GER-9253875, and DA 07741; Fluoxetine was a generous gift from Eli Lilly and Co.).

343.7

AGE-RELATED LOSS OF HIPPOCAMPAL SEROTONERGIC TRANSPORTER FUNCTION: CELLULAR ELECTROPHYSIOLOGICAL STUDIES WITH DULOXETINE. J.E. Smith* and J.M. Lakoski. Departments of Pharmacology and Anesthesia, Pennsylvania State University College of Medicine, Hershey, PA 17033.

Alterations in serotonergic neuronal system function have been documented with aging. To further understand synaptic responses which change with age, electrophysiologic techniques were used to study the sensitivity of hippocampal neurons to serotonin (5-HT) and antidepressants selective for the serotonin transporter (5-HTT). Duloxetine, a selective serotonin reuptake inhibitor, was utilized to determine age-related changes in neuronal responses mediated at the serotonergic transporter.

Female Fischer 344 rats divided into four age groups (young, middle, old, senescent; 3-8, 11-18, 20-24, >24 mo, respectively) were studied using an *in vivo* chloral hydrate anesthetized preparation. Drug solutions of 5-HT (0.01-0.04 M, pH 5.0-6.0), the 5-HT_{1A} agonist 8-OH-DPAT, duloxetine, and desipramine were applied microiontophoretically (5-60 nA, 1-5 min duration) to hippocampal neurons recorded extracellularly in pyramidal subfields CA1 and CA3; data collected were analyzed to compare the differences in the neuronal sensitivity between age groups. A decrease in the IT_{50} values was seen with aging, demonstrating an increase in sensitivity to 5-HT. Across all ages, microiontophoretic application of duloxetine potentiated the inhibitory effect of co-applied 5-HT and/or 8-OH-DPAT (n=40 cells). Presynaptic function was analyzed by calculation of RT_{50} values with a decrease found in the old and senescent groups. At 20-22 mo, the recovery time decreased with prolonged duloxetine co-application; this decrease was significant ($p<0.05$) in the 27 mo group. With aging, the potentiation of 5-HT by duloxetine is rapidly lost and results in a functional desensitization. In summary, these studies have identified a significant age-related change in the physiological function of the hippocampal presynaptic serotonergic transporter.

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343.8

CELL- AND SITE-SPECIFIC EFFECTS OF SEROTONIN ON THE MORPHOLOGICAL DEVELOPMENT *IN VITRO* OF ANTENNAL LOBE INTERNEURONS OF THE SPHINX MOTH *MANDUCA SEXTA*. A.R. Mercer[1,2], B.S. Kirchhof[2], and J.G. Hildebrand[1]*. [1]Dept. of Zool., Univ. Otago, Dunedin, NZ; [2]ARL Div. of Neurobiol., Univ. Arizona, Tucson, AZ 85721.

Serotonergic neurons in the primary olfactory centers (antennal lobes) of the brain of the sphinx moth *Manduca sexta* express their chemical phenotype early in development (Kent et al., *J. Neurobiol.* 19:451, 1987) and are well placed to influence events that occur during metamorphic adult development of the antennal-lobe neuropil. These neurons do not affect formation of the glomerular arrangement of the antennal lobes (Oland et al., *Soc. Neurosci. Abstr.* 19:443, 1993), but may regulate the structural or functional development of antennal-lobe interneurons. To begin to address this possibility, we have examined the effects of serotonin (5-hydroxytryptamine, 5HT) on the morphological development of *M. sexta* antennal-lobe interneurons in vitro. Cells were taken from pupae at stages 5 and 12 of the 18 stages of metamorphic adult development. 5HT (50 μM) added daily to the culture medium increased significantly the total neurite length of two groups of cells identified elsewhere as local interneurons. In contrast, the total neurite length of a group of neurons thought to be projection (output) neurons was not affected by 5HT, although the growth of branches arising from the primary neurites of these neurons was significantly enhanced by exposure to this amine. These results indicate that responses to 5HT are not only cell-specific but also site-specific in some neurons. Furthermore, responses of cells from stage-5 pupae were not identical to those of cells from pupae at stage 12. While supporting the hypothesis that 5HT influences the morphological development of antennal-lobe interneurons of the moth, the data suggest that the modulatory actions of this amine may change during the course of metamorphic adult development.

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343.9

ENHANCED SYNAPTOPHYSIN IMMUNOREACTIVITY IN RAT HIPPOCAMPAL CULTURE BY 5-HT_{1A} AGONIST, S100β AND CORTICOSTEROID RECEPTOR AGONIST.

M. Nishi*, P.M. Whitaker-Azmitia and E.C. Azmitia. 1. Dept. of Biology, New York Univ., New York, NY 10003, 2. Dept. of Psychiatry, SUNY, Stony Brook, NY 11794.

Serotonin (5-HT) has been shown to modulate brain maturation during development and adult plasticity. This effect may be due to activation of 5-HT_{1A} receptors and a corresponding increase in S100β and corticosterone. Synaptophysin, an integral protein of the synaptic vesicle membrane that correlates with synaptic density and neurotransmitter release, is reduced by depletion of 5-HT in the cortex and hippocampus of the adult rat. Injections of a 5-HT_{1A} agonist or dexamethasone can reverse the loss of synaptophysin immunoreactivity (IR).

In this study, we used morphometric analysis of synaptophysin-IR to evaluate the effects of the 5-HT_{1A} agonist, ipsapirone and the neuronal extension factor, S100β on hippocampal neurons grown in a serum and steroid free media. Both compounds increased the synaptophysin-IR. Ipsapirone (10^{-9} M) was more effective on neuronal cell bodies staining and S100β (10 ng/ml) was more effective in increasing the number of synaptophysin-IR varicosities on neuronal processes. In addition both types of corticosteroid receptor agonists, Ru28362 (10^{-8} M) and aldosterone (10^{-9} M) produced smaller increases compared to control groups in both the cell body staining and the number of varicosities.

The effect of these differentiating factors on the expression of synaptophysin-IR suggests multiple regulation sites for producing and maintaining presynaptic elements in the brain.

This work has been supported by funds from NIA program project #1 P01 AG10208.

343.10

DEPLETION OF SEROTONIN DURING PEAK SYNAPTOGENESIS IN IMMATURE RATS LEADS TO LOSS OF A DENDRITIC MARKER (MAP-2) AND LEARNING DISABILITIES AS ADULTS. P.M. Whitaker-Azmitia* A. Borella and J. Muneyyici. Dept. Psychiatry, SUNY, Stony Brook, NY 11794.

As a brain trophic factor, serotonin increases synaptogenesis in target regions. In the present study, we determined the longterm consequences of removing serotonin at the critical period for synapse formation. Rat pups were injected with PCPA, 100 mg/kg, from PND 10 to 20. On days 30 and 62, the animals were stained immunochemically for MAP-2 and staining density determined. At PND 62, animals were also tested for changes in reference and working memories in a maze with olfactory cues.

The cortex showed a significant dendritic loss at PND 30 (ROD's .299 vs .184, $p< .0001$) but not at PND 62. The hippocampus showed dendritic loss at both PND 30 (.436 vs. .344 $p<.01$) and PND 62 (.431 vs. .376 $p<.003$). Treated animals learned the maze as rapidly as controls (reference memory, sec. to goal 47 vs. 54), but failed to re-learn the maze, when the cues were changed (working memory, 54 vs. 97 sec. $p< .001$).

These results show that depletion of serotonin during a critical time for synaptogenesis leaves a permanent change in dendritic number and in a working memory task.

343.11

EFFECTS OF SHORT-TERM ADRENALECTOMY IN THE RAT HIPPOCAMPUS: CHANGES IN MAP-2, S-100 β AND 5-HT 1A RECEPTOR. Prashanth AK[1,2], Jose C. Poblete[2], Baolang Liao[2], Edgar E. Coons[2]* & E.C Azmitia[1,2]. 1-Center for Neural Science,NYU 2- Dept. Psychology, NYU 3-Lab. of Mol. Neuroplasticity, Dept. Biol. NYU and NY 10003.

Previous studies have indicated that long-term (LT) adrenalectomy (ADX) results in loss of mature glia and granule cells in the dentate gyrus with little effect on CA neurons. The LT-ADX effects were reversed by 72 hr. of dexamethasone. Recently, we observed that para-chloro-amphetamine (PCA) decreased 5-HT levels as well as S-100, MAP-2 and synaptophysin immunoreativity (IR) and these markers could be restored with a 5-HT-1A agonist or dexamethasone. In this study, adult female rats (125g-175g) were ADX and kept on saline (0.9%) for 10 days and given 5-HT_{1A} agonist ipsapirone (1 mg/kg IP) twice daily for two days before perfusion. All animals were perfused transcardially with 4% paraformaldehyde, 0.1% $MgCl_2$ in 0.1M phosphate buffered saline. The brains were sectioned on a vibratome, and stained 5-HT_{1A} receptor, S-100β and MAP-2. The sections were counter-stained with methyl green.

The ADX animals showed an increase in staining for the 5-HT_{1A} receptor. They also showed decreased staining for MAP-2 and for S-100β protein. The results are consistent with previous studies of short- and long-term adrenalectomy. Preliminary observations indicate that ipsapirone treatement may not have a major effect in reversing the ADX-induced changes. Supported NIA Grant # POI AG 10208

343.12

CORTICOSTEROIDS MODULATE NEUROTRANSMITTER RESPONSIVENESS OF RAT CA1 PYRAMIDAL NEURONS THROUGH STEROID RECEPTOR ACTIVATION.

W.S. Hesen and M. Joëls*. Dept. of Exp. Zoology, University of Amsterdam, Kruislaan 320, 1098 SM Amsterdam, the Netherlands.

The adrenal hormone corticosterone can bind to two different corticosteroid receptor types in the CNS: the high affinity mineralocorticoid receptor (MR, K_d~0.2 nM) and the lower affinity glucocorticoid receptor (GR, K_d~3-5 nM) which are both present in CA1 pyramidal neurons. With intracellular recordings in hippocampal slices, CA1 neurons were tested for their responsiveness to serotonin (5HT, 10 µM) and carbachol (CCh, 3 µM), which evoked hyperpolarizing and depolarizing responses respectively. Modulatory effects of MR and/or GR activation on these responses were studied using different protocols for steroid receptor activation. Male Wistar rats (120-200g) were adrenalectomized (ADX, 2-7d); subsequently steroid receptors were occupied i) *in vitro* by application of aldosterone (3 nM, MR-occupation) or corticosterone (30 nM, MR and GR occupation) to ADX-slices or ii) by *in vivo* application of a single doses of corticosterone (sc., 10-100 µg/100g body weight: mainly MR occupation and 1 mg/100g: MR and GR activated). It appeared that 5HT as well as CCh responses were relatively large under conditions where either no (ADX) or both MR and GR were activated. However these transmitter responses were suppressed when predominantly MRs were activated. Interestingly, large 5HT responses were observed in in adrenally intact rats with high levels of corticosterone induced by a short stress stimulus; pretreatment of rats with RU38486 (sc., 1 mg/100g) prevented the stress-induced increased 5HT-responsiveness. Similarly, low levels of corticosterone circulating in the morning were associated with smaller 5HT-hyperpolarizations. These data show that physiological variations in corticosteroid levels, and therefore MR/GR occupation, modulate neurotransmitter responsiveness in the rat hippocampus.

343.13

REPEATED INJECTIONS OF FLUOXETINE PRODUCE DELAYED AND GRADUAL REDUCTIONS IN THE ACTH AND OXYTOCIN RESPONSES TO THE 5-HT_{1A} AGONIST 8-OH-DPAT. Q. Li*, G. Battaglia, T. M. Cabrera, W. Pinto, A. Vicentic, C. B. Chambers and L. D. Van de Kar. Dept. Pharmacol. Sch. Med., Loyola Univ. Chicago, Maywood, IL. 60153.

Our previous studies indicate that long-term exposure to the 5-HT uptake blocker and antidepressant fluoxetine (PROZAC) reduces the ACTH, corticosterone and oxytocin responses to 5-HT_{1A} agonists. A single injection of fluoxetine did not produce this effect. In the present study, we examined the onset of the fluoxetine-induced reduction in the hormone responses to 8-OH-DPAT. Male rats were injected daily with fluoxetine (10 mg/kg, ip) for 3, 7, 14 or 22 days or with saline for 22 days. The rats were challenged with the 5-HT_{1A} agonist 8-OH-DPAT (0, 50, 200 or 500 µg/kg, sc) 18 hr after the last injection of fluoxetine. The rats were decapitated 15 min later and plasma was collected for hormone assays. 8-OH-DPAT dose-dependently increased the hormone levels. Repeated injections of fluoxetine decreased the ACTH and corticosterone responses to the 50 µg/kg dose of 8-OH-DPAT, but not to higher 8-OH-DPAT doses. The increases in plasma oxytocin after challenge with all three doses of 8-OH-DPAT were blunted by fluoxetine. A partial reduction of the hormone responses to 8-OH-DPAT appeared within 3 days and reached a maximum reduction after 14 days of injections of fluoxetine. The hormone responses to 8-OH-DPAT are mediated by postsynaptic 5-HT_{1A} receptors in the hypothalamus. Therefore, our results suggest that repeated injections of fluoxetine produce a delayed onset, gradual reduction in the function of 5-HT_{1A} receptors in the hypothalamus, which appear at 3 days and are maximal after 14 days (Supported by USPHS MH 45812 to LVdK).

343.14

EVIDENCE THAT THE PROLACTIN RESPONSE TO THE 5-HT_{1A} AGONIST 8-OH-DPAT IS MEDIATED BY G_i PROTEINS. L.D. Van de Kar*, Q. Li, A. Vicentic, G. Battaglia. Loyola U. Sch. Med., Dept. Pharmacol. 2160 S. First Av, Maywood, Il 60153.

5-HT_{1A} receptors are coupled to G_i proteins which inhibit adenylyl cyclase and reduce the intracellular levels of cAMP. To examine the hypothesis that the 5-HT_{1A} receptor-mediated increase in prolactin secretion is mediated by G_i proteins, the $G_{i/o}$ toxin pertussis toxin, or vehicle were injected into the lateral cerebral ventricle (ICV) of male rats, 48 hr before they were challenged with saline or with a single dose of 8-OH-DPAT (50 µg/kg sc, 15 min before sacrifice). The prolactin response to 8-OH-DPAT was significantly inhibited by pertussis toxin although the density of 5-HT_{1A} receptors in the hypothalamus was not altered. To examine the hypothesis that a reduction in intracellular cAMP is involved in prolactin release, rats received an ICV injection of 8-OH-DPAT (0, 0.1, 1 or 10 µg/kg ICV, 15 min before sacrifice) and two injections of saline or forskolin (10 nmol/kg ICV, 7 and 15 min before sacrifice). Since forskolin can directly activate adenylyl cyclase, elevating cAMP would counteract the effect of G_i protein activation, which would reduce the intracellular concentrations of cAMP. The ICV injection of 8-OH-DPAT dose-dependently increased plasma prolactin. Forskolin produced a shift to the right in the dose-response curve and reduced the maximal response. Combined, the data suggest that 8-OH-DPAT acts centrally to increase prolactin release by activation of 5-HT_{1A} receptors that are coupled through G_i proteins to adenylyl cyclase (supported in part by USPHS MH45812 to LVdK).

343.15

BIOCHEMICAL AND FUNCTIONAL IMPAIRMENT OF BRAIN 5-HT SYSTEMS FOLLOWING VARIOUS FENFLURAMINE DOSE-REGIMENS W. Pinto*, F. Garcia, T.M. Cabrera, Q. Li, L.D. Van de Kar and G. Battaglia Neuroscience Grad. Prog. and Dept. of Pharmacology, Loyola University of Chicago, Stritch School of Medicine, Maywood, IL 60153.

This study investigates the correspondence between the magnitude of biochemical and functional alterations following impairment of central serotonergic systems. 5-HT neurons which innervate most forebrain structures, also send collaterals to the hypothalamus (HYPO) where they can elevate several plasma hormones including ACTH, corticosterone (CORT), oxytocin and prolactin. Therefore, alterations in neuroendocrine responses to 5-HT releasers can provide a sensitive marker of the functional integrity of presynaptic 5-HT terminals. Adult, male Sprague-Dawley rats were treated with either saline or increasing doses of d,l-fenfluramine (2.5, 5.0 and 8.0 mg/kg, s.c. b.i.d, 4 days). 14 days post-treatment, the rats were challenged with a single dose of the 5-HT releaser p-chloroamphetamine (PCA) (8.0 mg/kg, i.p.). Low-dose fenfluramine treatment produced no reductions in 5-HT levels or in the density of 5-HT uptake sites in hypothalamic homogenates. However, a significant functional attenuation was observed in the PCA-mediated elevation of plasma ACTH (-21%) and oxytocin (-37%). In contrast, higher doses of fenfluramine reduced hypothalamic 5-HT levels (-40%), as well as 5-HT uptake sites (-56%) suggesting lesion of hypothalamic 5-HT terminals. Consequently, somewhat greater functional deficits were observed in the PCA-induced elevation of plasma ACTH (-30%) and oxytocin (-44%). These data suggest that low dose fenfluramine can produce a functional impairment of 5-HT systems in the absence of biochemical evidence for 5-HT axotomy. However, higher doses of fenfluramine can produce both biochemical and functional deficits which may be attributed to 5-HT axotomy and/or an impairment of remaining 5-HT terminals. (Supported by USPHS DA07741).

343.16

NEUROTENSIN BLOCKS ACTIVATION BY SOUND STRESS AND CORTI-COTROPIN RELEASING FACTOR OF MEDIAN RAPHE SEROTONERGIC NEURONS. M. C. Boadle-Biber*, K. C. Corley and T. H. Phan. Physiology Dept, Med. Coll. Virginia, VCU, Richmond, VA 23298.

Sound stress (SS) activates serotonergic (5-HT) neurons within the median (MRN) but not dorsal raphe nucleus (DRN) of the rat as shown by the enhanced 30 min accumulation of 5-hydroxytryptophan (5-HTP) after inhibition of aromatic amino acid decarboxylase with NSD 1015 (m-hydroxbenzylhydrazine, 100 mg/kg i.p.)(*J. Neurochem.* **64** (1995) S53A). In the present study, we show that neurotensin (NT) blocks activation of MRN 5-HT neurons by SS as well as corticotropin releasing factor (CRF), a peptide that mimics many of the actions of SS on 5-HT neurons (*Brain Res.*, **628** (1993) 105-114). Male Sprague Dawley rats, with indwelling bilateral ventricular cannulae, were divided into two groups; one received ICV NT (0.15 nmoles in 3 ul per side), the other saline. For the SS exposure, NSD 1015 was given 5 min after NT and both groups exposed to a 30 min SS (30 randomly presented, 100 msec, 2.9 kHz, 110 dB sound pulses) or no SS (sham SS controls). For CRF treatment, CRF (0.3 nmoles in 3 ul per side, or vehicle) was infused ICV, 10 min after NT; NSD 1015 was injected 30 min after CRF. Rats were killed by decapitation after accumulation of 5-HTP for 30 min, the MRN and DRN removed with 2 mm punches from a 3 mm coronal section of midbrain (*J. Neurochem.* **64** (1995) S35A) and stored at -70°C until analysed by HPLC-EC for 5-HTP content. Mean levels of 5-HTP, in ng/mg protein, increased 112 and 85% in MRN with SS and CRF respectively (from 9.3 to 19.7 ± 0.7 with SS and from 9.5 ± 0.4 to 17.6 ± 1.0 with CRF ($P < 0.01$) but were unchanged in DRN. NT had no significant effect on 5-HTP levels given alone, but blocked the increase in 5-HTP levels in MRN after SS or CRF infusion. Supported by NIH NINDS grant NS14090 to MCBB.

UPTAKE AND TRANSPORTERS: SEROTONIN

344.1

CLONING AND CHARACTERIZATION OF THE PROMOTER REGION OF THE MOUSE SEROTONIN TRANSPORTER GENE. N. Sakai*, O. Morikawa, N. Ikegaki, S. Kobayashi, M. Fujimoto and N. Saito Lab. of Mol. Pharmacol., Biosignal Research Center, Kobe University, Kobe 657, Japan

The serotonin transporter has been known to be the main target of the antidepressants and involved in the pathogenesis of the affective disorder. To study the transcriptional regulation of the serotonin transporter, we screened a mouse genomic library using a cDNA probe encoding the 5'-region of the rat serotonin transporter cDNA . We obtained an approximately 3 kbp fragment which contained the 5'-flanking region and exon I of the serotonin transporter. We sequenced the 0.9 kbp region upstream from the ATG and identified the translation-initiation site, 302 bp upstream from the ATG , by primer extension analysis. This promoter had a TATA box 84 bp upstream from the translation-initiation site and contained many transcription factor binding sites such as AP-2, α-INF, γ-IRE, NF-IL6, NF-κB and SP1. The transient chloramphenicol acetyltransferase (CAT) expression assay demonstrated that it functioned as a promoter in COS-7 cells. We examined the effects of phorbol ester and interferon on the promoter activity of the serotonin transporter using a CAT assay.

344.2

RANDOM MUTAGENESIS OF A LOOP REGION IN THE RAT BRAIN SEROTONIN TRANSPORTER. M. M. Stephan* and G. Rudnick. Pharmacology Dept., Yale School of Med., New Haven, CT.

The rat brain serotonin transporter is responsible for the re-uptake of serotonin from the synaptic cleft following neurotransmission. This transporter is predicted to contain twelve hydrophobic transmembrane a-helices, linked together by hydrophilic loops. Oligonucleotide-directed random mutagenesis was performed on a region of the transporter cDNA encoding 16 amino acid residues predicted to lie near the C-terminal end of the 7th transmembrane span (6 membrane-bound and 10 in the following extracellular loop). Random base changes were introduced into the mutagenic oligonucleotide by "dirty bottle" synthesis, in which low levels (1-2%) of the other three nucleotides were included in each of the A,T,C and G synthesizer bottles. Analysis of the transport abilities of these mutants has revealed at least one position which may be important for activity: F380, at which the relatively conservative substitution of V results in complete loss of activity. By contrast, several other positions were revealed as relatively unimportant for transport activity. D393 accepts nonconservative substitutions of G and H with no detectable loss of activity. I379 accepts the substitution of T with 95% activity remaining. Other nonconservative substitutions which do affect activity somewhat but are still well-tolerated (40-50% activity remaining) are R390M and E392G. Application of this method will allow relatively rapid identification of residues important for the transport mechanism as well as for other activities such as the binding of antidepressants and cocaine analogs.

344.3

SEROTONIN TRANSPORTER mRNA LOCALIZATION IN THE DEVELOPING RAT EMBRYO. Stefan. R. Hansson, É. Mezey@ and B.J. Hoffman*, Lab of Cell Biology, NIMH and @ Clinical Neurosciences Branch, NINDS, Bethesda , MD 20892

A role for serotonin (5HT) has been implicated in the developing rodent. For instance, blockade of the 5HT transporter (5HTT) leads to craniofacial and heart malformations of mouse embryos in culture. This suggests that the 5HTT may play a critical role in regulating the effects of 5HT in the embryo. We have used *in situ* hybridization histochemistry with RNA probes to delineate 5HTT gene expression during rat embryonic development. In the central nervous system (CNS), 5HTT mRNA was present in cells of the neural tube at embryonic day 11 (E11). By E15, mRNA was localized to midbrain and cingulate cortex. In addition, labeling was detected in retina and sympathetic ganglia. While the intensity of labeling in individual cells of raphe nuclei increased from E15 to E21, positive cells were also identified in the medulla, thalamus, caudate, hippocampus, subependyma and dura mater of E21 embryos. In addition to the CNS, 5HTT mRNA was detected in heart, bone, intestine, spleen, lung, aorta and adrenal medulla. In general, throughout embryonic development, those areas which expressed 5HTT mRNA continued to show labeling, either increasing in intensity or remaining the same. Expression of 5HTT suggests the importance of maintaining 5HT homeostasis even in tissues which may not synthesize 5HT. Further, presence of 5HTT mRNA in numerous sites throughout the embryo suggests a previously unappreciated role for 5HT in diverse physiologic systems during embryonic development. Whether 5HT provides important developmental cues, through which receptors these cues are relayed and whether 5HTT plays a critical role remains to be determined.

344.4

5-HT$_2$ RECEPTORS INCREASE 5-HT TRANSPORT VIA NITRIC OXIDE AND cGMP. Keith J. Miller* and Beth J. Hoffman, Lab of Cell Biology, NIMH; Bethesda, MD.

We have previously determined that the maximal rate of 5-HT transport can be regulated by nitric oxide and cGMP in an RBL 2H3 cell line and in brain synaptosomes. 5-HT receptors of the 5-HT$_2$ sub-family are coupled to elevations in calcium via IP3 and thus could be coupled to nitric oxide production. The 5HT$_{2C}$ receptor has been demonstrated to be coupled to cGMP in the choroid plexus. Therefore we determined whether 5HT$_2$ receptor activation could result in the regulation of 5-HT uptake by nitric oxide and cGMP. Brain slices were incubated with the 5-HT$_2$ receptor agonist DOI for 15 min. Nitrites and cGMP were measured or synaptosomes were prepared following treatment of the slices. DOI produced a dose-dependent increase in nitric oxide levels, cGMP levels, and ^{3}H-5HT uptake.^{3} H-5HT uptake was increased from 1700±96 to 4400±120 cpm/mg protein in hippocampal synaptosomes with an EC$_{50}$ of 6nM which was blocked by the antagonist mianserin. cGMP levels increased from 157 to 340 fmol/mg protein and nitrite levels rose from 22 to 31 µg/ml/mg protein. In the cortex the dose-response curve for ^{3}H-5HT uptake, cGMP and nitrite, was bell-shaped with maximal stimulation in uptake occuring at 0.1µM (from 10900±300 to 13400±150), with an EC$_{50}$ of 40nM. Nitrite levels rose from 8.6 to 9.9 µg/ml/mg protein and cGMP levels were elevated from 49 to 100 fmol/mg protein. Inhibition of uptake occured at 10µM DOI and was enhanced by 10µM mianserin. These results suggest that 5HT$_2$ receptors are coupled to nitric oxide and cGMP in the rat brain and that the activation of pre and/or postsynaptic 5-HT$_2$ receptors results in increased levels of 5-HT uptake. Further pharmacological characterization is necessary to identify which 5HT$_2$ subtype is involved. Alteration of the ability of the 5-HT transporter to remove 5-HT from the synapse may change the concentration of neurotransmitter in the synapse and thus may significantly alter serotonergic neurotransmission.

344.5

TOWARDS EXPRESSION OF THE HUMAN AND RAT BRAIN SEROTONIN TRANSPORTER (SERT) IN BACTERIA AND YEAST. [1]F. Titgemeyer, [1]S. Hilberer-Ehret, [2]K.N. Faber, [2]M. Veenhuis, [3]C. Tate, [4]P. Schloss, [4]H. Betz, [5]S. Boyce, [5]C. Williams, [2]S. de Boer* and [1]G.T. Robillard. [1]Dept. of Biochem., Nijenborgh 4, 9747AG Groningen; [2]Biology, Kerklaan 30, 9751NN Haren, Netherlands; [3]MRC Lab., Hills Road, Cambridge CB2 2QH, United Kingdom; [4]MPI Brain Res., Deutschordenstr. 46, 60528 Frankfurt, Germany; [5]Dept. of Biochem., Trinity College, Univ. Dublin, Dublin 2, Ireland.

SERT terminates the action of the neurotransmitter serotonin by reuptake into the presynapse. SERT is believed to be involved in mental diseases, i.e. depression. Antidepressants such as imipramine, fluoxetine and citalopram and the drugs cocaine and MDMA ('ecstasy') are acting directly on SERT. Therefore, it is of medical interest to elucidate the structure and function of SERT at the molecular level. Basis for structural analysis will be purification of mg quantities of protein. We have addressed this by studying overexpression in bacteria and yeast. We have expressed SERT in *Escherichia coli* taking advantage of the T7 phage expression system. *In vivo* labelled SERT protein migrates on SDS-PAGE at 55 kDa. Binding to [^{3}H]imipramine was detectable only when SERT was expressed as a fusion protein. The N-terminal 85aa protein domain (NSERT) was overexpressed of >10% of total protein. Purification to obtain protein quantities sufficient for structural work could be demonstrated. Using the methylotrophic yeast *Hansenula polymorpha*, we have studied homologous (PER9) and heterologous (SERT) expression of membrane proteins. Morphological changes of *H. polymorpha* integrants were observed under conditions when membrane protein was expressed.

344.6

SUBTYPES OF 5-HT AXONS DIFFER IN THEIR EXPRESSION OF SEROTONIN TRANSPORTER K.J. Axt*, M.E. Molliver[1], Y. Qian, and R.D. Blakely[2]. The Johns Hopkins Univ. Sch. Med., Baltimore, MD, 21205; Vanderbilt Univ., Nashville, TN, 37232.

p-Chloroamphetamine (*p*CA) and d-fenfluramine (FEN) are neurotoxic drugs since they ablate *fine* 5-HT axons, demonstrated by 5-HT and TPH immunocytochemistry (ICC). A subtype of 5-HT axons, *beaded* axons, is resistant to the neurotoxic effects of *p*CA and FEN. The 5-HT uptake inhibitor fluoxetine prevents the toxicity, indicating that the serotonin transporter (SERT) is critical for the neurotoxic effects of *p*CA and related drugs. It has been postulated that the differential vulnerability of *fine* and *beaded* axons might be explained by differences in the number or affinity of 5-HT uptake carrier sites. To test this hypothesis, we analyzed axon morphology using antisera to 5-HT or to SERT (CT-2). Control rats were compared to *p*CA- or FEN-treated rats, where *fine* 5-HT axons are ablated. In certain brain regions at 7-10 days after *p*CA or FEN, the 5-HT and SERT-IR axons are similar in morphology and density: in the glomerular layer of the olfactory bulb (MOB), layer III of lateral entorhinal cortex, and ventricular plexus, spared 5-HT axons are intensely reactive for both 5-HT and SERT. Since many spared 5-HT axons express high levels of SERT, neurotoxic resistance cannot be attributed solely to lack of the 5-HT transporter. However, in dentate gyrus (DG) and parietal cortex, where many 5-HT-IR axons survive, relatively few spared axons are SERT-IR, suggesting that some 5-HT axons may not express the transporter. Of the spared SERT-IR axons, many stain weakly and their varicosities are either not reactive or appear smaller than varicosities stained by 5-HT or TPH ICC, which may reflect a difference in distribution or density of the transporter. Compared with spared 5-HT-IR axons in DG and parietal cortex, the smaller number of surviving SERT-IR axons and the reduced staining of varicosities suggests the existence of multiple subtypes of drug-resistant 5-HT axons. [Support: [1]DA04431, NS15199, [2]DA07390]

344.7

PHOSPHORYLATION OF SEROTONIN TRANSPORTER DOMAINS AND THE ROLE OF PHOSPHORYLATION IN ACUTE TRANSPORTER REGULATION. Y. Qian*[1], H.E. Melikian [1], K.R.Moore [2], B.I. Duke and R.D. Blakely [3], [1] Grad. Prog. Neuroscience, [2] Dept. Anatomy & Cell Biol., Emory Univ., Atlanta, GA 30322 and [3] Dept. Pharmacol., Vanderbilt Univ., Nashville, TN 37232-6600.

Serotonin (5HT) transporters (SERTs) are responsible for efficient clearance of the amine from extracellular spaces. Although multiple reports demonstrate a capacity of SERTs to be acutely regulated by second messenger-linked systems, direct mechanisms have yet to be established. We examined the potential for SERT phosphorylation as a mechanism for acute regulation in two ways. We used PKC activators in HEK-293 cells stably transfected with human SERT cDNA. PMA treatment of these cells caused a rapid and dose-dependent inhibition of 5HT uptake. Similar results were obtained with the PKC activator PDBu. The inactive α forms of these agents were ineffective. Kinetic analysis show that reductions induced by PMA are principally mediated by a decrease in Vmax (46% of control) with little or no change in 5HT Km. SERT antibodies that can blot and immunoprecipitate posttranslationally modified SERT proteins are being used to assess the contribution of direct protein phosphorylation to PKC-mediated reductions. Human and rodent SERT proteins contain multiple, conserved sites for Ser/Thr-based protein phosphorylation, with most of these sites found in the presumed cytoplasmic NH2 and COOH termini. *In vitro* phosphorylation experiments were performed using glutathionine-S-transferase (GST) fusion proteins containing either the NH2 or COOH termini of rat SERT. Purified protein kinase C and protein kinase A phosphorylated both NH2 and COOH fusion proteins with little or no phosphorylation of the GST backbone. Interestingly, neither fusion protein is phosphorylated by purified cGMP dependent protein kinase *in vitro*. Additional studies seek to reveal residues phosphorylated under *in vitro* conditions and relate these changes to *in vivo* regulation of 5HT uptake activity. Support by NIDA DA07390 to R.D.B.

344.8

TRANSCRIPTIONAL REGULATION AND MESSAGE HETEROGENEITY OF THE HUMAN SEROTONIN TRANSPORTER GENE. C. C. Bradley*[1] and R.D. Blakely [2]. [1] Graduate Program in Neuroscience, Emory University School of Medicine, Atlanta, GA 30322 and [2] Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37235.

The human antidepressant sensitive serotonin transporter (SERT) terminates serotonergic transmission. Compromised SERT expression may contribute to neuropsychiatric disorders. We have used the human placental JAR cell line (Cool et al. JBC **266**:15750-7, 1991) as an *in vitro* model of chronic SERT regulation mediated by second messenger systems. Three SERT mRNAs can be detected in Northern blots of JAR and human brainstem mRNA. SERT-associated binding and uptake levels in JAR cells are markedly increased following chronic cholera toxin (CTX) or forskolin treatment, coincident with an increase in steady-state SERT mRNA levels (Ramamoorthy et al. JBC **268**:21626-31, 1993). We have employed a competitive RT-PCR strategy to quantitate SERT mRNA levels and applied it to CTX-treated JAR cells to assess changes in mRNA half-life. In this method, a single PCR product is amplified from JAR cDNA, consistent with multiple mRNAs derived from noncoding variants. RT-PCR of control JAR RNA revealed basal expression levels reflecting ~0.1pg of SERT RNA/µg total RNA whereas JAR cells treated with 100 ng/ml CTX for 16 hours showed mRNA levels of ~1.0pg/µg total RNA. Quantitation performed at different time points after actinomycin-D treatment indicate a SERT mRNA halflife of ~18 hours. CTX treatment caused little or no change in SERT mRNA halflife, consistent with increased nuclear initiation underlying elevations of SERT mRNA. Human SERT genomic fragments are presently being used to clarify the contribution of noncoding elements to SERT mRNA heterogeneity and CTX-induced transcriptional activation.

344.9

EMBRYONIC EXPRESSION OF MOUSE SEROTONIN TRANSPORTERS REVEALED BY [125I]RTI-55 AUTORADIOGRAPHY, IN SITU HYBRIDIZATION, AND IMMUNOCYTOCHEMISTRY. S. Schroeter* and R.D. Blakely. Dept. of Pharmacology, Vanderbilt University, Nashville, TN.

In addition to their role as neurotransmitters, biogenic amines such as serotonin (5-HT) have been implicated in morphogenetic signaling events in the early embryo. Embryos have been shown to accumulate neurotransmitters in a number of tissues during morphogenesis. For instance, chick and mouse embryos accumulate serotonin in the neural tube and at other morphogenetically active sites. 5-HT transport antagonists block this accumulation and lead to developmental defects. Due to difficulties inherent with *in vivo* and *in vitro* embryo preparations, a direct link to specific proteins involved in 5-HT accumulation has not been achieved. As part of our ongoing effort to characterize the role of transport proteins in early development, we determined 5-HT transporter (SERT) mRNA distribution with *in situ* hybridization and compared this with two functional measures of SERT protein expression: [125I]RTI-55 autoradiography and SERT immunocytochemistry. SERT's contribution to [125I]RTI-55 binding was assessed by parallel incubations with 10nM paroxetine or 100nM citalopram whereas the contribution of norepinephrine and dopamine transporters was assessed with 100nM nomifensine. As early as embryonic day 9, mouse embryos demonstrate SERT expression in the neural tube and in several peripheral tissues, consistent with a role of 5-HT in early developmental events. However, these sites appear to only partially overlap with sites of 5-HT accumulation previously defined *in vitro* and may indicate the involvement of additional processes or transporters in embryonic 5-HT accumulation. Supported by a Pharmaceutical Manufacturers Association Foundation fellowship to S.S. and by NIDA award DA-07390 to R.D.B.

344.10

SEROTONIN TRANSPORT KINETICS IN RATS GENETICALLY RESISTANT AND PRONE TO TASTE AVERSION CONDITIONING

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Synaptosomal serotonin (5-HT) transport kinetics were examined in two selectively bred lines of taste aversion prone (TAP) and taste aversion resistant (TAR) rats. Strain selection methodology paired a saccharin flavored conditional stimulus (CS) with the aversive unconditional stimulus (US) consequences of a cyclophosphamide injection. The selectively bred line differences in taste aversion (TA) conditionability are maintained with other injected US conditioning agents including ethanol. A range of ethanol doses produced dose dependent differences in TA acquisition in TAP rats without inducing TA in TAR rats (Elkins, et al., Alcohol Clin Exp Res, 16, 928-934, 1992). In a related study of naive free-choice ethanol acceptance, TAR rats consumed significantly more ethanol (presented as a 1% to 20% ethanol/water solution with plain water also freely available) than TAP rats (Elkins, et al., Alcohol Clin Exp Res, 17, 445, 1993). TAR rats also have been found to have lower levels of whole brain 5-HT than TAP rats (Orr, et al., Physiol Beh, 53, 495-500, 1992). In the present between lines comparisons of 5-HT transport kinetics, whole brain synaptosomal samples were examined in 12 TAP and 12 TAR adult rats. The TAR group was found to have a significantly ($p = < .01$) lower affinity for synaptosomal 5-HT transport (K_m = 1.42 mM, SD = 0.31) in comparison to the TAP group (K_m = 1.11 mM, SD = 0.16). No significant difference was found in V_{max} values between the groups. These results are consistent with observations that low 5-HT activity could constitute a vulnerability factor for alcohol drinking. They also point to the potential relevance of a mediating serotonergic link between taste aversion conditionability and the propensity to drink alcohol.

344.11

***IN VIVO* INHIBITION OF THE SEROTONIN TRANSPORTER BY THE ANTIDEPRESSANT NEFAZODONE IN THE RAT.** D. L. Knight*, M. J. Owens, K. J. Winders, J. Ieni and C. B. Nemeroff. Dept. Psychiatry & Behavorial Sciences, Emory Univ. Sch. Of Med., Atlanta, GA 30322.

Nefazodone HCl (Serzone®) is a new antidepressant with a chemical structure unrelated to SSRI's, tricyclics, tetracyclics, or MAO inhibitors. Nefazodone is active in a number of preclinical tests for antidepressant activity and shows clinical efficacy in the treatment of depression with a more favorable side-effect profile than the structurally similar antidepressant trazodone. Previous studies have shown that nefazodone is a potent antagonist of 5-HT_{2A} receptors and binds to the serotonin transporter *in vitro* and *in vivo*. To further investigate the ability of nefazodone to modify serotonergic transmission, the ability of systemically administered nefazodone to inhibit the serotonin transporter was assessed by measuring [3H]-5-HT uptake *ex vivo* and by preventing parachloroamphetamine (PCA) induced depletions of cortical 5-HT concentrations. In addition, the ability of *in vivo* nefazodone administration to inhibit 5-HT uptake following sub-chronic dosing was assessed.

Acute administration of nefazodone (100 and 150 mg/kg, sc) significantly increased the K_m for [3H]-5-HT uptake in rat cortical synaptosomes from 60 nmol/L in controls to 230 and 242 nmol/L in nefazodone-treated rats, respectively. Acute nefazodone administration (30, 100, and 150 mg/kg) antagonized PCA-induced depletion of cortical 5-HT concentrations in a dose-dependent manner as measured by HPLC at 1, 2, and 3 hours post treatment. In fact, 100 and 150 mg/kg nefazodone was equipotent with fluoxetine (10 mg/kg) over the course of the experiment. Sub-chronic administration of nefazodone (100 and 150 mg/kg, sc, b.i.d. X 11 days) reduced [3H]-5-HT uptake by 24% and 29%. Sub-chronic dosing with fluoxetine (5 mg/kg, sc, b.i.d. X 11 days) reduced [3H]-5-HT uptake by approximately 65%. These experiments confirm and extend previous reports concerning the ability of nefazodone to inhibit the 5-HT transporter *in vivo*. Supported by a grant from Bristol Myers-Squibb.

344.12

EFFECTS OF ACUTE PAROXETINE AND FLUOXETINE ON EXTRACELLULAR SEROTONIN IN THE RAPHE: AN IN VIVO MICRODIALYSIS STUDY. L. Malagié*, C. Jacquot and A.M. Gardier. Lab. Pharmacol. JE92-372, Fac. Pharm Univ. Paris-Sud, 92290 Chatenay-Malabry, France.

Activation of 5-HT_{1A} receptors located in median+dorsal raphe nuclei (RN) may be involved in the long delay of action of selective serotonin (5-HT) reuptake inhibitors (SSRI) in rodents and humans. High densities of these autoreceptors and of 5-HT transporter in RN may explain preferential effects of peripheral fluvoxamine in the raphe compared to those in serotoninergic terminal areas like the frontal cortex (FC) (Bel and Artigas, *Eur. J. Pharmacol.* (1992) 229:101). To know if this property is shared by other SSRIs and concerns other terminal areas, *in vivo* microdialysis was simultaneously performed in FC, ventral hippocampus (vHi) and RN after acute injection of fluoxetine (Flx, 1, 10 and 20 mg/kg i.p.) or paroxetine (Prx, 15 mg/kg s.c.) in anaesthetized rats. Basal extracellular 5-HT levels in vHi (m ± S.E.M. in fmol/20µl: 10.8±1.9, F2,59=5.17 P<0.01) were lower than in FC (23.7±2.4) and in RN (21.2±4.5). In the 3 brain area studied, extracellular 5-HT concentrations were increased following the 2 SSRI administrations. Prx induced a 7 fold increase in raphe 5-HT (+726%, P<0.001) compared to FC (+363%, P<0.001) and vHi (+446%, P<0.001). Flx (10 mg/kg) increased 5-HT preferentially in RN (+268%, P<0.001) compared to FC (+164%, P<0.001) and vHi (+205%, P<0.001). The regional specificity of Flx effects disappeared at higher dosage (20 mg/kg): similar increases were observed in the 3 structures, +252%, +267% and +236% in FC, vHi and RN, respectively, likely suggesting uptake site saturation. Thus, only a 10 mg/kg Flx dose qualitatively mimics those of Prx at 15 mg/kg inducing a preferential increase in raphe 5-HT: Flx and Prx presumably stimulated somatodendritic 5-HT_{1A} receptors, thus decreasing the firing rate and leading to small increases in FC and vHi extracellular 5-HT. However, quantitative differences in the effects of the 2 SSRIs may occur as the maximal 5-HT augmentation (% of baseline) in the RN induced by the 3 doses of Flx is limited to one-third of that induced by Prx. Distinct pharmacokinetic profiles of these SSRIs (a long elimination half-life for Flx, a short one for Prx; an active metabolite for Flx, an inactive one for Prx) may account for these differences.

344.13

IN VIVO STUDIES OF THE SEROTONIN TRANSPORTER WITH 5-IODO-6-NITROQUIPAZINE. A. Biegon*, J.L. Eberling, S.E. Taylor, H. F. VanBrocklin, S. Jordan, S.M. Hanrahan, J.A. Roberts, K.M. Brennan, C.A. Mathis, W.J. Jagust. Center for Functional Imaging, Lawrence Berkeley Laboratory, Berkeley, CA 94720.

In vivo imaging of serotonergic function is potentially useful in understanding several diseases of the nervous system. Promising preliminary results with the tracer 5-iodo-6-nitroquipazine (INQUIP) have prompted us to perform further studies designed to validate the use of the tracer as an in vivo ligand for the serotonin transporter. We studied 6 adult macaca mulatta in 8 experiments which involved single photon emission computed tomographic (SPECT) imaging at 17 to 24 hours post tracer injection, including 3 experiments with coinjection of the [123]I and [125]I radiolabelled tracer for direct comparison of SPECT and autoradiography, and 3 experiments in which animals were lesioned with the serotonergic neurotoxin (±)3,4 methylenedioxymethamphetamine (MDMA). In addition, we evaluated the metabolism of the tracer in the brain and periphery.

SPECT images obtained at 17 and 24 hours reflected both the known pattern of distribution of serotonin transporters and also showed close correspondence to the autoradiograms. The sites of greatest binding in both were brainstem (the raphe nuclei and colliculi) and thalamus (particularly central nuclei) followed by the basal ganglia, cerebral cortex, and cerebellum. SPECT ratios of brainstem to cerebellum were close to 3 at 17 h, and ratios for most regions changed little by 24 h. Autoradiograms from an MDMA treated animal showed 30 to 90% reductions of binding, which was also detectable in the SPECT data. At 17 h, virtually all of the tracer in the brainstem was in the form of unmetabolized parent compound. Plasma showed rapid peripheral metabolism of the tracer shortly after injection.

These results demonstrate that INQUIP SPECT images reflect binding to the serotonin transporter in vivo, with good target to background ratios and little brain metabolism. In addition, the tracer is sensitive enough to detect serotonergic lesions in the primate brain. These data suggest that further development of the tracer as a method for the in vivo study of serotonergic neurons in humans is warranted.

344.14

RELEASE OF $[^3H]MPP^+$ FROM C6 GLIOMA CELLS EXPRESSING THE HUMAN DOPAMINE OR SEROTONIN TRANSPORTERS. RA Johnson*, AJ Eshleman, TT Meyers, AK Evenson, KA Neve, and A Janowsky. Dept. Pharmacology, Oregon Health Sciences Univ. and VAMC, Portland, OR 97201.

C6 rat glioma cells, stably expressing the human dopamine transporter (C6-hDAT) or serotonin transporter (C6-hSERT), were used to examine the modulation of $[^3H]MPP^+$ release by an array of drugs via the transporters. Preliminary experiments revealed that both cell lines take up $[^3H]MPP^+$ in a time- and dose-dependent manner, with maximal uptake occurring after 120 minutes. For release experiments, cells were preloaded with $[^3H]MPP^+$, rinsed, and release buffer was added. After a 10 minute equilibration period, drugs were added to a final volume of 500 µl. For concentration-response experiments, the contents of the well were aspirated at 30 or 45 min for C6-hDAT or C6- hSERT, respectively. Spontaneous release in the absence of drugs was <10% for C6-hDAT and 10-20% for C6-hSERT after 45 min at 37°C. Preliminary results for the effects of an array of substrates and non-substrates are shown below:

Compound	C6-hDAT EC_{50} (nM)	C6-hDAT % release	C6 hSERT EC_{50} (nM)	C6-hSERT % release
RTI-55	45	40	10	50
Mazindol	100	40	240	57
CFT	156	40	750	55
Amphetamine	250	93	630	87
Cocaine	185	55	750	56
Dopamine	1200	86	8500	90
Fenfluramine	10,000	60	21800	80
Serotonin	100,000	60	78300	60

(Supported by NIDA Drug Development Contract; hSERT cDNA was kindly provided by Dr. Randy Blakely, Dept. Pharmacol., Vanderbilt Univ.)

344.15

HETEROGENEITY OF ANTIDEPRESSANT BINDING SITES ON THE RECOMBINANT RAT SEROTONIN TRANSPORTER SERT1. P. Schloss, S. Sur, S. Löwel* and H. Betz. Max-Planck-Institut für Hirnforschung, Deutschordenstrasse 46, 60528 Frankfurt, Germany

Antidepressants block the uptake of serotonin into serotonergic nerve terminals and blood platelets. Binding of the tricyclic antidepressant [^{3}H]imipramine to the recombinant rat serotonin transporter SERT1 expressed in HEK293 cells was found to be non-homogeneous. Scatchard analysis and competition experiments revealed the existence of two distinct antidepressant binding sites. At site 1, [^{3}H]imipramine binding was strictly sodium-dependent with an apparent K_D of about 10 nM. In contrast, [^{3}H]imipramine binding to site 2 occurred also in the absence of sodium and exhibited a lower affinity. Binding of the non-tricyclic antidepressant [^{3}H]citalopram was observed only at site2. Serotonin competitively inhibited antidepressant binding at both sites.

344.16

SHORT-TERM REGULATION OF GLIAL AND NEURONAL SEROTONIN UPTAKE. G. M. Anderson*, F. M. Vaccarino, and L. M. Hall, Child Study Center, Yale Univ. Sch.of Medicine, New Haven, CT 06510.

In previous studies we have demonstrated that 5-20 minute treatment with activators of protein kinase C (PKC) decreases the rate (Vmax) of serotonin (5HT) uptake in human platelets (Anderson and Horne, BBA , 1992). A similar effect has been reported for a rat basophilic cell line, and for human lung endothelium (Miller and Hoffman, JBC, 1994; Myers et al., AJP, 1989). Due to the potential importance of short-term regulation of brain 5HT re-uptake we have examined the short-term (10-12 min) effects of PKC activators on 5HT uptake by primary cultures of rat embryonic neurons and of rat neonatal glia

Glial cultures were obtained from the brainstem of neonatal (P1-P2) rats and cultured in 10 % fetal calf serum. Neuronal cultures were from the brainstem of embryonic (E13.5) rats. Preliminary experiments have indicated that glial uptake of 5HT is not affected by treatment with phorbol ester (B-TPA, 0.01-1.0 uM) or mezerein (0.01-1.0 uM). However, it appears that 5HT uptake in cultured rat embryonic neurons may be regulated in a complex fashion by phorbol ester. Low doses of B-TPA (0.01 uM) increased 5HT uptake, while high doses (1.0 uM) appeared to down-regulate uptake. The dose-response characteristics of the PKC activators with respect to 5HT uptake, as well as the effects of specific serotonergic agents, will be presented and the implications of possible short-term regulation of central 5HT uptake discussed.

344.17

MODULATION OF SEROTONIN TRANSPORTER ACTIVITY BY INTERFERON-α. N. Saito*, K. Seno, N. Sakai, N. Murakami and S. Honda. Laboratory of Molecular Pharmacology, Biosignal Research Center, Kobe University, Kobe 657, Japan

Molecular cloning revealed that various neurotransmitter transporters have similar structure with 12 putative transmembrane domains. Serotonin transporter (SET), a member of the neurotransmitter transporter family, is known to be a site of action for antidepressants. Functional experiment using mammalian cells expressing SET revealed that various antidepressants inhibit SET activity. To elucidate the molecular mechanism of depression, it is necessary to examine the modulation of SET activity in depression. Interferon(IFN)-α is commonly used as an antiviral drug in case of hepatitis, but the occurrence of depression after the treatment with IFN-α has been clinically attentioned. In the present study, we have examined the effect of IFN-α on the serotonin uptake through SET. The cDNA for SET (kindly provided from R.D. Blakely) was subcloned into pRC/CMV plasmids, and transfected by electroporation into COS-7 cells. Eadie-Hofstee analysis revealed that IFN-α inhibited the serotonin uptake by reducing the Vmax without affecting Km. This suggests that at least IFN-α does not cause depression by the direct modulation of SET. The molecular mechanism of inhibitory effect of IFN-α on SET was further examined.

344.18

INCREASED SEROTONIN TRANSPORTER GENE EXPRESSION IN RAPHE NEURONS OF THE AGED RAT. B. Meister*, H. Johnson and B. Ulfhake, *Department of Neuroscience, Karolinska Institute, 171 77 Stockholm, Sweden*

The action of serotonin (5-hydroxytryptamine; 5HT) in the nervous system is terminated by rapid Na^+/Cl^--dependent reaccumulation of the neurotransmitter into the presynaptic nerve terminal via a plasma membrane-bound transporter protein. The serotonin transporter is the major site of action for some antidepressants and drugs of abuse such as cocaine and amphetamine. Recently, cDNAs encoding serotonin transporter protein were cloned. Several studies suggest that the brain serotoninergic system undergoes changes with increasing age. Whereas the levels of 5HT in the spinal cord appear unchanged, the levels of 5HIAA, the major metabolite of 5HT, are increased in aged rodents. Serotoninergic nerve fibers show morphological signs of degeneration and a decrease in numbers. In the present study we have used *in situ* hybridization to study expression of serotonin transporter mRNA in the descending bulbospinal 5HT-system of adult (2-3 months) and aged (30 months) rats. In adult rats, serotonin transporter mRNA labelled neurons were detected in the nucleus raphe obscurus, pallidus and magnus. In all aged rats, the optical density of the serotonin transporter mRNA labelling of individual cell profiles was increased by 20-30% ($p < 0.001$) when compared with adult rats. Five of the six aged rats studied had various degrees of hindlimb motor dysfunctions. In rats with the most pronounced signs of hindlimb motor dysfunction the number of labelled neurons was increased by 25-75% ($p < 0.01$) when compared with young adult rats. It is concluded that an increased serotonin transporter gene expression is present in the bulbospinal 5HT system of aged animals.

344.19

SUBCELLULAR DISTRIBUTION OF A PUTATIVE TRANSPORTER FOR THE TRANSMITTER HISTAMINE IN AN ARTHROPOD PHOTORECEPTOR. J.R.Morgan and A.E.Stuart*. Dept. of Physiology, Univ. of North Carolina, Chapel Hill, NC 27599-7545.

Substantial evidence indicates that histamine (HA) is the neurotransmitter at many arthropod photoreceptor (PR) synapses. In barnacle, [^{3}H]HA is taken up into PR presynaptic terminals by a presumed transporter specific for HA over 5-hydroxytryptamine (5HT)(*Neurosci. Res.Suppl* , 1991, 15:S13-S23). We asked whether the PR axons and/or somata also take up [^{3}H]HA using light microscopic autoradiography as our assay. Incubations in [^{3}H]HA were for 15min in flashing light. Whole cells incubated in [^{3}H]HA took it up along their length, although their presynaptic terminals and axons labeled much more densely than their somata. To confine the [^{3}H]HA to particular cellular domains, preparations were placed in chambers in which the PR somata, axons or terminals could be separately exposed to [^{3}H]HA (20μM). When axons, but not the somata or terminals, were bathed in [^{3}H]HA, heavy label was found along the axon length. PR somata incubated in [^{3}H]HA labeled only moderately. Addition of 1mM unlabelled HA to the incubation saline blocked uptake in all regions. [^{3}H]5HT was not taken up into any cellular domain. [^{3}H]HA was not taken up into axons when Na was replaced by choline, indicating that uptake is Na-dependent. We conclude that a HA uptake mechanism, presumably a plasma membrane protein related to the Na-dependent aminergic transporters, exists on the barnacle PR's axons and somata membrane as well as at its presynaptic terminals. Experiments are in progress to determine the basis of quantitative differences in label along the length of the cell. Supported by NIH grant EY03347 to AES.

345.1

LOCALIZATION OF AN INS 1,3,4,5-P_4 RECEPTOR IN THE RAT CENTRAL NERVOUS SYSTEM BY IN-SITU HYBRIDIZATION.

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The recently identified 42-kDa high-affinity-InsP4 receptor is thought to play a crucial role in intracellular Ca2+ homeostasis [1]. Sequencing peptides of the receptor protein and PCR yielded partial sequence information [2]. Two antisense oligonucleotides (AO) were synthesized based on different sequences of the InsP4 receptor protein to analyze the cellular expression of the protein in the central nervous system. Nothern blot analysis of rat total poly A+ RNA using these AO revealed a band of approximately 1.3 kb. In-situ hybridization with both AO of rat brain cryostat sections showed a rather ubiquitous distribution of InsP4-receptor RNA. A very dense accumulation of silver grains was observed in the hippocampus, septum, cerebellum, cortex and the dorso-lateral geniculate (>80% of all cells positive). In the cerebellum the signal was most prominent in the granular cell layer. Also some cells in the molecular layer were found to be positive. The superior colliculus, most nuclei of the brain stem, the nucleus accumbens and the olfactory bulb exhibited a moderate signal intensity whereas the caudate nucleus, putamen and hypothalamus were weakly labelled. In rat retinal cryostat sections, silver grains were mainly associated with the inner nuclear and the ganglion cell layer. Thus, evidence is presented that prominent InsP4 receptor gene expression is found in cell types known to be especially vulnerable to excitotoxicity and Ca2+ overload. (1). Reiser G., Methods Neurosci., 18 1993, 280., (2) Hülser et al. Biol. Chem. Hoppe Seyler 375, 1994, 557.

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345.2

ACIDIFICATION OF 5-HT-CONTAINING VESICLES INDUCED BY A G-PROTEIN-COUPLED PLASMALEMMAL CALCIUM RECEPTOR. H. Tamir*, K.P. Liu, M. Adlersberg, S.H. Hsiung, & M.D. Gershon, NYS Psych. Inst. & Dept. Anat. & Cell Biol. Columbia Univ. P&S, NY, NY.

The neural crest-derived paratollicular (PF) cell secretes 5-HT and calcitonin in response to increased extracellular Ca^{2+} ($\uparrow[Ca^{2+}]_e$). PF secretory vesicles acidify in response to $\uparrow[Ca^{2+}]_e$. This acidification, which drives vesicular uptake of 5-HT, results from the stimulus-induced opening of vesicular Cl^- channels, which permits Cl^- to serve as a counterion for H^+ influx. PF cells express a plasmalemmal G protein-coupled receptor (CaR) that binds Ca^{2+} and Gd^{3+}. We tested the hypothesis that $\uparrow[Ca^{2+}]_e$ and Gd^{3+} activate the CaR, which mediates vesicle acidification. Radioactive Gi and Gq were specifically immunoprecipitated from PF membranes incubated with $\uparrow[Ca^{2+}]$ or Gd^{3+} in the presence of ^{35}S-GTPγS. Gd^{3+}-induced both vesicle acidification and $\uparrow[Ca^{2+}]_i$; both effects were antagonized by thapsigargin, but only vesicle acidification was blocked by calmodulin inhibitors. PF cells displayed NO synthase (NOS) immunoreactivity, NO donors acidified vesicles, and NOS inhibitors blocked Gd^{3+} induced vesicle acidification (but not $\uparrow[Ca^{2+}]_i$). Vesicles were acidified by dibutyryl-cGMP and LY83583, a guanyl cyclase inhibitor, antagonized Gd^{3+}-induced vesicle acidification. Our observations suggest the following signal transduction pathway: $\uparrow[Ca^{2+}]_e$ stimulates the CaR → activates Gi and/or Gq → activates PI-PLC → releases Ca^{2+} from the ER → $\uparrow[Ca^{2+}]_i$ complexes with calmodulin to stimulate NOS → NO activates guanylate cyclase → ↑ cGMP → opens the vesicular Cl^- channel. Supported by grants DK19743 and NS12969.

345.3

NITRIC OXIDE EVOKES THE RELEASE OF ADENOSINE FROM RAT HIPPOCAMPAL SLICES. R.M. Broad' and B.B. Fredholm Dept. of Physiology and Pharmacology, Karolinska Institutet, Stockholm, SWEDEN, 171 77.

Nitric Oxide (NO) has previously been shown to act as a neuromediator in the central nervous system, modulating the release of several classical and excitatory amino acid neurotransmitters. Adenosine is an endogenous inhibitory neuromodulator that has been shown to attenuate the release of many neurotransmitters in the CNS, and therefore may be responsible for the actions of NO. Using an electrically-stimulated, perfused rat hippocampal slice preparation, we have investigated the influence of NO on the release of adenosine. Following preincubation with [^{3}H]adenine, electrically-stimulated release of [^{3}H] (consisting of ATP and ATP breakdown products) was dramatically enhanced in the presence of the NO donor S-nitroso-N-acetylpenicillamine (SNAP). This effect was concentration dependent, becoming evident at 100μM SNAP and significant at 1mM SNAP. At this higher concentration, adenosine release in the presence of the NO donor was increased 80-fold above control. HPLC analysis revealed that the increase in [^{3}H] was accompanied by an increase in both [^{3}H]adenosine and endogenous adenosine as well as tritiated and endogenous inosine. SNAP also significantly enhanced the release of basal [^{3}H]purines and adenosine, although not to the same extent as that of electrically stimulated release. N-acetyl penicillamine (N-AP: 1mM), the breakdown product of SNAP, did not alter adenosine release. Sodium nitroprusside was considerably less potent than SNAP, evoking only a modest increase in release at the highest concentration used (1mM). These data directly demonstrate the ability of nitric oxide to evoke the release of adenosine. We propose that NO-mediated adenosine release may be responsible for some of the neuromodulatory effects of NO.

345.4

IMMUNOCYTOCHEMICAL STUDY OF THE DEVELOPMENTAL PROFILE OF NO-RESPONSIVE AND ANF-RESPONSIVE cGMP PRODUCTION IN THE FRONTAL CORTEX OF THE RAT. L. de Vente, M. Markerink-van Ittersum and H.W.M. Steinbusch*. European Graduate School for Neuroscience 'Brain and Behavior', Dept. of Psychiatry and Neuropsychology, University of Limburg, Maastricht, The Netherlands.

We have previously described that ANF stimulated cGMP accumulation in the cerebellum of the rat during maturation was partly inhibited by the NOS-inhibitor L-NAME, suggesting that an NO-activated guanylate cyclase (GNC) participated in the cGMP response (De Vente et al. Eur.J.Neurosci 2:845-862 (1990). In this study we investigated the localization of cGMP during development in slices of the frontal cortex of the rat, using nitroprusside (SNP) or ANF to stimulate sGNC or pGNC.

In unstimulated slices at day P4 we found cGMP-IR cell somata with extensive ramifications in layer 2 and 3 of the cortex. The number of these cells decreased sharply from day P8 till day P24. cGMP-IR was absent in slices incubated in the presence of L-NAME.

SNP stimulated cGMP production strongly in cell somata and fibers throughout the cortex. The number of cGMP-IR reactive cells decreased whereas the number of varicose fibers increased from day P8 till day P24.

The number of ANF-responsive cells increased from day P4 till day P8, thereafter a decline in the number of cGMP-IR cell somata was observed. At day P24 ANF-responsive, cGMP-producing cells were still present. L-NAME partly inhibited cGMP accumulation.

These results indicate differences in development for (NO responsive) sGNC and (ANF-responsive) pGNC. The data suggest that part of the cGMP-response to ANF stimulation is secondary to the stimulation of NOS. Both forms of the GNC may be present in the same cell.

345.5

DIFFERENTIAL REGULATION OF TETRAHYDROBIOPTERIN SYNTHESIS IN MONOAMINE AND NITRIC OXIDE-PRODUCING NEURONS. **M. Hahn*, M. Khalil, K. Hirayama and G. Kapatos. Cellular and Clinical Neurobiology Program, Department of Psychiatry and Behavioral Neurosciences, Wayne State University, School of Medicine, Detroit, MI 48201.**

Tetrahydrobiopterin (BH4) is the essential cofactor for the synthesis of monoamines (MA) and nitric oxide (NO). Although BH4 synthesis has been localized to MA neurons, no direct evidence exists for BH4 synthesis by NO-producing neurons. Cultures of postmitotic cerebellar granule neurons offer a model system to study BH4 synthesis within a homogenous population of NO-producing neurons. Cerebellar granule neurons maintained in culture were harvested after 1-21 days *in vitro* (DIV) and then assayed for BH4 content. BH4 levels increased 10-fold by 7 DIV and then declined slightly after 9 DIV. Following inhibition of its synthesis, BH4 declined with a $t_{1/2}$ of approximately 5 hours. Previous work, using primary cultures of the mesencephalon and hypothalamus, has shown that in these MA neurons the $t_{1/2}$ of BH4 is 4-5 hours, and that BH4 synthesis is stimulated by a cAMP-dependent mechanism that is sensitive to inhibitors of transcription or translation. Thus, the developmental profile and the rapid rate of turnover in cerebellar granule neruons prompted us to examine the regulation of BH4 synthesis. A 24-hour incubation with 8-Bromo-cAMP or IBMX elevated BH4 levels in cerebellar granule neurons by 30-50%. Unexpectedly, treatment with forskolin decreased BH4 levels by 25%. In addition, the ability of 8-Bromo-cAMP to stimulate BH4 levels was not prevented by actinomycin D or cycloheximide, and actinomycin D actually augmented BH4 levels. In conclusion, the rapid developmental increase in BH4 content and the turnover rate of BH4 in cultured cerebellar granule neurons suggest a high degree of regulation of BH4 synthesis in these postmitotic neurons. In addition, these findings argue that different modes of regulating BH4 synthesis are found associated with MA and NO neurotransmitter phenotypes. (supported by NIH NS26081)

345.6

CIRCADIAN CYCLING OF NITRIC OXIDE SYNTHASE (NOS) ACTIVITY AND CYTOSOLIC PROTEIN CONTENT IN RAT BRAIN. N. A. Ayers, L. Kapás, and J. M. Krueger*. Department of Physiology and Biophysics, University of Tennessee, Memphis, TN 38163.

Nitric oxide (NO) is implicated in sleep maintenance (1) and circadian rhythm regulation (2). To further elucidate the role of NO in sleep regulation and its association with circadian rhythms, we examined the circadian variation of NOS activity in the cerebellum, brain stem, hypothalamus, hippocampus, and "rest of brain" from male Sprague Dawley rats habituated to a 12:12 light dark cycle (lights on at 0900h). Six rats were decapitated at each of the following times: 1500h, 2100h, 0300h, 0900h. NOS activity was measured by the conversion of [^{3}H]-arginine to [^{3}H]-citrulline (3). Cytosolic protein concentrations were measured by standard bicinchoninic acid method (Pierce, Rockford, IL). There were significant differences of NOS activity among brain regions and in all five brain regions NOS exhibited significant changes with time ($p<0.0001$). For example at 2100h hypothalamus contained 51.0 ± 6.1 unit NOS activity/g wet tissue whereas the brain stem contained 20.5 ± 2.3 unit/g. Significant differences were also demonstrated between any two time points except between 0300h and 0900h. Basically NOS activity was higher during the dark phase and lower during the light period, e.g., for hypothalamus values at 1500h were 35.4 ± 4.2 compared to 77.9 ± 7.1 unit/g at 0300h. Protein concentrations also demonstrated circadian variation. Overall there was a significant increase in protein content in all brain regions, except "rest of the brain", during the dark period (ANOVA across 4 time points and 5 brain regions; time effect: $F(3,96)=17.69$, $p<0.0001$). These results augment the present evidence of NO as a key player in the physiological maintenance of biological rhythms and sleep.

1. Kapás, L., *et al* (1994) *Am. J. Physiol.* **226**: R151. 2. Watanabe, A., *et al* (1994) *Brain Res.* 646:161. 3. Bredt, D. S., Snyder, S. (1990) *Proc. Natl. Acad. Sci. USA* **87**: 682. Work supported by NIH NS-25378, NS-27250, NS-31453 and NS-30514.

345.7

PROTEIN TYROSINE NITRATION IN THE NERVOUS SYSTEM: A MARKER OF BRAIN NITRIC OXIDE PRODUCTION. R.R. Trifiletti, A.N. Bandele and E.A. Bolan. Departments of Neurology/Neuroscience and Pediatrics, New York Hospital-Cornell Medical Center, New York, NY 10021

Nitric oxide (NO) appears to be a key mediator of a variety of physiologic and toxic mechanisms in the brain. Based on *in vitro* studies with model proteins, we hypothesized that tyrosine nitration may be an important reaction of NO (or NO-derived peroxynitrite) with protein.

We utilized antibodies directed toward tetranitromethane-treated keyhole limpet hemocyanin to explore whether tyrosine-nitrated proteins (nitroproteins) exist in the rat central nervous system. We demonstrate at least 80 electrophoretically distinct nitroproteins in brain detected by 2-dimensional SDS/PAGE immunoblots. Nitroprotein immunostaining can be abolished by treatment of blots with sodium hydrosulfite, co-incubation with *in vitro* nitrated bovine serum albumin (BSA), 3-nitro, or 3,5-dinitrotyrosine, but not by co-incubation with native BSA, tyrosine or 4-O-phosphotyrosine. Nitroprotein staining does not simply parallel total protein staining.

Nitroprotein composition of various rat brain regions appear to be similar, with some minor regional and developmental differences noted; staining was also noted in a number of other tissues including liver and kidney.

Newborn rat pups were treated with 25 mg/kg N^G-nitroarginine, i.p. daily for 10 days, resulting in a marked diminution in nitroprotein immunostaining. Thus, pharmacologic blockade of *de novo* NO synthesis markedly depletes the brain and other tissues of nitroproteins, supporting the hypothesis that nitroproteins are produced by reaction of nitric oxide, or nitric oxide-derived free radicals (such as peroxynitrite) with proteins.

Nitroproteins may represent a useful "integrated" marker of NO production in the brain, and nitroproteins are potential targets for NO action or toxicity.

345.8

CEREBELLAR NITRIC OXIDE SYNTHASE (NOS) IS EXPRESSED WITHIN GRANULE CELL PATCHES INNERVATED BY SPECIFIC MOSSY FIBER TERMINALS: A DEVELOPMENT PROFILE J. Li[1], S. Chen[2], R.C.S. Lin[1] and S.S. Smith[1], [1]Dept of Anatomy and Neurobiology, [2]Mental Health Sci., MCP-Hahnemann Univ., Broad and Vine St., Phila. PA 19102

The nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) staining technique was utilized as a marker of nitric oxide synthase (NOS) to map NOS expression in developmental and adult rat cerebellum. Schilling et al.(1994) have recently demonstrated that NADPH-d positive granule cells in adult cerebellum were identified within irregular patches. In order to investigate the anatomical significance of this pattern of distribution, we have examined the relationship between NADPH-d stained patches and mossy fiber innervations. Mossy fiber projections were traced using cholera toxin B subunit (CTb) injected into the lateral reticular nuclei (LRtN). Then double labelling techniques were implemented to determine a possible co-localization of NADPH-d patches within the granule cell layer innervated by specific mossy fiber terminals. During development, NOS expression in both molecular and granule cell layers increased from post-natal day 5 to peak levels at PND 30, which were maintained in the adult. By PND 19 and in the adult, NADPH-d stained granule cells were visualized as "patches" throughout the cerebellum. Injection of CTb into the ventro-rostral LRtN resulted in clustered mossy fiber terminals matched (74-82%) with NADPH-d stained patches. In contrast, CTb injections into other areas of the LRtN were not as well overlapped with NADPH-d stained areas. These data suggest that patch-like localizations of NOS are innervated by mossy fiber projections from distinct areas of brainstem in adult cerebellum. It is possible that NOS patches may thus serve as defining specific functional or anatomical zones in adult cerebellum. (Supp. by USAF grant #93-1-0136 to S.S. Smith)

345.9

ACUTE LIVER FAILURE AND HYPERAMMONEMIA INCREASE NITRIC OXIDE SYNTHASE IN MOUSE BRAIN.
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Hyperammonemia and treatment of animals with thioacetamide (TAA) which causes acute liver failure, both reproduce many of the clinical and morphological features of hepatic encephalopathy (HE). There is evidence that the extracellular concentration of glutamate is elevated in HE and that ammonia impairs the function of the astrocytic glutamate transporter. Since stimulation of glutamate receptors activates the constitutive isoform of nitric oxide synthase (cNOS), we sought to investigate whether cNOS activity was affected in the brain of animals exposed to TAA and ammonium acetate. Mice were administered with either saline, TAA (100 mg/kg; i.p.) or ammonium acetate (5 mmole/kg; i.p.) once a day for 3 days. Mice were sacrificed 24 h after the last injection and the cerebellum was studied. Kinetics of cNOS activity was determined in the 10,000 *g* supernatant fraction by the conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline. Lineweaver-Burke plots showed a 30-40% increase in Vmax and a 40-50% decrease in Km values after TAA and ammonium acetate administration. Since the acute addition of ammonium acetate (up to 10 mM) to homogenates of normal cerebellum did not affect cNOS activity *in vitro*, it appears that ammonia acutely has no direct effect on cNOS activity. We suggest that the increase of cNOS in hyperammonemia and TAA-induced acute liver failure may contribute to the pathogenesis of HE. (Supported by NIH grants DK38153 and NS30291, VA and GRECC)

345.10

THE NO-cGMP PATHWAY IN RAT LOCUS COERULEUS: ELECTRO-PHYSIOLOGICAL AND IMMUNOHISTOCHEMICAL STUDIES. Z.-Q. Xu, O. Johansson*, H.W.M. Steinbusch#, S. Grillner and T. Hökfelt. Dept. of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden. *Dept. of Psychiatry & Neuropsychology, Univ. of Limburg, Maastricht, The Netherlands.

We have previously shown that application of nitric oxide synthase (NOS) inhibitors enhance the excitatory postsynaptic potential in locus coeruleus (LC) neurons, suggesting a functional role for nitric oxide in LC. Now we analyse the activation of guanylate cyclase (GC) by NO in the LC neurons *in vitro* using electrophysiology and immunohistochemistry. Local application of the NO-generating drug SIN-1 resulted in hyperpolarization of 25 out of 52 neurons. In 8 neurons, the action potential threshold was decreased and the firing rate was increased, while the membrane potential was not changed or slightly hyperpolarized. In 3 neurons, SIN-1 induced an increase in firing rate and/or a depolarization, but did not change the action potential threshold. In the remaining 16 neurons, no change in the membrane potential, firing rate or action potential threshold was observed. The SIN-1 derivative molsidomin did not exert any measurable effect in LC neurons (n=10). Another NO-generating substance, DEA/NO, mimicked the effects of SIN-1. Prior application of hemoglobin prevented or largely reduced the action of SIN-1. Under voltage-clamp conditions SIN-1 caused an outward current in SIN-1-inhibited cells (n=6), while it reduced the amplitude of the evoked exitatory postsynaptic currents. Local application of 8-bromo-cGMP mimicked the action of SIN-1 in SIN-1-inhibited neurons (n=6). It caused an increase in firing rate in 3 neurons, in which SIN-1 application increased the firing rate. 8-Bromo-cGMP had not effect on neurons which did not respond to SIN-1. Using immunohistochemical methods, the cGMP levels were measured in the slices with or without SIN-1 stimulation. Exposure to SIN-1 resulted in increased cGMP staining in 40% of the LC neurons. Almost all of these neurons were TH-positive, e.g. noradenergic neurons. These results suggest that the NO donors are acting through the NO-cGMP pathway to hyperpolarize the membrane potential, by stimulating GC and increasing endogenous levels of cGMP.

345.11

ROLE OF $[Ca^{2+}]_i$ IN THE NEURONAL EXPRESSION OF CALCITONIN GENE-RELATED PEPTIDE. M.D. Christensen* S.C. Supowit, B.N. Christensen, D.J. DiPette. The University of Texas Medical Branch at Galveston, Texas 77555

Preliminary whole animal data suggests that an increase in Ca^{2+} intake results in an increase in CGRP mRNA in dorsal root ganglia (DRG). The effect of Ca^{2+} supplementation on CGRP message could be the result of changes in $[Ca^{2+}]_i$ subsequent to an increase in extracellular Ca^{2+}. We tested the effect of altering Ca^{2+} (0.05 to 1.6 mM) on the expression of CGRP in primary cultures of adult rat DRG neurons. In the range of 0.05 to 1 mM Ca^{2+} there was a large attenuation of basal mRNA. At 1.0 and 2.0 mM, there was a 1.4 and 1.6 times increase in CGRP message respectively. To determine if the effect of Ca^{2+} on expression of CGRP message was mediated via changes in $[Ca^{2+}]_i$, we assessed the effect of (a) increased release from intracellular stores induced by thapsigargin (TH) and (b) increased influx of Ca^{2+} induced by Bay K 8644. In 1 mM extracellular Ca^{2+}, neither TH nor Bay K 8644 altered CGRP mRNA although both increased free $[Ca^{2+}]_i$ (determined by fura). We tested the hypothesis that Ca^{2+} is an important co-factor for a PKC-dependent second messenger pathway involved in CGRP expression. Activation of PKC by phorbol ester during a TH-induced increased in $[Ca^{2+}]_i$ enhanced CGRP mRNA by 1.7 times versus PMA alone ($n=3, p<0.01$). The PKC inhibitor bisindolylmaleimide significantly reduced the PMA induced response versus PMA alone ($n=4, p<0.05$). These results support the hypothesis that increased dietary Ca^{2+} enhances CGRP production through an extracellular Ca^{2+} dependent increase in mRNA production that involves a PKC-dependent pathway. This pathway could serve as a target for promoting CGRP mRNA production which may be useful for therapeutic intervention by increasing CGRP mRNA and protein production.

345.12

POSSIBLE REGULATION OF PLATELET SEROTONIN UPTAKE BY TYROSINE KINASES. D.M. Helmeste* and S.W. Tang. Department of Psychiatry, University of California, Irvine. Irvine, California 92717

Abnormalities in serotonin uptake and calcium mobilization in the platelets of affective disorder patients have fostered speculation regarding underlying mechanisms, but very little is known regarding biochemical pathways that regulate these functions. As phosphorylation is an important step in regulation of protein functions, we investigated the role of tyrosine kinases in the regulation of the serotonin uptake process. Our investigations demonstrate that platelet serotonin uptake appears to be closely regulated by tyrosine kinase activity, since three structurally distinct inhibitors of tyrosine kinase (genistein, herbimycin A and methyl 2,5-dihydroxycinnamate) cause rapid and dramatic reductions in tritiated serotonin uptake. Inhibition of uptake did not correlate with IC50's for inhibition of tritiated imipramine binding, suggesting that uptake inhibition was not due to direct binding of these drugs to the transporter. Investigation of drugs that cause release of intracellular calcium (thapsigargin, thrombin and agents that stimulate protein kinase C) were found by us to also inhibit platelet serotonin uptake. Since tyrosine kinase inhibition is also known to affect calcium fluxes in platelets, we wanted to determine whether changes in mobilizable calcium mediated the effects of genistein on serotonin uptake. Pretreatment of platelets with BAPTA-AM (10 micromolar, 37° C, 30 min.), a chelator of intracellular calcium, did not prevent genistein from inhibiting serotonin uptake. Additionally, measurement of changes in intracellular calcium (fura-2 method) after BAPTA-AM, genistein or methyl 2,5-dihydroxycinnamate administration did not show a clear correlation between reductions in intracellular calcium and effects on serotonin uptake. A more direct mechanism of tyrosine kinase-induced modulation of serotonin uptake (i.e., phosphorylation) is consistent with these results.

346.1

APLYSIA MAP KINASE IS ACTIVATED BY SEROTONIN AND PHOSPHORYLATES TRANSCRIPTION MODULATORS FOR LONG-TERM FACILITATION. D. Michael[1]*, D. Bartsch[1], M. M. Ning[1], R. Bastón[1], and R. Seger[2]. [1]HHMI, Ctr. Neurobiol. & Behav., Columbia Univ., NY, NY 10032; [2]Membrane Research & Biophys., Weizmann Inst. Sci, Rehovot, Israel.

Long-term facilitation of the sensory-motor synapse in *Aplysia*, which is induced by serotonin (5-HT), involves the growth of new synaptic connections by sensory cells and the activation of transcription. Two key transcription regulators of long-term facilitation have consensus sequences for phosphorylation by MAP kinase (MAPK): *Aplysia* C/EBP and *Aplysia* CREB-2. This has led us to clone the *Aplysia* gene for MAPK (ApMAPK) and to characterize its activity. The enzyme, which is 85% identical to the mammalian MAPK, was activated by 5-HT (20 μM, 30 minutes) in isolated ganglia. Both C/EBP and CREB-2 were phosphorylated effectively by partially purified fractions enriched for ApMAPK. Using "in-gel" kinase assays with C/EBP and CREB-2 as substrates, and using fully purified mammalian MAPK, we found that both proteins were phosphorylated by MAPK. However, MAPK targeted C/EBP more efficiently than CREB-2. Therefore, an additional kinase may be targeting CREB-2. To study the possible link between PKA and MAPK, we applied forskolin to isolated ganglia and found that it enhanced MAPK activity. Thus, PKA and MAPK might coordinately mediate the transcriptional changes responsible for the transition to long-term facilitation. Interestingly, the mammalian C/EBP is phosphorylated in vivo by the MAPK pathway, and this event may lead to its activation (Nakajima et al., PNAS 90:2207, 1993; Kowenz-Leutz, Gen. Dev. 8:2781, 1994). It is therefore possible that ApMAPK directly mediates transcriptional activation in *Aplysia*.

346.2

REGULATION BY VIP AND NORADRENALINE (NA) OF GLYCOGEN SYNTHASE mRNA EXPRESSION.
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Glycogen is the single largest energy reserve of the brain. Brain glycogen is predominantly localized in astrocytes and its metabolism is under the control of two principal enzymes: glycogen synthase (GlyS) and glycogen phosphorylase (GlyP). VIP and NA promote two opposed cAMP-dependent and temporally regulated actions on glycogen levels in primary cultures of cortical astrocytes: glycogenolysis within a few minutes (Sorg and Magistretti, (1991) Brain Res. 563:227-233), and a subsequent resynthesis of glycogen, resulting within 8-10 hours in a 6-10 fold increase in glycogen content (Sorg and Magistretti (1992) J. Neurosci. 12: 4923-4931). The long-term action of VIP and NA is sensitive to protein synthesis inhibitors, suggesting that the synthesis of one or more enzymes involved in glycogen metabolism are modulated by VIP and NA. Two potential candidates for this regulation are GlyS and GlyP. We have cloned and characterized the astrocyte GlyS isozyme. The 3.5 kb clone isolated contains an open reading frame of 2214 bp encoding a protein of 737 amino acids. The cellular distribution of the GlyS mRNA, studied by Northern blot, shows the presence of a single transcript of 4 kb in cultures of astrocytes and, to a lesser degree, of neurons. As revealed by Northern blot analysis, VIP 1 μM and NA 100 μM increase the level of GlyS mRNA within 8-10 hours, while GlyP mRNA levels were not affected by VIP or NA. The same treatment in the cortical neuron cultures did not modulate the levels of GlyS mRNA. This study indicates that VIP and NA, by increasing cAMP levels, simultaneously trigger a short-term effect (glycogenolysis), as well as a delayed one that is transcriptionally regulated (glycogen resynthesis). This long-term effect ensures that sufficient substrate is available for the continued expression of the short-term action.

346.3

THE TRANSCRIPTION FACTORS C/EBPβ AND C/EBPδ ARE INDUCED BY VIP, PACAP AND NORADRENALINE IN MOUSE CORTICAL ASTROCYTES: A ROLE IN ENERGY METABOLISM REGULATION.
J-R. Cardinaux* and P.J. Magistretti. Institut de Physiologie, Université de Lausanne, Lausanne, Switzerland.

We have previously described a transcription-dependent induction of glycogen resynthesis by VIP or noradrenaline (NA) in astrocytes, which is mediated by cAMP (Sorg and Magistretti, 1992, J. Neurosci. 12:4923). Since it has been postulated that the regulation of energy balance in hepatocytes and adipocytes may be channeled at least in part through the CCAAT/enhancer binding protein (C/EBP) family of transcription factors, we tested the hypothesis that C/EBP isoforms could be expressed in astrocytes and their level of expression regulated by VIP, the VIP-related peptide PACAP, or by NA. By Northern and Western blot analysis, we observed that C/EBPβ and C/EBPδ mRNA and protein are induced by VIP 10 nM as well as PACAP 1 nM or by NA 100 nM in astrocytes prepared from mouse cerebral cortex. A pharmacological analysis indicated that the induction of C/EBPβ and -δ by NA is mediated by β-subtype adrenergic receptors. VIP, PACAP and NA therefore probably increase C/EBPβ and -δ expression via the cAMP second-messenger pathway. This is further confirmed by the induction of both proteins by 8-(4Br)-cAMP or forskolin. Induction of C/EBPβ and -δ mRNA by VIP occurs in the presence of a protein synthesis inhibitor. Thus, *c/ebpβ* and *c/ebpδ* can be considered as cAMP-inducible immediate-early genes in mouse astrocytes. Overexpression of C/EBPδ by transient transfection of astrocytes with an expression vector containing its cDNA sequence results in a marked increase of glycogen over basal levels (227 ± 3 vs 104 ± 7 nmol of glycogen/mg of protein). These results suggest that C/EBPδ may regulate gene expression of energy metabolism-related enzymes in astrocytes.

346.4

MOLECULAR MECHANISMS OF cAMP RESPONSE ELEMENT BINDING PROTEIN (CREB) REGULATION IN A LOCUS COERULEUS-LIKE (CATH.a) CELL LINE. K.L. Widnell*, P.A. Iredale, R.S. Duman, and E.J. Nestler. Laboratory of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508

Studies of the regulation of gene expression by the cAMP intracellular signaling pathway have focused on CREB and related proteins. It is generally believed that the CREB gene is constitutively expressed and not subject to regulation. However, in the course of investigating effects of chronic morphine on cAMP pathway proteins in the locus coeruleus (LC), a brain region important for physical aspects of opiate addiction, we observed that levels of CREB immunoreactivity and CRE binding are increased by chronic morphine administration [Widnell et al. (1994) *PNAS* **91**:10947]. CREB expression is also subject to dynamic regulation by the cAMP pathway in an LC-like (CATH.a) cell line. We are currently investigating the molecular mechanisms underlying cAMP regulation of CREB expression in the LC-like cell line using forskolin, an activator of adenylyl cyclase. These studies demonstrate that the forskolin-induced decrease in CREB message depends upon protein synthesis. Furthermore, a decrease in the rate of CREB gene transcription, as assessed by nuclear run-on assays, could account for the forskolin-induced decrease in CREB message. Interestingly, while forskolin results in a long-term down-regulation of CREB protein, there is a transient increase in CREB immunoreactivity that occurs in the absence of any observed increase in CREB mRNA levels. The observed up-regulation of CREB protein is not dependent upon protein synthesis, and may represent a phosphorylation-induced stabilization of CREB protein. These studies, which will delineate the mechanisms controlling CREB expression, should contribute to our understanding of the precise molecular steps by which morphine produces addiction via actions on specific target brain regions.

346.5

EXPRESSION OF A CORTEX SPECIFIC PKA ISOFORM CONFERS ON CEREBELLAR GRANULE CELLS HIGH SENSITIVITY TO THE cAMP-INDUCED GENE TRANSCRIPTION. M. Paolillo*, C. Ventra, G. Schettini and V.E. Avvedimento. Dipartimento di Neuroscienze, Dipartimento di Biologia e Patologia Cellulare e Molecolare, II Facoltà di Medicina, 80131 Napoli, Casa di Cura "Villa Chiarugi", 84014 Nocera Inf. ITALY.

The subunits composition of PKA varies greatly in the CNS. The expression of the regulatory subunit RIIβ and of the relative anchor protein AKAP is almost undetectable in the brainstem and cerebellum while is maximal in the cortex. Cortical neurons show a poor dissociation of PKA cytoplasmic holoenzyme following a cAMP pulse and an efficient accumulation of catalytic subunit in the nucleus. On the other hand, cerebellar granule cells show a marked holoenzyme dissociation after cAMP stimulation while nuclear translocation is almost undetectable. The functional correlate of the catalytic subunit nuclear translocation is the phosphorylation of CREB (cAMP responsive element binding protein) which is strong in cortical neurons and undetectable in cerebellar granule cells. To verify whether the different nuclear responses to cAMP in these areas could be due to the expression of different PKA isoforms, we reconstituted the cortical PKA in cerebellar granule cells by microinjecting the cells with the expression vectors carrying RIIβ, AKAP, PKA catalytic subunit and a reporter gene (lac-Z) under the control of a cAMP responsive element (CRE). The injection of of RIIβ and AKAP genes efficiently stimulated the cAMP-induced expresion of of lac-Z gene. RIIβ alone was able to slighlty stimulate lac-Z transcription, AKAP and catalytic subunit alone or in combination were ineffective. These data indicate that the expression of the PKA regulatory subunit RIIβ andof the anchor protein AKAP can amplify cAMP signals to the nucleus and dictate different responses to cAMP in the brain.

346.6

Differential display PCR of mRNA expression after sustained expression of FOS in neurons. McPhie, D.L.*1, Lim, F.L. 2, Geller, A.I. 2, and Neve R.L.1 (1. Laboratory of Molecular Neurogenetics, McLean Hospital, Belmont, MA 02178 and 2. Laboratory of Endocrinology Childrens Hospital, Boston MA. 02115) Numerous models of neuronal plasticity feature alterations in expression of the immediate early gene c-fos. However, the significance of these fos expression changes is not known. Sustained increases in FOS protein levels in neurons and resultant increases in neurotransmitter release, can be achieved by infecting primary rat cortical neurons in culture with a Herpes Simplex Virus One (HSV-1) vector expressing recombitant Fos (Lim et al., this meeting). To study the downstream genetic changes accompanying the experimentally-induced long-term increases in neuronal Fos expression, we used differential-display reverse transcription PCR (DDRT-PCR) to examine the mRNA populations in neurons infected with HSVfos or with HSV expressing a non-functional Fos protein. Total RNA was prepared and a subset of the expressed mRNA's were reverse transcribed using an anchored $T_{12}VN$ (V=A, G, or C; N= A, G, T, or C). CDNAs were then amplified by PCR using random 10mers after which the PCR products were examined on 5% denaturing polyacrylamide gels. At least 9 PCR fragments were differentially expressed. The bands were isolated, reamplified and cloned into vectors. These probes will be used to screen RNA blots to confirm differential expression after which they will be sequenced and characterized functionally. Supported by NIH Grant HD24236.

346.7

ENHANCED DNA BINDING ACTIVITY OF AP-1 BY THE ANTICONVULSIVE AND ANTIMANIC AGENT VALPROIC ACID IN VITRO. G. Chen*, W. Z. Potter, D. B. Hawver and H. K. Manji, Dept. of Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine, Detroit, MI 48201, and Sec. Clin. Pharmacol. NIMH, NIH, Bethesda, MD 20892.

Despit extensive research, the mechanisms by which lithium, carbamazepine and valproic acid (VPA) produce their clinical antimanic actions are still unclear. Recently, we found that chronic, but not acute, VPA treatment has marked effects on the cAMP second messenger generating system, protein kinase C (PKC) isozymes, and PKC's substrate MARCKS in vitro. These effects of VPA are similar to the effects of lithium which we and other investigators have observed both in vitro and in vivo. Since both VPA's biochemical effects and clinical antimanic action require chronic administration, we investigated the possible role of VPA in the modulation of gene expression. DNA binding activities of activator protein 1 (AP-1) and cAMP responsive element binding protein (CREB), which are regulated by PKC and PKA catalyzed phosphorylations, were studied in acute (hours) and chronic (days) VPA treated rat C6 glioma cells. VPA did not affect CREB DNA binding activity, but concentration- and time-dependently increased AP-1 DNA binding activity. The activity was raised at 2 hours (the shortest time examined) and remained high after 6 days (the longest time used) of continuing VPA treatment. VPA also enhanced AP-1 DNA binding activity in human neuroblastoma SH-SY5Y cells. Addition of VPA to the binding mixture had no effect on the activity. Co-treatment of cells with either bisindolylmaleimide or chelerythyrine, the PKC inhibitors, did not block the increasing effects of VPA. VPA had no effects on protein phosphatase (PP) 1 or PP2A activity in vitro. The mechanism underlying VPA's effect on AP-1 DNA binding activity is unclear, but currently under investigation. The present study provides evidence for the modulation of gene expression by antimanic agents, which may be one of the reasons for late onset of clinical antimanic action.

346.8

CONVULSANT AGENTS INDUCE c-FOS EXPRESSION IN PRIMARY CORTICAL NEURONS THROUGH DIFFERENT PATHWAYS. J.M.Tusell[1], S.Barrón* and J.Serratosa, Depts. Neurochem[1]. and Pharmacol. & Toxicol. CID-CSIC. Barcelona. Spain.

In neurons, an increase in calcium levels after an external signal represents the initial step in many signalling pathways. The goal of this work has been to determine the possible role of L-type calcium voltage sensitive channels (VSCC) and the calcium binding protein, calmodulin (CaM), in the induction of c-fos expression following administration of different convulsant drugs.These drugs act at the level of L-type VSCC (BAY K 8644 (BK)) and γ-hexachlorocyclohexane (γ-HCH)), NMDA and non NMDA receptors (NMDA and kainic acid) and at the level of the GABAergic system (picrotoxinin (PTX) and pentilenetetrazole(PTZ)). The inhibition of c-fos induction elicited by these drugs was studied using the L-Type VSCC antagonist, nifedipine, and the CaM antagonist W-7. We also determined the inhibitory effect of the nervous system depressor δ-hexachlorocyclohexane (δ-HCH). Primary cortical neurons were obtained from rat embryos (E17). c-fos protein and mRNA were detected by means of immunocytochemistry and Northern Blot. Approximately 30% of the neurons displayed c-fos nuclear immunoreactivity under basal condition. This value was increased to about 55% after treatment with any convulsant used. Nifedipine and δ-HCH acted in a similar way reducing c-fos induction due to BK, γ-HCH and PTZ to basal or below levels. However, they were not able to inhibit the proto-oncogen induction elicited by PTX. W-7 abolished c-fos induction elicited by BK, γ-HCH, PTZ and kainic acid. The CaM antagonist did not affect NMDA or PTX-mediated increases in c-fos expression in neurons. The results obtained with δ-HCH, nifedipine and W-7 suggest that PTX and NMDA activate c-fos expression through calcium-dependent intracellular mechanisms, that are different from those activated by the other convulsant, which at least in part, act via L-type VSCC and CaM.

346.9

REPEATED AMPHETAMINE ADMINISTRATION INDUCES A PROLONGED AUGMENTATION OF PHOSPHORYLATED-CREB AND FOS-RELATED ANTIGEN IMMUNOREACTIVITY IN RAT STRIATUM. J.N. Simpson*, J.Q. Wang and J.F. McGinty. Dept. Anatomy and Cell Biology, School of Medicine, East Carolina University, Greenville, NC 27858-4354.

Semi-quantitative immunocytochemistry was used to investigate the levels of CREB-, phosphorylated-CREB (P-CREB)-, Fos-, and Fos-related antigen (FRA)-immunoreactivity (IR) in the striatum of rats after acute or repeated amphetamine (AMPH) administration. Rats were perfused 20 min (P-CREB) or 2 h (CREB, P-CREB, Fos, FRA) after a single injection (5 mg/kg, i.p.) or 5 daily injections of AMPH. The latency to onset of stereotypical behaviors was significantly reduced in rats exposed to repeated amphetamine as compared to acute AMPH, indicating development of behavioral sensitization. CREB-IR was not altered in the dorsal or ventral striatum following acute or repeated AMPH. P-CREB-IR was significantly induced 20 min, but not 2 h, following acute AMPH whereas a significant induction of P-CREB-IR was found 20 min and 2 h after repeated AMPH in the dorsal striatum only. Fos-IR was significantly induced in the dorsal striatum following acute and repeated AMPH. Fos-IR in the core of the nucleus accumbens (NAC) was significantly increased following repeated AMPH only. Acute AMPH induced, and repeated AMPH further augmented, FRA-IR in the dorsal striatum while not affecting FRA-IR in the NAC. These data demonstrate that repeated AMPH administration results in a prolonged induction of P-CREB- and FRA-IR in the dorsal striatum, indicating that alterations in striatal gene expression associated with the development of behavioral sensitization may be mediated, in part, by these transcription factors. Supported by DA 05470 (JNS) and DA 03982 (JFM).

346.10

A NEW GTPase-ACTIVATING PROTEIN GENE FAMILY IN BRAIN. H.Baba[1], B.Fuss[2], P.Puollet[3], J. Urano[3], F.Tamanoi[3], and W.B.Macklin[2]*.
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GAPIII is a newly identified GTPase-activating protein (GAP), which is a class of proteins that acts as negative regulators of p21ras. Amino acid sequence studies suggest that GAPIII and another known mammalian GAP, Gap1^m, are members of a new gene family, which may be the mammalian homologs of Drosophila Gap1. This protein family has several functional protein domains such as the Ca^{2+}-dependent phospholipid-binding domain (C2), the Pleckstrin homology domain (PH), Bruton's tyrosine kinase domain (btk) and the GAP-related domain (GRD). The protein structures of GAPIII and Gap1^m are unique from other mammalian GAPs, such as p120GAP and neurofibromin, which suggests that there may be unique mechanisms by which these proteins interact with cell surface receptors and p21ras. To investigate the functional elements of GAPIII, constructs containing different segments of GAPIII were transformed into *ira1* mutant yeast. In such yeast cells, which lack their own GAP, IRA1 protein, GAPIII was tested for complementation of the missing GAP activity. Constructs containing either the GRD domain alone or the GRD domain with the full PH/btk domain complement the *ira* mutation and induce down-regulation of ras proteins, whereas constructs containing the GRD domain and the PH/btk domain with no btk domain have only a weak ability to complement this mutant. These data suggest that the bik domain may have some influence on the ability of the GRD domain of GAPIII to activate ras GTPase in yeast. Since GAPIII is expressed mainly in the adult brain, especially in neurons, it may play a significant role for ras-mediated signal transduction in mature neurons. (Supported by the Natl. Mult. Scler. Soc.)

346.11

NEURONAL LOCALIZATION OF THE *dbl* GENE PRODUCT (*Dbl*)

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The *dbl* oncogene was initially identified from a human diffuse B-cell lymphoma DNA in NIH3T3 transfection assay. Its normal cellular homologue codes for a 115 kD protein which is known to have GDP/GTP exchange protein activity towards the *rho* family small GTP-binding protein members including *RhoA* and *Cdc*42HS. Several proteins with *dbl* homology regions have been identified and some of these are enriched in brain. We have used a polyclonal rabbit antibody generated against a peptide sequence (residues 818-831) found uniquely in mouse *Dbl* and outside of the *dbl* homology region to map the immunohistochemical distribution of *Dbl* in rat brain. This antibody specifically recognized *Dbl* in transformed NIH3T3 fibroblasts stably overexpressing this protein. Rat brain *Dbl* immunoreactivity was confined to neurons with high level of expression in hippocampus, cortex and brainstem. Cerebellar neurons, however, showed minimal immunoreactivity. Neuronal *Dbl* staining was eliminated by competition with the *Dbl* specific peptide (818-831). Western blots of hippocampal extracts identified a 115 kD band which was eliminated by *Dbl* peptide competition. Whole body sections of neonatal rat pups also showed prominent neuronal immunoreactivity. The high level of expression and regional heterogeneity of *Dbl* immunoreactivity in rat brain suggests a role for this protein in neuronal signalling pathways.

347.1

ORAL CONTRACEPTIVE USERS SHOW BLUNTED FREE CORTISOL RESPONSES TO PSYCHOLOGICAL AND PHYSICAL STRESS. C. Kirschbaum, K.-M. Pirke and D.H. Hellhammer*. Center for Psychobiology and Psychosomatic Research, University of Trier, D-54286 Trier, Germany.

In three studies the effect of oral contraceptives on free cortisol responsiveness was investigated. In the first two studies, saliva cortisol responses to the psychological stress of public speaking and mental arithmetic were investigated in women using oral contraceptives (OC; n=28) and in control women (n=29). While no significant differences in baseline levels were observed, altered adrenocortical responses were found in OC users. These women showed significantly attenuated cortisol responses to the experimental stressor in both studies, with peak cortisol levels only slightly elevated above baseline levels. These differences could not be attributed to affective responses as indicated in ratings on visual analogue scales assessing subjective stress responses (Study 2). A comparision between control women and men (n=19) again revealed the previously reported result of larger cortisol responses to psychological stress in males. In a third study, free cortisol, heart rate and affective responses were studied in 31 women using oral contraceptives (OC) and 22 control women performing bicycle ergometry until exhaustion. While OC users and control women showed significant increases in both free cortisol and heart rate, OC users had significantly attenuated cortisol responses. Individual work load, peak heart rates and affective responses were similar in both groups. Menstrual or pill cycle phase did not affect any of the parameters studied. It is concluded that the use of OC may interfer with the adrenocortical response to psychological and physical stress and should therefore be viewed as an important intervening variable. While it appears that differences at a supra-adrenal site is responsible for the observed cortisol hyporesponsiveness in OC users, the physiological mechanisms remain to be elucidated.

347.2

NON-GENOMIC MECHANISM OF GLUCOCORTICOID INHIBITION ON ADRENOCORTICOTROPHIN EXPRESSION: POSSIBLE INVOLVEMENT OF PERTUSSIS TOXIN-SENSITIVE GTP-BINDING PROTEIN.

Y. Iwasaki, Y. Oiso, Y. Aoki, H. Nagasaki, A. Oka* and H. Saito. First Department of Internal Medicine, Nagoya University School of Medicine, Nagoya 466; Department of Pediatrics, University of Tokyo, Tokyo 113, Japan.

We investigated the cellular, especially non-genomic mechanism of glucocorticoid negative feedback on corticotroph cells using the AtT-20 mouse corticotroph tumor cell line. Dexamethasone (DEX; 100 nM) potently suppressed forskolin-induced cAMP increase / ACTH release, and proopiomelanocortin gene expression. When *de novo* protein synthesis was blocked by cycloheximide (CHX; 100 μM), DEX still suppressed cAMP increase and ACTH secretion, although less potently than without CHX. The inhibition may be at the level of adenylate cyclase and distal to cAMP generation. Interestingly, pre-treatment of the cells with pertussis toxin (PTX; 50 ng/ml) abolished the cAMP decrease and partially eliminated ACTH inhibition by DEX, and combined treatment with CHX and PTX completely abolished the DEX suppression of ACTH secretion. These results suggest that non-genomic as well as genomic mechanisms are involved in the glucocorticoid negative regulation of adrenocorticotropin expression, and PTX-sensitive GTP-binding protein (G-protein) might, at least partly, participate in the non-genomic effect. This may be the first report showing the functional involvement of G-protein in some of the biological effects of glucocorticoid hormone.

347.3

GLUCOCORTICOIDS REGULATE SYNTHESIS OF HEAT-INDUCIBLE PROTEINS IN RAT BRAIN. C.S. Barr * and L.A. Dokas. Departments of Neurology and Biochemistry/ Molecular Biology, Medical College of Ohio, Toledo, OH 43699-0008.

Age-related increases in the secretion of glucocorticoids are thought to be involved in the reduction in cognition which is associated with aging, dementia, and other neuropathologies involving damage to the limbic system. It is thought that corticosteroids place neurons in "endangerment", thus rendering them susceptible to various metabolic insults, with a progressive reduction in the number in hippocampal neurons concomitant with aging. Using heat shock of rat brain slices as a model for cellular insult, we have found the synthesis of several proteins to be altered 4 hr following a single corticosterone injection: a 25 kDa protein is down-regulated in the synaptosomal-mitochondrial fraction of the hippocampus; a 47 kDa protein is down-regulated in the hippocampus, cortex and cerebellum; and a 28kDa, cytosolic protein is up-regulated in the cerebellum. Since these effects are mimicked by the administration of RU-23862, a specific GR or Type II receptor agonist, it is likely that the regulation of these proteins occurs predominantly in the presence of elevated titers of circulating glucocorticoids. It is, therefore, possible that these corticosteroid-sensitive proteins are molecular markers for the endangered state which is thought to occur in the senescent brain as a result of increases in the cumulative exposure to glucocorticoids.

347.4

ANDROGENS DECREASE GLUCOCORTICOID RECEPTOR mRNA IN THE CA1 REGION OF THE RAT HIPPOCAMPUS. J.E.Kerr*, G. Hejna, S.G.Beck, R.J.Handa. Depts. of Pharmacol. and Cell Biol., Neurobiol., and Anat. Loyola Univ. Chicago, Stritch School of Medicine, Maywood, IL 60153.

Androgen, mineralocorticoid and glucocorticoid receptors (AR, MR and GR) are ligand-activated transcription factors that influence gene expression in CNS neurons. High levels of expression of AR, MR and GR mRNA have been found in the CA1 region of the rat hippocampus and suggest that cross-talk between these receptors could occur at some level of transcription. To begin to investigate this hypothesis, we examined the regulation MR, GR and AR mRNA expression in the rat hippocampus by both androgens and glucocorticoids. Three month old male Sprague-Dawley rats were either castrated for three weeks, castrated and immediately implanted with two Silastic capsules filled with dihydrotestosterone propionate (DHT), or left gonadally intact. Four days prior to sacrifice, these animals were either adrenalectomized (ADX'd), or sham operated. GR, MR and AR mRNA levels were measured in the CA1, CA3 and dentate gyrus (DG) cell regions using *in situ* hybridization. In the CA1 region, DHT treatment decreased GR mRNA levels to 69 percent of levels found in gonadally intact rats ($p < 0.01$) and prevented the ADX-induced increases in GR mRNA observed in the gonadally intact and castrated animals ($p < 0.01$). No changes in were observed in the DG, where AR expression is low. ADX also increased MR mRNA expression in the CA1 region, and there was no effect of DHT on MR mRNA levels. AR mRNA levels in the CA1 region were unchanged across all treatment groups. *In vitro* binding studies revealed almost complete nuclear occupancy of hippocampal AR in DHT-treated castrates and no appreciable binding of DHT to hippocampal GRs ($K_i > 1000$ nM) which suggest that androgen regulation of GR mRNA is occurring through AR binding. These data demonstrate an interaction between AR and GR which may prove critical in hippocampal development, memory and cell survival.

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347.5

CHRONIC GABAERGIC DYSFUNCTION IN THE DORSOMEDIAL HYPOTHALAMUS DISRUPTS THE NORMAL DIURNAL RHYTHM OF CORTICOSTERONE SECRETION IN THE RAT. S.R. Keim* and A. Shekhar, Dept. of Psychiatry, Indiana University Medical Center, Indianapolis, Indiana 46202.

Previous studies have demonstrated that acute $GABA_A$ receptor blockade in the dorsomedial hypothalamus (DMH) of rats elicits a panic-like response and significant increases in plasma ACTH and corticosterone (CS) levels. It has been suggested that the suprachiasmatic nucleus may regulate the circadian rhythm of corticotrophin-releasing hormone secretion by the paraventricular nucleus via a pathway that has an intermediate synapse within the DMH (Buijs et al., J.Comp.Neurol.335:42-54,1993). Therefore, the present study was conducted to test if chronic dysfunction of GABA inhibition within the DMH would disrupt the normal circadian rhythm of CS secretion. At baseline, plasma samples for CS measurement were taken at 8.00 A.M., 12.00 Noon, and 4.00 PM in a group of rats fitted with femoral arterial catheters. These rats were then implanted with stereotaxically placed chronic Alzet mini-pumps filled with the GABA synthesis inhibitor, l-Allylglycine (l-AG) into the DMH. After 4 days of chronic l-AG infusion into the DMH, plasma CS samples were once again obtained at above time intervals. The levels of plasma CS were significantly increased at times 8.00 A.M. and 4.00 P.M. compared to baseline, showing a clear disruption of CS diurnal rhythm. (Supported by MH 45362)

347.6

FEEDBACK INHIBITION OF cAMP-INDUCED POMC GENE TRANSCRIPTION BY GLUCOCORTICOIDS: VARIATION IN cFOS PHOSPHORYLATION

A-L Boutillier* and J. L. Roberts. Fishberg Research Center for Neurobiology. Mount Sinai Medical Center, NY, NY 10029.

In the anterior pituitary, the POMC gene is transcriptionally activated by Corticotropin Releasing Hormone (CRH), whose receptors are positively coupled to adenylate cyclase, and down-regulated by glucocorticoids. Using a corticotroph-derived cell line, the AtT20 cells, we have previously shown that CRH induces cFos, thus leading to the activation of POMC gene transcription through an AP1 site within the first exon. Several regulatory interactions between the AP1 and the glucocorticoid receptor (GR) have been reported and we addressed these interactions in AtT20 cells.

Our results show that overexpression of cFos counteracts the inhibitory effect of dexamethasone on CRH-induced POMC gene transcription. This inhibition is mediated at least in part through GR's ability to block AP1 function at the exonic site. Moreover, co-treatment of AtT20 cells by CRH and dexamethasone strongly reduces the AP1 binding activity. In an attempt to decipher the molecular mechanisms by which this interaction occurs, we investigated whether it is dependent on the phosphorylation status of the cFos protein. We show that cFos is rapidly phosphorylated in response to CRH. Interestingly, co-treatment of the cells with CRH and dexamethasone significantly reduced the phosphorylation of cFos. 2D tryptic map analysis of phosphorylated cFos revealed a different pattern when dexamethasone is co-applied with CRH.

We propose that the interaction of the GR with cFos masks a functional phosphorylation site, resulting in the inactivation of AP1 activity. cFos mutation analyses are currently under investigation in order to localize these phosphorylation sites.

347.7

DIAZEPAM'S INHIBITORY EFFECT ON STRESS-INDUCED CORTICOSTERONE LEVELS IS INFLUENCED BY PRIOR EXPOSURE TO REPEATED RESTRAINT AND DIAZEPAM. B.A. Kalman*, M. A. Cole, P.J. Kim, R.L. Spencer. Behavioral Neuroscience Div., Dept. of Psychology, University of Colo., Boulder, CO 80309.

Prior research has shown diazepam acts as a potent anxiolytic in stressful contexts and, paradoxically, as a stimulator of HPA axis activity. We have examined the effects of diazepam on plasma corticosterone (CORT) levels in the rat utilizing a repeated restraint paradigm. Consistent with most literature, it was revealed that diazepam administered IP (1.5, 3.0, or 6.0 mg/kg) 1 hr prior to restraint increased non-stress, baseline plasma CORT levels in a dose-dependent fashion. CORT levels of diazepam-injected rats did not differ from the stress levels of controls during the 1 hr restraint-stress procedure. However, diazepam was able to dramatically attenuate the stress-induced increase in CORT when preceded by 4 days of repeated diazepam injections combined with daily restraint. This decrease was greater than that produced by 4 days of prior restraint stress alone (i.e., habituation to a repeated stressor) and was apparent even when we failed to observe significant habituation in repeatedly restrained controls. Furthermore, diazepam by itself given once a day for 4 consecutive days prior to administering diazepam in conjunction with restraint was not sufficient to decrease stress-induced levels of CORT. These results indicate that the inhibitory effect of diazepam on the HPA axis stress response is not observed initially but is apparent following exposure to repeated restraint and diazepam. Supported by the UROP, JFDA and GIA programs of the Univ. of Colorado.

347.8

OSMOLALITY-DEPENDENT STEROID FEEDBACK REGULATION OF VASOPRESSIN GENE EXPRESSION. L.T. Knapp, K.A. Berghorn†, W.-W. Lei* G.E. Hoffman†, and T.G. Sherman. Depts of Neuroscience and †Neurobiology, University of Pittsburgh, Pittsburgh PA 15260 USA.

We have recently demonstrated the *de novo* induction of glucocorticoid receptors (GR) in AVP magnocellular neurons in the chronic hypoosmolar rat (Endo. 136: 804, 1995). We have investigated the possible role for this GR in establishing osmolality-dependent steroid feedback inhibition on AVP expression, and whether this feedback regulation constitutes a direct or indirect action of GR on the AVP gene. Chemical adrenalectomy with metyrapone, an inhibitor of adrenal glucocorticoid synthesis, results in an induction of nuclear AVP hnRNA levels in hypoosmolar, but not in normoosmolar, rats, as measured by intron-directed *in situ* hybridization and a competitive RT-PCR assay. Preliminary results indicate, however, that the actions of metyrapone are not mimicked by RU486 treatment. Because RU486 does not antagonize the repressive actions of GR on transcription, these results may provide evidence for the direct actions of GR on the AVP gene. Furthermore, we hypothesize that the hypoosmolar inhibition of AVP expression and the hypoosmolar induction of GR expression are coordinately mediated by decreased nuclear levels of phosphoryl-CREB (pCREB). As measured by western analysis and immunocytochemistry, levels of pCREB were increased 2.5-fold in magnocellular AVP neurons with acute hyperosmolality and were undetectable with hypoosmolality. Treatment of hypoosmolar rats with naloxone predominantly blocked the induction of magnocellular GR and re-established detectable levels of pCREB (60% of control). These data support the hypothesis that pCREB is an important reciprocal regulator of AVP and GR expression. The induction of GR expression in hypothalamic AVP neurons represents a novel mechanism for the establishment of physiology-specific steroid feedback control. (NSF grant BNS-9021307)

347.9

Glucocorticoids block the compensatory release of inhibitory neurotransmitters following an excitotoxic insult to the hippocampus. R. Dash, H. Maecker, A. Smith, F. Weiss, A. Ngai, R. Winn, R. Sapolsky* Dept. Biol. Sciences, Stanford Univ., Stanford, CA 94305-5020; Dept. Neuropharm., The Scripps Research Institute, La Jolla, CA 92037; Dept. Neurol. Surgery, Univ. Washington School of Medicine, Seattle, WA. 98195

Glucocorticoids (GCs), the adrenal steroid hormones released during stress, can endanger hippocampal neurons, impairing their capacity to survive excitotoxic insults. Recent data suggest a mechanism for this endangerment. GCs inhibit glucose uptake into hippocampal neurons and, consequently, such cells are less capable of carrying out the costly tasks of controlling concentrations of synaptic glutamate and of free cytosolic calcium during excitotoxic insults; the result is a greater likelihood of the neuron dying. We now report an additional way in which GCs might compromise hippocampal neurons during an insult. A number of events have been characterized in neurons that can be viewed as protective, compensatory responses to excitotoxic insults. Among them are the release of inhibitory neurotransmitters such as GABA, adenosine and taurine. We observe that GCs block this protective response. Rats were adrenalectomized and maintained either GC-free or exposed to constant GC concentrations in the upper physiological range. Three days later, microdialysis of the hippocampal CA3 cell field was carried out. Excitotoxic seizures were induced with kainic acid in the perfusate; extracellular accumulation of GABA, adenosine and taurine was measured by HPLC. Seizures triggered a significant accumulation of all three neurotransmitters in adrenalectomized rats; GCs blocked the GABA and adenosine responses. Thus, GCs may exacerbate excitotoxic insults by preventing the protective release of these inhibitory neurotransmitters.

347.10

RATE-SENSITIVE GLUCOCORTICOID FEEDBACK INHIBITION IN NORMAL HUMANS AND DEPRESSED PATIENTS. D.K.Kim*, B.L.Kim, B.H.Yu, K.S.Hong, J.Ritchie, S.Austin, B.J.Carroll. Department of Psychiatry, Samsung Medical Center, Seoul 135-230, Korea.

This study aims to demonstrate the rate-sensitive glucocorticoid fast-feedback(FFB) inhibition of adrenocorticotropic hormone(ACTH) with ovine corticotropin-releasing factor(oCRF) administration, and to determine the possible site(s) on which glucocorticoid exerts its FFB inhibition of ACTH secretion in humans. A third objective is to document the importance of this FFB as a confound in the CRF stimulation test in depressed patients(DEP) by comparing the ACTH response to oCRF between DEP and normal controls.

We examined the post-CRF secretory patterns of ACTH, arginine vasopressin(AVP), and cortisol in 7 normal subjects and 3 DEP with a sampling schedule of plasma at three-minute intervals. All three hormones demonstrated significant secretory responses to submaxial dose of oCRF (1 μg/kg body weight), with AVP increaing from 1.33 to 7.77 pg/ml, ACTH from 2.98 to 14.52 fmol/ml, and cortisol from 4.44 to 25.93 μg/dl. The decline in plasma ACTH followed by its initial peak coincided with the rising phase of the cortisol response in normal subjects, then a rebound of ACTH after plasma cortisol reached a high plateau level. The ACTH response to oCRF was significantly reduced by the ramp of cortisol, however, the effect of oCRF on ACTH release was more affected by the cortisol preinfusion. The DEP patients appear to shut off secretion earlier, leading to a significantly blunted integrated ACTH response in comparison with normal subjects.

This early data suggests that FFB in the oCRF test is common in human subjects. FFB is a major, uncontrolled confound in studies to date of the CRF stimulation procedure in DEP, and Pituitary gland might be the site of action of this FFB. Additional data is being collected and will be presented.

347.11

FOOD RESTRICTION ALTERS CIRCADIAN VARIATION OF HIPPOCAMPAL CORTICOSTEROID RECEPTOR BUT NOT 5-HT2C RECEPTOR mRNA EXPRESSION. M.C. Holmes*, K.L. French and J.R. Seckl, Dept. of Medicine, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, Scotland, UK.

Animals housed in pairs, in a light controlled room (lights on 07.00-19.00h) and given food and water *ad libitum* exhibited a monophasic rhythm in plasma corticosterone levels, glucocorticoid receptor (GR) and 5-HT2C receptor mRNA expression in the hippocampus. To determine factors involved in the circadian regulation of these receptor transcripts, a set of animals were only allowed access to food for 2h (10.00-12.00h). The rats were entrained to food restriction for 3 weeks and showed a biphasic rhythm in plasma corticosterone levels (peaking at 08.00 and 20.00h). The restriction of food to the daylight hours produced a shift in normal feeding and behaviour, both thought to be mediated by 5-HT2C receptors. However, the diurnal variation of 5-HT2C mRNA expression in the ventral hippocampus (vCA1 and subiculum) was not altered by food restriction, suggesting that 5-HT2C receptor mRNA is regulated by light-dark cues rather than feeding behaviour. GR mRNA expression was altered in the food-restricted animals to give a biphasic rhythm similar to the rhythm in plasma corticosterone but displaced by 4h. In animals with no diurnal variation of plasma corticosterone (adrenalectomized + corticosterone pellet s.c.) the rhythm in both GR and 5-HT2C receptor mRNA expression was abolished. Hence, we suggest that while circadian variations of hippocampal GR mRNA expression are closely regulated by plasma glucococorticoid levels, 5-HT2C receptor mRNA expression is regulated by glucocorticoid levels in conjunction with other factors associated with light-dark cues.

347.12

EFFECT OF NEONATAL GONADECTOMY AND ADULT HORMONE REPLACEMENT ON HYPOTHALAMIC-PITUITARY-ADRENAL STRESS RESPONSE AND BRAIN CORTICOSTEROID RECEPTOR LEVELS IN RATS. B.F. Furey, M. Child, & C.M. McCormick*, Department of Psychology, Bates College, Lewiston, ME 04240, USA.

Hypothalamic-pituitary-adrenal (HPA) function is influenced by circulating levels of sex steroids. However, the consequences of neonatal gonadectomy (GDX) on adult HPA function is unknown. Male and female rats were either GDX on the first day of life (neoGDX) or sham-operated. At three months, half of the shams were gonadectomized (adultGDX). All received jugular catheters and s.c. implants of either cholesterol (sham), testosterone (T: males), or estradiol benzoate and progesterone (EB-P: females). Five days following surgery, HPA function was assessed by determining plasma corticosterone (CORT) levels prior to, and at intervals following, 20 min of restraint stress. NeoGDX and adultGDX both resulted in increased levels of CORT in males, and decreased levels in females, compared to same-sex shams. EB-P increased plasma CORT levels in both neoGDX and adultGDX. In contrast, T implants caused a greater reduction of CORT levels in adultGDX than neoGDX. These differences in CORT response to stress are not explained by differences in corticosterone binding globulin (CBG) levels across the groups. Further, the differences are not related to Type I and Type II receptor levels in various brain areas: NeoGDX and intact animals did not differ in [3H]CORT or [3H]dexamethasone binding in the hippocampus, septum, frontal cortex, or hypothalamus. These data suggest that the HPA of females remains sensitive to the influence of sex hormones in adulthood despite a lack of ovarian secretions throughout development. In contrast, neoGDX in males reduced sensitivity of the HPA to testosterone. Longer regimens of T replacement are necessary to determine whether or not there are organizational effects of neonatal androgens on the development of the HPA.

347.13

PROTEIN LEVELS OF THE GLUCOCORTICOID RECEPTOR, MINERALOCORTICOID RECEPTOR AND HEAT SHOCK PROTEIN-90 IN THE RAT HIPPOCAMPUS: EFFECT OF ADRENALECTOMY.

M.I. Morano*, C.A. Caamaño, D.M. Vázquez, S.J. Watson and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, MI.

The hippocampal corticosteroid receptors (glucocorticoid receptor or GR and mineralocorticoid receptor or MR) are involved in the negative feedback of the hypothalamic-pituitary-adrenal axis. Furthermore, the association of these cortiocosteroid receptors with a protein complex which involves several heat shock proteins, including hsp-90, is thought to be critical to their ability to bind steroids. Previous *in vitro* studies demonstrate that the removal of circulating corticosterone by adrenalectomy (ADX) increases the hippocampal binding capacity of both receptors, GR and MR. In the present study we examined the GR and MR binding capacities and the protein levels of GR, MR and hsp-90 by Western blot analysis in hippocampal cytosols of rats corresponding to the following groups: 1) unhandled intact, 2) 12 h ADX, 3) 12 h SHAM, 4) 7 d ADX, 5) 7 d SHAM, 6) 7 d ADX + Dexamethasone (Dex) in the drinking water. No change in protein levels was observed in GR or MR in the SHAM-operated groups or after 12 h ADX with respect to the intact group. However, 7 d ADX up-regulated the GR and MR protein levels to more than 100 %. Dex replacement decreased the ADX effect over GR to 50 % of the control group. In contrast, the same cytosolic preparation showed similar hsp-90 protein levels in all the groups except in the ADX + Dex which exhibited a significant decrease. In sum, we conclude that the increase of GR and MR binding after adrenalectomy is due to the up-regulation of the protein levels of these receptors in the absence of glucocorticoid.

347.14

PRENATAL ETHANOL EXPOSURE AND GLUCOCORTICOID RECEPTORS.

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One effect of prenatal ethanol exposure is offspring hyperresponsiveness to stressors. We have previously shown that prenatal ethanol exposure does not alter hippocampal MR (mineralocorticoid receptor, Type I) or GR (glucocorticoid receptor, Type II) densities or binding affinities of male and female rats under nonstressed conditions. Furthermore, following stress, prenatal ethanol exposure did not differentially down-regulate hippocampal GRs, nor alter densities of hippocampal MRs or hypothalamic MRs and GRs in females. This study examined MR and GR densities in the hippocampus, anterior pituitary, hypothalamus and prefrontal cortex in rats that were nonstressed or exposed to chronic intermittent stress (3 wks, twice daily). The subjects were adult male and female offspring from prenatal ethanol exposed (E, 36% EDC), pair-fed (PF) and ad libitum-fed control (C) rats.

Chronic stress caused decreased body weights and increased adrenal/body weight ratios in both sexes. In males, hippocampal GR densities were lower in E than PF rats following stress, overall hypothalamic GR densities were higher in E than PF rats, and overall pituitary GRs were up-regulated in all groups following stress. In females, overall hippocampal GRs were marginally up-regulated following stress, with the up-regulation being significant only in the C group; overall hippocampal MRs were up-regulated and overall hypothalamic GRs were down-regulated in all groups following stress. Thus, this stress regimen differentially affected GR and MR densities in males and females at the different CNS sites. Importantly, there was a subtle effect of prenatal ethanol exposure on GR densities.

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347.15

BINDING CHARACTERISTICS OF CORTICOSTEROID RECEPTORS IN THE RAT HYPOTHALAMUS ARE SEXUALLY DIFFERENTIATED.

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We recently reported sexually differentiated responses of the hypothalmo-pituitary-adrenal (HPA) axis (Patchev et al., FASEB J., 9: 419). In this work we examined whether hypothalamic neurons cultured from juvenile (21 d.o.) male and female rats, as well as from female rats that had been neonatally-estrogenized in vivo (according to a protocol known to defeminize the brain), display differences in Type I and II corticosteroid receptor (CR) binding. After 10-12 days in vitro, binding CR sites were characterized in intact cells. We used ^{3}H-aldosterone and ^{3}H-dexamethasone to label Type I and II CR, respectively. Hypothalamic cells from male, female and neonatally-estrogenized female rats showed no specific 3H-aldosterone binding sites. Specific and saturable dexamethasone-binding sites were found to occur in greater numbers (25-30%) in female-derived cells than in male-derived cells; this result is consistent with that already published for Type II CR gene transcription in the hypothalamus. The characteristics of dexamethasone binding sites in cells originating from neonatally-estrogenized females were similar to those found in the cells from males. These findings provide further support for the notion that the brain-organizing effects of gonadal steroids during perinatal life encompass not only the well-known neural mechanisms controlling reproduction, but also extend to the regulation of the HPA axis.

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347.16

DEXAMETHASONE SUPPRESSION OF HPA AXIS RESPONSE TO ACUTE STRESS: RELATIONSHIP TO ESTIMATES OF CORTICOSTEROID RECEPTOR OCCUPANCY IN BRAIN AND PITUITARY.

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Dexamethasone (DEX) is a potent synthetic glucocorticoid that has been widely used in biological psychiatry as a probe (DEX Suppression Test; DST) for testing HPA axis sensitivity to corticosteroid negative feedback. We have examined in the rat the dose-related ability of s.c. injections of DEX (1-50 µg/kg) to suppress the corticosterone response to a subsequent (90 min interval) acute stress (1 hr restraint). The 50µg/kg dose of DEX almost completely eliminated the corticosterone response (80% reduction), and the 25µg/kg dose had a partial effect (60% reduction). We also examined the effect of the high dose of DEX on available Type I and Type II corticosteroid receptors in the pituitary and the brain of rats that had been adrenalectomized for 24 h (in order to remove competing endogenous adrenal steroids). We found that DEX (50µg/kg), 30 and 90 min after injection, had no effect on available Type I or Type II corticosteroid receptor binding in the hippocampus, whereas DEX produced a selective and significant time-related decrease in available Type II receptors in the pituitary. From these studies we infer that a dose of DEX that was near maximal effectiveness in suppressing an acute stress-induced rise in corticosterone, acted by selectively occupying Type II receptors in the pituitary. These results support other studies suggesting that DEX in the context of a DST may act as a selective probe of the effects of acute activation of pituitary Type II corticosteroid receptors on HPA axis activity. Supported by UROP, JFDA and GIA programs of the Univ. of Colorado.

347.17

TIME COURSE OF CORTICOSTEROID AND SEROTONIN RECEPTOR GENE EXPRESSION IN THE RAT HIPPOCAMPUS FOLLOWING ACUTE MDMA.

J.L.W. Yau*, J. Noble and J.R. Seckl Dept. Medicine. Western General Hospital Edinburgh, U.K.

Glucocorticoids and serotonin (5-HT) interact in the hippocampus, altering electrophysiological, neuroendocrine and behavioural parameters. Chronic methylenedioxymethamphetamine (MDMA) causes selective 5-HT neuronal degeneration, decreases mineralocorticoid (MR) and glucocorticoid (GR) receptor and increases 5-HT$_{2C}$ receptor gene expression in the hippocampus. Acutely, MDMA releases 5-HT from nerve terminals, prior to damaging axons. In the present study, we examined the effects of acute MDMA on hippocampal corticosteroid and 5-HT receptor subtype mRNA expression using *in situ* hybridisation histochemistry. Rats were killed 9h, 16h and 48h after a single injection of MDMA (20mg/kg, s.c). Hippocampal neuronal MR mRNA expression decreased (13% fall in CA3 and CA4, $p < 0.05$) at 9h, increased (69% rise, $p < 0.01$) at 16h and returned to control levels by 48h. In contrast, hippocampal neuronal GR mRNA expression was decreased (41% fall, $p < 0.01$) at 9h, remaining decreased at 16h and returned to control levels by 48h. 5-HT$_{1A}$ receptor mRNA expression was decreased in CA1(20% fall, $p < 0.05$) at all time points. 5-HT$_{2A}$ receptor mRNA expression remained unaltered. Effects on other 5-HT receptor gene expression are currently being examined. These results show a differential regulation of hippocampal MR and GR after acute MDMA and that induction of MR mRNA expression requires several hours following acute MDMA-induced 5-HT release. The decreased GR mRNA expression might be a consequence of the MR changes or reflects the direct effects of increased corticosterone secretion.

347.18

EVIDENCE FOR REGION-SPECIFIC VARIATION IN GLUCOCORTICOID RECEPTOR EXPRESSION IN BRAIN TISSUE FROM NEONATAL PIGS.

S. Weaver[1], D. O'Donnell[1], W.T. Dixon[1], and M.J. Meaney[2]. [1]Dept. Animal Sci., Univ. of Alberta, Edmonton, Alberta, Canada T6G 2P5. [2]Depts. of Neurology & Neurosurgery, and Psychiatry, Douglas Hosp. Res. Ctre., McGill Univ., Montreal, Quebec, Canada H4H 1R3.

Brain glucocorticoid receptors (GR) play a crucial role in the negative feedback effect of cortisol during and after exposure to a stressor. Western blotting has been used to detect the glucocorticoid receptor (GR) in a variety of tissues. Most tissues/cells exhibit a doublet with an apparent molecular weight of 90 to 97 KDa. In this study the pituitary gland, hypothalamus, frontal cortex, and hippocampus was collected from 14 day old pigs and western blotting was performed for GR receptor detection. A protein doublet of 95 and 87 kDa was detected in all regions examined with a more intense signal for the 87 kDa band in both the hypothalamus and pituitary gland. In the frontal cortex and hippocampus both bands exhibited a more uniform signal density. Hollenberg et al. (1985, Nature. 318:635) have shown that there are two isoforms of GR designated α and β with the α form electrophoretically separating at 95 kDa and β separating more rapidly at a lower molecular weight. In the current experiment the upper band may represent the α form in that it separates at approximately 95 kDa with the lower band, which separates at 87 kDa, representing the β form. The two bands we have detected may represent a neural source of the two isoforms of GR and these isoforms may have distinct roles in the differential regulation of GR in the hypothalmus and pituitary gland compared to the hippocampus and frontal cortex.

347.19

INTRACEREBROVENTRICULAR INJECTIONS OF DEXAMETHASONE DO NOT PROMOTE INSULIN RELEASE IN LAN/CP ADRENALECTOMIZED RATS. K. DeOre, K. Kamara, C. Hansen, O. Michaelis and T. Castonguay*Univ. of Maryland - College Park, USDA - Beltsville, MD and NIH, Bethesda, MD

Genetically obese (ob/ob) adrenalectomized (ADX) mice injected with dexamethasone ICV increase circulating insulin within 30 min of treatment. The present studies were conducted to learn if ADX LAN/CP rats would also respond to ICV dexamethasone by increasing circulating insulin. Lean and obese LAN/CP rats were individually housed in standard hanging cages and allowed free access to Purina Chow and water. Rats were assigned to one of two experimental treatments, with one half given bilateral adrenalectomies. The remaining rats were given sham operations. After surgery, all ADX rats were given saline in place of drinking water. They were allowed to recover from surgery for 4 days and were then injected ICV with 250 ng dexamethasone in 2 ul CSF. Control rats were injected with 2ul CSF. Tail blood samples were collected at the time of injection, and again 15, 30 60, 120 and 180 minutes post injection. Blood was centrifuged and plasma was assayed for insulin via RIA. Lean sham operated rats had basal insulin concentrations of 158 ± 30 uU/ml. By contrast, obese sham operated rats had basal insulin concentrations of 438 ± 87 uU/ml. Adrenalectomy decreased obese basal insulin concentrations (160 ± 23 uU/ml $p<.05$) but not lean values (95 ± 20 uU/ml; $p>.05$). Dexamethasone injections failed to promote increases in basal levels in either lean or obese ADX or sham operated groups. These data suggest that although ADX suppresses insulin in both mice and rats, the extent of glucocorticoid control over pancreatic insulin release differs between these two rodent species. Supported in part by the Howard Hughes Foundation and by NIH grant DDK42446 and a grant from the Maryland Agricultural Experiment Station Competitive Grants Program.

347.20

11ß-HYDROXYSTEROID DEHYDROGENASE (11ß-HSD) IN HIPPOCAMPAL CELL CULTURES IS A REDUCTASE WHICH REACTIVATES INERT 11-DEHYDROCORTICOSTERONE THUS POTENTIATING KAINIC ACID NEUROTOXICITY. J. R. Seckl* and V. Rajan. Dept. Med., West. Gen. Hosp. Edinburgh, UK.

11ß-HSD converts corticosterone to inert 11-dehydrocorticosterone (DHC). Hippocampal 11ß-HSD is induced by glucocorticoids in vivo, suggesting it may attenuate glucocorticoid neurotoxicity. There are 2 isozymes; 11ß-HSD1, a bi-directional, NADP-dependent enzyme, and 11ß-HSD2, an NAD-dependent exclusive 11ß-dehydrogenase (corticosterone-inactivating). Primary fetal rat hippocampal cell cultures (~85% neurons) had 11ß-HSD activity. This was NADP-, not NAD-dependent, and 11ß-HSD1 mRNA was expressed. Unexpectedly, the reaction direction in intact cells was 11ß-reduction (reactivation of inert DHC), although homogenization revealed the enzyme capable of 11ß-dehydrogenation when removed from its cellular context. Dexamethasone (10^{-7}M) increased 11ß-HSD activity (102% rise), increasing only 11ß-reduction in intact cells. To determine the functional relevance of hippocampal 11ß-reductase, glucocorticoid potentiation of kainic acid (KA, 10^{-5}M) neurotoxicity was examined. Pretreatment of hippocampal cells with corticosterone (10^{-5}M) reduced survival with KA. 11ß-HSD activity was inhibited by carbenoxolone (10^{-6}M). Carbenoxolone alone had no effect on cell survival after KA, nor altered corticosterone efficacy. DHC (10^{-5}M) also potentiated KA neurotoxicity, however this effect was lost if 11ß-HSD was inhibited with carbenoxolone. Measures to attenuate hippocampal 11ß-reductase may reduce neuronal vulnerability to glucocorticoids.

OSMOREGULATION: MAGNOCELLULAR ENDOCRINE NEURONS

348.1

EFFECTS OF CASTRATION AND TESTOSTERONE ON VASOPRESSIN RELEASE. Kerry L. Swenson*, C.D. Sladek. Finch University of Health Sciences/The Chicago Medical School, North Chicago, IL 60064.

Previous experiments demonstrated that gonadectomy prevented the increase in hypothalamic VP mRNA content induced by chronic administration of an oral salt load. However, neither posterior pituitary VP content nor hypothalamic VP mRNA content in hydrated rats was altered. Subsequent studies demonstrated that treatment of male gonadectomized rats with testosterone restored the hyperosmolar-induced increase in VP mRNA (Crowley and Amico, Endocrinology 133:2711-2718, 1993). Experiments were performed to determine if castration or testosterone altered osmotically stimulated VP release in vitro. Perifused explants of the hypothalamo-neurohypophyseal system were obtained from sham and gonadectomized male rats. There was no significant difference in VP release (determined by RIA of perifusate fractions) stimulated by a ramp increase in osmolality of the culture media (40 mosm/6 hrs) between the two groups. RIA also showed that there was no testosterone detectable in the media. Therefore, experiments were then performed to determine if the addition of testosterone to the perifusion media altered osmotically stimulated VP release. Explants were exposed to physiological levels of testosterone, 3 ng/ml, throughout the perifusion experiment. Testosterone completely inhibited the osmotically stimulated increase in VP. In contrast to the in vivo studies, these results suggest that castration has no effect on the osmotically induced increase in VP release and that it is inhibited by testosterone in vitro. Supported by NIH Grant #NS27975.

348.2

A ROLE FOR NON-NMDA EXCITATORY AMINO ACID RECEPTORS IN THE OSMOTIC REGULATION OF VASOPRESSIN mRNA. C.D. Sladek* and H.E. Sidorowicz. Finch University of Health Sciences/Chicago Medical School, North Chicago, IL, 60064.

Vasopressin (VP) mRNA content is increased by chronic dehydration. This effect is mimicked by exposure of hypothalamo-neurohypophyseal explants to a ramp increase in osmolality for 6 hours. Previous studies demonstrated that this induction of VPmRNA content requires synaptic input and was blocked by kynurenic acid, a non-specific antagonist of excitatory amino acid receptors. The later observation implicated excitatory amino acids as one of the neurotransmitters involved in this response. Therefore, experiments were initiated to indentify the type of excitatory amino acid receptor involved. Perifused hypothalamo-neurohypophyseal explants were exposed to a ramp increase in osmolality (40mOsm over 6 hours achieved by increasing NaCl) in the presence and absence of 10µM 6,7-dinitroquinoxaline-2,3-dione (DNQX), an antagonist of non-NMDA excitatory amino acid receptors. VP release (measured by RIA in timed fractions of the perifusate) and VP mRNA content (measured by RNase protection assay at the end of the 6 hour osmotic stimulus) were significantly increased by exposure to the osmotic stimulus. DNQX inhibited osmotically stimulated VP release ($F=16.65$, $p=0.0008$) without significantly reducing basal release. It also prevented the osmotically stimulated increase in VP mRNA content ($p<0.05$). These results suggest that the synaptic regulation of VPmRNA content by osmotic stimulation is mediated at least in part by non-NMDA excitatory amino acid receptor activation. *Supported by NIH grant NS27975.*

348.3

VASOPRESSIN ANTISENSE SPECIFICALLY INHIBITS OSMOTIC STIMULATION OF VASOPRESSIN, BUT NOT OXYTOCIN RELEASE. M. Ludwig*, M. Morris, and C. D. Sladek, Bowman Gray School of Medicine, Winston-Salem, N.C., 27157, and Finch Univ. of Health Sciences/Chicago Med Sch, North Chicago, IL, 60064.

Rapid effects of antisense oligodeoxynucleotide (ODN) on the function of the neurohypophyseal system have been reported. In this study, the effect of a vasopressin (VP) antisense ODN on osmotically stimulated release of VP and oxytocin (OT) from perifused hypothalamo-neurohypophyseal explants was evaluated. Following a 2 hr equilibration period, either an 18-mer phosphorothiodated ODN targeted to the translation start site of VP mRNA or a mixed base sequence (8µM) were added to the perifusate. 2 hrs later all explants were exposed to a ramp increase in osmolality (40mosm/6 hr). VP and OT release were measured by RIA in timed fractions of the perifusate. VP antisense ODN inhibited VP, but not OT release in response to the osmotic stimulus. VP release was significantly increased in explants perifused with the mixed base ODN in comparison to antisense ODN ($F=18.54$, $p=0.0026$). OT release was not significantly different between the mixed base and antisense groups. These observations indicate that acute exposure to VP antisense ODN interupts osmotically stimulated VP release. The effect was specific for VP release as the OT response was not inhibited. These results are consistent with data demonstrating that antisense ODNs inhibit neurohypophyseal function in a hormone specific manner. Further studies are required to ascertain the mechanism underlying the inhibition of hormone release. *Supported by NIH grants NS27975(CDS), HL43178 (MM) and NC Heart NC-92-GB-16.*

348.4

EXPRESSION OF VASOPRESSIN MRNA DURING MATURATION IN THE RAT. M.D. Fitzsimmons*, A.G. Robinson. Dept. of Endocrinology. Univ. of Pittsburgh, PA 15261.

Between 3 and 12 weeks of age in the rat, total vasopressin (AVP) content in the posterior pituitary increases substantially; the accumulation does not result from feedback from the posterior pituitary, nor as a consequence of increasing body weight or gonadal steroid secretion. In this experiment, we examined the pattern of AVP mRNA expression during maturation to determine the relationship between mRNA level and the accumulation of hormone in the posterior pituitary. Expressed in relative units, AVP mRNA was: 55±5 at 3.5 weeks, 97±9 at 6 weeks, 126±11 at 9 weeks, and 134±14 at 12 weeks. In order to assess the relative contribution of transcription and mRNA stability to the change in mRNA level, we measured the downregulation of AVP mRNA at 0, 1, 2, 3, 4, and 7 days after inducing hyponatremia in rats at the same ages. Since this experimental design incorporates a higher time resolution than earlier studies, we were able to detect an initial tendency for hyponatremia to stimulate AVP mRNA transcription in some age groups. This paradoxical stimulation, which may result from stress associated with surgical implantation of DDAVP osmotic minipumps, resolved within 2 days in all groups. Thereafter, all age groups exhibited similar patterns of AVP mRNA downregulation during hyponatremia; the physiologic half-life of vasopressin mRNA appears to be independent of age, to the resolution of the technique employed. We infer from these observations that the increase in hypothalamic AVP mRNA during maturation results from increased transcription and not changes in mRNA stability. In spite of higher transcription, older animals exhibit less pituitary AVP accumulation, indicating that absolute AVP secretion rate increases with age. Since the rate of pituitary accumulation is highest when mRNA content is lowest, the quantitative relationship between transcription and secretion may depend on age, with younger animals storing a higher fraction of their total synthesis. Supported by DK 16166.

348.5

ENDOGENOUS HYPERINSULINEMIA SUPPRESSES VASOPRESSIN MRNA SYNTHESIS IN THE HYPOTHALAMUS. R.H. Rao, M.D. Fitzsimmons, M.I. Sandberg, A.G. Robinson*. Dept. of Endocrinology, University of Pittsburgh, Pittsburgh PA 15261.

Vasopressin (AVP) secretion is known to be increased in insulin deficient Type I diabetes mellitus, a phenomenon that has been attributed to volume depletion from osmotic diuresis induced by hyperglycemia. Nevertheless, we and others have shown that, even with volume repletion, AVP secretion is increased in insulin-deficient hyperglycemic rats. We studied the relationship between insulin and AVP in a rat model of endogenous hyperinsulinemia resulting from chronic (10 days) administration of 10% fructose in the diet. AVP mRNA (measured by RNase protection assay) was suppressed compared to rats fed a regular diet (2516±184 CPM vs. 3289±230 respectively). In a second model of endogenous hyperinsulinemia, induced by administering 10% glucose in the drinking water for 10 days, AVP mRNA was similarly suppressed compared to rats given water sweetened with saccharin (2819±249 vs. 3936±211 respectively). The relationship of these observations to peripheral insulin resistance was studied by performing euglycemic hyperinsulinemic clamps. Rats fed a 10% fructose diet had lower glucose disposal rates under equivalent clamp conditions, indicating significant insulin resistance. 10.6±5.1 µmol/kg/min vs. 31.8±7.6 in controls (n=5, p<0.05). However, glucose disposal rates during steady-state euglycemia were not impaired in rats given 10% glucose in their drinking water (23.4±3.7 µmol/kg/min, p=NS vs controls), indicating that hyperinsulinemia in these rats was not associated with insulin resistance. In summary, we have observed decreased expression of AVP mRNA after two separate hyperinsulinemia protocols (glucose feeding and fructose feeding); only the fructose diet, however, also induced peripheral insulin resistance. On the basis of these observations, and of previous studies showing that insulin deficiency increases AVP secretion, we conclude that AVP synthesis and release are responsive to insulin. Furthermore, our results indicate that the insulin responsiveness of the brain is preserved in the presence of peripheral insulin resistance.

348.6

INCREASED NMDAR1 EXPRESSION IN THE HYPOTHALAMUS WITH HYPEROSMOTIC STIMULATION: QUANTITATIVE IMMUNOHISTOCHEMISTRY AND WESTERN BLOT ANALYSIS. M.C. Curras* and C. Decavel *Dept. of Neuroscience, University of California, Riverside.*

Functional NMDA receptors have been described in rat supraoptic (SON) neurons (Hu and Bourke, 1992; Curras et al 1992). We have recently detected the NMDAR1 subunit on immunohistochemically identified magnocellular neurons of the SON (Soc. Neurosci. Abstr. 20 (1):347, 1994). Quantification of NMDAR1 immunolabeling in SON showed that expression of this subunit was increased during chronic dehydration (2% saline drinking water: 7-10 days). Another hypothalamic nucleus, which participates in water balance, the paraventricular nucleus (PVN) was tested for changes in NMDAR1 expression. Immunohistochemical detection of NMDAR1 in sections from control and saline-treated rats was done using silver-intensified gold (SIG) as a marker. Computerized analysis of labeling was performed over the posterolateral magnocellular subnucleus of the PVN of both groups. Labeling density was calculated as the ratio of the mean gray levels of PVN/ n. reuniens of the thalamus, which has not been shown to be involved in water balance. This ratio was significantly greater in saline rats (1.146 + 0.038; n=11) than in control rats (1.035 + 0.012; n=7) at 0.01 level. Although NMDAR1 is ubiquitous in the CNS the increased NMDAR1 labeling in saline-treated rats was specifically observed in the hypothalamo-neurohypophyseal system. Western blots were performed to test if increased NMDAR1 expression would be detectable in hypothalamic homogenates from saline-treated rats. Cortex, hypothalamus and liver homogenates were loaded on a 7.5% gel (SDS-PAGE). After electrotransfer onto membrane, NMDAR1 immunolabeling was found between 100-125kD. NMDAR1 expression was significantly greater in hypothalamus from saline vs control rats. Cortex showed NMDAR1 expression but no difference with saline treatment. (Quantitative image analysis was done using Quantimet 500 (Leica) customized by A. Lubarsky).

348.7

DIFFERENTIAL EXPRESSION OF NMDAR2 (NR2) RECEPTOR SUBUNITS IN VASOPRESSIN AND OXYTOCIN NEUROENDOCRINE CELLS W.M. Al-Ghoul, R.S. Greenwood and R.B. Meeker*, Dept. of Neurology and Neurobiology Curriculum, University of North Carolina, Chapel Hill, NC 27599

NMDA receptors consist of NR1 and NR2 subunits assembled in heteromeric configurations to form ligand gated ion channels. While the NR1 subunit of the NMDA receptor is abundant throughout the CNS, the NR2 subunits show substantial regional variation. Within the supraoptic nucleus, it has been suggested that vasopressin (VP) and oxytocin (OT) neuroendocrine cells display different NMDA receptor activities. Since NMDA receptor characteristics may be determined by NR2 subunit composition, we used immunocytochemical-*in situ* hybridization double-labeling to evaluate NR2 subunit expression in VP and OT neuroendocrine cells. Sections of rat brain were reacted with antibodies against neurophysin II or neurophysin I to identify VP and OT cells, respectively, then processed for *in situ* hybridization using ^{35}S-labeled oligonucleotides specific for NR2A, -B, -C or -D. Silver grain densities from high resolution autoradiograms were quantified over immunocytochemically identified cells to determine the relative expression of each NR2 subunit. Whereas the NR1 subunit was ubiquitously expressed in VP and OT cells, the NR2 subunits were differentially distributed with the following abundance: VP: 2B = 2C ≥ 2D >> 2A; OT: 2B >> 2D>> 2C > 2A. VP cells were five times as likely to express the NR2C subunit and only half as likely to express the NR2B relative to OT cells. An average of 41-96% of the cells had detectable levels of each subtype indicating that some cells probably express multiple NR2 subtypes. These data indicate that VP and OT cells are likely to be controlled by distinct subtypes of NMDA receptors. Supported by NIH Grants NS13411 and NS30923

348.8

LACK OF ACUTE VOLUME REGULATION IN SUPRAOPTIC NUCLEUS (SON) NEURONS ISOLATED FROM THE ADULT RAT. S.J. Gentles, S.H.R. Oliet, P. Drapeau* and C.W. Bourque. Centre for Research in Neuroscience, Montreal General Hospital and McGill University, Montreal, PQ, H3G 1A4, Canada.

The intrinsic osmosensitivity of SON neurons is believed to result from the modulation of stretch-inactivated cationic channels by osmotically-evoked changes in cell volume [Oliet & Bourque *Nature* 364, 1993]. To behave as non-adapting osmometric transducers, however, SON neurons must lack the ability to volume-regulate. Osmotically-evoked (±mannitol) changes in cell volume were therefore examined by laser scanning or phase microscopy of neurons acutely isolated from adult rats. Examination of 16 SON neurons during strong osmotic stimulation (±93 mOsmol/kg; 64 minutes) revealed no time-dependent decay of initial osmotically-evoked volume changes. Over a more physiological range (±40 mOsm) the volume of SON neurons decreased as a linear function of external osmolality with a slope of -0.29%/mOsmol/kg (R=0.99; n=11), a value comparable to strict osmometric behaviour (-0.34%/mOsmol/kg). The volume of cortical neurons was much less sensitive to changes in external osmolality (-0.10%/mOsmol/kg; R=0.66; n=17), indicating that these cells partially regulate their volume when faced with an osmotic challenge. These observations suggest that SON neurons do not volume regulate and are consistent with a role for stretch-inactivated cationic channels in osmoreception. *Supported by the Canadian Heart & Stroke Foundation and the Medical Research Council of Canada.*

348.9

GENERATION OF BURST DISCHARGE IN HYPOTHALAMIC SUPRAOPTIC NEURONS *IN VITRO*. G. Böhmer, W. Greffrath, E. Martin and K. Behrend*. Dept. of Physiology and Pathophysiology, Gutenberg University, 55099 Mainz, Germany.

In vasopressinergic neurons of the hypothalamic supraoptic nucleus hyperosmotic stimulation results in a transition from tonic to phasic discharge. The present study aimed at closer examining mechanisms involved in rhythmogenesis of burst discharge. Membran potentials of supraoptic neurons of male rats were intracellularly recorded from hypothalamic slices at 32 °C using glass microelectrodes filled with 2 M potassium acetate. In supraoptic neurons spiketrains induced by current injection were succeeded by a sequence of hyperpolarizing and depolarizing afterpotentials (AHP and ADP, respectively). These afterpotentials were blocked by Cd^{2+} and thus were considered Ca^{2+}-activated. A fast component of the AHP was blocked by apamin whereas a slow component of the AHP was blocked by charybdotoxin. The amplitude of the posttrain depolarization depended on the interaction of the ADP and the slow AHP. Application of histamine or N-methyl-D-aspartate to the bath resulted in an increase of the amplitude of the ADP and eventually in a spiketrain. Further depolarization induced repetitive bursts of action potentials. In many supraoptic neurons fast oscillations of the membranc potential (30 to 60 Hz) occurred on top of nearthreshold ADPs and during nearthreshold depolarization induced by current injection. In spontaneously bursting neurons bursts were preceded by fast oscillations of the membrane potential. The induction of these oscillations was voltage-dependent (threshold: -58 to -48 mV). These low voltage-activated oscillations (LVAO) were blocked by TTX suggesting the activation of a TTX-sensitive, persistent Na^+-current underlying LVAO. Further depolarization during continued application of TTX induced high voltage-activated oscillations (HVAO; threshold: -45 to -35 mV). HVAO were blocked by Cd^{2+} suggesting the activation of a HVA Ca^{2+}-current underlying HVAO. The Na^+-dependent LVAO and the Ca^{2+}-activated ADP may be important for the induction and maintenance of burst discharge in supraoptic neurons. The fast and slow Ca^{2+}-activated AHP may be important for the termination of burst discharge and the delay of onset of the succeeding burst of action potentials.

348.10

EFFECTS OF A SALT LOAD ON ENSEMBLE UNIT ACTIVITY IN THE PARAVENTRICULAR NUCLEUS (PVN) OF THE AWAKE, BEHAVING RAT. *JM Paris*, RV Subrahmanyam, MF Callahan, DJ Woodward.* Dept. Physiology/Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC 27157-1083

Oxytocin and vasopressin neurons in the PVN respond to osmotic stimuli and to changes in plasma Na^+ concentrations. We hypothesized that the neural activity of a sub-population of PVN neurons should be altered in response to a chronic salt load. Three male rats were implanted with microwire recording electrodes in the PVN. Neural activity was recorded in three 2 hr sessions: 1) under control (normal water) conditions; 2) following 48 hr exposure to a 1.1% NaCl solution; and 3) after a 48 hr recovery period (normal water). Of 31 neurons simultaneously isolated in the PVN, 16 had a 20% or greater increase in firing rate after a 48 hr salt load (range 25-3260%); 11 units had a decreased firing rate, and 4 were unchanged. The increases in firing rate were associated with a mean 69±7% decrease in inter-spike interval (ISI) and a 134±60% increase in burst rate. Also, salt exposure recruited 1-4 quiescent neurons in each rat. Following 48 hrs of recovery, only 3 neurons returned to baseline firing patterns, suggesting that salt exposure may induce long-term alterations in the activity of these cells. These results represent the first characterization of osmosensitive neurons in the PVN of the awake rat. In addition to a more in-depth examination of the ensemble firing patterns of these neurons, future studies will explore concurrent alterations in blood pressure associated with various osmotic challenges. *Supported by DA02338 (DJW) and NC Governor's Inst. (JMP). WWW Server: http//biogfx.bgsm.wfu.edu/*

348.11

c-FOS INDUCED BY ANGIOTENSIN AND HYPEROSMOLALITY IN SON AND PVN NEURONS EXPRESSING AT1 RECEPTOR.
Z. Ying*, S. Livreri, and J. Buggy. Dept. of Physiology, University of South Carolina, Columbia, SC 29208.

Supraoptic and paraventricular nuclei (SON, PVN) receive excitatory afferent input from subfornical organ (SFO) and organum vasculosum of the lamina terminalis (OVLT). Angiotensin II (ANG) may serve as a transmitter in these pathways and ANG type 1 (AT1) receptor immunoreactivity has been demonstrated in SON and PVN. We investigated whether neurons expressing AT1 receptors express Fos after hyperosmotic or i.v. ANG treatment in rats. Confocal laser scanning microscopy was used to co-localize fluorescent double-immunolabeling for c-fos and AT1 receptor. After i.p. hypertonic saline injection, 97.2% of AT1 neurons in SON and 97.4% in PVN expressed Fos. After ANG infusion, Fos immunoreactivity was detected in 84% of AT1 neurons in SON and 93.6% in PVN. Hyperosmotic treatment preferentially induced Fos in the OVLT cap and SFO rim whereas ANG preferentially induced Fos in the OVLT wall and SFO core and rim. AT1 receptor immunoreactivity was dense throughout SFO and OVLT but was not resolved into individual neurons for co-localization with Fos. These results demonstrate that neurons expressing AT1 receptor in SON, PVN, OVLT, and SFO were activated for Fos expression by hyperosmolality and intravenous infusion of angiotensin.

348.12

SOMATOSTATIN ANALOG INDUCES FOS ACTIVATION IN RAT MAGNOCELLULAR NEURONS.
M.I. Sandberg*, M.D. Fitzsimmons, R.H. Rao, G.E. Hoffman[1], A.G. Robinson. Depts. of Endocrinology and [1]Neurobiology, University of Pittsburgh, Pittsburgh PA 15261.

Elevated secretion of vasopressin occurs in diabetes mellitus secondary to glucose-induced osmotic diuresis, but clinical and experimental evidence indicate that, in addition, glucose acts as an osmotic agent under insulin-deficient conditions. In order to study the relative contributions of glucose and insulin on vasopressin secretion, we have developed a rat model in which octreotide (a somatostatin analog) was infused peripherally to inhibit insulin secretion with and without glucose-induced hyperglycemia. In this model, we have shown that peripheral vasopressin levels are elevated only in the hyperglycemic, insulin-deficient rats. In the present experiment, we investigate the effect of peripherally infused octreotide on sites of vasopressin synthesis in the rat brain. Non-fasted, awake, chronically catheterized 325-350g male Sprague-Dawley rats received an intravenous infusion of 140 mEq NaCl (12 ml/kg/hr) for 110 minutes. Rats also, received either a 10% dextrose infusion (12 ml/kg/hr), octreotide (250 µg/kg/hr) or both. Blood was sampled from a jugular catheter at the end of the infusion. Animals were then perfused transcardially with buffered 4% paraformaldehyde/2% acrolein. The brains were removed, microtome sectioned at 25 µm, and stained for c-fos immunoreactivity. Rats receiving octreotide alone exhibited plasma glucose levels near 150 mg/dl and insulin levels of 7 ng/ml. Infusion of 10% dextrose increased plasma glucose to 950 mg/dl. Octreotide blunted the insulin response to dextrose infusion: 16 ng/ml in octreotide treated versus 39 ng/ml in rats receiving dextrose alone. Strong fos activation was found in the magnocellular neurons in the rats which received octreotide or octreotide plus dextrose, but only marginal activation in rats receiving dextrose alone. From these observations, we suggest that the peripheral octreotide infusion either stimulates magnocellular neurons directly or that a state of relative insulin deficiency triggers an osmotic response to glucose in osmosensitive neurons. Supported by DK 16166.

348.13

c-FOS AND c-JUN mRNA EXPRESSION IN RAT SUPRAOPTIC NEURONS (SON) AFTER ACUTE *VERSUS* REPEATED OSMOTIC STIMULATION.
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The proto-oncogenes, c-*fos* and c-*jun* are rapidly and transiently induced after a variety of stimuli under *in vitro* and *in vivo* conditions. Using a double-label immunofluorescence method, previous work has demonstrated that 90 min after acute hypertonic saline injection, Fos and Jun are co-expressed in 80% of SON neurons. However, when rats receive repeated hypertonic stimulation, only 17% of SON neurons have co-existent Fos and Jun immunostaining [NEUR. ABST. '94, 1567]. The induction of *c-fos* and *c-jun* mRNA was assessed using Northern blots and *in situ* hybridization. mRNA levels of both transcription factors increased within 5 min, peaked at 30-60 min, and disappeared by 180 min after acute hypertonic saline injection. c-*fos* mRNA induction was always stronger than c-*jun*. The magnitude of increased c-*fos* and c-*jun* mRNA expression was different following acute and repeated hypertonic saline injection. Similar to the response pattern observed for Fos and Jun immunocytochemistry, the induction of c-*fos* mRNA was less after repeated stimulation, and c-*jun* mRNA induction was essentially nonexistent. Gel-shift DNA binding assay supports the idea that SON protein extracts from injected rats form a complex with AP-1 oligomers.

OSMOREGULATION: MECHANISMS

349.1

ANTAGONISTIC EFFECT OF ANGIOTENSIN II AND NITRIC OXIDE ON RAT SUBFORNICAL ORGAN NEURONS
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Confirming previously published results on duck subfornical organ (SFO) neurons (Schmid et al., 1995, Neurosci. Lett. 187:149-152), 90% (n=20) of rat SFO neurons showed an excitatory response to 10^{-7}M angiotensin II (ANGII) and were additionally inhibited by the nitric oxide donor sodium nitroprusside (SNP, 10^{-4}M). Those data were derived using the same extracellular recording technique from slices of the rat SFO as used for the duck experiments. The antagonistic effect of ANGII and SNP on the activity of SFO neurons might represent the cellular basis for the antagonistic effect of both substances on water intake, when directly applied into the rat SFO. The inhibitory effect of SNP is presumably mediated by an activation of guanylate cyclase, because superfusion of the membrane permeable analog 8-Br cGMP (10^{-4}M) caused an inhibitory effect on neurons which could be inhibited by SNP. To further investigate the transduction mechanism underlying the effects of ANGII and SNP on those neurons, cells were acutely dissociated from the SFO of 8-16 days old rats and investigated with patch clamp recording techniques in the whole cell recording mode. After a gigaohm seal had been established, the reactivity of the cells was tested by applying a brief puff of glutamat (10^{-3}M) or ANGII (10^{-7}M). In contrast to non-spiking glial cells, the cells which showed an increased action potential frequency to glutamat and/or ANGII displayed a rapidly desensitizing, TTX-sensitive (0.5mM) inward current under voltage clamp conditions and a rapidly and a slowly decaying outward current component. At a holding potential of -70mV glutamat induced a rapidly desensitizing inward current, while ANGII caused an inhibition of the rapidly and slowly desensitizing potassium current component. Supported by DFG Si 230/1.

349.2

COLOCALIZATION OF ANGIOTENSIN II AND NEUROPHYSIN II/ VASOPRESSIN IMMUNOREACTIVITY IN THE PRIMARY CULTURE OF RAT SUBFORNICAL ORGAN.
M. Jurzak*, R. Gerstberger, F. Fahrenholz and A. K. Johnson. Dept. Psychology and Pharmacology, Univ. of Iowa, Iowa City, IA 52242-1407.

To characterize the peptide content of cultured subfornical organ (SFO) cells, specific antibodies were employed to localize angiotensin II (ANGII), neurophysin II (NPII) and vasopressin (AVP). After 5-7 days in culture, between 50 and 98 % of SFO cells with neuronal morphology revealed strong ANG immunoreactivity (n=12). Cells exhibiting the same morphology stained positive with a neuron specific enolase (NSE) antibody. In double-labeling experiments, colocalization of NPII and ANG was found in 94 ±3.9 % of cells. The two antibodies revealed a differential staining pattern, with ANG reactivity extending from the cell body into processes and NPII staining restricted to the cytoplasma. In ELISA tests the polyclonal rabbit ANGII antibody used revealed the following reactivity pattern: ANGII = ANGIII >>ANGI. The specificity of the monoclonal NPII antibody was determined in ELISA. In the tissue slice, NPII reactivity completely colocalized with AVP in magno- and parvocellular neurons of the hypothalamus. In a control experiment performed in cell culture, AVP immunoreactivity paralleled the NPII staining (> 95% colocalization). These results demonstrate the capability of cultured SFO cells to synthesize ANGII and NPII/AVP. In addition, the high percentage of colocalization suggests a strong functional relationship of both transmitter systems. Supported by A. v. Humboldt Foundation.

349.3

ANGIOTENSINERGIC PATHWAYS FROM THE SUBFORNICAL ORGAN TO THE MEDIAN PREOPTIC NUCLEUS IN NORMOTENSIVE AND HYPERTENSIVE RATS. J. Tanaka*, A. Ushigome, I. Muguruma, S. Shimamune and M. Matsuda. Dept. of Human Dev., Naruto Univ. of Educ., Tokushima 772, Japan.

Electrophysiological properties of angiotensinergic pathways from the subfornical organ (SFO) to the median preoptic nucleus (MnPO) were investigated in normotensive Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) under urethane anesthesia. (1) The activity of SFO neurons that were antidromically activated by electrical stimulation of the MnPO was compared between WKY (n = 28) and SHR (n = 27). No significant differences were observed between WKY and SHR in the latency, conduction velocity or thresholod of antidromic activation. The spontaneous firing ratio was significantly higher and the refractory period was significantly shorter in SHR. (2) Sixteen out of 46 MnPO neurons in WKY and 15 out of 47 MnPO neurons in SHR were excited by both angiotensin II (ANG II) applied iontophoretically and electrical stimulation of the SFO, and the excitatory responses were abolished by the ANG II antagonist saralasin applied iontophoretically. In these MnPO neurons that exhibited the excitation to both ANG II and SFO stimulation, the spontaneous discharge rate was significantly higher and the threshold current for the SFO stimulus-evoked excitation was significantly lower in SHR. The sensitivity to SFO stimulation was much greater in SHR than in WKY. These results provide evidence that there are marked alterations in the physiological properties of the angiotensinergic pathways from the SFO to the MnPO between WKY and SHR.

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349.4

MEDIAN PREOPTIC NEURONS PROJECTING TO THE PARAVENTRICULAR NUCLEUS IN NORMOTENSIVE AND HYPERTENSIVE RATS. K. Kariya[1]*, M. Oda[1], M. Kurumi[1], K. Hori[2], M. Nomura[2] and J. Tanaka[3]. [1]Res. Lab., Torii Co., Chiba 267, [2]Dept. of Physiol., Saitama Med. Sch., Saitama 350-04, [3]Dept. of Human Dev., Naruto Univ. of Educ., Tokushima 772, Japan.

Twenty-one median preoptic nucleus (MnPO) neurons in normotensive Wistar-Kyoto rats (WKY) and 18 MnPO neurons in spontaneously hypertensive rats (SHR) were antidromically activated by electrical stimulation of the hypothalamic paraventricular nucleus (PVN) under urethane anesthesia. No significant differences in the latency, conduction velocity, or threshold of antidromic activation were observed between WKY and SHR. The spontaneous firing ratio was significantly higher in SHR than in WKY. The activity of these identified MnPO units was examined for a response to intracarotid injections of isotonic (0.15 M NaCl solution, 0.05 ml) or hypertonic (0.3 M NaCl solution, 0.05 ml) saline. Injections of isotonic saline did not cause any change in the activity of all the units tested. Injections of hypertonic saline elicited an increase in the firing of 14 units in WKY and 12 units in SHR and a depression in the excitability of 3 units in WKY and 4 units in SHR, but did not affect the remaining 4 units in WKY and 2 units in SHR. The duration and frequency of excitatory response, but not the inhibitory response, evoked by the osmotic stimulation was much greater in SHR than in WKY. These results show that MnPO neurons projecting to the PVN may carry the information from osmosensitive elements, and that there is the difference between WKY and SHR in the responsivity of these MnPO neurons to the osmotic stimulation.

349.5

ISOTONIC VOLUME CHANGES AND OSMOTIC STIMULI EVOKE SIMILAR RESPONSES IN NEURONS OF THE RAT OVLT. H. Hiruma, D. Richard and C.W. Bourque*. *Centre for Research in Neuroscience, Montreal General Hospital & McGill University, Montreal, P.Q. Canada H3G 1A4.*

Osmotically-evoked changes in membrane potential control the electrical activity of neurons in the organum vasculosum lamina terminalis (OVLT). Whole cell recordings examined the possibility that changes in volume mediate osmosensitivity in OVLT neurons acutely isolated from adult rats. Hypertonic and hypotonic stimuli (±10-30 mM mannitol; n=32) evoked depolarizing and hyperpolarizing responses, respectively. Voltage-current analysis indicated that such effects result from increases and decreases in membrane conductance, with associated reversal potentials of -41±3 (n=8) and -38±2 (n=5) mV. Isotonic increases or decreases in cell volume caused by the application of positive or negative pressure to the inside of the patch pipette also respectively evoked depolarizing and hyperpolarizing responses. The latter were associated with increases and decreases in input conductance, with reversal potentials of -36±6 (n=9) and -39±7 mV (n=4). Thus, variations in cell volume evoked by either changes in pipette pressure or external osmolality appear to modulate a similar conductance pathway. Since E_{Cl} was <-90 mV in our conditions, the osmosensitivity of OVLT neurons may be mediated by volume-gated nonselective cation channels. *Supported by Yokohama City, the Ministry of Education, Science & Culture of Japan, The Canadian M.R.C., and the Heart & Stroke Foundation of Canada.*

349.6

ROLE OF Na^+/H^+ EXCHANGE IN SWELLING OF GLIAL CELLS GROWN UNDER DIABETIC KETOACIDOTIC CONDITIONS. R.W. Putnam, P.B. Douglas, N. Sheibani and J.B. Dean*. Dept. of Physiology and Biophysics, Wright State Univ. Sch. of Med., Dayton, OH 45435.

A small number of children treated for diabetic ketoacidosis (DKA) develop neurologic symptoms within hours of treatment and many of these children die. This effect has been attributed to the release from inhibition of glial cell Na^+/H^+ exchange (NHE) upon correction of pH_o, resulting in increased NHE activity and glial cell swelling (Van der Meulen et al., 1987, *Lancet* ii:306-308). We tested this hypothesis by growing C6 glioma cells for 48 hr under DKA conditions: normal media + 50 mM glucose (total of 55 mM) + 6 mM β-OH butyrate (βHB) + 2 mM acetoacetate (AA). The day that cells were to be used, pH_o was lowered from 7.4 to 6.5 (decreased HCO_3^- at constant 5% CO_2) for 2 hr. Changes in cell volume were measured using near-right angle laser light scattering and pH_i was measured using the pH-sensitive dye BCECF in an SLM DMX1000 Spectrofluorometer. All measurements were made in confluent monolayers of C6 cells. All experiments were done at 37°C in NaHEPES buffer in the nominal absence of CO_2/HCO_3^-. Correcting external glucose (from 55 to 5 mM) resulted in glial cell swelling with no change of pH_i. Removal of all external ketoacids resulted in a small decrease in cell volume and a marked (~0.67 unit) pH_i increase. This pH_i increase was largely due to the removal of βHB and, to a much smaller extent, to the removal AA. Correcting external pH (6.5→7.4) resulted in a small volume decrease and a rapid increase of pH_i that was largely unaffected by the NHE inhibitor amiloride. Exposure of C6 cells to insulin (up to 10 nM) did not affect pH_i nor did it increase the activity of NHE in response to cell acidification. **These data indicate that neurologic symptoms associated with treatment of juvenile DKA are unlikely to be due to glial cell swelling associated with NHE activity.** [Supported by the Juvenile Diabetes Foundation, International.]

349.7

LARGE ANION CHANNELS ARE NOT ACTIVATED DURING ASTROCYTE REGULATORY VOLUME DECREASE (RVD). E. Scemes, A. Andrade-Rozental* and D.C. Spray. University of Sao Paulo, Sao Paulo, Brazil and Albert Einstein College of Medicine, Bronx, NY 10461

Large conductance anion channels have been described in a variety of cell types, including astrocytes. We have investigated the question of whether these maxi-anion channels are involved in cell volume regulation in primary cultures of mouse astrocytes. These cells were exposed to moderate hypo-osmotic shocks (20%) and the time course of RVD was evaluated by the dye-dilution method using real time confocal microscopy. Channel activity was recorded using the cell-attached patch-clamp technique. Large conductance anion channels were observed in 17% of inside-out patches (7 of 41) obtained from astrocytes in symmetrical 140 mM NaCl, with slope conductances of 370-380 pS. Open probabilities of the channels were high at 0 mV and quite low at ±40 mV. Such channel activity was not observed in cell-attached recordings from astrocytes exposed to 140 mM NaCl solution but appeared in only 1 of 55 of the patches at 10 min after application of the hypo-osmotic shock. Slope conductance of this channel was 150-160 pS in the cell-attached mode and after excising the patch the slope conductance was 350-360 pS. Since the time course of astrocyte RVD was 3 min, whereas channel activity was seen only once and then only at 10 min after the hypo-osmotic shock, the maxi-anion channel does not appear to play an obligatory role in astrocyte RVD under our recording conditions.

349.8

LACK OF DELAYED VOLUME REGULATION IN RAT HIPPOCAMPAL SLICES DURING OSMOTIC STRESS. M.E. Lobinowich, E.P. Osehobo and R.D. Andrew*. Dept. of Anatomy and Cell Biology, Queen's Univ., Kingston, Ontario K7L 3N6.

Brain cells in culture respond to severe and maintained osmotic stress by passive volume changes followed over many seconds by some compensation. There is little evidence for or against this form of volume regulation in the intact mammalian brain. In the hippocampal slice where the neuron-glia relationship is maintained, we imaged changes in light transmittance (T)[1] as well as tissue resistance (both measures of cell volume change) during physiological extremes of osmotic change to detect evidence for cellular volume regulation. During superfusion of hypo-osmotic aCSF (-40 mOsm) T increased 20 to 25% in CA1 RAD (n = 6 slices). The response was maintained throughout hypo-osmotic stress over 45 min. This 'plateau' response argues against a delayed volume compensation. Moreover T did not 'undershoot' but immediately returned to baseline following re-introduction of control aCSF. A similar increase in T (27.7 ± 2%) occured at 35°C in 7 slices. In addition, the -40 mOsm aCSF reversibly increased the relative tissue resistance (R_{REL}) measured across CA1 RAD (n = 9 slices) with a time course identical to the increase in T. Conversely, mannitol (+ 40 mOsm) decreased R_{REL} by 8% (n = 12 slices) and decreased T by 15.5%, again with no volume compensation over 20 min. When Cl^- was removed from the aCSF, a volume reduction occurred following initial swelling. This 'delayed' regulatory response may reflect extreme and unphysiological conditions.

To conclude, there was no evidence in the hippocampal slice for volume compensation over many minutes in the face of maintained osmotic stress at physiological extremes.

1. Andrew and MacVicar, 1991. *Neuroscience* **62**, 371-383.

350.1

ANATOMIC CHARACTERIZATION OF THE VENTRAL MEDIAL PREOPTIC AREA: INVOLVEMENT IN THE FEBRILE REACTION. J. K. Elmquist*, T. E. Scammell, J. E. Sherin, C. D. Breder, and C. B. Saper. Dept. of Neurology, Harvard Medical School/Beth Israel Hospital, Boston, MA 02115, and Committee on Neurobiology, Univ. of Chicago, Chicago, IL 60637.

Intravenous (iv) administration of endotoxin (LPS) induces the expression of Fos-like immunoreactivity (Fos-IR) in nuclear groups of the rat brain involved in regulation of autonomic homeostasis. One of these regions, the ventral medial preoptic area (VMPO) may be an important site for the initiation of fever. The VMPO is a small cell group located immediately adjacent to the organum vasculosum of the lamina terminalis (OVLT) and is near thermoregulatory cells of the preoptic area. Immunohistochemical analysis of VMPO revealed a GABAergic (GAD-67) cell population and the presence of neuronal cyclooxygenase-II (COX-II), the enzyme responsible for the production of prostaglandins. Furthermore, LPS administration induced COX-II-like immunoreactivity in perivascular cells in VMPO and the OVLT. The afferent and efferent projections of VMPO were assessed using small injections of a mixture of biotinylated dextrans and cholera toxin-b (CTb) into the region of VMPO. These injections revealed that VMPO neurons project to many sites including the medial preoptic nucleus, dorsomedial hypothalamic nucleus (DMH), lateral hypothalamic area, parabrachial nucleus, and the paraventricular hypothalamic nucleus (PVH). Retrogradely labeled cells were observed in regions including the DMH, suprachiasmatic nucleus, ventrolateral medulla, and the PVH. Anatomic and physiological evidence indicates that the PVH is essential for thermoregulation and initiation of fever. The potential role of the VMPO-PVH projection during the febrile response was assessed by injecting CTb into the PVH of rats, followed by iv febrile doses of LPS (5 or 125 µg/kg) 5-7 days later. Analysis of these brains demonstrated numerous retrogradely labeled cells in VMPO, many of which also contained Fos-IR. These results indicate that VMPO is activated following LPS challenge and some of these cells project directly to the PVH. These observations indicate that VMPO may be involved in the initiation of the febrile response following LPS.

350.2

PREOPTIC PROSTAGLANDINS AND FEVER

T.E. Scammell*, J.K. Elmquist, and C.B. Saper. Dept. of Neurology, Harvard Medical School / Beth Israel Hospital, Boston, MA 02115

Prostaglandin E2 (PGE2) is essential in the production of fever, yet the necessary sites of PGE2 synthesis and action are unknown. To determine precisely the brain regions at which PGE2 produces fever, we have developed a microinjection technique that does not alter normal thermoregulation and allows temperature recording in unrestrained animals. Microinjection of PGE2 into the preoptic area of male, Sprague-Dawley rats rapidly induced fever, and the largest fevers were caused by injections close to the organum vasculosum of the lamina terminalis (OVLT) and the adjacent ventromedial preoptic area. The pattern of neural activation induced by intrapreoptic PGE2 was determined using Fos immunohistochemistry. As is seen during lipopolysaccharide (LPS) fever, many autonomic regulatory regions contained neuronal Fos-like immunoreactivity (Fos-IR) including the ventromedial preoptic nucleus, median preoptic nucleus, paraventricular nucleus, parabrachial nucleus, nucleus of the solitary tract, and ventrolateral medulla. In contrast, injection of artificial CSF into the preoptic area had no effect on body temperature and did not induce significant Fos-IR in any autonomic control nuclei. To determine if PG synthesis in the region of the OVLT is essential for the production of fever, we have begun to study whether preoptic injections of ketorolac, a water-soluble inhibitor of prostaglandin production, blocks LPS-induced fever. Injection of ketorolac close to the OVLT suppressed LPS fever, but injections more than 800 mm away from the OVLT had little effect on fever. Taken together, these experiments demonstrate that prostaglandins produced in the region of the OVLT contribute to the production of fever, and preoptic injection of PGE2 produces fever via neural pathways similar to those activated during endotoxin fever.

350.3

MORPHOLOGICAL CHARACTERISTICS OF HYPOTHALAMIC THERMOSENSITIVE AND TEMPERATURE INSENSITIVE NEURONS. J.D. Griffin* and C.B. Saper. Department of Neurology, Beth Israel Hospital and Harvard Medical School, Boston, MA 02115

Tight-seal, whole-cell recordings were made from rat hypothalamic neurons in horizontal or coronal tissue slices during changes in temperature. The intracellular stain biocytin (0.5%) was added to the tip of all recording electrodes. Neurons were classified as warm sensitive, cold sensitive, or temperature insensitive based on firing rate responses to a change in temperature. Neurons which did not show spontaneous firing rate activity were classified as silent. At the end of a recording session, tissue slices were fixed and biocytin histochemistry was done to determine the exact location and morphological characteristics of each neuron. Warm sensitive neurons in the medial preoptic nucleus (MPN), anterior hypothalamic area (AHA), and perifornical region displayed branching bipolar dendrites projecting in lateral and medial directions. Temperature insensitive neurons in the medial preoptic area, MPN, AHA, and perifornical region had primary dendrites projecting in parallel to the edge of the third ventricle (rostral/caudal or dorsal/ventral). Neurons classified as silent were located in the MPN, lateral hypothalamic area, perifornical region, and the septum. They had multipolar dendritic arborizations, averaging four primary dendrites projecting in all directions. The results of this study indicate that the dendritic orientation of neurons in the preoptic area correlates closely with their thermoreceptive properties. We hypothesize that dendritic orientation may reflect the organization of hypothalamic neurons into specific networks for thermal and related regulation.

350.4

MODULATORY ROLE OF CYCLIC AMP IN RAT HYPOTHALAMIC NEURONAL THERMOSENSITIVITY. A.R. Chow and J.A. Boulant*. Department of Physiology, The Ohio State University, Columbus, OH 43210

Using rat hypothalamic tissue slices, our previous studies have shown that, in the preoptic/anterior hypothalamus, neuronal warm-sensitivity can be enhanced by drugs that elevate intracellular cyclic adenosine 3',5'-monophosphate (cAMP). The present experiments used whole-cell patch clamp recordings to examine the mechanisms of cAMP's effect on neuronal thermosensitivity. Unlike the low-slope temperature insensitive neurons, in warm sensitive neurons and moderate-slope temperature insensitive neurons, the cAMP analog, 8-bromo-cAMP (250-500 µM), enhanced the effect of temperature on the rate of depolarization of prepotentials that precede each action potential. Also, in some neurons, cAMP altered the effect of temperature on the depolarization that follows the after-hyperpolarizing potential. Both of these cAMP effects produce decreased interspike intervals and increased firing rates during warming; and, thus, they provide mechanisms for cAMP-enhanced neuronal thermosensitivity.

(Supported by NIH grant NS14644.)

350.5

GLUTAMATE AND GLUTAMINE ANTAGONISTS ALTER HEAT-SHOCK PROTEIN EXPRESSION AND THERMAL PROFILES IN RATS EXPOSED TO MICROWAVE HEATING. P.A. Mason[1*], K.E. Purdy[2], T.J. Walters[3], J.M. Doyle[3], J.L. Kane[3], and R. Escarciga[4]. Operational Technologies Corp., San Antonio, TX, 78229, [2]Trinity University, San Antonio, TX, 78212, [3]Systems Research Laboratories, San Antonio, TX, 78235, and [4]Armstrong Laboratory, Radiofrequency Radiation Division, Brooks AFB, TX, 78235

Glutamine and glutamate have putative roles in inducing heat-shock protein (hsp) expression. The effects of MK-801, a selective non-competative N-methyl-D-aspartate (NMDA) receptor antagonist, and L-methionine sulfoximine (L-MSO), a glutamine synthetase inhibitor, on the expression of hsps (72/73 kDa) in the rat brain after brief periods of hyperthermia were examined. L-MSO decreased basal temperatures. High-powered microwaves (2.06 GHz) delivered at 1.7 W/cm^2 were used to induce hyperthermia in male Sprague-Dawley rats. These rats were euthanized 6 hr after exposure. Hsp expression was quantitated in immunohistochemically-stained sections. When tympanic temperatures exceeded 40.3°C, hsp was expressed in the brains of saline-injected rats with some regions revealing dense hsp expression. MK-801 (3.0 mg/kg, i.p.), injected 90 min prior to exposure, attenuated hsp expression. MSO (150 mg/kg, i.p.), injected 3 hr prior to exposure, also attenuated hsp expression. No hsp expression was observed in sham-exposed rats receiving saline injections. MK-801 induced hsp expression in layers III & IV of the retrosplenial granular cortex of sham-exposed rats.

350.6

THERMAL RESPONSIVENESS OF VENTROMEDIAL HYPOTHALAMIC NEURONS (VMH) OF COLD-AND ROOM TEMPERATURE-ACCLIMATED RATS. **Q. Li* and J. Thornhill. Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, Sask. Canada. S7N 5E5.**

Previous work has shown that neurons in the ventromedial hypothalamic nucleus (VMH), a well-known central site that governs non-shivering thermogenesis, specifically respond to scrotal thermal stimulation(10-40°C) of room temperature(21°C) acclimatized rats(RA). The present experiments were conducted to examine the responsiveness of VMH neurons in cold-acclimated rats(CA)(4°C for 4wks) to scrotal thermal stimulation. VMH extracellular activity was recorded with a glass-micropipette filled with 0.5 M sodium acetate in urethane-anaesthetized, male cold-acclimated Sprague-Dawley rats, when scrotal temperature was incrementally changed via a localized thermode perfused with water. $T_{SCROTUM}$, T_{TAIL} and intrascapular brown adipose tissue temperatures (T_{IBAT}) were continuously monitored on the computer via thermistor probes and a temperature monitor. Colonic (core) temperature was maintained at 37.5°C via a servo-controlled heating blanket. Based on their thermal coefficients, VMH neurons were classified as warm-responsive (WRN), cold-responsive (CRN) and temperature non-responsive neurons (TNRN). CA-rats, but not RA-rats, showed a significant increase ($P<0.05$) in T_{IBAT}s after scrotal cooling. Compared to neurons from RA-rats, recorded VMH neurons of CA-rats displayed a higher control basal firing rate and VMH CRNs of CA-rats increased their thermosensitivity to scrotal thermal stimulation. The neuronal activity of VMH TNRNs was not altered by thermal stimulation. The results indicated that cold acclimation (1) increased T_{IBAT}s to scrotal cooling, unlike RA rats and (2) caused associated changes in VMH neuronal activity, suggesting that VMH neurons are involved in the observed adaptive response of IBAT to cold acclimation. ***Supported by the MRC of Canada.***

350.7

PROJECTIONS FROM THE PREOPTIC AREA TO THE MIDBRAIN FOR THERMOREGULATORY VASOMOTOR CONTROL IN RATS. Y.-H. Zhang, K. Yamada, M. Yanase-Fujiwara, T. Hosogno, X.-M. Chen, and K. Kanosue* Dept. of Physiology, Dept. of Basic Laboratory Science, Osaka Univ. Med. Sch., Osaka 530, Japan.

To investigate the midbrain sites innervated by neurons of the preoptic area for thermoregulatory vasomotor control we measured paw skin and tail skin temperature while stimulating midbrain areas with electrical current and with 1 mM DL-homocysteinic acid and we evaluated the effects of various microknife cuts on the skin vasomotion elicited by warming the preoptic area. Experiments were made in rats anesthetized with ketamine (200 mg/kg i.p.). Electrical or chemical stimulation of sites in the ventral part of the periaqueductal grey(PAG) and in the reticular formation caused vasodilation, whereas stimulation of sites in the ventral tegmental area produced vasoconstriction. Microknife cuts rostral to the ventral tegmental area or in the PAG eliminated vasodilation by preoptic warming, whereas cuts caudal to the ventral tegmental area elicited sustained skin vasodilation. Warm-sensitive neurons in the preoptic area contribute to vasomotor control more than do cold-sensitive neurons (Zhang et al., J. Physiol., 1995), and it seems that these warm-sensitive neurons send excitatory signals to the vasodilator neurons in the PAG and in the reticular formation, while they send inhibitory signals to vasoconstrictor neurons in the ventral tegmantal area.

350.8

SYMPATHETIC OUTFLOW TO INTERSCAPULAR BROWN ADIPOSE TISSUE AND NONSHIVERING THERMOGENESIS IN COLD ACCLIMATED MICE. S.A. Kirov* and M.I. Talan. Laboratory of Behavioral Sciences, National Institute on Aging, NIH, Gerontology Research Center, Baltimore, MD 21224.

Efferent sympathetic nervous activity (SNA) from a small branch of the intercostal nerve, which ended in the interscapular brown adipose tissue, and heat production from nonshivering thermogenesis (HP) were measured in male, C57BL/6J mice under urethane anesthesia, vecuronium bromide myorelaxation, and artificial ventilation. All measurements were conducted before and during rapid (15-18 min) whole body cooling (8°C below control level). Three groups of mice were tested: (1) cold acclimated (ACCLIM) (subjected to three, consecutive, cold stress tests that resulted in improvement of cold tolerance); (2) failed acclimated (FAILED) (repeated cold stress tests did not result in improvement of cold tolerance); and (3) NAIVE.

SNA and HP increased significantly during body cooling in all mice. Animals from the FAILED group had lower SNA and lower HP before and during cold stimulation than mice from the ACCLIM group. Maximal responses to cold stimulation of ACCLIM mice exceeded those of FAILED mice by 57% for SNA and by 24% for HP. SNA and HP in the FAILED group were similar to that of NAIVE mice. These findings indicate that the sympathetic nervous system plays a primary role in cold acclimation-related changes of nonshivering thermogenesis.

350.9

INVOLVEMENT OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPARs) IN COLD ADAPTATION. H. M. Guardiola-Diaz*, S. Rehnmark, J.-Å. Gustafsson and S. E. H. Alexson Ctr. for Biotechnology, Karolinska Inst. at NOVUM; Dept. of Metabolic Research,Wenner Gren Institute at Stockholms Univ.; and Div. of Clinical Chemistry at the Karolinska Inst., S-141 57 HUDDINGE, Sweden.

PPARs are nuclear receptors responsive to peroxisome proliferators such as clorfibric acid, nafenopin and WY-14,643 as well as fatty acids. Endogenous ligand(s) for these proteins have not been identified, therefore PPARs are considered orphan nuclear receptors. Several isoforms of this receptor (α,γ,γ2, nuc1(β,δ)) have been isolated from rodent, human and amphibian tissues. Previous reports suggest that the PPARγ2 and nuc1 isoforms may play important roles in adipose tissue differentiation. Since this process is pivotal to the mammalian adaptation to cold we hypothesize that PPAR isoforms could be mediating important metabolic changes during cold exposure. We have tested this hypothesis by analyzing PPAR mRNA expression in rodent brown adipose tissue as a function of time in the cold (4^0 C). Our results demonstrate that PPARγ2 and nuc1 mRNA levels increase after one hour exposure to cold and are then rapidly repressed (after 6 hours) below control levels. These data imply that PPARs are involved in the initial molecular changes in the cold adaptive response probably by modulating transcription of PPAR-responsive genes.

NEUROENDOCRINE REGULATION: THYROID AND GONADAL AXES

351.1

LITHIUM AND CARBAMAZEPINE AFFECT INTRACELLULAR THYROID HORMONE METABOLISM IN THE RAT CNS. U. Gaio, G. Pinna, M. Eravci, C. Hessenius, A. Campos-Barros, A. Musa, J. Mihic*[b], H. Meinhold and A. Baumgartner[a]. Depts Nuclear Medicine and Psychiatry[a], Clinic B. Franklin, Free Univ., 12200 Berlin, Germany and Dept. Pharmacol.[b], Univ. of Colorado H.S.C., Denver, CO 80262, USA.

Severe hypothyroidism may induce a host of affective symptoms in humans. Interactions between antidepressants and thyroid hormone (TH) production and metabolism are reported. Supply of T3 in brain depends mostly on the cellular uptake and intracellular deiodination of T4 by the type-II 5' deiodinase (5'D-II). 5-III-deiodinase (5D-III) deiodinates T3 to 3,3'-T2. The effects of antidepressant drugs on the activity of the two enzymes 5'D-II and 5D-III are dependent on the time of day. We investigated at two different times during the 24 hrs cycle whether subchronic (14 days) administration of lithium (Li) and carbamazepine (CBZ) exerts an influence on TH metabolism in rat CNS. Three groups comprising 16 rats each were fed a diet containing 0.3% Li or CBZ or the same without Li or CBZ for 14 days, respectively. Eight Li- or CBZ-treated rats and 8 control rats were killed by decapitation between 4 and 5 AM, and the 8 remaining rats in each of the three groups were sacrificed between 7–8 PM. The measurement of deiodinase activities was based on the release of radioiodide from ^{125}I -labelled substrates. CBZ, but not Li, enhanced 5'D-II deiodinase activity in 2 brain areas at 4 AM. In rats sacrificed at 8 PM, however, both Li and CBZ significantly increased 5'D-II activity in 7 and in 9 out of 10 brain areas, respectively. At 4 AM, 5D-III activities were inhibited after Li treatment in 6 brain areas and only in hippocampus after CBZ. At 8 PM, while CBZ had no effect, Li decreased 5D-III activities in frontal cortex and striatum. The mean serum Li concentrations were 1.30 ± 0.13 mmol/L and 1.37 ± 0.10 mmol/L, for CBZ 5.8 ± 0.5 mg/L and 5.3 ± 0.51 mg/L for the groups sacrificed in the morning and evening respectively. These results demonstrate that antidepressant treatment with Li and CBZ affect intracellular thyroid hormone metabolism in various rat brain areas. These effects are dependent on the circadian rhythm and probably result in increased T3 production. Whether or not the changes in the activities of 5'D-II and 5D-III are linked to the prophylactic properties of Li and CBZ in the treatment of affective disorders remain to be further elucidated.

351.2

EFFECTS OF LITHIUM ON GENE EXPRESSION OF THYROID HORMONE RECEPTORS (THRS) IN RAT BRAIN. C-G. Hahn, A. C. Pawlyk, P.C. Whybrow* and S. M. Tejani-Butt. Dept. of Psychiatry, Univ. of Penn. Sch. of Med., Phila., PA 19104.

Even though lithium has received wide attention in the treatment of manic depressive illness, the mechanisms by which lithium causes its mood stabilizing effects are not yet understood. Lithium is known to interact with the thyroid axis and causes hypothyroidism in a subgroup of patients which compromises its antimanic effects. Since subchronic lithium was recently reported to alter thyroid hormone metabolism in the rat frontal cortex, the present study determined whether these effects of lithium were mediated through regulation of THR gene expression. Adult male euthyroid rats were given either a diet containing 0.25% lithium or one without lithium for 14 days. Rats were sacrificed in the evening and different brain regions were isolated and frozen. RNA was isolated from the cortex, hypothalamus and cerebellum. To quantitate the isoform specific mRNAs of THRα1 and THRα2, RNAse protection assay as well as Northern blot analysis was employed. Repeated treatment with lithium increased THRα1 mRNA in the cortex, decreased THRα1 mRNA in the hypothalamus and had no effect in the cerebellum. In contrast, THRα2 gene expression was found to be unaltered in these brain regions. Thus it appears that repeated lithium regulates THR gene expression in a subtype and tissue specific manner in the rat brain. It remains to be determined whether the observed effects of lithium on THR gene expression is related to its therapeutic value in the treatment of bipolar disorder.

351.3

THYROID HORMONE RECEPTOR α2 mRNA LEVELS ARE INCREASED IN THE HYPOTHALAMUS OF ADULT HYPOTHYROID RATS. A. C. Pawlyk, P. A. Rittenhouse*, E. Redei and S. M. Tejani-Butt. Depts. of Psychiatry and Pharmacology, Univ. of Penn. Sch. of Med., Phila., PA 19104.

The role of thyroid hormones (TH) on brain function has been thought to be restricted to developmental influences. Although it has been suggested that thyroid hormone receptor (THR) gene expression is insensitive to alterations in thyroid status in the adult rat brain, the expression of prepro-TRH has been found to be altered in the hypothalamus in response to thyroidectomy. Therefore, changes in thyroid status may affect expression of TH responsive genes. In the present study, we examined the status of THRα2 gene expression in the hypothalamus of adult male rats that were rendered hypothyroid by the addition of 0.05% 6-propyl-thiouracil in their drinking water for 4 weeks. Following this period, rats were sacrificed and hypothalamic tissue was dissected and frozen. RNA was isolated and probed for THRα2 using Northern blots. Hypothalamic THRα2 mRNA levels were found to be significantly increased in hypothyroid rats when compared to euthyroid rats. Our results indicate that THRα2 can be regulated in the hypothalamus in response to decreased availability of TH. Since unliganded THRα2 can regulate gene expression, the observed increase in THRα2 gene expression in hypothyroid rats could have functional significance in hypothalamic gene regulation.

351.4

NGF LEVEL IN THE PITUITARY GLAND DURING ADULT HYPOTHYROIDISM. L. Calzà*, °L. Giardino, §L. Aloe. Inst. of Human Physiology, University of Cagliari; §Inst. of Neurobiology, CNR, Rome, Italy; °Inst. Otolaryngol., University of Milano, Italy.

The involvement of nerve growth factor (NGF) in neuroendocrine regulation is supported by several lines of evidences. Recent reports indicate that NGF and NGF high affinity receptors are present in all the different cell lines in the rat pituitary gland and NGF is secreted by anterior pituitary cells *in vitro* . Adult hypothyroidism is characterized by neurochemical abnormalities involving neurotransmitters, neuropeptides, neuronal proteins and also trophic factors in different brain areas including hypothalamus and in peripheral tissues including pituitary gland. Moreover, adult hypothyroidism is characterized by other major endocrine abnormalities involving the adrenal axis, the prolactin and the growth hormone secretion. In this paper we investigated the NGF levels in several endocrine organs of adult hypo- and hyperthyroid rats. Hypothyroidism was induced by thyroidectomy after 60 days from birth, hyperthyroidism by treatment with T4 (.18mg day, added to the food pellets). The experiment has been performed after 6 weeks of hypo- and hyperthyroidism. NGF levels were measured using a two-site immunoenzymatic assay. We found a 6 fold increase of NGF-IR in the pituitary gland of hypothyroid rats *(control: pg/g tissue 96.6+-29.1; hypothyroid: pg/g tissue 620.0+-95.5)*, whereas no differences were found in testis, adrenal gland and blood. Long term hyperthyroidism does not modified NGF levels in the investigated tissues. These data could support a possible role of thyroid hormone in the regulation of NGF expression in the pituitary gland. The NGF level modification could be also an indirect consequence of long-term hypothyroidism, suggesting a possible role of NGF in the microenvironment regulation in the anterior pituitary.

351.5

LIFELONG TREATMENT OF RATS WITH ANGIOTENSIN-CONVERTING ENZYME INHIBITOR DELAYS PUBERTY IN FEMALE RATS. R.C. Speth*, E.E. Nilsson, D. Edwards and K.H. Bereceek Washington State University, Pullman, WA 99164-6520 and Dept Physiology & Biophysics University of Alabama at Birmingham, Birmingham, AL 35294

Inhibition of the brain renin-angiotensin system (BRAS) disrupts luteinizing hormone (LH) release and ovulation in normal cycling rats. Angiotensin II (AII) receptors in regions of the brain that regulate LH release are lower in prepubertal females than in mature females, suggesting that stimulation of AII receptors in these brain areas is critical for puberty to occur. To investigate this question, female Wistar-Kyoto rats were treated throughout their lives with the angiotensin-converting enzyme (ACE) inhibitor captopril, to inhibit formation of AII. Prior to conception and through weaning, the dams were maintained on 400 mg/ml of captopril in their drinking water. Upon weaning, the pups were maintained on captopril containing water until the time at which they were sacrificed. The age at which the captopril-treated rats underwent vaginal opening and first estrus was monitored and compared to a control group of rats. Vaginal opening occurred later in the captopril treated rats, 38.6±0.8 (Mean±SD) versus 34.9±1.1 days of age in the control group, $p<0.001$. The age of first estrus was also similarly delayed, 43.3±1.3 versus 39.0±2.3 days of age ($p<0.001$) for the captopril-treated and control groups, respectively. The body weight of the control rats at time of sacrifice (the first proestrus day after the first estrus) was significantly greater($p<0.025$) than that of the captopril-treated rats, 102±7.6 versus 93±6.3 grams, respectively, despite the slightly earlier age at the time of sacrifice of the control rats. These results suggest that inhibition of ACE delays puberty and slows development. Formation of AII is a major function of ACE, however, this enzyme also acts to degrade bradykinin, opioid peptides and substance P. Thus while the effects of captopril to delay puberty suggests that AII stimulation of brain AII receptors is important for the timely occurrence of puberty, other peptide substrates of ACE may also be involved in this process. Supported by NIH grants, NS21305, HL31515, HL46554.

351.6

DOES CORTICOSTERONE PLAY A ROLE IN THE EFFECT OF PROGESTERONE UPON GABAb RECEPTORS IN THE NEOCORTEX OF RAT? M.I. Al-Dahan, and R.H. Thalmann* Dept. of Cell Biology and The Div. of Neuroscience, Baylor College of Medicine, Houston, TX 77030

We found that injection of 0.1–2.0 mg progesterone/100g body weight significantly increased the apparent GABAb receptor density that is assayed by the binding of the GABAb agonist [^{3}H]–baclofen to well–washed membranes prepared from the cerebral cortex of ovariectomized (OVX) rats. The progestin/glucocorticoid antagonist RU 486 injected *in vivo* reduced this binding a similar amount whether or not progesterone had been injected, suggesting either a nonspecific effect, or perhaps an antagonism of the effect of endogenous corticosterone as well as injected progesterone. When progesterone was injected in adrenalectomized, ovariectomized rats (ADX,OVX), there was an increase in baclofen binding, as in the case of OVX alone. This was the case in ADX,OVX animals whether plasma corticosterone was 850nM (presumably incomplete ADX) or 245nM. Injections of corticosterone alone that produced plasma levels of 1700nM at sacrifice 4 hours later did not increase baclofen binding. Thus, so far, we have been able to implicate only progesterone in regulating GABAb receptors in a manner similar to their regulation during the estrous cycle (Brain Res. v. 640,p33). Supported by NINDS grant NS–21713.

351.7

SUBCELLULAR LOCALIZATION OF SPECIFIC MEMBRANE ESTROGEN BINDING SITES IN FEMALE RAT BRAIN. J. Zheng* and V. D. Ramirez, Neurosci. Program and Dept. of Physiol. and Biophys., Univ. of Illinois, Urbana, IL 61801

In crude synaptosomal fractions (P2), we previously identified one (23 kDa) of three major (23, 28, 32 kDa) membrane estrogen-binding proteins as oligomycin-sensitivity conferring protein (OSCP), a subunit of mitochondrial FoF1 ATP synthase. This unexpected finding prompted us to examine the subcellular distribution of membrane estrogen-binding proteins. We prepared crude nuclei (P1), mitochondrial (mP2) and microsomal (P3) fractions from adult female rat brain. The estrogen-binding sites were determined by radioligand binding assay using 17β-estradiol-6-carboxymethyl)oxime: [125I]-bovine serum albumin conjugate (17β-E-6-[125I]BSA). The competition assay showed Kd of 12, 15, 38 nM and Bmax of 21, 36, 55 pmol/mg protein for mP2, P1, and P3, respectively. The specific membrane estrogen-binding proteins were further analyzed by ligand blotting using 17β-E-6-[125I]BSA. In the mP2 fraction, a 23 kDa protein was strongly labeled and was identified as OSCP. An additional 40 kDa estrogen-binding protein was also uniquely labeled in mP2. In the P3 fraction, three (28, 32, and 46 kDa) proteins were highly concentrated with much less presence in mP2 and P1. In addition, a 130 kDa protein was present in all fractions but highly concentrated in P1. These data suggest that, unlike the 23 kDa protein (OSCP), the 28, 32, and 130 kDa proteins are not mitochondrial proteins, but most likely plasma membrane proteins.

351.8

KILLING OF GH_4C_1 PITUITARY TUMOR CELLS BY 17-αIODOVINYL ESTRADIOL ($^{125}IVE_2$) IS CELL CYCLE INDEPENDENT C.T. Moore*, G.S. Schneiderman, M.H. Schneiderman, E.R. DeSombre, J.F. Rodriguez-Sierra, Depts. Cell Biology and Anatomy, Radiology, UNMC,Omaha,NE and The Ben May Institute, Univ.Chicago, IL

An asynchronously growing population of the pituitary tumor cell line, GH_4C_1, was exposed to 17-αIodovinyl Estradiol ($^{125}IVE_2$) to determine whether the radiotoxic potential of ^{125}I labeled estrogen, through its estrogen receptor (ER)-mediated association with DNA , was related to the cell cycle. GH_4C_1 cells are estrogen responsive cells and secrete both growth hormone and prolactin. Estrogen binds to DNA at the site of estrogen responsive elements (EREs), thereby altering transcription of genes. Radiolabeled IUdR (^{125}IUdR) is an analog of the nucleoside thymidine and is incorporated into the DNA of proliferating cells during the S-phase. The percentage of cells in S-phase, the labeling index (LI), can be determined by autoradiography. ^{125}I incorporated into DNA is lethal to cells so after treatment with ^{125}IUdR the survival is equal to 1 minus LI. Therefore, we tested whether $^{125}IVE_2$, via mediation of ER, could deliver the ^{125}I to DNA sites thereby killing the steroid sensitive cells and whether such an effect was cell cycle dependent. GH_4C_1 cells were grown in suspension, washed, then incubated for 70 h. Suspension cultures were divided into four groups and exposed as follows: 4.92 μCi/ml $^{125}IE_2$, 2.00 μCi/ml ^{125}IUdR, 2.27 x 10^{-8}M E_2 or 100μl 100% ethanol. Two experiments were run. In the first, cultures were exposed to $^{125}IVE_2$ for 30 min at 37°C, washed to remove any unbound treatment, then frozen at -80°C to accumulate ^{125}I decays. At various times after treatment, cells were plated, incubated for two weeks, stained and colony survival determined. In the second experiment, cultures were continuously exposed to treatment for 95 h. At various times samples were harvested, prepared for autoradiography, and plated to determine colony survival. The LI was approximately 30% after 30 min in ^{125}IUdR, indicating that approximately 30% of the cells were in S-phase during exponential growth. The length of the cell cycle, determined from the increase of the LI with time, was approximately 40 h: G1+M+G2 phases = 25 h , S-phase = 15 h. Because the survival of cells that were frozen to accumulate ^{125}I-decays from $^{125}IE_2$ declined to near zero with increasing decays, we conclude that radiotoxicity of $^{125}IE_2$ to GH_4C_1 cells is cell cycle independent. It has not escaped our attention that radiolabeled estradiol can be used as a labeling tool as well as a therapeutic agent against cells responsive to estrogen therapy in cancer.

351.9

11ß-HYDROXYSTEROID DEHYDROGENASE TYPE 2 (11-HSD2) EXPRESSION IN RAT: *IN SITU* HYBRIDIZATION STUDIES.

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The enzyme 11-HSD allows aldosterone occupancy of mineralocorticoid receptors (MR) by inactivating endogenous glucocorticoids. The present study examined the distribution of 11-HSD2 mRNA in rat brain and peripheral tissues by *in situ* hybridization. An ^{35}S-labeled cRNA probe was generated from a 770 base pair cDNA clone of rat 11-HSD2, from coding region 283-1052. This was linearized with Hind III and synthesized with T7 RNA polymerase. The 11-HSD2 probe was hybridized to 30μm brain sections and 7μm sections of paraffin-embedded peripheral tissues. High levels of expression of 11-HSD2 mRNA were found in kidney, parotid, ovary and rostral medulla of the brain, with moderate levels in uterus and submandibular gland and very low levels in colon and adrenal. No expression was found in liver or testis. These results, together with anatomical studies on MR distribution, suggest that 11-HSD2 may have a functional relationship with MR in some but not all of these tissues, although the specific role of 11-HSD2 is yet to be elucidated.

351.10

A DOSE RESPONSE STUDY OF DEHYDROEPIANDROSTERONE SULFATE ON DENTATE GYRUS LTP. J. Harris*, A. Yoo, and B. Dubrovsky. Neurophysiology Laboratory, McGill University, Montréal, Québec, Canada H3A 1A1

Neurosteroids are produced peripherally by endocrine glands, as well as enzymatically in the glia from steroid hormone substrates. Control of GABA receptor sites and Ca^{++} channel currents are prime targets for neurosteroid actions, and their effects are concentration dependent. For this reason, and the fact that treatment with one of them, DHEAS, improves performance in tasks involving memory in aged rats, we explored the effect of this hormone on dentate gyrus LTP in a dose response mode.

Intact anaesthetized rats (urethane, 1.5 g/kg) were used. Electrodes were stereotaxically positioned in the perforant path and dentate gyrus for stimulation (bifocal) and recording (monofocal). DHEAS (10, 20, and 30 mg/kg, dissolved in Nutralipid 10%) was injected into the femoral vein. Twenty control animals were randomly interspersed within the experimental groups and were injected solely with Nutralipid.

The results showed a significant increase in LTP at all doses in relation to baseline values. Further, there were significant increments in amplitude at 20 and 30 mg in relation to 10 mg. However, the data did not reveal significant differences between the 20 and 30 mg treated rats. While enhancing a putative memory phenomena such as LTP, caution should be applied to the potential long-term beneficial effects of DHEAS on the consequences of aging, especially in the CNS. Neurosteroids at high concentrations behave like glucocorticoids on Ca^{++} channel currents (ffrench-Mullen 1994, 1995). Activation of this channel by glucocorticoids has been linked with aging phenomena in the CNS.

351.11

A SMALLER FORM OF ANDROGEN RECEPTOR mRNA IN BRAIN CONTAINS A TRUNCATED 5'-UNTRANSLATED REGION. R.H. Price Jr.*, M.E. Wilson and R.J. Handa. Depts of Cell Biology, Neurobiology and Anatomy and Molecular Biology, Loyola Univ. of Chicago, Maywood, IL 60153.

The androgen receptor (AR) is a steroid hormone receptor present in both brain and peripheral tissues. In brain, the AR is encoded by mRNA species of two different lengths, the larger of which is approximately 11 kb and the smaller being 9.3 kb. The two forms of AR mRNA are distributed in different proportions in various brain regions. Previous results suggest that the 9.3 kb form contains a truncated or different 5' untranslated region (5'UTR). We have utilized a 5' RACE technique in an attempt to detect and amplify the 5' ends of both AR mRNA species in various brain regions and reproductive tissues of the male rat. Reverse transcription was performed using an oligo primer directed against nucleotides 2772 - 2791. ds cDNA representing both forms of AR was synthesized from ventral prostate, testis, anterior pituitary, hippocampus, cerebral cortex, and hypothalamus. PCR using AR primers targeting nucleotides 1004 - 1024 and 1228 - 1238 (both near 5' end of the coding region) and primers specific for a ds cDNA Adaptor (Clontech) ligated to the 5' ends of the AR ds cDNAs revealed two predominant amplified products. One product corresponds in size to the previously described 1 kb 5'UTR and the other reflects a significantly smaller 5'UTR of less than 100 bases. The relative concentrations of both forms of AR mRNA in the brain regions corresponds to previous findings. The existence of a shorter 5'UTR presents the possibility that there is an alternate transcription start site proximal to the amino-terminal end of the coding region. Sequence data will determine the similarity of the shorter 5'UTR with the existing, previously cloned 1 kb 5'UTR. These data may help determine region-specific regulation of AR expression in the brain. Supported by NSF IBN 9408890.

351.12

ESTROGEN PROTECTS PRIMARY CORTICAL NEURONS FROM GLUTAMATE NEUROTOXICITY. C.A. Singer*, T.M. Strickland, K.L. Rogers and D.M. Dorsa. Departments of Pharmacology and Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98195 and GRECC, VA Medical Center, Seattle, WA 98108

Estrogens have been shown to exert neurotrophic effects in the brain by regulating processes involved in neuronal growth, differentiation and survival. The ability of estrogen to promote neuronal survival has been shown in primary neuronal cultures from the amygdala, as well as in neuroblastoma and glial cells during serum deprivation or hypoglycemia. This leads to the possibility that estrogen might exert neuroprotective effects during other types of cytotoxic insults. Neurotoxicity mediated by the excitatory neurotransmitter glutamate has been extensively studied in primary cortical neurons and the present experiments were designed to examine the possibility that estrogen may protect these neurons from glutamate neurotoxicity. Rat primary cortical neurons were cultured for 7 to 12 days in a serum-containing medium. Twenty-four hours prior to glutamate exposure the cells were shifted to a serum-free medium containing various concentrations of 17β-estradiol, 17α-estradiol or cholesterol. The neurons were then exposed to 50 μM or 100 μM glutamate for 5 minutes and maintained in a serum-free medium prior to assay. Cytotoxicity was determined by measuring lactate dehydrogenase (LDH) in the medium twenty-four hours after glutamate exposure. Results from these experiments demonstrated that 10 nM 17β-estradiol protects cortical neurons from exposure to glutamate. Treatment with 10 nM 17α-estradiol or 10 nM of the steroid precursor cholesterol did not protect the neurons. These results demonstrate that 17β-estradiol can protect neurons from glutamate toxicity. The molecular basis of this protective effect, however, remains to be elucidated. Supported by the VA, AG05136, NS20311, NS07332, and NARSAD.

351.13

EFFECT OF *IN VITRO* 17ß-ESTRADIOL (E2) ON CONCENTRATIONS OF GLUCOSE TRANSPORTER (GLUT1) IN RAT BLOOD-BRAIN BARRIER (BBB). J. Shi and J. W. Simpkins*. Department of Pharmacodynamics & Center for Neurobiology of Aging, College of Pharmacy, University of Florida, Gainesville, FL 32610

Glucose provides the primary source of fuel in mammalian brains and a 55kDa GLUT1 glucose transporter is responsible for the passage of glucose across blood-brain barrier endothelial cells. We previously reported that 17ß-estradiol (E2) caused a 40% increase in glucose transport across the BBB. The present study was designed to determine if E2 increases GLUT1 protein in brain endothelial cells in vitro. Method: 7 female rats (initial body weight 200-225gm, from Charles River, Inc.) were decapitated 14 days after ovariectomy (OVX). Cortex homogenates were separated into Ringer's solutions containing either 544 pg/ml E2 or vehicle. Microvessels were isolated and then incubated at 37°C for 4 hours in the presence or absence of E2. Protein samples were isolated, separated on SDS-PAGE gel and electrically transfered to nitrocellulose membrane. Rabbit anti- rat brain glucose transporter and ECL Western blotting analysis system were used for immunoblotting and chemiluminescence, respectively. The same sample from intact rats were used as the standard for quantification. Red blood cell glucose transporter were used as reference because its motility is identical to BBB GLUT1. Results: (1) the only protein observed was 55 kDa band; (2) total cellular GLUT1 increased in the E2 group (mean +/- S.E.M., 4.917+/-.16) vs. vehicle group (3.739+/-.209) ($p<0.001$); cytosol GLUT1 increased similarly to total cellular GLUT1 (2.616+/-.148 vs. 2.021+/-.148, $p<.05$); plasma membrane GLUT1 changed little between two groups (OVX+E2 vs. OVX, 3.477+/-.244 vs. 3.239+/-.273, $p=.52$); . This study shows that in vitro treatment with E2 increases total GLUT1 in the brain microvessel endothelial cells and that over the 4 hours incubation, this increase is confined to the cytosol with little change in the plasma membrane. (Supported by NIA grant AG 10485)

351.14

PHARMACOLOGICAL SPECIFICITY OF ESTROGEN-MODULATED ENHANCEMENT OF AMPHETAMINE-INDUCED STRIATAL DOPAMINE RELEASE. L. Xiao* and J. B. Becker, Dept. of Psychology, Univ. of Michigan, Ann Arbor, MI 48104.

While the striatum does not appear to contain classical intracellular estrogen receptors, estradiol rapidly enhances amphetamine (AMPH)-induced striatal dopamine (DA) release. This has led to the hypothesis that estradiol acts within the striatum via a novel estrogen receptor-based mechanism(s). To determine the specificity of estrogen-modulated DA release, several estrogen analogs, metabolites and the classical antiestrogen, tamoxifen (TAM), were applied to superfused striatal tissue. Striatal tissue obtained from ovariectomized female rats was superfused with an estrogen metabolite/analog for 30 min followed by 2.5 min AMPH to induce DA release. TAM was tested alone and in the presence of 17ß-estradiol to determine its agonistic and antagonistic properties. We demonstrate that the catechol estrogens, 2-hydroxyestradiol and 4-hydroxyestradiol, at a concentration equimolar to the effective dose of 17ß-estradiol enhanced AMPH-stimulated striatal DA release. In contrast, estrone and estriol did not mimic the effect of estradiol. The methylated metabolite of 2-hydroxyestradiol, 2-methoxyestradiol, attenuated striatal DA release at the equimolar concentration to that of estradiol. Furthermore, TAM displayed a partial agonistic effect when administered alone, and antagonistic effect when applied with estradiol. Dose response studies of these compounds are currently underway to further examine the relative potencies of the effect. Results from this study are believed to have important implications with respect to the mechanism(s) underlying the hormonal regulation of neurochemical and behavioral indices of the mesostriatal DA system as well as the role of DA in reproductive activity in the rat. Supported by NSF #BNS9021966; and NIH #T32-HD07048.

351.15

THE EFFECTS OF EXOGENOUS RELAXIN ADMINISTRATION ON *C-FOS* EXPRESSION IN THE BRAIN OF PREGNANT RATS. T.A. Heine, S. Di*, L.L. Anderson, C.D. Jacobson. Departments of Veterinary Anatomy and Animal Science and the Neuroscience Program, Iowa State University, Ames, IA 50011.

Relaxin is a hormone involved in remodeling the gravid cervix. It is also thought to play a role in parturition. Our laboratory has been studying the effects of relaxin on *c-fos* activation in the brain. *C-fos* is a nuclear second messenger which is activated through stimulation of cellular receptors. Peak levels of expression are reached two hours after initial activation. In this study, eight adult virgin female rats were divided into two groups. Following achievement of timed pregnancy, rats were ovariectomized at eleven days of gestation, and pregnancy maintained with subcutaneously placed estradiol and progesterone capsules. Jugular cannulas were also placed at this time. On day nineteen of gestation, one group of rats received intravenous (IV) relaxin (10 µg) and the other group received an equivalent volume of isotonic saline IV. Two hours post-injection, the rats were euthanized followed by intracardiac perfusion with saline and 4% paraformaldehyde. Immunocytochemistry was used to visualize areas in the brain in which *c-fos* had been activated by the relaxin or saline administration. The supraoptic nucleus (SON) had extensive *c-fos* activity in the rats receiving relaxin, whereas minimal activity was seen in the SON of rats receiving isotonic saline. Analysis of other hypothalamic nuclei will be discussed. Studies are currently being conducted to determine if relaxin is specifically affecting oxytocinergic cells in the SON.

351.16

ESTRADIOL INHIBITION OF RAT STRIATAL CALCIUM CHANNELS: G-PROTEIN MECHANISM OF ACTION. P.G. Mermelstein*, J.B. Becker and D. J. Surmeier. Neuroscience Program, Psychology Dept. and Reproductive Sciences, Univ. Michigan, Ann Arbor, MI and Dept. Anatomy and Neurobiology, Univ. Tennessee, Memphis, TN.

Steroid hormones act upon cells containing intracellular receptors. However, recent evidence suggest steroids can have specific and rapid effects at the cellular membrane. Using whole-cell patch clamp techniques, 17β-estradiol reversibly blocked calcium currents in acutely dissociated and primary cultured striatal neurons. The effects were sex specific: the reduction of calcium currents was greater in neurons taken from female tissue. 17β-Estradiol primarily targeted L-type calcium channels and its inhibition was reliably seen within seconds of administration. Maximum block of 17β-estradiol occurred at picomolar concentrations. 17β-Estradiol, when conjugated to BSA, was also effective in reducing calcium current, suggesting the effects occurred on the membrane surface. Dialysis of striatal neurons with GTPγS demonstrated inhibition of calcium currents by 17β-estradiol was through G-protein activation. 17α-estradiol also reduced calcium currents, but its maximum block was significantly less then when compared to 17β-estradiol. Estriol and 4-hydroxyestradiol were found to reduce calcium currents with similar efficacy to 17β-estradiol, while estrone and 2-methoxyestriol were less effective. Tamoxifen served as a partial agonist as well, but did little to occlude 17β-estradiol's reduction in calcium current. These results suggest that at physiological concentrations, 17β-estradiol has immediate and specific actions upon striatal neurons altering signaling pathways independent of genome activating receptors.

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NEURAL-IMMUNE INTERACTIONS: CYTOKINES II

352.1

COMPARISON OF IL1α DISTRIBUTION IN CVO AND NON-CVO TISSUES AFTER IV AND ICV INJECTION. S.R. Plotkin, W.A. Banks, and A.J. Kastin*. Veterans Affairs Medical Center and Tulane Neuroscience Interdisciplinary Program, New Orleans, LA, 70146.

Interleukin-1α (IL-1α) has been implicated in many processes within the central nervous system (CNS). IL-1α could interact with CNS tissues either by crossing the blood-brain barrier (BBB) or by interacting directly with circumventricular organs (CVOs), regions within the brain lacking a BBB. In this study, Swiss-Webster mice were given intravenous (IV) and intracerebroventricular (ICV) injections of radioactively labeled IL-1α (^{125}I-IL-1α) and albumin, and samples of CVO tissues (pineal, median eminence, and area postrema) and non-CVO tissues (cortex and cerebellum) taken for analysis. When compared with cortex and cerebellum after IV or ICV injection, CVOs contained higher amounts of ^{125}I-IL-1α on a per gram basis, but significantly smaller amounts as a percent of total brain radioactivity. When unlabeled IL-1α was included in the injection, there was a decrease in the levels of ^{125}I-IL-1α in CVO and non-CVO tissues after IV injection but not after ICV injection, indicating that uptake can be inhibited in the blood to brain direction. These results suggest that both CVO and non-CVO brain tissues selectively take up blood-borne ^{125}I-IL-1α, although the basis for this selectivity may not be the same for both types of tissue. The results also suggest that diffusion is an important process for centrally administered cytokines. Overall, this study supports current views that blood-borne cytokines can exert some of their CNS effects either by acting directly at CVOs or by crossing the BBB and interacting with cells isolated from the periphery.

352.2

SOMATOSTATIN MODULATION OF INTERLEUKIN-2 SECRETION BY ACTIVATED HUMAN T CELLS INVOLVES A SUBCLASS OF SPECIFIC RECEPTOR THAT ARE NOT DIRECTLY COUPLED BUT REQUIRES INHIBITION OF ADENYLATE CYCLASE. S. Krantic*, A. Cardoso, C. Rabourdin-Combe. Lab. Biol. Mol. Cell., ENS de Lyon, France.

Neuropeptide somatostatin (SRIF) displays immunomodulating properties such as complex regulation of T cell proliferation. We assessed whether such regulation involves a modulation of interleukin-2 (IL-2) secretion.

IL-2 content was quantified in the supernatants of human T cells of Jurkat line incubated in the presence of mitogens (phorbol ester 10 ng/ml+phytohaemagglutinin 2 µg/ml) and SRIF. Our data show that SRIF potentiates mitogen-stimulated IL-2 secretion with an EC_{50} of $1X10^{-8}$M.

We further analyzed kinetic and pharmacological properties of relevant SRIF binding sites by performing ^{125}ITyr^{1}-SRIF14 binding assays on semi-purified plasma membrane preparations of Jurkat cells. Three types of binding sites were identified (Kd_1=$7.8X10^{-11}$M, Kd_2=$3.7X10^{-10}$M and Kd_3=$1.24X10^{-8}$M). The latter binding site was a good candidate for mediating SRIF effects on IL-2 secretion since its Kd is identical to EC_{50} of SRIF action on IL-2 secretion.

Finally, we examined the coupling of Jurkat cell SRIF binding sites to adenylate cyclase. IC_{50} value for SRIF inhibition of the enzyme was $2.2X10^{-11}$M thus suggesting the involvement of high affinity receptors.

Therefore, the putative SRIF receptor involved in regulation of IL-2 secretion is pharmacologically defined as a low-affinity receptor that is not directly coupled to adenylate cyclase. However, at SRIF concentration (about 10^{-8}M) necessary for activation of receptor involved in neuropeptide action on IL-2 secretion, adenylate cyclase appears already maximaly inhibited via activation of high-affinity receptors.

352.3

INTERLEUKIN 2 (IL-2) SUPPRESSES AFFERENT SENSORY TRANSMISSION IN THE PRIMARY SOMATOSENSORY (SI) CORTEX K.H. Lee[1]*, C.K. Won[2], H.J. Park[2], K.H. Pyun[3] and H.C. Shin[2] Dept. of Neurosurgery[1], Dept. of Physiology[2], Coll. of Med., Hallym Univ. Chunchon, Immunology Lab[3], KRIBB, KIST, Taejeon, Korea

Although IL-2-like immunoreactivity and IL-2 receptors are present in laminar IV of the frontoparietal cortex in the rat, the functional role of IL-2 in this brain region has not been characterized. In this study the effect of topical application of IL-2 on afferent sensory transmission to neurons in the SI cortex was determined in anaesthetized rats. Quantitative determination of the effect of IL-2 on the afferent sensory transmission to the neurons in the SI cortex was carried out by generating poststimulus time histograms of single unit responses to the subcutaneous electrical stimulation of the receptive field located in the whisker area of the face. IL-2 (0.1, 1.0, 5.0 units) significantly suppressed afferent sensory transmission in SI cortical neurons (n=19) in a dose dependent manner (0.1 U: -6.00±2.9%, $p < 0.01$; 1.0 U: -12.14±1.6%, $p < 0.01$; 5 U: -25.06±3.5%, $p < 0.01$; 0.1 vs. 1.0 U: $p < 0.05$; 1 U vs. 5 U, $p < 0.01$). IL-2 induced suppression fully recovered by 60 min after drug. In control experiments, saline solution containing 0.2% bovine serum albumin, used as a vehicle, did not affect afferent sensory transmission. The results of this study provide evidence that IL-2 and its receptors present in the SI cortex may participate in the processing of afferent sensory information arising from the face (supported by KOSEF 941-0700-009-2).

352.4

POSSIBLE INVOLVEMENT OF CYTOSOLIC PHOSPHOLIPASE A_2 IN THE TNFα SIGNALING PATHWAY IN ASTROCYTES. W. Tong, M. Hannink and G. Y. Sun* Biochem. Dept., Univ. Missouri, Columbia, MO 65212

We have recently shown that addition of tumor necrosis factor (TNFα) to cultured astrocytes resulted in activation of DNA-binding by a RelA homodimer (JBC, 270:2703, 1995) and the induction of the group II phospholipase A_2 mRNA (Mol. Chem. Neuropath., in press). Although in some cells, TNFα signaling is known to involve the stimulation of an acidic sphingomyelinase and release of ceramide as second messenger, our studies suggest little or no involvement of the sphingomyelinase pathway in the TNFα signaling in astrocytes. On the other hand, our results demonstrated the ability of arachidonic acid (AA) (10 µM), lysophosphatidylcholine (LPC) and lysophosphatidic acid (LPA) (10 µM) to induce RelA/DNA binding as well as release of secretory PLA_2. LPC *together with AA gave a synergistic effect on PLA_2 release and* together, they also potentiated the effect of TNFα on PLA_2 release. The involvement of PLA_2 is further supported by the observation that bromophenacylbromide, a PLA_2 inhibitor, resulted in complete inhibition of RelA/DNA binding induced by TNFα. These results support the notion that cytosolic PLA_2 leading to the release of lysophospholipids and AA is an early event associated with TNFα activation of RelA/DNA binding and PLA_2 release in astrocytes.

352.5

THE MAJOR SOURCE OF PLASMA INTERLEUKIN-6 (IL-6) ELICITED BY IMMOBILIZATION (IM) STRESS IN RAT. A.Takaki*, S. Shioda, Q.-H. Huang, A. Arimura. U.S.-Japan Biomed Res Labs, Tulane Univ, Belle Chasse, LA 70037, USA; Dept of Physiol, Fac of Medicine, Kyushu Univ, Fukuoka 812, Japan.

We have recently reported that IM-induced plasma IL-6 elevation was mediated by both central and peripheral catecholaminergic mechanisms, and that adrenal corticoid suppressed this response (Takaki et al. 1995). However, the source for increased plasma IL-6 during IM stress remains unknown. Our previous study excluded the spleen, which contains many immune competent cells, as well as the adrenal and pituitary gland, as possible candidates. Since Kupffer (K) cells in the liver were suggested as a major source for the hemorrhage shock-induced plasma IL-6 (O'Neill et al. 1993), the liver was focused as the next candidate. Male CD rats (300g BW) were partially (70%) hepatectomized under anesthesia 4 days before IM stress. Awake animals were restrained for 60 min in the supine position. Plasma IL-6 response to IM was significantly attenuated by partial hepatectomy ($p<0.01$). To examine the involvement of the sympathetic innervation in the liver, the rats underwent surgical denervation of the hepatic nerve. IM-induced plasma IL-6 response in denervated rats was again attenuated as compared with the sham-operated animals ($p<0.01$). Furthermore, the tissue distribution of IL-6 like immunoreactivity (IL-6-LI) was examined histochemically using polyclonal anti-murine IL-6 antiserum (R&D system, MN) which appeared to cross-react with rat IL-6. Some K and endothelial (E) cells in the liver obtained from rats immobilized for 1hr showed significant IL-6-LI. In addition, norepinephrine stimulated IL-6 production in K cells obtained by *in vitro* culture preparation. These findings suggest that the liver cells, especially K and E cells, are the major source of increased plasma IL-6 during IM stress, and that sympathetic innervation in the liver plays a critical role in the production of IL-6 in these cells.

352.6

BIOTINYLATED INTERLEUKIN-1 RECEPTOR ANTAGONIST BINDS TO VAGAL PARAGANGLIA IN DIVERSE VERTEBRATE SPECIES. L.E. Goehler*[1,3], J. Relton[2], M. Laudenslager[4], S.F. Maier[1], & L.R. Watkins[1]. [1]Dept Psych, Univ. Colorado, Boulder, CO 80309; [2]Amgen, Boulder, CO.; Depts. of C & S Biology[3] & Psychiatry[4], UCHSC, Denver CO.

Activation of the immune system elicits a variety of physiological & behavioral effects that are mediated by the central nervous system (CNS). This immune-to-brain communication results from the release of cytokines such as interleukin-1 (IL1) during the initial phases of the immune response. IL1 is key since IL1 receptor antagonist (IL1ra) blocks illness responses. Intriguingly, section of the abdominal vagus can also block illness responses, including elevated steroid, hypothalamic norepinephrine depletion, hyperalgesia, & fever (Watkins, Maier & Goehler, Life Sci., Minireview, 1995, in press). This suggests that the immune system must somehow activate vagal afferents in order to signal the brain. Indeed, we recently reported that biotinylated IL1ra (bIL1ra) selectively binds to glomus cells within abdominal vagal paraganglia of the rat. These putative chemoreceptors may thus be a key link between the immune system & vagal afferents to the CNS.

Because vagal paraganglia are present in diverse vertebrate species, we investigated whether glomus cells from other species also bind bIL1ra. Whole vagus nerves from monkey (Macaca nemistrina), bullfrog (Rana catesbeiana) & goldfish (Carassius auratus) were dissected without fixation, & incubated overnight in bIL1ra (2.95 mg/ml). Following immersion fixation & washing, the nerves were processed for avidin-biotin complex (ABC) histochemistry. Biotinylated IL1ra binding occurred in glomus cells of vagal paraganglia in all three species. Omission & competition controls confirmed specificity of binding. Taken together, these results suggest that vagal sensitivity to cytokines evolved early in the vertebrate lineage, & that vagal mediation of immune to brain communication may be a fundamental feature of vertebrate bodily defenses.NIH Grant MH45045, NIH Grant NS31569, & Amgen.

352.7

EFFECTS OF CHRONIC INTRACEREBROVENTRICULAR ADMINISTRATION OF INTERFERON-α IN THE RAT BRAIN. M. Sanchez, G.E. Farrar and D. Saphier*. Dept. of Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130.

Interferon-α (IFN-α) is an immunopotentiating cytokine produced by virus-activated leukocytes that is used clinically for it's antiproliferative and antiviral activity. However, chronic administration of IFN-α has been found to cause cognitive, behavioral and neurological deficits that resemble those seen in HIV-disease. In fact, increased plasma and CSF concentrations of IFN-α have been detected in this disease, suggesting that this cytokine may be at least partially responsible for the neuropathology found in such patients. There are some changes characteristic of neurodegeneration, including: proliferation of astrocytes, changes in neuronal processes, alterations in the cytoskeleton of neurons and neuronal cell death. Thus, the aim of the present study was to further characterize the neuropathological effects of chronic i.c.v. administration of IFN-α (10^3 U/5 µl, three times per week), using histological and peroxidase-antiperoxidase immunohistochemistry techniques. Paraformaldehyde-fixed, frozen tissue sections of the brains of treated rats were used, and the following monoclonal antibodies were employed for the immunohistochemical studies:

a) Proteins identified as components of neuronal intermediate filaments: either to the non-phosphorylated (SMI32) or phosphorylated (SMI35) epitopes in the heavy molecular weight neurofilament.

b) Glial fibrillary acidic protein (GFAP).

c) Heat shock protein-70 (HSP-70): both constitutive (HSP73) and inducible (HSP72).

Preliminary results show some alterations in the immunoreactivity for SMI32 and/or SMI35 in the hippocampus and frontal cortex of the IFN-α-treated rats, although no signs of neuropathology were evident in Nissl-stained sections. In some IFN-α-treated animals, increased GFAP immunoreactivity was found in the substantia nigra, which paralleled some locomotor activity deficits. In order to determine if this reflects an effect on the dopaminergic cells of the region, we are currently carrying out a tyrosine hydroxylase immunohistochemical study.

352.8

IL-6 DOES NOT AFFECT THE VIABILITY OF RAT PRIMARY NEURONS IN CULTURE. N.S. Ranciat, L. Ramonda-Rocha, Y. Xia, R. Pal, M.L. Michaelis, E.K. Michaelis*, Dept. of Pharmacol.& Toxicol. and the Higuchi Biosci. Ctr., Univ. of Kansas, Lawrence, KS 66045

Interleukin-6 (IL-6) is a pleiotropic cytokine which mediates signals to its target cells by binding to a receptor composed of a ligand-binding unit (IL-6R) and a signal transducer (gp130) shared with several other cytokine receptors. The presence of IL-6 in amyloid plaques of Alzheimer's patients and an IL-6 response element in the Amyloid Precursor Protein (APP) promoter led to the hypothesis that IL-6 may be involved in neurodegenerative events. We investigated the effects of IL-6 on the viability of neurons in culture and assessed expression of IL-6R's in these cultured cells and in adult rat brain. Primary cortical neurons were treated with IL-6 (25-150 U/ml) for 24 to 72 hrs. Viability was assessed by morphological criteria and 2 biochemical assays, LDH release into the medium and MTT reduction by the mitochondrial enzyme succinate dehydrogenase. Expression of the IL-6R was monitored by immunoblot analysis. IL-6 treatment did not affect the viability of rat primary cortical neurons under any of the experimental conditions tested. Immunochemical analyses revealed that the IL-6R is expressed in adult rat brain, but no immunoreactive IL-6R could be detected in cultured neurons. This lack of expression of IL-6R in cultured neurons may be due to the culture conditions or may reflect developmental regulation of the IL-6R in the CNS. These 2 hypotheses are currently under investigation in our laboratory (Supported by Alzheimer's Assoc. grant #94-128 and MMD-Sci. Ed. Partnership.)

352.9

BEHAVIORAL ACTIVATING EFFECTS OF INTERLEUKIN (IL)-2 AND IL-6. S. Zalcman*[1], D.M. Nance[2], and A.H. Greenberg[2]. [1]Center for Studies in Behavioral Neurobiology, Dept. of Psychology, Concordia University, Montreal, Qc, Canada H3G 1M8 and [2]Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, Mb, Canada R3E 0V9.

We have previously demonstrated that recombinant IL-1, IL-2 and IL-6 induce cytokine-specific neurochemical alterations. Consistent with this observation is the novel finding that these cytokines differentially alter behavior. In particular, increases in the number of rears and digging episodes were evident in IL-6-treated BALB/c mice (200 or 400 ng, ip) exposed to a novel environment (i.e., a new shoebox cage). Moreover, IL-6-treated animals placed into a circular environment (i.e., a round beaker) exhibited turning behavior, tail gnawing and an increased tendency to scratch the walls of the beaker compared with vehicle-treated controls. Dopamine (DA) concentrations in the nucleus accumbens and in the caudate nucleus were reduced in IL-6-treated animals compared with controls. Hypothalamic or extra-hypothalamic norepinephrine (NE) levels were not appreciably altered in these mice. In contrast, IL-1 induced a depression of behavior that appeared to be independent of central DA changes. Moreover, IL-2-treated mice (200 or 400 ng, ip) appeared to be hypervigilant in that an increased number of nose pokes (into a hole drilled into a side of a new cage) was evident in these animlas compared with control responses. These effects appeared to be associated, at least in part, with alterations of hypothalamic and extra-hypothalamic NE activity. Furthermore, exposing IL-2-treated animals to a mild stressor resulted in malformed nests 24-48 hr after treatment. It is suggested that cytokine-specific behavioral activating effects serve an adaptive purpose during an orchestrated immune response. However, pathological elevations of these cytokines (such as may occur during an autoimmune response) or cytokine-stressor interactions could result in behavioral profiles that are associated with psychopathological states. (Supported by NIMH, MRC of Canada)

352.10

EXPRESSION OF TYPE 1 AND TYPE 2 TUMOR NECROSIS FACTOR RECEPTORS BY RAT GLIA. J.M. Dopp[1]*, A. Mackenzie-Graham[1], G.C. Otero[1], and J.E. Merrill[1,2]. Dept. of Neurology[1], UCLA, Los Angeles, CA, 90024; and Berlex Biosciences[2], Richmond, CA, 94804.

Tumor necrosis factor alpha (TNFα) and lymphotoxin alpha (LTα) induce pleiotropic cellular effects through low-affinity 55-kDa type 1 receptors (TNFR1, CD120a) and high-affinity 75-kDa type 2 receptors (TNFR2, CD120b). TNFα is present in multiple sclerosis (MS) lesions, and its secretion by microglia may contribute to oligodendrocyte injury during MS. *In vitro*, TNFα and LTα induce a diversity of effects in glial cells, ranging from proliferation to cytotoxicity. To determine whether differential expression of TNFR1 versus TNFR2 underlies the unique response of a given type of glia to TNFα and LTα, primary cultures of enriched astrocytes (99% $GFAP^+$), microglia (97% $AcLDLR^+$), and oligodendrocytes (95% $GalC^+$) were used in Northern and Western blot analyses of TNFR expression. Northern blots of poly-A^+ RNA revealed a single 2.5-kb TNFR1 transcript in all types of glia and in the RAW 264.7 cell line; transcript levels were increased by IFNγ. TNFR2 transcripts of approximately 3.3, 4.4, and 5.7 kb were detected in unstimulated RAW cells, in TNFα-stimulated microglia and, less intensely, in TNFα-stimulated oligodendrocytes. In Western blots, anti-TNFR1 antibodies detected a 55-kDa protein in whole-cell lysates of all types of glia and RAW cells. Anti-TNFR2 antibodies detected a 69-kDa protein in RAW cells and all glia; protein levels were downregulated by TNFα in astrocytes. Both antibodies also bound a 73-kDa protein in all types of cells. Cytokine-induced TNFR shedding and TNFR functions in glia are presently being analyzed. This work was supported by the Conrad N. Hilton Foundation and the Norman Cousins Program in Psychoneuroimmunology at UCLA.

352.11

CENTRAL COADMINISTRATION OF THE CYTOKINES IL-2 AND IL-3: EFFECTS ON HYPOTHALAMIC-PITUITARY-ADRENOCORTICAL (HPA) FUNCTION. U.-K. Hanisch*[1], W. Rowe[2], J. Neuhaus[1], D. van Rossum[2], M.J. Meaney[2] and R. Quirion[2]. [1] MDC Berlin, 13122, Germany, [2] Douglas Hospital Research Centre, McGill University Montreal, H4H 1R3, Canada.

Chronic intracerebroventricular (i.c.v.) administration of IL-2 in rats leads to permanent HPA hyperfunction and causes damage to both glial and neuronal cells (Hanisch et al., *Endocrinology* **135**: 2465-2472, 1994). In the present study, male Sprague-Dawley rats were i.c.v. infused via osmotic minipumps over a period of 14 days with either IL-2 (3 ng/hr), IL-3 (7 ng/hr), both IL-2 and IL-3, or vehicle only (as controls). IL-2 given alone led to persistently increased plasma levels of corticosterone (CORT). IL-3 itself did not alter HPA function. When IL-3 is chronically co-infused along with IL-2, IL-3 significantly inhibited the IL-2-induced raise in CORT levels. These HPA effects of central IL-2 infusion versus IL-2/IL-3 coadministration thus parallel the clinical observation that *systemic* IL-3 administration can block the increase in adrenal cortisol release as induced in cancer patients by high doses of *systemic* IL-2 (Lissoni et al., *J. Biol. Regul. Homeost. Agents* **6**: 113-115, 1992). While attenuating the effect of IL-2 on the HPA axis, coadministration of IL-3 at this dose (ratio) apparently could not abolish the neural tissue injury resulting from chronically elevated central levels of IL-2. Animals receiving IL-2 only or both IL-2 and IL-3 showed brain infiltration by peripheral cells accompanied by CNS lesions, as previously reported for IL-2-infused rat CNS (Hanisch et al., *Soc. Neurosci. Abstr.* **20**: 954, 1994). IL-3 alone did not affect brain tissue integrity. Taken together, the data suggest that IL-3 - although not having significant effects on its own - can modulate the neuroendocrine activity of IL-2 likely by a central mechanism. These results may, therefore, be relevant to the clinical application of IL-2 during immunotherapies. Supported by the BMFT of Germany and the MRC of Canada.

352.12

INTERLEUKIN-15 GENE EXPRESSION IN HUMAN ASTROCYTES AND MICROGLIA. Y.-B. Lee*, J.-I. Satoh, D.G. Walker, and S.U. Kim. Div. of Neurology, Univ. of British Columbia, Vancouver, BC, Canada

Recent studies have suggested that inflammatory cytokines such as IL-1, TNF-α, IFN-γ, and IL-6 are involved in the pathogenesis of several inflammatory and degenerative neurological diseases by activating astrocytes and microglia. These cytokines also could accelerate the progression of inflammatory processes mediated by infiltrating T cells. However, the mechanisms of entry and proliferation of T cells in the CNS and possible involvement of cytokines in these processes remain unknown. Interleukin-15 (IL-15), a newly cloned cytokine which shares T cell growth activity and components of the receptor with IL-2, could play a role in T cell activation in the CNS.

To investigate the expression and cytokine-mediated induction of IL-15 in the human CNS, enriched preparations of astrocytes and microglia were established from cultures of human fetal brains (12-20 weeks' gestation). The expression of IL-15 gene by these cells was determined by reverse transcription-polymerase chain reaction (RT-PCR). Low levels of IL-15 mRNA were detected in both untreated microglia and astrocytes. Upregulation of IL-15 mRNA was demonstrated in microglia following IFN-γ treatment and in astrocytes by IFN-γ and IL-1β. In addition, IL-15 mRNA expression was downregulated by IL-6 in astrocytes. These results suggest that IL-15 produced by astrocytes and microglia plays a role in the regulation of T cell activation in the human CNS. (Supported by the Medical Research Council and the MS Society of Canada)

352.13

ASTROCYTES EXPRESS REGIONAL HETEROGENEITY AFTER LIPOPOLYSACCHARIDE INDUCTION OF THE IMMUNO-REACTIVE PHENOTYPE

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Lipopolysaccharide (LPS) has been shown to be a strong inducer of the immuno-reactive phenotype in cultured cerebral astroglial cells. This reactive state is characterized by a strong expression of class I and II MHC antigens, an increased production of TNF-α, IL-1 and IL-6, and as recently shown the expression of an inducible nitric oxide (NO)-synthase. Numerous studies document that cerebral astrocytes do not constitute a homogeneous population inside the brain (for review, see Wilkin *et al.*, 1990 TINS 13:43-46).

In this *in vitro* study, we analyzed the regional heterogeneity of primary rat astrocytes after induction of the immuno-reactive phenotype by a 48 hour-incubation in the presence of LPS. We measured the expression of MHC class II molecules, the production of NO° as nitrites, and the synthesis of TNF-α in astroglial cultures originating from the following brain regions: cortex, hippocampus, septum, striatum and brain stem. After LPS-induction, class II expression is highest in hippocampal and lowest in septal cultures the other regions showing intermediate values. NO° production is most intense in hippocampal and cortical astrocytes, the lowest levels appearing in cultures from brain stem. TNF-α synthesis was readily induced in all regions by LPS. Brain stem astrocytes show however much lower amounts than the other tested regions. We conclude that immunological activation reveals specific regional differences in the cerebral astroglial population.

352.14

DIFFERENT MECHANISMS INVOLVED IN THE INHIBITORY EFFECTS OF VIP ON IL-2 AND IL-4 PRODUCTION. Z. Xin*[1], L. Sun[2], H.Y. Wang[1], H. Tang[1], and D. Ganea[1]. [1]Rutgers University, Newark, NJ 07102 and [2] American Cyanamid, Pearl River, NY 10965.

Neuropeptides have been reported to modulate the immune response *in vivo* and *in vitro* and specific neuropeptide receptors have been reported on a variety of immune cells. Previous results from our laboratory indicated that VIP inhibits both IL-2 and IL-4 production in murine splenocytes and thymocytes. Here we investigate the mechanisms underlying the inhibition of IL-2 and IL-4 by VIP. Balb/c spleen cells and thymocytes are cultured with anti-CD3 plus PMA in the presence of VIP and Northern blots or competitive PCR are carried out for spleen cells and thymocytes, respectively. Our results indicate that VIP inhibits IL-2, but not IL-4 steady-state levels of mRNA in both spleen cells and thymocytes, suggesting transcriptional and post-transcriptional regulation for IL-2 and IL-4, respectively. Previous studies involved cAMP as a transduction pathway for IL-2 transcriptional inhibition. We compared VIP and forskolin, a known cAMP inducer, in terms of cAMP induction and IL-2 inhibition and concluded that an additional transduction pathway, besides cAMP, participates in IL-2 inhibition. Actinomycin D experiments indicate that VIP does not affect IL-2 mRNA stability, suggesting that the IL-2 transcriptional rate is affected by VIP. Posttranscriptional regulation of IL-4 production by VIP could involve different mechanisms Our present results indicate that VIP does not affect secretion, uptake, or intracellular IL-4 stability, suggesting a specific inhibitory effect on IL-4 translation.

352.15

GANGLIOSIDE GT1b AFFECTS CONSTITUTIVE AND INDUCIBLE EXPRESSION OF TRANSCRIPTION FACTORS NF-κB, IRF-1 AND IRF-2 IN ASTROCYTES. P.T Massa* and H. Wu Dept. of Neurology, SUNY-HSC, Syracuse, N.Y. 13210.

Neural-specific regulation of cytokine-inducible genes(ISGs) is controlled by transcription factors active at the interferon regulatory factor-element(IRF-E) and/or κB site in the promoter of these genes. A four hour treatment with IFN-γ or TNF-α led to an increase in transcription factors NF-κB and IRF-1, but loss of IRF-2. Simultaneous treatment with GT1b caused a reversal of this cytokine effect. However, short-term treatment (10 minutes) with IFN-γ or TNF-α with GT1b led to activation of γ-activated factor(GAF) and NF-κB, respectively, comparable to levels seen with cytokines alone, indicating no effect of GT1b on immediate cytokine signal transduction and transcriptional activation pathways. Therefore, GT1b may affect longer-term downstream events related to transcriptional or postranslational modification of IRF-1, IRF-2, NF-κB, and GAF. The effect of GT1b may be related to inhibition of constitutive or cytokine-inducible proteolytic processing of these factors, because a calpain I/proteosome inhibitor had similar effects to GT1b. This may indicate a specific target for GT1b in neural cells. (This investigation was supported by a grant from the National Multiple Sclerosis Society).

352.16

CYTOKINE RESPONSES FOLLOWING HERPES SIMPLES VIRUS INFECTION IN DIFFERENTIALLY HOUSED MALE BALB/C MICE. J. D. Karp* and J. A. Moynihan. Center for Psychoneuroimmunology Research and Department of Psychiatry, Univ. Rochester, Rochester, NY 14642.

The severity of primary and recurrent herpes simplex virus type-1 (HSV) infections have been associated with demands of the psychosocial environment in humans and animals. The immunological mechanisms responsible for this interaction are not known. Differential housing is a psychosocial manipulation that establishes neurochemical and immunological distinctions between animals. This report investigates the influence of differential housing on lymphocyte production of cytokines following sublethal infection with HSV.

BALB/c mice were housed 1 or 4 per cage for several wks before infection with HSV. Thirty-two mice were challenged with HSV in the hind footpad six and 13 days after infection and delayed type hypersensitivity (DTH) responses recorded. Splenocytes and popliteal lymph nodes from thirty-three mice were harvested seven days after infection, washed and prepared for *in vitro* analyses.

DTH responses were greater in individually-housed compared to group-housed mice after each challenge ($p<.03$). HSV-stimulated ^{3}H-thymidine incorporation by splenocytes was higher in individually-housed than group-housed mice ($p<.01$). Splenocytes from individually-housed mice produced more HSV-stimulated interleukin (IL)-2, IL-4 and interferon (IFN)-γ than cells from group-housed mice after 24 hrs (each $p\leq.01$). Housing condition did not influence IL-2, IL-4 or IFN-γ cytokine production by splenocytes at 48 or 72 hrs. No cage differences were observed in cytokine production by popliteal lymph node cells after 24, 48 or 72 hrs.

These results confirm that the psychosocial environment can influence cytokine responses of BALB/c mice to sublethal HSV infection and suggest that these influences are lost after >24 hrs of *in vitro* conditions.

352.17

SELF-STIMULATION FROM THE MESENCEPHALON FOLLOWING INTRAVENTRICULAR INTERLEUKIN-2 ADMINISTRATION. A. L. O. Hebb and R. M. Zacharko*. Dept. Psychology, Carleton Univ., Ottawa, Canada

Central availability of the cytokine, interleukin-2, has been associated with altered dopamine (DA) turnover and electrophysiological activity in the medial prefrontal cortex and the mesencephalon. The alterations of central DA activity induced by IL-2 administration are, in some instances, reminiscent of the neurochemical variations induced by mild, uncontrollable stressors. These data suggest that the immune and the central nervous system communicate, in part, via a cytokine interface which may contribute to the induction of behavioral pathology. Data collected in this laboratory have revealed that mild, uncontrollable stressors provoke anhedonia from mesolimbic and mesocortical sites including the medial prefrontal cortex, the nucleus accumbens and the ventral tegmental (A10) area. The present investigation assessed whether intraventricular administration of IL-2 would provoke alterations in self-stimulation thresholds among CD-1 mice responding for brain stimulation from the A10 region. CD-1 mice were implanted with a bipolar stimulating electrode and a cannula in the lateral ventricle. Mice were trained to respond for brain stimulation at a constant current (70 uA) and a frequency range of 20-80 Hz in an ascending and descending order. Determination of half-max responding was evaluated for each animal prior to and following central IL-2 (5 ng in 1 ul PBS) or vehicle administration. Self-stimulation determinations were accomplished 15 minutes as well as 48, 72 and 168 hours following intraventricular drug application. IL-2 adminstration increased half-max stimulation frequencies among animals responding for brain stimulation from the dorsal A10 region while decreasing these values among ventral A10 placements. These data are consistent with the notion that sub-areas of the VTA are not only differentially responsive to stressor application but also to cytokine administration. Supported by the Gustavus and Louis Pfeiffer Research Foundation and NSERC.

352.18

THE EFFECTS OF THE HIV-1 ENVELOPE PROTEIN GP120 ON THE PRODUCTION OF NITRIC OXIDE AND PROINFLAMMATORY CYTOKINES IN UNPRIMED OR INTERFERON-γ PRIMED GLIAL CELLS. L.-Y. Kong*, M. K. McMillian, G. Bing, P. M. Hudson, Z. Feng, Q. Qi and J.-S. Hong. Laboratory of Environmental Neuroscience, NIEHS/NIH, Research Triangle Park, NC 27709.

The neurotoxicity of the HIV envelope protein gp120 has been implicated in the neuropathology of AIDS. Nitric oxide (NO) and proinflammatory cytokines produced by microglia and astrocytes may contribute to the gp120-related toxicity. This study examined the effects and possible mechanisms of gp120 obtained from two strains, HIV-1_{IIIB} and HIV-1_{SF2}, of the HIV-1 virus on the production of NO, TNFα, IL-1α, and IL-6 in unprimed or interferon-γ (IFNγ) primed glial cells. Mouse glial cells exposed to HIV-1_{IIIB} gp120 (10 pM to 100 nM) released increasing amounts of NO, TNFα and IL-6 in a dose-dependent manner, whereas IL-1α was undetectable. The cells exposed to HIV-1_{SF2} gp120 only increased the release of IL-6. The HIV-1_{IIIB} gp120-induced NO or cytokine productions were significantly enhanced by priming glial cells with IFNγ (200 U/ml). However, while HIV-1_{SF2} gp120-induced IL-6 production was increased in the IFNγ-primed cells, the production of NO in the IFNγ-primed cells was slightly decreased by the exposure to HIV-1_{SF2} gp120. Tyrphostin A25, a potent inhibitor of tyrosine kinases, inhibited both HIV-1_{IIIB} gp120- and HIV-1_{SF2} gp120-induced cytokine production, indicating that tyrosine kinases are involved in the gp120-induced signal transduction pathway(s). These results demonstrate that the production of NO, TNFα, IL-1α, or IL-6 from both unprimed and IFNγ-primed glial cells are differentially regulated by HIV-1_{IIIB} and HIV-1_{SF2} gp120, which may provide potential insights into the roles of NO and proinflammatory cytokines in the neurotoxicity of gp120 and the neuropathology of different strains of HIV-1 viruses.

352.19

SOMATOSTATIN MODULATES THE RELEASE OF INTERLEUKIN 6 FROM RAT CORTICAL TYPE I ASTROCYTES: pharmacological and molecular clues. M. Grimaldi, T. Florio and ^G. Schettini*. Dip. Neuroscienze Sez. Farm., Univ. Napoli "Federico II", Via S. Pansini 5, I-80131 Napoli; ^Chair of Pharm., Ist. Oncologia Clinica e Sperimentale, Univ. Genova, Viale Benedetto XV 10, I-16132 Genova; ITALY.

Astrocytes release substances critical to the development of central nervosus system. Among these, cytokines, such as interleukin 6, have been recently ascribed to the family of neurotrophic agents. Therefore, the study of the regulation of the release of these substances is growing in relevance. We have previously demonstrated that agents, such as VIP, that increase cAMP and activate protein kinase A, increased the release of interleukin 6 from rat cortical type I astrocytes. Here we studied the effect of the well-known neurohormone somatostatin known to inhibit adenylate cyclase, to stimulate phosphatase activity, to cause hyperpolarization in neurons, aiming to reveal its role in the regulation of interleukin 6 release by rat cortical type I astrocytes.

Somatostatin induced a concentration-related inhibition of resting interleukin 6 release with a maximal effectiveness at 100 nM. The inhibitory action of somatostatin was more effective in the presence of the direct activator of adenylate cyclase catalyst, forskolin. Indeed, forskolin stimulation of interleukin 6 release was completely blunted by somatostatin, that decreased the release of the cytokine below the basal value. This powerful inhibitory effect of somatostatin was achieved with a maximum at 1 uM concentration.

To test whether this inhibition was achieved via a G-protein-coupled mechanism we pretreated astrocytes with pertussis toxin, known to block the inhibitory G-protein mediating the inhibition of adenylate cyclase. Pertussis toxin abolished the inhibitory action of somatostatin both in basal and in forskolin stimulated conditions.

Finally we pharmacologically characterized the type of somatostatin receptor involved in this inhibitory action and typized astrocytic receptor subset by PCR, with the aim to correlate the somatostatin receptor type responsible of this inhibitory action and its coupled transduction mechanism. (This work was supported by MURST 40% '93; AIDS '94 to G.S.)

352.20

EXPRESSION OF IMMUNOLOGICALLY RELEVANT MOLECULES IN THE NERVOUS SYSTEM. M.L. Baker, B.M. Gebhardt, C.L. Weill* Neuroscience Center of Excellence, LSUMC, New Orleans, LA 70112

Neurons lack MHC Class II expression, but have a low basal expression of Class I molecules. Herpesvirus (HSV-1) infection and MHC expression are modulated by cytokines produced by lymphocytes. Interferon gamma (IFN-γ) has antiviral properties and is known to induce MHC expression in some cell types. Cortex and trigeminal ganglionic tissues from latently infected and uninfected mice were examined for the expression of immunologically significant markers (MHC Class II, IFN-γ, IFN-γ receptor, and macrophage marker) using immunohistochemical staining techniques. None of the markers examined were seen in freshly obtained cortex or ganglion tissue with or without prior infection. In the second experiment, both latent and uninfected cortex and trigeminal ganglion tissue were incubated in the presence or absence of IFN-γ for 72 hours. After 72 hour incubation, both IFN-γ and IFN-γ receptors were expressed in cortex of latent and uninfected mice in the presence or absence of IFN-γ. Macrophage marker was seen only in uninfected cortex treated with IFN-γ. MHC Class II was not detected in cortex or ganglion tissue regardless of treatment. IFN-γ treatment induced IFN-γ receptor expression in both uninfected and latent ganglia. The results in the trigeminal ganglion (Table) show that IFN-γ treatment of uninfected ganglia induces the expression of macrophage marker, IFN-γ, and IFN-γ receptor. (NEI EY08701, NIH).

Immunologically relevant molecule expression in TG

	Control		IFN-γ	
	Uninfected	Latent	Uninfected	Latent
Macrophage marker	-	+	+	+
IFN-γ	-	+	+	+
IFN-γ receptor	-	-	+	+

CARDIOVASCULAR REGULATION: VAGAL PHARMACOLOGY AND PHYSIOLOGY

353.1

INVOLVEMENT OF NITRIC OXIDE ON BAROREFLEX REGULATION. C.J. Tseng*, H.W. Liu, H.C. Lin, W.J. Lo and C.S. Tung. Dept. of Medical Education and Research, VGH-Kaohsiung and Dept. of Physiology, National Defense Medical Center, Taipei,Taiwan, R.O.C..

We reported recently that nitric oxide (NO) is involved in central cardiovascular regulation of rat brainstem nuclei. In the present study, we evaluated further the baroreflex response of NO in the nucleus tractus solitarii (NTS) of rats. Male Sprague-Dawley rats were anesthetized with urethane, blood pressure and heart rate was monitored intra-arterially. Intramedullary microinjection (60 nl) of NO synthase inhibitor N^G-methyl-L-arginine (L-NMMA) or normal saline was made into the NTS. Baroreflex response were elicited by increasing doses of phenylephrine (10-30 μg/Kg i.v.) before and after intra-NTS administration of L-NMMA or normal saline. The reflex bradycardia elicited by phenylephrine was significantly inhibited by the pretreatment of L-NMMA. Furthermore, the inhibitory effects on baroreflex activation by L-NMMA were significantly reversed by pretreatment with L-arginin. These results suggest that central endogenous NO is involved in the medullary regulation of blood pressure and that NO synthase inhibitor attenuated baroreflex activation.

353.2

CARDIOVASCULAR EFFECTS OF NITRIC OXIDE IN THE NUCLEUS TRACTUS SOLITARII OF RATS. H.C. Lin*, H.W. Liu, W.J. Lo, C.S. Tung and C.J. Tseng. Depts. of Pharmacology and Physiology, National Defense Medical Center, Taipei, and Dept. of Medical Education & research, Veterans General Hospital-Kaohsiung, Taiwan, R.O.C..

Nitric Oxide (NO) is an endogenously synthesized effector molecular which acts as a neurotransmitter with novel properties in both central and peripheral nervous system. We previously reported that NO was involved in central cardiovascular regulation. The purpose of the present study was to elucidate the involvement of other endogenous mechanisms that could contribute to the final hemodynamic response to NO in the nucleus tractus solitarii (NTS). In normotensive Sprague-Dawley rats, intra-NTS microinjection of L-arginine (1-100 nmol/60nl) produced a dose-dependent decrease in blood pressure and heart rate. These effects were blocked by prior adminstration of the NO synthase inhibitor N^G-methyl-L-arginine (L-NMMA) and by NMDA receptor antagonist MK-801. Similarly, prior administration of L-NMMA siginificantly attenuated the depressor and bradycardic effect of glutamate or NMDA. The specificity of the NO-NMDA interaction in the NTS was domonstrated with adrenergic and angiotensin receptor antagonist that did not affect the L-arginine response in the NTS. These results demonstrate a reciprocal attenuation of NO synthase inhibitor and NMDA receptor antagonist on NMDA and L-arginine responses respectively in the brainstem, and suggest that NO and NMDA may interact in central cardiovascular regulation.

353.3

HEMODYNAMIC EFFECTS ELICITED BY MICROINJECTION OF GLUTAMATE (GLU) INTO THE NTS OF CONSCIOUS RATS. E. Colombari*, R.A. Shaffer, W.T. Talman, and S.J. Lewis. Depts. of Pharmacol. and Neurol., Univ. of Iowa & VAMC, Iowa City, IA, 52242, USA.

Microinjection of GLU into the NTS of conscious rats elicits pressor and bradycardiac responses. We sought to determine the regional hemodynamic effects that are associated with these cardiovascular effects. Blood flow in different vascular beds was measured by pulsed Doppler flowmeters in conscious rats. Relative vascular resistance (Renal: RR, Mesenteric: MR, and Hindquarter: HQR) was calculated by dividing arterial pressure (mmHg) by the Doppler shift (Khz). Microinjection of GLU (1 nmol/100 nL) into the NTS produced pressor and bradycardiac responses. The three different beds demonstrated significant vasoconstriction immediately after the microinjection (RR:+96±14%, MR:+123±21%, and HQR: +301±43%), but HQR demonstrated delayed dilatation (-29±5%, n=7). Prazosin (1mg/Kg, i.v.) essentially abolished vasoconstriction produced by GLU microinjected into the NTS (RR:+1±4%, MR:+5±4%, and HQR: +62±12%) and the pressor response observed before prazosin was replaced by hypotension (+26±5% vs -21±5%). In contrast, prazosin did not alter the delayed hindquarter dilatation elicited by GLU. However, nitric oxide synthase inhibitor L-NAME (25 µmol/Kg, i.v.) caused use dependent reduction in hindquarter dilatation. This study suggests that microinjection of GLU into the NTS of conscious rats activates cardiac parasympathetic nerves and both sympathetic constrictor and dilator fibers to arteries in the hindlimb. The use dependent reduction in hindquarter vasodilatation is consistent with release from preformed stores of a compound that causes vasodilatation and whose synthesis depends on nitric oxide synthase. Support: CNPq (200006-93); VA merit review and career award, NIH HL32205 and 14388.

353.4

EVIDENCE THAT NMDA RECEPTORS IN NTS AND CVLM MEDIATE BAROREFLEX INHIBITION OF RVLM PRESYMPATHETIC NEURONS. W-J Yuan, W-F Rong, J-J Wang, W-Z Wang, and X-Y Shen*. Department of Physiology, Faculty of Basic Medical Science, Second Military Medical University, Shanghai 200433; Department of Anatomy, Shanghai Medical University, Shanghai 200032, China.

Spontaneously active neurons in the RVLM were extracellularly recorded in urethane-anesthetized and artificially ventilated rats. Eighty-five units were defined as presympathetic neurons because they were inhibited by baroreceptor activation through aortic nerve stimulation or bolus intravenous injection of phenylephrine and there were cardiac rhythms in their spontaneous firing. We found that baroreflex inhibition of these presumptive presympathetic neurons were blocked by: 1) intravenous administration of ketamine(10 mg/kg), which is known to be a non-competitive NMDA receptor blocker; 2) microinjection of CPP(50 mM, 100 nl), a specific NMDA receptor antagonist, into the aortic nerve projection sites in the NTS and 3) microinjection of CPP(50 mM, 100 nl) into ipsilateral CVLM. We conclude that the baroreceptor-sympathetic reflex is mediated in the NTS and CVLM by NMDA receptors.

353.5

IN VITRO RECORDINGS FROM AREA POSTREMA NEURONS DEMONSTRATE RESPONSIVENESS TO ANGIOTENSIN II CHOLECYSTOKININ AND GLUTAMATE Kaiqi Sun, Zhongsheng Han*, Vicki L. Lowes and Alastair V. Ferguson. Dept. of Physiology, Queen's Univ., Kingston, Ontario, Canada, K7L 3N6.

The area postrema (AP) lacks a normal blood brain barrier and is thus ideally suited to integrate hormonal and neuronal signals. It has been shown to play roles in regulation of cardiovascular function and food intake. Evidence suggests that angiotensin II (ANG II), glutamate and cholecystokinin (CCK) mediate these functions through AP. This study was designed to examine the responses of AP neurons to these substances in rat brain slices. Extracellular recordings were obtained from AP neurons and effects of ANG II, CCK and glutamate were examined. 106 AP neurons were tested with ANG II (10^{-8}M to 10^{-6}M), of which 38.7% were excited and 13.2% inhibited. Following blockade of synaptic transmission with a low Ca^{++}, high Mg^{++} solution, effects of ANG II were maintained in all cells retaining spontaneous activity. Losartan (10^{-6}M) blocked the excitatory effects of ANG II in all 4 cells tested demonstrating these effects to be mediated by the AT_1 receptors. CCK (10^{-7}M) was found to enhance the activity of 47.1% of the 34 AP neurons tested. In addition, 16 neurons were tested with glutamate (10^{-7}M), of which 7 were excited and 9 unresponsive. These results demonstrate that AP neurons are sensitive to ANG II, glutamate and CCK, findings which correlate well with the proposed role of this structure in cardiovascular function and food intake.

Supported by The Heart and Stroke Foundation of Ontario

353.6

RESPONSES OF NEURONS IN THE DORSAL MOTOR NUCLEUS TO AREA POSTREMA ACTIVATION BY ELECTRICAL STIMULATION AND ANGIOTENSIN II IN ANESTHETIZED RABBITS. L. Qu, M. Hay, E. Hasser and V.S. Bishop*. Dept. of Physiology, Univ. TX Hlth. Sci. Ctr., San Antonio, TX 78284-7756.

We previously demonstrated that the area postrema (AP) projects to the dorsal motor nucleus (DMN). This study was designed to determine if activation of the AP neurons by angiotensin II (AngII) alters neuronal activity of the DMN. In anesthetized rabbits, spontaneous action potentials were extracellularly recorded from DMN and neuronal activity of the DMN was examined before and after microinjection of AngII into the AP (1ng/nl, 10nl). In 27 cells, microinjection of AngII into the AP produced predominantly an inhibitory effect and reduced the mean baseline firing rate from 4.3 to 3.5 spikes/sec($P<0.05$). Seven of the cells inhibited by AngII (n=12) received excitatory baroreceptor input, suggesting that those neurons inhibited by AP injection of AngII are involved in the baroreflex regulation of heart rate. Also, electrical stimulation of the AP produced an overall inhibitory effect on an additional 32 spontaneous DMN's and reduced the mean firing rate by 27.8% ($P<0.05$). In the DMN's evoked by AP stimulation (n=32), the mean latency was 15.3±1.8ms, ranging from 2 to 40 ms. Five cells were also antidromically activated by stimulation of the vagus nerves, suggesting that neurons responding to AP activation may innervate the heart. The results of this study indicate that the AP projects to the DMN and that activation of AP neurons by circulating AngII reduces neuronal activity of the DMN's. The results suggest that AngII may reset heart rate by activation of AP projections to the DMN. (Supported by NIH Grants HL36080-HL12415)

353.7

Angiotensin inhibits M-type K^+ currents in putative aortic baroreceptor neurons of the adult rat. A.M. Allen*, J.T. Cunningham, M.J. Sullivan, R.E. Wachtel and F.M. Abboud. Cardiovascular and VA Medical Centers, University of Iowa College of Medicine, Iowa City, I.A. 52242.

Angiotensin (Ang) receptors occur in the nodose ganglion and application of Ang to the isolated, whole nodose ganglion produces a small depolarization (1). We examined the effect of Ang on M-type K^+(M) currents in isolated adult rat nodose ganglion neurons co-cultured with rabbit aortic smooth muscle cells for 1-3 days. All cells studied were labelled with DiI, which had been injected into either the fat pad surrounding the aortic arch or a cuff around the aortic depressor nerve, 10-14 days prior to culture. M currents were studied under voltage clamp using whole cell patch clamp techniques. The holding voltage was -30 mV and a step was applied to -60 mV for 500 ms before returning to -30 mV. The magnitude of the M current was determined as the difference between the current at the beginning and end of the step to -60 mV. M currents, observed in 66 % of labelled cells, ranged from 45 to 310 pA. Bath application of Ang (10^{-6}M) produced a 31±7% decrease in the current in 8/11 cells. The remaining cells showed a small (6%) increase. The inhibition by Ang was maintained for greater than 5 min. After exposure to the angiotensin receptor antagonist, Losartan (10^{-4}M) for 5-7 min, Ang produced an 11±3% inhibition of the M current (9 cells). We demonstrate that Ang inhibits the M current in putative aortic baroreceptors of the rat, providing a mechanism for the small depolarizing effect of Ang in the nodose ganglion.

1. Widdop,RE et al., Clin.Exper.Hypertens., 14: 597-613 (1992).

353.8

MECHANOSENSITIVE (MS) ION CHANNELS IN PUTATIVE AORTIC BARORECEPTOR NEURONS (ABNS). J.T. Cunningham*, S.A. Kraske, M.J. Sullivan, R.E. Wachtel & F.M. Abboud. Departs. of Medicine and Anesthesia, the VAMC and The Cardiovascular Center, Univ. of Iowa.

We have previously demonstrated that slow hypoosmotic stretch increases the conductance of putative ABNs in culture. This conductance was inward at and below the resting membrane potential of the ABNs and was blocked by gadolinium. In these experiments, we report the effect of rapid and direct mechanical stimulation of the neurons on the single channel. Cell attached patch clamp experiments were performed on dissociated nodose ganglia neurons from adult rats. Putative ABNs were identified by labeling the adventitia of aortic arch with the carbocyanine dye DiI 7 to 14 day prior to dissociation. Cells were tested for MS channels by applying negative pressure on the patches through the recording pipettes. In putative ABNs, MS channels were observed which were inward at the resting membrane potential of the cell using high NaCl or KCl recording solution. The activity of these channels was increased by appling 30 to 60 mmHg on the patch. The slope conductance is 114 pS and its reversal potential is 0 mV. These channels were not blocked by TEA (40 mM), TTX (1-10 µM), charybdotoxin (200 nM) or 4-AP (4 mM) but were sensitive to gadolinium (20 µM). Using standard culturing conditions, this channel was found in less than 20% of the patches tested. These observations are consistent with the macroscopic currents produced by hypoosmotic stretch. This suggests that ABNs have MS ion channels that may participate in the mechanotransduction of baroreceptor neurons.

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353.9

Presynaptic Factors Regulating Tetanus-Induced Sustained Potentiation of Monosynaptic GABAergic Transmission in Medulla

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Stimulation in the region of the tractus solitarius evokes monosynaptic IPSCs (evIPSCs) in dorsomedial nucleus tractus solitarius (dmNTS) neurons recorded in the presence of glutamate receptor antagonists (DNQX, 10μM; D-AP5, 50μM) with whole cell (CsCl) electrodes in coronal rat medullary slices. Tetanus (2s, 20-50Hz) reproducibly increases the amplitude of subsequent IPSCs for 20-40 min (Glaum and Brooks, *JANS*, in press, 1995).

Recent findings: Spontaneously occuring IPSCs are increased in frequency immediately following tetanus, however this increase does not persist for as long as the evIPSC remains potentiated. The mean^2/variance of posttetanus evIPSC amplitude increased > > mean amplitude of posttetanus evIPSCs. Examination of the effects of tetanus on miniature IPSCs (mIPSCs) in the presence of tetrodotoxin (1μM) indicates that tetanus increases mIPSC frequency without shifting the cumulative frequency distribution.

Tetanus-induced sustained potentiation of evIPSCs was evident in slices treated (>7 min) with the N-type voltage dependent Ca^{2+} channel (VDCC) antagonist ω-conotoxin-GVIA (1μM) or L-type channel blocker nimodipine (10μM), but not in those slices treated with either AGA-IVA (200nM, 20 min) or ω-contotoxin-MVIIC (2μM). High Mg^{2+}/low Ca^{2+} solutions reversibly block induction of sustained potentiation.

Tetanus produces sustained potentiation of the inhibitory component of mixed EPSP/IPSP complexes recorded in dmNTS neurons in recordings (K^+ gluconate electrodes) made in the absence of glutamate receptor antagonists.

These results, together with earlier data, indicate a likely presynaptic locus of tetanus-induced sustained potentiation of inhibitory transmission in dmNTS which depends, in part, upon Ca^{2+} influx through P/Q-type VDCCs. Tetanus appears to induce a sustained increase in interneuronal excitability. Underlying mechanisms and physiological relevance will be discussed.

353.10

INFLUENCE OF GABA IN THE NUCLEUS TRACTUS SOLITARIUS ON BLOOD PRESSURE IN BARORECEPTOR-DENERVATED RATS.

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We have previously reported that injection of the GABA agonist muscimol into the nucleus tractus solitarius (NTS) of chronically arterial baroreceptor-denervated (BD) rats has no effect on blood pressure, in contrast to the marked increase in blood pressure it elicits in baroreceptor intact rats (Schreihofer and Sved, Am. J. Physiol. 263:R258, 1992). We had interpreted that result as suggesting a lack of tonic excitation of NTS neurons in BD rats; alternatively, it could indicate that in BD rats there is tonic maximal GABA-mediated inhibition of neurons in the NTS. The present study aimed to distinguish between these two possibilities, by examining the changes in blood pressure elicited by injection of the GABA antagonist bicuculline methiodide (BMI) into the NTS of BD and control rats. In chloralose-anesthetized, paralyzed, ventilated rats, injection of 10 pmol BMI (in 100 nl of artificial CSF) into the NTS elicited minimal changes in blood pressure (-4$\pm$2 mm Hg following unilateral injection, from a baseline of 117$\pm$4 mm Hg, n=8). In contrast, in BD rats (complete sino-aortic denervation 7-10 days prior to experiment) injection of BMI into the NTS elicited marked reductions in blood pressure (-52$\pm$6 mm Hg following unilateral injection, from a baseline of 116$\pm$6 mm Hg, n=6). The maximal decrease in blood pressure elicited by BMI in BD rats was equivalent to the maximal decrease in blood pressure that could be evoked by the local injection of L-glutamate (200 pmol; -58$\pm$8 mm Hg; n=6). These results suggest that the lack of a tonic role of the NTS in the regulation of blood pressure in BD rats, is a result of maximal GABA-mediated inhibition of relevant NTS neurons.

353.11

SEROTONIN EXCITES NUCLEUS TRACTUS SOLITARIUS (NTS) NEURONES RECEIVING INPUTS FROM CARDIAC VAGAL C-FIBRE AFFERENTS IN RATS.

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In rats microinjection 5-HT into the NTS decreases blood pressure and heart rate by acting on 5-HT_{1A} receptors (Itoh & Bunag, J. Pharmacol. Exp. Ther. **256**, 1147-1153, 1991). In view of the role of the NTS in cardiovascular regulation, studies were carried out in artificially ventilated, sodium pentobarbitone-anaesthetized rats to study the effects of the ionophoretic application of 5-HT (50 mM, pH 5) and a 5-HT_{1A} agonist, 8-OH-DPAT (20 mM, pH 4), on NTS neurones receiving either cervical vagual or cardiac vagal C-fibre inputs.

Of the 16 neurones excited by cervical vagal stimulation, ionophoretic application of 5-HT (0-10nA) increased the ongoing activity of 8, depressed the activity of 1 and did not affect the remaining. Application of 8-OH-DPAT (5-30nA) produced a similar effect. Of the 35 neurones tested, activity of 18 was increased, of 14 was depressed and 3 were unaffected. In contrast, NTS neurones excited by stimulating the cardiac vagus nerve were usually excited by iontophoretic application of 5-HT (3 of 3) and 8-OH-DPAT (4 of 5).

These results demonstrate that 5-HT has both facilitatory and depressant actions on NTS neurones but has mainly an excitatory effect on NTS neurones with cardiac vagal C-fibre afferent input. This effect is mimicked by a 5-HT_{1A} agonist suggesting that 5-HT acts on 5-HT_{1A} receptors in the NTS to modulate C-fibre input from the heart..

This work was supported by the Wellcome Trust.

353.12

CAPSAICIN DEPRESSES RAT BARORECEPTOR AFFERENTS PERIPHERALLY AS WELL AS SYNAPTIC RESPONSES TO TRACT ACTIVATION IN MEDIAL NUCLEUS TRACTUS SOLITARIUS (mNTS).

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Capsaicin (CAP) potently depresses a range of different sensory neurons with slowly conducting axons. Of cardiovascular sensory afferents, mechanoreceptors including baroreceptors have been thought to be resistant to CAP. Here we tested the effects of CAP on axonal transmission in the aortic depressor nerve (ADN) which in the rat contains large numbers of C-fiber baroreceptors. 100-1000 nM CAP selectively depressed and then reversibly blocked the slow conduction velocity volleys in the ADN compound action potential. Reflex blood pressure and heart rate responses corresponding to C-fiber activation of ADN by low frequency, high intensity electrical stimulation were similarly blocked by CAP. Excitatory synaptic responses (EPSP) to solitary tract activation were recorded in mNTS neurons in a horizontal slice. Superfusion of the slice with 100-1000 nM CAP reduced the EPSP by 20-60%. There were additional variable effects of CAP on membrane potential and resistance. In conclusion, CAP appears to block sensory transmission at axonal sites both in the peripheral and central processes of visceral sensory neurons. The synaptic depression of sensory afferent input to mNTS is partial and is consistent with a selective, complete blockade of sensory axons with unmyelinated or lightly myelinated axons.

353.13

Do Substance P Immunoreactive Afferent Nerve Terminals Influence Negative Dromotropic Vagal Motoneurons In The Rostral Nucleus Ambiguus (rNA)?

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Microinjections of substance P (SP) into the NA cause bradycardia. Ultrastructural evidence indicates that SP terminals directly synapse upon negative chronotropic neurons.These morphological data suggest an anatomical substrate for the inhibitory effect of SP on cardiac rate. We have used a dual labeling histochemical and immunocytochemical method to look for potential synaptic contacts of SP terminals upon negative dromotropic neurons. Negative dromotropic neurons were labeled in the rNA of three cats following injections of a retrograde tracer into a left atrial fat pad ganglion that selectively inhibits AV conduction. Injections into the adjacent myocardium, or into the pericardium (N=2) did not result in labeling in the brain. Labeled neurons were elongated and multipolar, with a round uninvaginated nucleus, and abundant cytoplasm containing dense masses of rough endoplamsic reticulum. They also showed distinctive somatic and dendritic spines. Labeling was most commonly found in perikarya and proximal dendrites, however, distal dendrites as small as 0.8μ in diameter were also labeled. SP nerve terminals were also readily detected in these tissues. However, only 2.2% (3 of 138) could be defined as being in close apposition to negative dromotropic neurons. In no case was a clearly identified synapse formed between a SP terminal and a retrogradely labeled profile. By contrast, over 120 unlabeled terminals were observed to make synaptic contact with retrogradely labeled profiles. These data indicate that SP terminals in rNA do not synapse upon negative dromotropic neurons. The data further suggest that parasympathetic preganglionic vagal neurons controlling cardiac rate or AV conduction are modulated by CNS afferent neurons utilizing different neurotransmitters.

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353.14

CARDIOVASCULAR RESPONSES TO MICROINJECTION OF ARGININE VASOPRESSIN INTO THE NUCLEUS TRACTUS SOLITARIUS OF THE SPONTANEOUSLY HYPERTENSIVE RAT.

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Radioimmunoassay has demonstrated a decrease in the amount of arginine vasopressin (AVP) present in the brain stem of the spontaneously hypertensive rat (SHR). The significance of this finding for cardiovascular processing in the nucleus tractus solitarius (NTS) has not been fully investigated. Previous results from this laboratory have shown that endogenous AVP in the NTS influences baroreflex control of RSNA. To determine if the cardiovascular responses to AVP are altered in the SHR, AVP was microinjected into the caudal NTS of 14 week old SHR and Sprague-Dawley rats (SDR). Changes in mean arterial pressure (MAP), heart rate (HR) and renal sympathetic nerve activity (RSNA) were recorded. In SDR (n=10), a single microinjection of 10 ng AVP in 20 nl artificial CSF into the dorsomedial NTS did not change HR (-1.1$\pm$2.7 beats/min) or RSNA (-6.2$\pm$2.7%) but slightly decreased MAP (-7.1$\pm$2.2 mmHg). In SHR (n=4), a single microinjection of 10 ng AVP induced a significant decrease in RSNA (-16.6$\pm$3.9%) and MAP (-11.5$\pm$3.3 mmHg) in SHR but did not change heart rate (-6.5$\pm$6.4 beats/min). In a second experiment, 4 10 nl microinjections of AVP (5 ng each) were placed in the left and right NTS 200 μm lateral to calamus and 600 μm lateral and rostral to calamus. Bilateral microinjection of AVP decreased HR in SDR (-35.9$\pm$12.3 beats/min; n=5) and SHR (-41.4$\pm$17.6 beats/min; n=4) without markedly changing MAP and RSNA. These results suggest that NTS neurons modulating RSNA may be more sensitive to exogenous AVP in the SHR than in the normotensive rat.

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353.15

C-FOS ANTISENSE IN THE NUCLEUS TRACTUS SOLITARIUS ALTERS BLOOD PRESSURE RESPONSE TO CHRONIC ARGININE VASOPRESSIN INFUSION. M.G. Sanderford, M. Hay* and V.S. Bishop. Dept. Physiology, Univ. TX Hlth. Sci. Ctr., 7703 Floyd Curl Dr., San Antonio, TX 78284

Previous studies have shown that arginine vasopressin (AVP) inhibits its own vasoconstrictor activity through area postrema mediated sympathoinhibition resulting in little or no change in mean arterial blood pressure (MAP) during AVP infusions. Because Fos immunoreactivity increases in cells of the medial nucleus tractus solitarius (mNTS) following infusions of AVP, we postulated that *c-fos* expression in these cells might contribute to the inability of AVP to sustain an increase in MAP. To examine the potential role of mNTS *c-fos* expression during AVP infusion on the control of MAP, 200nl of vehicle (0.1M PBS), 1mM *c-fos* antisense oligonucleotides or 1mM sense oligonucleotides was injected bilaterally into the medial NTS of anesthetized rabbits instrumented for the measurement of MAP and renal sympathetic nerve activity (RSNA). Five hours after NTS injections, AVP was infused at a rate to decrease RSNA by approximately 20% and the infusion was maintained at that level. In animals receiving no *c-fos* antisense into the mNTS, 3 hour infusions of AVP (1.67 ± 0.58 mU/kg/min) resulted in a MAP change of -11.5 ± 8.0 mmHg (n=2). In contrast, in animals receiving *c-fos* antisense into the mNTS, 3 hour infusions of AVP (1.77 ± 0.18 mU/kg/min) resulted in a MAP change of 7.1 ± 9.3 mmHg (n=3). The observed responses were correlated with decreased *c-fos* expression in the NTS. These results suggest that expression of *c-fos* in the mNTS is involved in AVP's central regulation of cardiovascular function. (Supported by NIH HL36080 and HL12415)

353.16

IN VITRO RECORDINGS FROM AREA POSTREMA NEURONS DEMONSTRATE RESPONSIVENESS TO ADRENOMEDULLIN, A NEWLY DISCOVERED VASOACTIVE PEPTIDE. Mark A. Allen, Kaigi Sun, and Alastair V. Ferguson*. Dept. of Physiology, Queen's Univ., Kingston, Ontario, Canada, K7L 3N6.

Adrenomedullin (ADM) is a recently discovered 52 amino acid peptide that was originally found in human pheochromocytoma. It circulates in fmol concentrations in normal plasma and has been shown to exhibit potent and long lasting vasodilatory actions *in vivo*. On the basis of these findings, it has been suggested that ADM may play a role in cardiovascular control. ADM mRNA is expressed in several tissues including the adrenal medulla, ventricle, lung, kidney and brain and ADM has been shown to exert CNS influences over the control of fluid balance. One potential site for such central actions of ADM is the area postrema (AP), a circumventricular organ which plays a significant role in cardiovascular regulation. In this study, extracellular recordings were obtained from AP neurons in rat brain slices and the effects of bath perfusion of ADM (10^{-7} M, for 200s) were examined. Of the 24 cells tested with ADM, 8 were found to increase their spontaneous spike frequencies by greater than 30% (mean increase: 107 ± 25%). The remaining cells were unaffected. These results suggest that in addition to its peripheral vasodilatory actions, ADM may exert its central actions by influencing AP neurons and thus cardiovascular control.

Supported by The Heart and Stroke Foundation of Ontario

353.17

MELANOCORTIN-4 RECEPTORS (MC4-R) IN THE DORSAL VAGAL COMPLEX (DVC) MEDIATE HYPOTENSIVE AND BRADYCARDIC EFFECTS OF α-MELANOCYTE STIMULATING HORMONE (αMSH) S-J. Li, K. Varga, R.D. Cone, V.J. Hruby & G. Kunos* Dept. Pharmacol. Tox., Med. Coll. VA, Richmond, VA 23298

Proopiomelanocortin (POMC)-containing neurons projecting from the arcuate nucleus to the DVC are involved in depressor cardiovascular regulation (Neuroscience, 33:559-566, 1989). The DVC is rich in αMSH and contains high levels of MC4-R, the roles of which have not been clarified. We tested the effects on blood pressure (BP) and heart rate (HR) of αMSH microinjected unilaterally into the DVC of urethane-anesthetized rats. αMSH, 250 pmol/100 nl, lowered BP (-23±8 mmHg) and HR (-41±11 beats/min). Changes in BP and HR peaked at 5 min and lasted >40 min. In other animals, α-MSH was tested 10 min after the microinjection into the same site of either 10 pmol of SHU9119, a novel selective antagonist of MC4-R, or the opiate antagonist l-naloxone (140 pmol). SHU9119 did not alter BP or HR, but blocked the effects of αMSH (-3±2 mmHg, +4±12 beats/min, $P < 0.001$). Naloxone did not alter BP or HR, nor did it influence the effects of αMSH (-17±6 mmHg, -36±9 beats/min). ß-endorphin (ßE, 1.25 pmol intra-DVC) also decreased BP (-19±2 mmHg) and HR (-26±3 beats/min), with peak changes occurring at 30-40 min postinjection. In separate animals, 10 pmol SHU9119 did not alter responses to subsequently microinjected ßE (-20±3 mmHg, -21±3 beats/min), whereas 140 pmol l-naloxone blocked the effects of ßE (-5±2 mmHg, +14±3 beats/min, $P<0.001$). These findings indicate that αMSH and ßE, both products of the precursor POMC, cause hypotension and bradycardia by acting at distinct MC4-R and opiate receptors in the DVC, respectively.

CARDIOVASCULAR REGULATION: MEDULLARY RETICULAR FORMATION

354.1

DISTRIBUTION OF NEURONS IN THE CAUDAL VENTROLATERAL MEDULLA THAT MEDIATE THE BARORECEPTOR REFLEX RESPONSE. E. Badoer†, M. McKinley, B. Oldfield, C. Chen* and R. McAllen. Howard Florey Institute, University of Melbourne, Australia.

Until recently, electrophysiology has been the only tool available to detect barosensitive neurons in the caudal ventrolateral medulla (CVLM). A major disadvantage is that only small samples of cells can be studied. The use of the protein Fos as a marker of activated neurons has overcome this problem. The present study has utilised the Fos technique to determine the distribution of the population of barosensitive neurons in the CVLM. In anaesthetised male, sprague-dawley rats, the retrogradely transported tracer, rhodamine beads, was microinjected into the pressor area of the rostral VLM. At least 5 days later, baroreceptors were either activated in the conscious rats by raising blood pressure with phenylephrine (10µg/kg/min iv for 2hrs, n=5), or unloaded by decreasing blood pressure with diazoxide (30mg/kg subcutaneously, n=5). Saline was used in control rats (n=4 and 5 respectively). Ninety to 120 minutes after the start of the experiment, the rats were perfused with 4% paraformaldehyde. The medulla was sectioned and examined between 1mm caudal and 0.4mm rostral to the obex. Neurons that contained rhodamine (indicating they projected to the RVLM) were counted and the proportion of those that expressed Fos (ie barosensitive) was determined. Rhodamine-containing neurons and Fos positive cells were located at all rostrocaudal levels of the CVLM examined. Compared to controls, phenylephrine infusion induced a significant increase in the proportion of those neurons that expressed Fos (14% vs 1% $P<0.0001$), whilst diazoxide had no significant effect. After phenyleprine the rhodamine-containing barosensitive neurons were found mainly at the level of the obex. The results suggest there is a subpopulation of barosensitive neurons in the CVLM, confined to a small region of the rostral part of the CVLM, which are the likely interneurons mediating the baroreceptor reflex response.

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354.2

ROLE OF THE ROSTRAL VENTROLATERAL MEDULLA IN THE MAINTENANCE OF ARTERIAL PRESSURE IN CHRONICALLY BARORECEPTOR DENERVATED RATS. A.M. Schreihofer* and A.F. Sved. Department of Neuroscience, University of Pittsburgh, Pittsburgh PA 15260.

Chronically baroreceptor denervated (BD) rats, produced either by selective sino-aortic denervation or by electrolytic lesion of the projection site of all baroreceptor afferents (the nucleus tractus solitarius; NTS), have normal resting mean arterial pressure (MAP) although the lability of MAP is markedly exaggerated. The present study sought to determine whether MAP in BD rats is maintained by supraspinal drive to sympathetic vasomotor outflow, as it is in intact rats. Because the rostral ventrolateral medulla (RVLM) is the site of tonic supraspinal drive to sympathetic vasomotor outflow in intact rats, we compared the effects of inhibiting the RVLM on MAP in urethane-anesthetized BD rats and control rats. Bilateral injection of muscimol (100 pmol) into the RVLM in control rats decreased MAP from 114±13 mm Hg to 47±4 mm Hg (n=7); MAP was not further decreased by subsequent ganglionic blockade by chlorisondamine (2 mg/kg iv). Similar results were obtained in BD rats. In sino-aortic denervated rats MAP decreased from 90±5 mm Hg to 49±3 mm Hg (n=7) and in NTS-lesioned rats MAP decreased from 99±5 mm Hg to 49±2 mm Hg (n=3); chlorisondamine did not have an additional effect on MAP in either group. Dose-response curves for phenylephrine-evoked increases in MAP in ganglionic blocked BD rats and control rats were the same, indicating that sympathetically-evoked changes in vascular resistance were not altered in BD rats. These results indicate that resting MAP in BD rats is supported by sympathetic vasomotor outflow to the same extent as in intact rats. Futhermore, in BD rats, as in control rats, the RVLM is the primary source of this tonic vasomotor drive.

354.3

ROLE OF VENTROLATERAL MEDULLARY PRESSOR AREA (VLPA) AND INTERMEDIOLATERAL CELL COLUMN (IML) IN MEDIATING CARDIOVASCULAR RESPONSES ELICITED FROM THE A5 REGION.

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Microinjections (50 nl) of L-glutamate (1.77 nmol) into the A5 neuronal pool elicited depressor and bradycardic responses. It was hypothesized that these responses may be mediated by noradrenergic projections from the A5 neurons to the VLPA neurons or IML neurons because norepinephrine (NE; 50, 100 & 400 pmol) elicited a depressor and bradycardic response when injected into the VLPA and a bradycardic response when injected into the IML at T2. The purpose of the present study was to identify which of the afore-mentioned neuronal pools mediate the responses elicited from A5. Experiments were done in pentobarbital-anesthetized and artificially ventilated rats. Injections of muscimol (150 pmol; used as a pharmacological tool to depress reversibly the activity of neurons) into the VLPA did not alter the cardiovascular responses elicited by injections of glutamate into the A5 region. Injections of idazoxan (IDZ; 1 nmol; an alpha-2 adrenergic receptor blocker) into the VLPA also did not alter the cardiovascular responses elicited by injections of glutamate into the A5 region. On the other hand, injections of IDZ (1 nmol) into the IML at T2 blocked the bradycardic responses to injections of glutamate into the A5 region. These results suggest that: (1) cardiovascular responses elicited by injections of L-glutamate into the A5 region are not mediated by inhibition of VLPA neurons, (2) on the other hand, inhibition of IML neurons at T2 by noradrenergic projections from A5 may be responsible for the bradycardic responses elicited from A5 region and these responses are mediated by alpha-2 adrenergic receptors.

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354.4

ACTIVATION OF $GABA_B$ RECEPTORS HYPERPOLARIZES RAT RVLM NEURONS AND DEPRESSES SYNAPTIC RESPONSES.

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Whole-cell patch recordings were made from rostroventrolateral medulla (RVLM) neurons of brainstem slices prepared from 8-12 day old rats. Superfusing the slices with the $GABA_B$ receptor agonist (-) baclofen (3-10 μM) hyperpolarized and decreased input resistance of RVLM neurons. Membrane hyperpolarization decreased the baclofen-hyperpolarization. Baclofen depressed the excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) evoked by focal stimulation near the recording neuron. Returning the membrane potential to the resting level by injection of DC current during baclofen-induced hyperpolarization did not restore the amplitude of EPSPs and IPSPs. Spontaneous miniature EPSPs and IPSPs were recorded in some of the RVLM neurons and baclofen effectively and reversibly eliminated these spontaneous synaptic responses. Pretreating the slices with the putative $GABA_B$ receptor antagonist 2-hydroxysaclofen (20-30 μM) partially antagonized the hyperpolarizing and synaptic depressant action of baclofen. It is concluded that $GABA_B$ receptors are present in post- and pre-synaptic sites of RVLM neurons and that their activation hyperpolarizes RVLM neurons and depresses synaptic responses. (Supported by NIH Grant HL51314).

354.5

MONOPHASIC AND MULTIPHASIC GABA RESPONSES IN NEURONS OF THE RAT ROSTRAL VENTROLATERAL MEDULLA *IN VITRO*.

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The GABAergic system plays an important role in baroreflex and cardiovascular regulation. The rostral ventrolateral medulla (RVL) contains reticulospinal sympathoexcitatory neurons which receive a tonic inhibition from the caudal ventrolateral medulla and from local GABAergic interneurons. Using intracellular recording in rat medullary slices, we studied the responses to pressure applied GABA agonists in electrophysiologically identified neurons within the RVL; moreover, we examined which group of neurons receives a significant GABAergic synaptic input. Neurons were classified into regular pacemaking, irregular spiking, and silent neurons. With KAc-filled electrodes, GABA (20 mM) evoked responses that ranged from a monophasic hyperpolarization to a combination of hyperpolarizing and depolarizing responses. The nature of these responses depended on the amount of the GABA delivered and on the type of neuron recorded. In regular pacemaker neurons, it consisted of a fast hyperpolarization followed by a slower depolarization that were both sensitive to SR95531 and picrotoxin. A GABA dose-dependent long lasting hyperpolarization sensitive to the $GABA_B$ antagonist CGP35348, terminated the response. In irregular spiking and silent neurons, GABA application produced usually a monophasic hyperpolarization and depolarization, respectively. The fast component of these responses was sensitive to the $GABA_A$ antagonists. Inhibitory synaptic potentials were recorded in particular in silent and regular pacemaker neurons. They sometimes occurred with a regular frequency and were reduced or blocked by the $GABA_A$ antagonists. Our data show that different groups of neurons exhibit different GABA responsiveness and that at least some of the regular pacemaker neurons recorded in vitro might act as local GABAergic internerneurons to provide tonic inhibitory input to sympathoexcitatory neurons.

354.6

GLYCINE RELEASE IN THE RAT VENTROLATERAL MEDULLA DURING BARORECEPTOR STIMULATION.

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Although glycine has long been known as a potential neurotransmitter in the ventrolateral medulla its function remains unknown. The efflux of isotopically labelled glycine during baroreceptor stimulation in the *in vivo* superfused ventral medullary surface (VMS) was investigated. A small acrylic plastic chamber (internal volume = 30 μL was sealed in place over the desired position in the VMS. Isotope labelling of tissue was achieved by a 50 min closed cycle superfusion with artificial cerebrospinal fluid (CSF) containing 2 μCi/mL of (^{14}C)-glycine. Subsequently the VMS was superfused during 45 min with isotope-free CFS and the superfusate collected in 3 min samples. Quench corrected radioactivity (DPM) was estimated by liquid scintillation spectometry. Baroreceptor stimulation (BS) was carried out by increasing mean arterial blood pressure to ≥ 160 mmHg with phenylephrine (200 μg/min, i.v.) during 6 min. Results obtained demonstrated that: a) baroreceptor stimulation induced a 59 ± 14,5% increase in the spontaneous efflux of glycine in the rostral VMS (n=10); b) in previously baroreceptor denervated animals BS produced only 7 ± 1,8% increase in spontaneous glycine efflux (n=6); c) when the superfusion chamber was placed over the caudal-medial VMS the increase in glycine efflux in response to BS was reduced to 3 ± 0,9% (n=6). Results obtained support the hypothesis that glycine is a neurotransmitter in the VMS and suggest that it might be part of the baroreceptor reflex arch.

Supported by FAPESP

354.7

Rhythmic membrane events in presympathetic neurones of the rat rostral ventrolateral medulla oblongata (RVLM) A. Zagon* and K. M. Spyer, Department of Physiology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, U.K.

Presympathetic neurones of the RVLM play a major role in generating tonic and reflex sympathetic activities. In the present study we have examined their electrophysiological characteristics that might underlie the generation of these drives.

In vivo intracellular recordings were made from cells of α-chloralose anaesthetised, artificialy ventilated Spraque-Dawley rats. Spinal projections and inputs from aortic nerve and/or vasodepressor region of the nucleus of the solitary tract (NTS) were tested. The discharge pattern of the cells and the underlying rhythmic membrane events were analysed off-line (CED 1401 system).

45 neurones received inhibitory, excitatory or excitatory-inhibitory input following aortic nerve stimulation. Based on a combination of criteria, they were defined as sympatho-excitatory or -inhibitory cells of the RVLM and were encountered both with and without direct spinal projections. A 22-26 and 33-36 Hz rhythmic oscillation of the membrane potential was observed in the majority of cells. The amplitude of the 22-26 Hz rhythm, that was most pronounced during lung deflation, could be enhanced by aortic nerve and NTS activation and cessation of ventilation. The 33-36 Hz rhythm was enhanced during depolarization when, together with an increase in firing rate, the cardiac related activity pattern of the cells diminished.

We suggest, that a 22-26 Hz oscillation is due to synaptic input while the 33-36 Hz rhythm is more likely to be generated internally.

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354.8

DO SYMPATHETIC PREMOTOR NEURONS CROSSTALK?

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The bursting rhythms that characterize sympathetic whole nerve discharge may originate in the brainstem. Most ongoing sympathetic vasomotor drive is attributable to premotor neurons in the brainstem, particularly the rostral ventrolateral medulla (RVLM). One possibility is that sympathetic bursts are generated by cross-connections between, or involving, premotor neurons. Alternatively, premotor neurons may transmit bursts generated by antecedent neurons. Either way, one would expect to see short-term firing correlations between RVLM premotor neurons. We generated cross correlograms from the spontaneous activity of 35 pairs of barosensitive RVLM neurons extracellularly recorded in chloralose-anesthetized cats (by single or 2 electrode methods). One or both of each pair were proven to be bulbospinal. We found no clear evidence of direct short-term (1/few millisecond) synaptic interactions. Two of the 35 neuron pairs showed strong synchrony within +/-100ms, which was independent of the cardiac cycle, indicating common inputs other than baroreceptors. Five further pairs showed such synchrony very weakly. We conclude that direct crosstalk between these cells is rare, weak or absent, and that only a minority is synchronized by common drives. (Supported by NH&MRC)

354.9

MODULATION OF THE CARDIOVASCULAR RESPONSES TO STATIC MUSCLE CONTRACTION BY BLOCKADE OF AMPA-GLUTAMATE RECEPTORS IN THE LATERAL RETICULAR NUCLEUS IN ANESTHETIZED RATS. R. DiGiacco, D. Mokler* and A.Ally, Departments of Pharmacology & Biochemistry, University of New England College of Medicine, Biddeford, ME 04005

The effects of microdialyzing CNQX (an AMPA-Glutamate receptor antagonist) into the lateral reticular nucleus (LRN), magnocellular part, on the cardiovascular responses evoked by static contraction of the triceps surae muscle were studied using chloral hydrate-anesthetized rats (n = 5). A microdialysis probe was inserted into the LRN (AP:-5.8; L:1.5; H:-8.0) ipsilateral to the muscle being contracted using a stereotaxic guide. Contraction, elicited by stimulation of the tibial nerve (3 X motor threshold, 0.1 msec, 40 Hz) for 30 sec, increased mean arterial pressure (MAP), heart rate (HR), and tension by 27 ± 4 mmHg, 32 ± 12 bpm, and 181 ± 20 g, respectively. Microdialysis of CNQX (2 mM) for 40 min attenuated the contraction-evoked responses, as MAP and HR increased by 10 ± 4 mmHg and 10 ± 8 bpm, respectively ($P < 0.05$). Developed tension did not change after the administration of the drug. This suggests that neurotransmission in this area of the medulla might be involved in the central regulation of cardiovascular responses to muscle contraction. Furthermore, these results demonstrate that AMPA-Glutamate receptors within the LRN appear to play a role in mediating the cardiovascular responses to static muscle contraction.

354.10

RESPONSES OF NUCLEUS RETICULARIS PARVOCELLULARIS TO HYPOXIA AND HYPERCAPNIA. L.M. Sexcius*, R.M. Douglas, E.T. Anderson, and C.O. Trouth. Dept. of Physiol. & Biophysics, Howard University Coll. Med., Washington, D.C. 20059.

Maximal pressor responses to stimulation of the nucleus reticularis parvocellularis (NRP) have been reported. The integrity of the NRP is essential for the expression of the cerebral ischemic response (CIR) (Kumada et al., Circ. Res., 45:63-70, 1979). In this investigation extracellular NRP neuronal activity and blood pressure were recorded from spontaneously breathing cats exposed to hypoxic (12% O_2) hypercapnic (5% CO_2) conditions. Cerebral ischemia (clamp. carotid and vertebral art.) leads to an increase in blood pressure and in neuronal activity recorded at NRP. Hypoxia with hypercapnia caused an increase in NRP neuronal activity and blood pressure similar to that observed with cerebral ischemia. However, hypercapnia (5% CO_2) alone did not exhibit either an increase in neuronal activity or blood pressure. It appears that ischemia is more effective in inducing the CIR than hypercapnia alone.

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354.11

DIRECT PROJECTIONS FROM THE VENTROMEDIAL MEDULLARY REGION AND A5 PONTINE CELL GROUP TO AUTONOMIC PREGANGLIONIC NEURONS EXPRESSING NEUROKININ1-RECEPTOR IMMUNOREACTIVITY IN THE SPINAL CORD OF RATS. E. Polgár, J. Nagy, M. Petkó and M. Antal,* Department of Anatomy, University Medical School of Debrecen, H-4012 Hungary.

The spinal projections of neurons in the ventromedial medullary region (VMM) and A5 pontine cell group (A5) that play a major role in the regulation of autonomic functions in general and of cardiovascular control in particular were studied in the rat by using the anterograde neural tracer Pheseolus vulgaris leucoagglutinin (PHA-L). After injecting PHA-L into the VMM or A5, numerous labelled axon terminals were detected in the intermediolateral cell column (IML) of the thoracic and lumbar spinal cord. To study the postsynaptic targets of these descending fibres sections were stained for both PHA-L and calbindin-D-28k (CaB), a calcium-binding protein that has been reported to be a marker of spinal autonomic preganglionic neurons. CaB-immunoreactive neurons in the IML were found to receive multiple contacts from fibres descending from both the VMM and A5. Synaptic contacts as well as GABA- and glycine-immunoreactivity of the presynaptic terminals were identified in a correlative electron microscopic study by using postembedding immunocytochemical methods.

Combining the PHA-L labelling with the immunocytochemical detection of neurokinin1-receptor (NK1-R; kindly provided by S. Vigna), a well known receptor for substance-P, it was found that autonomic preganglionic neurons receiving monosynaptic contacts from VMM and A5 terminals express a strong immunoreactivity also for NK1-R. Synaptic and extrasynaptic localization of NK1-R-immunoreactive membrane compartments as well as the relations of VMM or A5 terminals and NK1-R-immunoreactive membrane divisions of autonomic preganglionic neurons were investigated in a correlative electron microscopic study.

354.12

MULTIPLE 5-HYDROXYTRYPTAMINE RECEPTORS ON RAT RVLM NEURONS. L. L. Hwang* and N. J. Dun. Dept. of Anat. & Neurobiol., Medical College of Ohio, Toledo, OH 43614

Rat rostroventrolateral medulla (RVLM) exhibits a dense network of 5-HT-immunoreactive fibers. Effects of 5-HT on RVLM neurons were studied by the whole-cell patch technique in brainstem slices removed from 8-12 day old rats. 5-HT applied either by superfusion or pressure ejection elicited three types of currents: a brisk inward current, a slow inward current associated with decreased conductance, and a slow outward current associated with increased conductance. All three types of 5-HT-induced currents were blocked by the non-specific antagonist methysergide. The brisk inward current was blocked by the 5-HT_3 receptor antagonist MDL-72222 (10 μM). The slow outward and inward current was made smaller with membrane hyperpolarization. The 5-HT_{1A} agonist 8-OH-DPAT (10 μM) caused a slow outward current similar to that of 5-HT. The $5\text{-HT}_2/5\text{-HT}_{1A}$ antagonist spiperone (1 μM) and the $5\text{-HT}_2/5\text{-HT}_{1C}$ antagonist ketanserin (1 μM) partially blocked the slow outward and inward current. Spontaneous miniature excitatory postsynaptic currents (mEPSCs) were recorded in some RVLM neurons. 5-HT depressed mEPSCs in some but increased the amplitude in other RVLM neurons. The result shows that 5-HT may modify the electrical activity and synaptic transmission of RVLM neurons by interacting with multiple 5-HT receptors. (Supported by NIH Grant HL51314).

354.13

CORRELATION BETWEEN THE ACTIVITY OF RESPIRATORY-MODULATED RAPHE-SPINAL NEURONES AND SYMPATHETIC NERVE DISCHARGE S-Y. Zhou* and M.P. Gilbey, Dept. Physiology, Royal Free Hospital School of Medicine, London NW3 2PF, UK.

A previous study from this laboratory demonstrated that in the rat some raphe-spinal neurones have their activity modulated by central respiratory drive (Gilbey, Futuro-Neto and Zhou, J.Auton.Nerv.Syst. 50, 263-273, 1995). In this study the correlation between the activity of these neurones and sympathetic nerve discharge was examined in the 2-6 Hz frequency band. Recordings were made in chloralose anaesthetized, paralysed, vagotomized and artificially ventilated rats. Correlations between the discharge of single units in either raphe pallidus, obscurus or magnus and cervical sympathetic nerve activity were assessed using coherence analysis (25 pairs). Peak coherence values occurred at frequencies ranging from 2.1 - 5.7 Hz. The mean peak coherence value was 0.18 ± 0.02 (range 0.08 - 0.40, 32 windows). Thus all coherence values although low were significantly greater than zero (see Benignus, IEEE Trans. Audio Electroacoustics, AU-18, 320, 1970). Of the neurones used in the analysis sixteen of the eighteen which were tentatively identified as having axons projecting to the intermediolateral cell column had respiratory-modulated discharge patterns. Interestingly, only 2 of 19 pairs involving neurones recorded from raphe obscurus yielded coherence values of > 0.2 , whereas 4 of 6 pairs comprising of neurones recorded from pallidus or magnus produced coherence values > 0.2. These data are consistent with the idea that respiratory-modulation and 2-6 Hz rhythms are relayed to the sympathetic outflow by some caudal raphe neurones. The tendency for raphe pallidus and raphe magnus neurones to have higher coherence with sympathetic nerve discharge than raphe obscurus neurones indicates that the influence of the various caudal raphe nuclei on sympathetic activity may differ. (Supported by the Wellcome Trust)

354.14

AN INTRACELLULAR STUDY OF RESPIRATORY-MODULATED CAUDAL RAPHE NEURONES AND THEIR RELATIONSHIP TO SEROTONIN-CONTAINING NEURONES IN THE RAT MEDULLA. M.P. Gilbey*, J. Li, P.N. Izzo and S-Y. Zhou, Department of Physiology, Royal Free Hospital School of Medicine, Rowland Hill St, London NW3 2PF, UK.

Our previous extracellular studies have demonstrated that neurones in caudal raphe (2.50 to 3.50 mm caudal to interaural line) may be involved in relaying respiratory modulation to sympathetic preganglionic neurones (Gilbey, Futuro-Neto and Zhou, J.Auton.Nerv.Syst. 50, 263-273, 1995). However, whether these neurones contain serotonin has not been examined. The aim of present studies was to investigate whether raphe neurones with respiratory-related activity contained serotonin. Experiments were performed on male Sprague-Dawley rats (250-350 g) anaesthetized with sodium pentobarbitone (60 mg/kg I.P.) and supplemented with i.v. chloralose (5-10 mg). Animals were paralysed (gallamine triethiodide 16 mg/kg), vagotomized and artificially ventilated. Intracellular recording and labelling were achieved using glass microelectrodes filled with 5% Lucifer Yellow (in 1 M lithium citrate) or 10% Fluorescein-Dextran (in 1.5 M potassium methyl sulphate). The brainstem was removed and post-fixed overnight. Serial coronal sections (50 μm) of the medulla were cut on a vibrating microtome and all sections were then incubated to detect serotonin immunoreactivity using immuno-fluorescence techniques. Both intracellularly labelled and serotonin immunoreactive neurones were examined by a confocal microscope. Eight of sixteen labelled neurones located in caudal raphe had respiratory-related activity (5 expiratory- , 2 inspiratory- and 1 post-inspiratory-modulated). All neurones were identified as non-immunoreactive for serotonin. These data indicate that some raphe neurones which have respiratory-related activity do not contain serotonin. (Supported by the Wellcome Trust and British Heart Foundation)

354.15

CARDIOVASCULAR RESPONSES ELICITED FROM THE LOCUS COERULEUS ARE ABOLISHED BY MICROINJECTIONS OF 6-HYDROXYDOPAMINE INTO THE NUCLEUS AMBIGUUS/ VENTROLATERAL MEDULLARY DEPRESSOR AREA. H.N. Sapru* and V.K. Malhotra, Depts. of Neurosurgery & Pharmacology, New Jersey Medical School, Newark, NJ 07103.

Microinjections (50 nl) of L-glutamate (1.77 nmol) into the locus coeruleus (LC) neuronal pool elicited depressor and bradycardic responses. It was hypothesized that these responses may be mediated by noradrenergic projections from LC to the NA/VLDA neurons because small doses of norepinephrine (NE; 0.75, 1.5, 3 & 6 pmol) elicited a depressor and bradycardic response when injected into the NA/VLDA. The purpose of the present study was to determine pharmacologically if the destruction of noradrenergic terminals (and possibly noradrenergic neurons) can abolish the cardiovascular responses elicited from the LC. Experiments were done in pentobarbital-anesthetized and artificially ventilated rats. A solution of 6-hydroxydopamine (6-OH-D, 2 µg/µl) containing ascorbic acid (0.1 mg/ml) was used for the destruction of noradrenergic nerve terminals. Five microinjections (100 nl each) were made; the interval between injections was 5 min. After a waiting period of 1 hour, tyramine (25 nmol/50 nl) was injected into the NA/VLDA; usual depressor and bradycardic responses of tyramine were absent indicating that noradrenergic terminals and/or neurons were destroyed. Subsequent injections of glutamate into the LC did not elicit the usual depressor and bradycardic responses. These results suggest that cardiovascular responses elicited by injections of L-glutamate into the LC are mediated by noradrenergic nerve terminals and/or noradrenergic neurons located in the NA/VLDA region.

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354.16

RELATIONSHIP BETWEEN THE REGULATION OF BLOOD PRESSURE AND *IN VIVO* NORADRENERGIC NEURAL ACTIVITIES IN THE LOCUS COERULEUS OF YOUNG SPONTANEOUSLY HYPERTENSIVE RATS. K.H. Ko[1]*, Y.T. Kim[1], J.H. Lee[1], E.K. Lee, M.J. Song[1], J.W. Dailey[2] and P.C. Jobe[2]
[1]Department of Pharmacology, College of Pharmacy, Seoul National University, Seoul 151, Korea. [2]Department of Basic Sciences, University of Illinois College of Medicine at Peoria, Peoria, Il 61656, U.S.A.

The purpose of the present study was to determine whether *in vivo* noradrenergic neural activity in the locus coeruleus is related to the development of hypertension. Two groups of animals were prepared, 1) young spontaneously hypertensive rats (SHR) and 2) age-matched normotensive wistar kyoto rats (WKY). At 6 weeks of age, the release of norepinephrine (NE) and 3,4-dihydroxyphenylglycol (DOPEG) from locus coeruleus of young SHR and WKY as an index of neural activity were determined by *in vivo* microdialysis along with blood pressure (BP) at three conditions : 1) normal; 2) elevated BP by systemic injection of phenylephrine and 3) α_1-adrenoceptor stimulated by perfusion of phenylephrine into the locus coeruleus through microdialysis probe. Basal releases of NE and DOPEG from the locus coeruleus were 0.415 ± 0.089 pg/20min, 1.311 ± 0.293 pg/20min in SHR and 0.204 ± 0.078 pg/20min, 1.492 ± 0.365 pg/20min in WKY respectively. Basal release of NE from the locus coeruleus of SHR was significantly greater than that of WKY. Phenylephrine systemic injection caused elevation of BP in both SHR and WKY in a dose related manner. Following phenyephrine injection, the releases of NE and DOPEG from the locus coeruleus of SHR were significantly decreased, whereas there were no significant changes in the releases of NE and DOPEG in young WKY. α_1-Adrenoceptor stimulation in the locus coeruleus by perfused phenylephrine through microdialysis probe caused pressor responses in both SHR and WKY, but the magnitude of pressor response in SHR was larger compared with that in WKY. The result from the present study suggests that noradrenergic neural activity in locus coeruleus may contribute as one of triggering factors for the expression of hypertension in young SHR.

354.17

BRAINSTEM INTERNEURONS NECESSARY FOR VESTIBULAR INFLUENCES ON SYMPATHETIC OUTFLOW. B.C. Steinbacher, Jr.* and B.J. Yates. Depts. Otolaryngology & Neuroscience, Univ. Pittsburgh, Pittsburgh, PA 15213.

The vestibular system has prominent influences on the sympathetic nervous system and cardiovascular function, and may participate in making adjustments in circulation during movement and changes in posture. Vestibular signals are relayed to sympathetic preganglionic neurons in the spinal cord through reticulospinal neurons located in the rostral ventrolateral medulla (RVLM, Yates et al., *Am. J. Physiol.* 268, 1995). However, both anatomical and physiological studies have shown that RVLM neurons receive labyrinthine signals through polysynaptic pathways (Yates et al., *Brain Res.* 552, 1991), suggesting that vestibulosympathetic reflexes are mediated by brainstem interneurons. The objective of this study was to determine which brainstem regions contain interneurons that are critical for producing vestibulosympathetic responses.

Experiments were conducted on decerebrate cats, and responses were recorded from the splanchnic nerve following electrical stimulation of the vestibular nerve. Brainstem lesions were produced by microinjecting the neurotoxin kainic acid or by mechanical methods. The areas targeted for lesions included the lateral reticular formation near the obex, nucleus tractus solitarius, and the pontine parabrachial nucleus. Lesions of the caudalmost lateral medullary reticular formation abolished vestibulosympathetic reflexes, indicating that interneurons critical for these responses are located in this area. The same lesions also attenuated most components of somatosympathetic responses elicited by stimulation of the sciatic nerve, raising the possibility that a common pool of interneurons integrates signals from muscle, skin and the vestibular system that reflect body position in space. Lesions of other brainstem areas, including more rostral parts of the lateral reticular formation, nucleus tractus solitarius, or the parabrachial nucleus had little effect on either somatosympathetic or vestibulosympathetic reflexes, suggesting that interneurons that mediate these responses are confined to a small part of the caudal medulla.

Supported by NIH grant DC00693.

354.18

PROCESSING OF VESTIBULAR, HINDLIMB AND BARORECEPTOR INPUTS BY NEURONS IN THE CAUDAL VENTROLATERAL MEDULLA (CVLM). B.J. Yates* and B.C. Steinbacher, Jr. Depts. Otolaryngology & Neuroscience, Univ. Pittsburgh, Pittsburgh, PA 15213.

Lesions of the lateral reticular formation caudal to the obex, including the CVLM, abolish vestibulosympathetic reflexes and substantially reduce the amplitude of somatosympathetic responses. Thus, interneurons located in this region are a critical part of the circuitry that relays vestibular and other somatic signals to descending cardiovascular-regulatory pathways. Neurons in the CVLM also play an essential role in the baroreceptor reflex pathway (Jeske et al., *Neuroscience* 65, 1995; Blessing, *News Physiol. Sci.* 6, 1991), and make direct inhibitory connections with neurons in the rostral ventrolateral medulla that project to sympathetic preganglionic neurons in the spinal cord. The purpose of this study was to determine if common neurons in the CVLM mediate baroreceptor, vestibulosympathetic and somatosympathetic reflexes.

Recordings were made from CVLM neurons in cats anesthetized using α-chloralose and urethane. Vestibular inputs were elicited by electrical stimulation of the vestibular nerve, somatic afferents were activated by stimulation of the sciatic nerve, and neurons with baroreceptor inputs were identified by having discharges correlated with spontaneous fluctuations in blood pressure during the cardiac cycle. Many neurons in the CVLM responded to vestibular nerve stimulation, frequently at short latency (1.1-1.5 ms from the effective shock). However, only a small fraction of neurons with baroreceptor inputs received labyrinthine signals. Thus, different interneurons mediate vestibulosympathetic and baroreceptor reflexes. Many CVLM neurons with baroreceptor inputs responded to sciatic nerve stimulation, as did about half of the neurons with vestibular inputs. These data suggest that somatosympathetic responses are mediated both by neurons involved in producing baroreceptor reflexes and by neurons which lack baroreceptor inputs but which integrate a number of inputs relating to body position.

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PAIN PATHWAYS: GENE EXPRESSION

355.1

LOCALIZATION OF ENKEPHALIN AND THE DELTA OPIATE RECEPTOR IN THE PONTINE PARABRACHIAL NUCLEUS: POSSIBLE INVOLVEMENT IN CFA-INDUCED NOXIOUS PERIPHERAL INFLAMMATION. *Lisa L. Bellavance* and Alvin J. Beitz; Neuroscience Program, University of Minnesota, St. Paul, MN 55108.*

Previous work from our laboratory has shown that a CFA-induced peripheral inflammatory model of nociception results in an increase in the number of cFos immunoreactive nuclei in the pontine parabrachial nucleus (PBN) of the rat. This increase in cFos-like immunoreactivity (cFos-LI) is restricted to the Kolliker-Fuse and lateral subdivisions of the PBN and suggests a role for these areas in nociception and/or antinociception. One neurochemical that is likely to participate in nociceptive/antinociceptive processes in the PBN is the neuromodulator, enkephalin (ENK). We have used both immunocytochemistry and *in situ* hybridization to determine the distribution of ENK in the lateral PBN (PBNl). Moreover, we have utilized immunocytochemical techniques to determine the comparative distribution in the PBNl of the delta opiate receptor (antibody generous gift from Dr. R. Elde; see Dado *et al*, 1993 and Arvidsson *et al.*, 1995) through which ENK is likely to exert some of its cellular effects. Interestingly, the distribution of ENK mRNA and immunoreactivity is more pronounced in the medial dorsal aspects of the PBNl while immunoreactivity for the delta opiate receptor is more noticeable in the lateral ventral aspects of this subdivision. The distribution of cFos-LI seen after inflammation partially overlaps with the distributions of both ENK and the delta opiate receptor in the PBNl. Further experiments are necessary to ascertain if those neurons expressing cFos in the PBNl in response to pain also contain ENK and/or delta opiate receptor mRNA's. These experiments are in progress and will provide important data defining the neurochemical nature of the PBN's involvement in nociception and antinociception. This work was supported by NIH grant DA06687 and NIDA research fellowship DA05639.

355.2

LIMBIC AND CORTICAL EXPRESSION OF C-FOS FOLLOWING PERIPHERAL NEURECTOMY IN RATS. Anthony L. Vaccarino, Jane E. Magnusson, Kathryn L. Ruck and Richard D. Olson*. Department of Psychology, University of New Orleans, LA 70148.

Peripheral nerve damage often leads to pathological pain processes which persist long after healing has occurred. There is evidence that persistent pain following nerve injury is related to changes in CNS function occurring at the time of nerve damage. In the present study, the expression of c-fos was examined in various limbic and cortical areas following peripheral neurectomy. Male Long-Evans rats were anesthetized with sodium pentobarbital and the sciatic nerve from one hind paw was sectioned free and cut. In half the rats, the sciatic nerve and surrounding tissue and muscle were anesthetized with bupivicaine (0.75%) prior to nerve section. Immediately, 1 h, or 2 h after nerve section, the animals were perfused through the heart with saline followed by 4% paraformaldehyde. Brains were removed and placed in 4% paraformaldehyde for 24 h and in 30% sucrose paraformaldehyde for an additional 48 h. Frozen coronal sections (40 um) were placed in PBSx and stained with Sheep polyclonal antibody to fos. Peripheral neurectomy resulted in c-fos expression in various limbic and cortical areas, including the periventricular nucleus of the hypothalamus, cingulate cortex and amygdaloid complex. C-Fos expression was most pronounced 2 hours after nerve section. Anesthetizing the sciatic nerve prior to nerve section resulted in decreased c-fos expression in the pariventricular nucleus and cingulate cortex. This research was supported by a Louisiana Education Quality Support Fund (LEQSF).

355.3

PREPROCHOLECYSTOKININ mRNA EXPRESSING NEURONS IN THE RAT PARABRACHIAL NUCLEUS. Ola Hermanson*, Martin Hallbeck, Dan Larhammar and Anders Blomqvist. Dept Cell Biology, University of Linköping, S-581 85 Linköping and Dept Medical Pharmacology, University of Uppsala, S-751 24 Uppsala, Sweden.

The parabrachial nucleus (PB) is a major termination site for ascending fibers from nociceptive-responsive neurons in the spinal and trigeminal dorsal horn. Nociceptive stimuli induce the expression of the transcription factor FOS in neurons in the dorsal part of the lateral PB (Hermanson et al., Soc. Neurosci. Abstr., 1992). FOS may regulate the transcription of several neuropeptide genes, such as the CCK gene, which contains the AP-1 site. PB has been reported to contain preprocholecystokinin (ppCCK) mRNA but its distribution within PB is unknown. The aim of this study was to analyse the subnuclear localization of ppCCK-expressing neurons in PB, particularly in relation to the distribution of noxiously evoked FOS .

Male Sprague-Dawley rats were killed by transcardial perfusion with 4% paraformaldehyde. The brain was cut at 20 µm on a freezing microtome. In situ hybridization was performed with a ^{35}S-labeled 0.5 kBp RNA probe complementary to ppCCK (Deschenes et al., Proc. Natl. Acad. Sci., 1984). The sections were pretreated with proteinase K, hybridized for 20 hours at 59°C, and then posthybridized under high stringency conditions, followed by autoradiography.

Several subnuclei contained ppCCK-expressing neurons. A large number of ppCCK-positive neurons was present in the superior lateral subnucleus. Many labeled neurons were also seen in the dorsal lateral subnucleus, while a moderate number of ppCCK-expressing neurons was seen in the ventral lateral subnucleus. Other parts of PB, such as the Kölliker-Fuse nucleus and the external lateral subnucleus, contained only scattered labeling.

The present findings show that the distribution of ppCCK-expressing neurons in the dorsal parts of PB is similar to the pattern of FOS-expression seen after noxious stimulation, suggesting that noxiously-evoked FOS in PB may be related to ppCCK-expressing neurons.

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355.4

FOS PROTEIN IN PARAVENTRICULAR THALAMIC (PVT) AND DORSAL RAPHE (DRN) NUCLEI OF MONOARTHRITIC RATS.

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It has been established that Fos protein appears after extracellular stimulation. We induced acute arthritis, in the same way as in the model known as Pain-Induced Functional Impairment in the Rat (PIFIR), in order to determine which brain areas of awake rats are activated. Groups (n=4) of Male Wistar rats (250-350 g) were used: i)manipulated control group (M), ii)rats injected with vehicle (V); and iii)rats injected with 30% uric acid (UA). The injection was performed in the knee joint of the right hind limb of the rats under brief anesthesia with halothane. After an exposure period of 2.5 or 4.5 h to V or UA, the animals were anesthetized with ketamine-xylazine and then perfused. Brains were removed and cut in coronal sections (40µm) which were exposed to Fos antibody and processed by the Avidin-Biotin technique. The number of positive cells was counted and analyzed by Student's t-test. Among the areas analyzed, we found a significant increment of the number of Fos positive cells in DRN and PVT.

	DRN		PVT	
	2.5 h	4.5 h	2.5 h	4.5 h
V	38 ± 6	41 ± 4	47 ± 4	38 ± 4
UA	50 ± 3	58 ± 4*	80 ± 6*	84 ± 10*
M	33 ± 4		43 ± 7	

The temporal changes observed in DRN and PVT were closely related to functional impairment of the hind limb as registered with the PIFIR model. Thus, Fos could be used as a marker in DRN and PVT in the PIFIR model. Our data suggest that these regions might participate in a nociceptive neural pathway from knee joint.

355.5

DISTRIBUTION OF C-FOS IN THE BRAIN OF RODENTS AFTER NOXIOUS STIMULATION OF VISCERA. G.F. Gebhart*[1], A. Solodkin[2] and R.J. Traub[1]. Pharmacology[1] and Anatomy and Neurology[2]. University of Iowa College of Medicine, Iowa City, IA, 52242

There is much to learn yet about the circuitry responsible for the perception of visceral pain. Identification of Fos-ir neurons has become a powerful tool to detect populations of neurons activated by different stimuli. In an attempt to elucidate some of the supraspinal areas involved in the integration of noxious stimulation from viscera, we examined c-Fos expression after noxious colorectal distention (CRD) in rats.

Using standard immunocytochemical techniques, we compared the distribution of c-Fos after three different treatments: a) CRD + loose restraint, b) loose restraint, c) no treatment.

CRD induced Fos in several brain areas. Fos was also present in some areas in the stress group but not in controls. The areas showing Fos induction after CRD are: parabrachial nucleus, ventro-lateral PAG, VPL, anterio-medial and posterior nuclei in thalamus, medial and cortical amygdaloid nuclei, mammillary hypothalamic nuclei, lateral septal nucleus, infragranular cingulate and insular cortices. Areas with induction of Fos in stress and CRD conditions, with the latter showing a greater density of stained nuclei were: dorso-lateral PAG, paraventricular and suprafascicular nuclei in thalamus, cingulate, frontal, perirhinal, entorhinal and piriform cortices and hippocampal formation.

These observations show a widespread activation of brain areas after noxious stimulation of the colon. Even though there were some areas specifically activated after noxious stimulation and not stress, there were many which were activated under both conditions. These results suggest that the perception of pain is the result of parallel activation in several limbic and non-limbic areas. Supported by: NS 30604 and NS 19912.

355.6

FORMALIN-INDUCED C-FOS EXPRESSION IN THE SPINAL CORD OF RAT FETUSES. D. Yi* and G. Barr. Biopsychology Doctoral Program, Dept. of Psychology, Hunter College-CUNY, NY, NY 10021 and New York State Psychiatric Institute, 722 W. 168th Street, NY, NY 10032.

Understanding the ontogeny of pain processing is an important first step in controlling and alleviating pain in neonates. It has been shown that the basic connections in nociceptive pathways are present before birth in both rats and humans. The primary afferents and the dorsal horn cells that are involved in processing nociceptive information, however, are immature at birth and undergo considerable postnatal maturation. Following formalin injection into the forepaw or hindpaw, the c-fos oncogene is expressed in the spinal cord dorsal horn as early as the day of birth. In order to study functional maturation of pain pathway before birth, we examined the expression of c-fos gene in the spinal cord of rat fetuses following formalin injection into the forepaw or hindpaw. An adult female rat was placed with a male rat for 14 hours overnight. On the following day, a vaginal smear was taken and with the presence of sperm, that day was designated as embryonic day (ED) 0. On ED 19 or ED 20, the dam underwent chemomyelotomy at L1/L2, and was placed in a holding devise submerged in a warm saline bath. The uterine horns were externalized through an abdominal incision into the saline bath, and either the forepaw or the hindpaw of the fetal animal was injected with 5 µl of 10% formalin or saline. Fetuses remained in the uterine horn for 2 hours post-formalin injection, and were sacrificed at the end of the 2 hour period. ED 20 fetuses were perfused, and all animals were fixed or post-fixed by immersion in 4% paraformaldehyde. The lumbar or cervical portion of spinal cord was dissected out, sectioned and processed for Fos immunocytochemistry. No Fos labeled cells were apparent in the spinal cord of ED 19 animals. However, stained Fos nuclei were observed bilaterally in the spinal cord of ED 20 fetuses. Furthermore, very few cells in the superficial laminae were labeled and most of the labeling occurred in the deeper layers of the dorsal horn. These data indicate that the dorsal horn cells are expressing Fos protein before birth although different than the Fos staining pattern of postnatal animals. Supported by RO1 DA-07341.

355.7

ONTOGENY OF FORMALIN INDUCED C-FOS EXPRESSION IN THE RAT BRAIN. G.A. Barr* and D.K. Yi. Biopsychology Doctoral Program, Dept. of Psychology, Hunter College-CUNY, NY, NY 10021 and New York State Psychiatric Institute, 722 W. 168th Street, NY, NY 10032.

The processing of painful stimulation differs in the young, and although it has been shown that the basic connections in nociceptive pathways are present before birth in both rats and humans, the functional ontogeny of nociception is poorly understood. In the rat, the primary afferents and the dorsal horn cells of the spinal cord that are involved in processing nociceptive information develop prenatally but undergo considerable postnatal maturation. Virtually nothing is known of the functional maturation of brain structures that, in the adult, are known to process this noxious afferent input. Following noxious stimulation of a variety of body regions, the c-fos oncogene is expressed in the spinal cord and brain of adult animals. In the infant the Fos protein is also induced in the spinal cord dorsal horn by various types of noxious stimulation as early as the day of birth. In order to study functional maturation of pain processing by brain structures, formalin or saline was injected into the forepaw or hindpaw of 0, 3, and 14 day old rat pups. After 2 hours, the pups were overdosed with a barbiturate, and perfused with 4% paraformaldehyde.The brain was dissected out, sectioned and processed for Fos immunocytochemistry. Fos-like immunoreactive cell nuclei were found in a variety of brain sites comparable to those reported by others in the adult animal. These include but are not limited to the rostral ventral medulla, periaqueductal gray of the midbrain, medial thalamic nuclei, and the hypothalamus. There was also Fos staining in some brain regions in the control rat pups. These data indicate that the brain is capable of expressing c-fos in response to a noxious stimulus as early as the day of birth. Supported by RO1 DA-07341.

355.8

EVALUATION OF FOS-LABELING IN THE RABBIT BRAIN FOLLOWING INDUCTION OF SINUSITIS. A.K. Roche* and K.C. Kajander. Depts. of Pharmacology, Oral Science, Cell Biology and Neuroanatomy, and Graduate Program in Neuroscience, University of Minnesota, Minneapolis, MN 55455.

The purpose of this study was to evaluate Fos-labeling in the rabbit brain following induction of sinusitis. The bacterium *Bacteroides fragilis* was used to induce sinusitis.

We used adult, male, New-Zealand White rabbits (2.5-3.5 kg). After onset of anesthesia, the left maxillary sinus was opened by drilling through the bony ceiling of the nasal dorsum. To prevent spread of bacteria to adjacent nasal areas, the maxillary ostium was closed using a cotton plug and cyanoacrylate ester. For induction of inflammation, $1x10^8$ colony forming units of *Bacteroides fragilis*, suspended in 0.5 ml phosphate buffered saline, were injected onto a plug of sterile cotton placed in the left maxillary sinus. The opening in the nasal dorsum was then closed using dental resin; the skin incision was sutured, and the animal was allowed to recover. Animals were sacrificed 24 hours later for immunohistochemical localization of Fos protein.

Following induction of sinusitis, a significant increase (Student's *t*, $p<.05$) in Fos-labeled nuclei was observed in the ipsilateral subnucleus caudalis of the spinal trigeminal tract. In a previous study using WGA-HRP, we traced the central projections of nerves innervating the sinus to this subnucleus (Neurosci. Abst., 20:1571, 1994). A complete analysis of Fos-labeling in other regions of the brain is in progress.

In this study, we used Fos-labeling to map the distribution of neurons activated by sinusitis. Our study appears to be the first to evaluate alterations in the central nervous system following induction of sinusitis.

355.9

DESCENDING AFFERENTS PARTIALLY SUPPRESS SPINAL DYNORPHIN mRNA AND PEPTIDE UPREGULATION INDUCED BY PERIPHERAL INFLAMMATION M.A. Ruda, K. Ren, E. Pfaffenroth, D. R. Kenshalo, Jr.*, L. MacArthur. Neurobiology & Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892

Inflammation and hyperalgesia induce a dramatic upregulation of opioid and non-opioid mRNA and peptide levels in the spinal dorsal horn. The role of descending modulation in these events is not known. To elucidate the role of descending mechanisms in regulation of dynorphin (DYN) levels induced by tissue injury, rats received a hindpaw injection of 0.3 ml complete Freund's adjuvant (CFA) seven days after spinal transection. The L4-5 spinal segments were prepared for RNA blot analysis and immunocytochemistry 3 days after CFA. Comparisons were made between 3 groups of CFA treated rats: spinal transected (SX); sham surgery (SH); and naive (N). Paw edema was similar in all three groups (11.5mm ipsi vs 4.8mm contra). The inflamed hindpaw of SH and N rats demonstrated hyperalgesia to a radiant heat stimulus, while SX rats exhibited a withdrawal reflex to the same heat stimulus. RNA blot analysis demonstrated an 810%, 527% and 500% ipsilateral upregulation in DYN mRNA in the SX, SH and N groups respectively, as compared to the contralateral side of the SH group. Immunolabeling of colchicine treated rats showed an average of 35.8 DYN immunoreactive (DYN-IR) cell bodies /30μm section ipsilateral to the inflammation in SX rats and 34.5 cells in SH rats. These data demonstrate that descending afferents to spinal neural circuits partially suppress the DYN mRNA and peptide upregulation that occurs in response to peripheral inflammation and hyperalgesia. Since a similar number of neurons showed DYN-IR in both the SX and SH groups, it is likely that the increased DYN mRNA in the SX group reflects a more dynamic response of the same neuronal population to the painful stimulus rather than a recruitment of more neurons to the response.

355.10

EFFECTS OF AGING ON EXPRESSION OF DYNORPHIN IN RAT SPINAL CORD FOLLOWING PERIPHERAL INFLAMMATION AND HYPERALGESIA. **R.X. Zhang and M.A. Ruda*. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.**

Dynorphin (DYN), a *kappa* opioid, is thought to play an important role in the modulation of nociceptive spinal neural networks. Complete Freund's adjuvant (CFA) injected into the rat hindpaw results in hyperalgesia and upregulation of DYN mRNA and peptide in the ipsilateral spinal cord. The aging process is associated with a number of physiological and biochemical changes that may impact neuronal processing of noxious inputs. This study aimed to identify age-related changes in the behavioral response and regulation of DYN mRNA and peptide in the spinal cord after CFA-induced inflammation. CFA was injected unilaterally into the hindpaw of male young (3 month, n=15) and aged (18 month, n=14) Sprague-Dawley rats. The withdrawal latency of the hindpaws to radiant heat was measured from 2 hr to 3 days after CFA. The post-CFA paw withdrawal latencies were significantly shorter ($P<0.05$, ANOVA) in aged rats while paw diameters, a measure of edema, were significantly larger ($P<0.05$, ANOVA) in aged than in young animals. At day 3, the L 4-5 spinal segments were removed and total RNA extracted. RNA blots were hybridized with a ^{32}P-labeled DYN cDNA probe. In aged rats there was a significant increase (2-fold, $P<0.05$, ANOVA) in DYN mRNA levels in the spinal cord on the inflamed side as compared to the inflamed side in young rats. There was also a significant ipsilateral increase (3-fold, $P<0.05$, ANOVA) in the number of detectable DYN immunostained neurons in aged as compared to young rats. No significant differences in DYN mRNA and peptide levels were observed in the contralateral spinal cord of aged as compared to young rats. These data indicate that aged rats exhibit a more robust induction of DYN mRNA and peptide as a result of peripheral inflammation than that produced in young rats.

PAIN MODULATION: ANATOMY AND PHYSIOLOGY—NEUROPATHIC PAIN

356.1

IMPORTANCE OF THE LOCATION OF INJURY ON SYMPATHETIC DEPENDENCY OF RAT MODELS OF NEUROPATHIC PAIN. D.H. Lee*, K. Chung and J.M. Chung, Marine Biomed. Inst., Depts. of Anat. & Neurosci. and Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX 77555.

The aim of this study was to test a hypothesis that the location of injury in the peripheral nerve is an important determinant for sympathetic dependency in rat neuropathic pain models.

Under halothane anesthesia, neuropathic injury was produced at the left L5/6 spinal nerves in three groups of rats by: 1) tightly ligating them with 6-0 silk; 2) placing loose ligatures with 5-0 chromic gut; and 3) tightly ligating half of the nerves with 6-0 silk. Behavioral tests for ongoing and evoked pain were conducted for the next 4 weeks and the degree of sprouting sympathetic fibers in the L5/6 dorsal root ganglia (DRG) was assessed.

Rats in all three groups displayed behavioral signs of ongoing and evoked pain with a similar time course. The L5/6 DRG of all three groups of rats showed large numbers of sprouting sympathetic postganglionic fibers as visualized by immunohistochemical staining with antibody against tyrosine hydroxylase. These numbers are significantly higher than those seen after distal injuries made by the same methods.

The data suggest that the proximal nerve injury, regardless of the method of injury, triggers sprouting of sympathetic postganglionic fibers into the DRG and produces sympathetically dependent neuropathic pain behaviors. (Supported by NIH grants NS31680 and NS11255)

356.2

SYMPATHETIC SPROUTING IN AXOTOMIZED RAT DRG: ULTRASTRUCTURE. M. Devor*, V. Shinder and R. Govrin-Lippmann, Department of Cell and Animal Biology, Life Sciences Institute, Hebrew University, Jerusalem, Israel 91904

Using tyrosine hydroxylase (TH) immunocytochemistry at the light microscopic level we confirmed the prior finding that following peripheral nerve injury, sympathetic postganglionic axons sprout within the corresponding dorsal root ganglia (DRGs) and in some cases form a basket-like plexus around LL sensory neuron somata. At long survival times, some axotomized DRG neurons acquire an onion bulb-like crust of glial cells which form a loose net around the normal single-layer of closely adherent satellite cells. Ultrastructural analysis, including 3D reconstruction, of TH-positive axonal baskets indicated that sympathetic axons grow on the surface of the glial cells that surround the neuron, and grow preferentially on onion-bulb layers when present. Sympathetic fibers do not contact the neuronal membrane surface directly. However, lanthanum tracer studies showed that small molecules in the bulk extracellular space have access to the soma membrane by diffusion into the seam that separates adjacent satellite cells in the adherent sheath, with subsequent spread into the satellite cell-neuron cleft. Light and EM immunocytochemistry using an antibody that specifically recognizes α2A adrenoreceptors (provided by D. Rosin, Univ. Virginia Med. Sch.) indicated that many LL and SD DRG neurons express these receptors on intracytoplasmic vesicles and on the soma membrane. There was no clear indication that nerve injury triggers receptor upregulation. Together, these observations are consistent with the hypothesis that functional sympathetic-sensory coupling in DRGs is mediated by noradrenalin, released non-synaptically from sprouted sympathetic fibers in the ganglion, which acts on adrenoreceptors on the soma surface after diffusion through the seam/cleft system that ensheaths the neuron.

356.3

SOMATOTOPICAL REDISTRIBUTION OF C-FOS NEURONS IN THE SUPERFICIAL DORSAL HORN AFTER PERIPHERAL NERVE INJURY. C. Molander*, J. Hongpaisan & P. Shortland, Dept. of Neuroscience, Karolinska Institutet, S-171 77 Stockholm, Sweden.

The functional somatotopic organization in the lumbar spinal cord dorsal horn after peripheral nerve injury was studied by mapping neuronal C-FOS distribution. Electrical stimulation of the saphenous nerve at C-fiber strength was performed 5-150 days following either resection of the sciatic nerve, or combined sciatic nerve resection and saphenous nerve crush. Stimulation of the intact saphenous nerve on the contralateral side was made for comparison. Electrical stimulation of the normal saphenous nerve resulted in numerous labeled neurons in the L2-4 superficial dorsal horn. Sparse labeling was seen also in deeper laminae. Bilateral stimulation of the saphenous nerves after unilateral sciatic nerve section combined with saphenous crush resulted in an increased number of labelled cells in laminae I-II and larger distribution (in the mediolateral & caudal directions) on the injured side compared to the uninjured control side, at all survival times. Bilateral saphenous nerve stimulation after sciatic transection alone resulted in similar but less profound changes. The results indicate that chronic nerve injury is accompanied by an increased capacity of neighbouring intact or regenerating (crushed) nerves to excite postsynaptic neurons in the dorsal horn, including part of the territory of the adjacent chronically injured nerve. The timecourse of these alterations indicates that several different mechanisms, including unmasking as well as peripheral & central collateral sprouting, may be operating. Supported by Tore Nilsons fond.

356.4

NMDA, AMPA AND KAINATE RECEPTORS ARE PRESENT IN AXONS IN THE GLABROUS SKIN OF THE RAT HINDPAW. S.M. Carlton* and R.E. Coggeshall, Dept. of Anatomy and Neurosciences, Marine Biomeical Institute, UTMB, Galveston, TX 77555.

It has been previously shown that AMPA, KA and NMDA receptors are expressed in the dorsal root ganglia (DRG) of rat. Based on cell size, small DRG cells immunohistochemically label with antibodies to subunits associated with all 3 glutamate receptor subclasses while large DRG cells were found to label for subunits associated with AMPA and NMDA, but not Kainate receptors. The present study addressed whether glutamate receptors were transported to the periphery, and thus could be involved in sensory processing in cutaneous axons.

Rats (male, Sprague-Dawley) were deeply anesthetized and perfused with saline, followed by mixed aldehydes. The glabrous skin from the third toe of each rat was removed and cut with a razor blade into 100um slabs. These slabs were immunostained free-floating with antibodies which recognized the GluR1 (AMPA), GluR5,6,7 (Kainate) or the NMDAR1 (NMDA) subunits. Following immunostaining, the tissue was osmicated, dehydrated and embedded in plastic for ultrathin sectioning. At the EM level, bundles of unmyelinated axons and accompanying Schwann cells were found in the dermis and at the dermal-epidermal junction. Each bundle contained a varying number of labeled as well as unlabeled axons. The label consisted of dense reaction product within the axoplasm, or in some cases, along the axoplasmic membrane. It is unknown at this time if the labeled axons originated from unmylinated parent axons or if some were myelinated axons which had lost their sheaths. These results indicate that the 3 glutamate receptors subtypes previously localized in the DRG cell bodies are transported out to the periphery. The presence of glutamate receptors on presumed sensory axons suggests that they play a role in sensory transmission. (Supported by NS11255 and 27910).

356.5

SYSTEMIC INJECTION OF KETAMINE ALLEVIATES PAIN BEHAVIORS IN A MODEL OF PERIPHERAL NEUROPATHY. J. Qian*, S. Brown and S. M. Carlton. Dept. of Anatomy and Neurosciences, Marine Biomedical Inst., UTMB, Galveston, TX, 77555.

Painful neuropathies which develop after nerve injury involve central sensitization that is thought to result from activation of NMDA receptors. This hypothesis predicts that NMDA receptor antagonists will be therapeutic in alleviating neuropathic pain. In the present study, the efficacy of ketamine (KET), a non-competitive NMDA receptor-channel blocker, is assessed for relieving neuropathic pain behaviors in rats.

Male Sprague-Dawley rats (125-175 g) with tight ligations of the left L5 & L6 spinal nerves develop, within a week, increased sensitivity on the plantar surface of the left hindpaw. The nerve-injured hindpaws withdraw to innocuous and noxious von Frey hair stimulation (mechanical allodynia and hyperalgesia), application of acetone (cold allodynia), and display frequent and sustained paw withdrawals in the absence of any stimuli (ongoing pain) or when placed on a cold plate (stress-induced pain). Systemic bolus injection of 0.01 mg/kg KET transiently (15-30 min) decreases these nociceptive behaviors. KET at 1.0 mg/kg consistently attenuates all the neuropathic pain behaviors by about 50% without any noticeable side effects. Higher doses (25 & 50 mg/kg) cause motor impairment for 15-30 min, which masks KET's fast-acting analgesic properties. However, the antinociceptive effects out-lasts the motor deficits, reducing the pain behaviors for over an hour. In all cases, the antinociceptive effects of KET are dose- and time-dependent, reaching maximal effect within 15 min, and decaying over the next 1-2 hrs. Mechanical allodynia and hyperalgesia are most efficiently attenuated by i.p. KET, followed by cold allodynia, stress-induced pain, and ongoing pain.

The present results demonstrate that blockade of NMDA receptors can effectively alleviate pain behaviors in neuropathic rats, substantiating the important role of NMDA receptors in the mechanisms that underlie the development and maintenance of painful neuropathies. In addition, the potent analgesic efficacy of ketamine suggests that this clinically safe drug can be applied in pain management of neuropathic patients. (Supported by NS11255 and NS27910.)

356.6

IDENTIFICATION OF α-ADRENORECEPTOR SUBTYPES EXPRESSED IN RAT DORSAL ROOT GANGLIA. S.L. Knock*, S.G. Widen and S.M. Carlton. Depts. Humanities & Basic Science, Sealy Center for Molecular Science, Anatomy & Neuroscience, University of Texas Medical Branch, Galveston, TX 77555

Following peripheral nerve injuries, patients can develop painful neuropathies that often become chronic and disabling. In some of these patients, the pain is sympathetically maintained and unresponsive to narcotic analgesics. Pharmacological studies strongly suggest that α-adrenoreceptors play a major role in the development of this sympathetically maintained pain (SMP). Recent development of a rat model characterized by SMP and cloning of α-adrenoreceptor genes provide the necessary framework for the investigation of changes in the expression of α-adrenoreceptors during SMP. We hypothesize that an increase in transcription of α-adrenoreceptor gene(s) provides the mechanism that results in activation of nociceptive afferents in response to adrenergic stimulation.

Using Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) techniques, we have analyzed for $\alpha_{2A,2B}$ and $_{2C}$-adrenoreceptor messenger RNA (mRNA) in dorsal root ganglia, L_4, L_5 and L_6. In normal rats, we have been able to detect the expression of α_{2A} and α_{2B}-adrenoreceptor mRNA, but not α_{2C}-adrenoreceptor mRNA. Thus, changes in the expression of these receptors in neuropathic animals may underlie SMP. (Supported by the Pearl and Aaron Forman Research Foundation, and NS11255, NS27910)

356.7

SPROUTING OF B-HRP FILLED FIBERS OF THE L5 SPINAL NERVE FOLLOWING L5 AND L6 SPINAL NERVE LIGATION IN THE RAT. H.A. LEKAN*, S.M. CARLTON, AND R.E. COGGESHALL. Marine Biomedical Institute, The University of Texas Medical Branch, Galveston, TX 77555-1069.

An experimental peripheral neuropathy was induced in rats by ligating the L5 and L6 spinal nerves approximately 5mm distal to the dorsal root ganglia. Previous behavioral and electrophysiological studies indicate that these animals consistently exhibit heat hyperalgesia and mechanical allodynia for a period of at least one month. To address the question of possible anatomical changes in the spinal distribution of large diameter afferent fibers, cholera toxin B subunit-horseradish peroxidase conjugate (B-HRP) was injected into the L5 spinal nerve of neuropathic animals. Three days following injection of the B-HRP, the animals were sacrificed and perfused with mixed aldehydes. Appropriate spinal cord segments were sectioned on a vibratome, and the tissue sections were processed for B-HRP labelling using tetramethylbenzidine histochemistry.

In normal rats, B-HRP filled axons (shown to be predominantly Aβ fibers) enter the medial 2/3rds of lamina I and III-V of the dorsal horn, but not lamina II. Our preliminary results indicate that 2 weeks following spinal nerve ligations, B-HRP labelled fibers are now present in lamina II. If further studies support these preliminary data, then the results would suggest sprouting of Aβ fibers into a region of the dorsal horn normally occupied by small diameter fibers subserving pain and temperature, similar to what has been previously reported for sciatic nerve ligation. We will now test whether the behavioral changes that characterize this model are correlated with the entrance of the Aβ fibers into lamina II. This work is supported by NIH grants NS11255, NS10161, NS27910 and NS07185.

356.8

GLUTAMATE INJECTED INTO THE RAT HINDPAW PRODUCES MECHANICAL ALLODYNIA AND HEAT HYPERALGESIA. G.L. Hargett*, J.F. deGroot and S.M. Carlton, Department of Anatomy and Neurosciences, Marine Biomedical Institute, UTMB, Galveston, TX 77555.

It has been demonstrated in our laboratory that AMPA, Kainate and NMDA receptors are present in axons in the rat hindpaw (see Carlton and Coggeshall). In the present study, we addressed whether activation of these peripheral receptors with exogenous glutamate (Glu) played a role in sensory transduction.

To assess mechanical allodynia, naive rats (male, Sprague-Dawley, 125-200gm) were placed on a mesh grid and behaviorally tested with von Frey filaments (9.4, 28, and 47mN). The number and quality (mild, average, robust) of each withdrawal response per 10 stimulations was recorded. Following baseline testing, animals were briefly anesthetized with halothane for injection of 20μl of Glu (0.01, 0.1, 1, and 5mM, n≥5 for each dose) or vehicle (phosphate buffered saline, PBS) into the plantar skin. Animals were tested post-injection for 60 mins. at 5 min. intervals. To assess heat hyperalgesia, rats were placed on a 45± 5° C plate for 1 min. intervals and the elevation time of the left hindpaw recorded. Following baseline testing, animals were anesthetized as described above and 50μl 0.1mM Glu (n=3) or PBS (n=3) was injected into the foot pad. Rats were retested every 5 mins. for 40 mins.

Injection of PBS resulted in little change in behavioral responses to von Frey or heat stimuli. However, injection of .01mM Glu produced an increase in withdrawal to the 9.4 and 28mN filament while 0.1mM Glu produced an increase in withdrawal to the 28 and 47mN filament. Responses peaked within 15 mins. post-injection. Compared to PBS, 0.1mM Glu caused an increase in elevation time of the injected foot on the 45°C plate. The results suggest that 1) there are functional glutamate receptors in the glabrous skin and 2) these receptors play a role in the transduction of mechanical and heat stimuli. (Supported by NS11255 and NS27910.)

356.9

MORPHINE REDUCES BEHAVIORAL SIGNS OF NEUROPATHIC PAIN IN A RAT TAIL MODEL. **Y.W. Yoon*, H.S. Na, K.H. Ko, S.K. Hong and S.H. Nahm. Dept. of Physiology, Korea Univ. Med. Coll., Seoul, KOREA., 136-705.**

The aim of this study was to see the effects of morphine on neuropathic pain in an experimental rat model.

Neuropathic injury was produced by resection of the inferior caudal trunk between the S3 and S4 spinal nerves unilaterally under halothane anesthesia (Neurosci. Lett., 177;50-52, 1994). Behavioral tests were performed to examine the presence of mechanical allodynia and thermal allodynia.

Operated rats showed tail-flick responses to innocuous mechanical stimuli applied to certain spot(s) of the tail with von Frey hairs as well as tail flicks or tail flick-like responses following immersion of the tail to warm (40 °C) or cold (4 °C) water. One week after the nerve injury, we examined the effects of intraperitoneally injected morphine. At 1, 3, 5 mg/kg doses, morphine reduced the responses in a dose-dependent fashion. The analgesic effect of 5 mg/kg morphine was almost completely reversed by naloxone (0.5 mg/kg; IP injection).

The present data clearly show that specific activation of opioid receptors by morphine can alleviate the neuropathic pains in our model, which seems to contradict the classical view that neuropathic pain is opioid-resistant.

356.10

CRITICAL ROLE OF CAPSAICIN-SENSITIVE NERVE FIBERS IN THE DEVELOPMENT OF THE NEUROPATHIC SYMPTOMS IN A RAT MODEL. S.K. Hong*, H.S. Na, Y.I Km and K.H. Ko Dept. Physiol., Med. Coll., Korea Univ., Seoul, KOREA.

We investigated the role of capsaicin-sensitive small diameter fibers in the development of the pain behaviors in a rat model, produced by transecting some but not all of the nerves innervating the tail.

Capsaicin (50 mg/kg, sc.) injected neonatally produced thermal hypoalgesia in the tail the degree of which was variable across individual rats. When subjected to partial denervation of the tail during adulthood, the animals with moderate thermal hypoalgesia exhibited signs of pain to normally innocuous mechanical stimuli applied to the tail with von Frey hairs (4.9-mN or 19.6-mN bending force), but not to thermal stimuli given by immersion of the tail into cold (4°C) or warm (40°C) water. The animals with marked thermal hypoalgesia, on the other hand, exhibited no signs of pain either to the mechanical or to the thermal stimuli.

These results suggest that the capsaicin-sensitive fibers are critical in the development of mechanical, as well as thermal, allodynia. (Supported by NON DIRECTED RESEARCH FUND, KRF)

356.11

THE ROLE OF SIGNALS FROM DORSAL ROOT GANGLION IN NEUROPATHIC PAINS INDUCED BY NERVE INJURY.

H. S. Na*, Y. I. Km, K.H. Ko and S.K. Hong. Dept. Physiol., Med. Coll., Korea Univ., Seoul, KOREA.

We have developed a new rat model for peripheral neuropathy by resecting the superior caudal trunk at the level between the S1 and S2 spinal nerves under halothane anesthesia. These animals displayed much shorter tail-flick latencies following immersion of the tail to warm (40° C) or cold (4°C) water and tail-flick responses to normally innocuous mechanical stimulation to the tail with von Frey hairs. Using this new model, we examined which of the signals from the injured site of the nerve and from the dorsal root ganglion cells of the injured nerve is critical for generation of the neuropathic pains.

To block transmission of the electrical and/or chemical signals arising from the very injured site of the nerve, the neuroma produced in this site was severed with scissors or soaked with bupivacaine or colchicine. None of these manipulations resulted in alleviation of the signs of neuropathic pain. In contrast, cutting the dorsal root at the S1 level (i.e., S1 dorsal rhizotomy) significantly reduced or completely removed the neuropathic signs.

The data suggest that signals from the dorsal root ganglion cells, not from the injured site, are crucial for generation of the neuropathic pains. (Supported by NON DIRECTED RESEARCH FUND, KRF)

356.12

NORADRENALINE (NE) INCREASES THE EXCITABILITY OF NEURONS IN THE S1-S3 DORSAL ROOT GANGLIA (DRG) FROM A RAT MODEL OF NEUROPATHIC PAIN. Y. I. Kim, H. S. Na, Y. W. Yoon, M. K. Lee, H. J. Kim, J.-M. Chung*, S. H. Nahm and S. K. Hong. Dept. of Physiol., Coll. of Med., Korea Univ., Seoul, Korea.

Our rat model of neuropathic pain, produced by unilateral transection of some of the peripherally projecting nerve fibers from the sacral DRG (Na et al., 1994, Kim et al., 1995), is characterized by the noradrenergic fiber sprouting in the DRG that contain the cell bodies with the injured nerve fibers and by the sensitivity to the α-receptor blocker phentolamine (unpublished observation). In the present study we tested if bath-applied NE (30 or 100 μM) altered the excitability of neurons in the sacral DRG maintained *in vitro* by examining the injected current-voltage response (I-V) relationship. Cells in both ipsi- and contralateral DRG to the transection were subjected to the test. In 7 of 8 ipsilateral neurons, NE reduced the typical outward rectification and/or made the cells produce more spikes in response to a given level of depolarizing current step. In 3 of 6 contralateral neurons, similar or slightly weaker effects of NE were observed. The NE effects were reversible, not associated with significant changes in resting membrane potential, input resistance or time-dependent inward rectification and detected in cells exhibiting hump in the repolarizing phase of the action potential (i.e., "hump" cells) as well as in "non-hump" cells.

The present results, coupled with our unpublished observations stated above, point to the possibility that the sympathetically maintained neuropathic pain in our model is at least partly due to the NE-mediated increased excitability of cells in the sacral DRG, which involves depression of outward rectification. In addition, the data indicate that the NE effects are not limited to a certain cell type. (Supported by KOSEF and Biotech 2000)

356.13

NEUROPATHIC NERVE INJURY INDUCES SPROUTING OF A FIBRE PRIMARY AFFERENTS INTO SUBSTANTIA GELATINOSA OF THE ADULT RAT SPINAL CORD. P. Shortland*[1], L.C. Yu[2], T. Lundeberg[2], & C. Molander[1]. Depts. of (1) Neuroscience & (2) Physiology and Pharmacology, Karolinska Institutet, S-171 77 Stockholm, Sweden.

Two animal models have recently been developed for the study of neuropathic pain mechanisms. Rats that have a chronic constriction injury (CCI, Bennett & Xie, '88) or ligation of spinal nerves (SNL, Kim & Chung, '92) show signs of spontaneous pain, allodynia & hyperalgesia. These changes are thought to arise from altered activity levels in the peripheral nerve, dorsal root ganglion (DRG) & the dorsal horn (DH). This study investigated the central effects of neuropathic nerve injury on the A fibers, known to sprout into lamina II after nerve section (Woolf et al., '92). Adult male rats were subjected to either sciatic nerve CCI (4-0 chromic sutures or silk) or tight ligation of the L5/6 spinal nerves (4-0 silk). In CCI rats, the nerve was injected with 1% BHRP 10-14 days later proximal to the constrictions. In SNL rats, BHRP was either injected into the L5 DRG (to label injured afferents) or the sciatic nerve (to label intact L4 afferents). The contralateral uninjured side, used for control injections, showed labelling in laminae I and III-V, leaving lamina II devoid of labelling. In CCI rats, BHRP label was seen throughout laminae I-V in the L4-5 DH by 10days post injury. There were, however, regions in lamina II that were incompletely labeled by BHRP. Adjacent sections stained for TMPase showed depletion, but not a total loss, in the sciatic region. In SNL rats, BHRP injection into the L5 DRG produced sprouting into lamina II whilst injection into the L4 spinal nerve did not. The results indicate that injured Aß afferents sprout into SG in these models whilst intact adjacent afferents do not. Thus, injured Aß fibers may contribute to the generation of abnormal sensations in neuropathic rats. Supported by Åke Wibergs stiftelse.

356.14

SPROUTING SYMPATHETIC FIBERS FORM SYNAPTIC ENDINGS IN THE DORSAL ROOT GANGLION OF THE NEUROPATHIC RAT. K. Chung* and J.M. Chung, Marine Biomed. Inst., Depts. of Anat. & Neurosci. and Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX 77555.

The purpose of this study was to dtermine whether sprouting sympathetic postganglionic fibers form synaptic endings/contacts in the dorsal root ganglion (DRG) of the neuropathic rat. Sympathetic axons and endings in the DRG were visualized under electron microscopy by immunohistochemical staining with antibody against tyrosine hydroxylase (TH).

Under halothane anesthesia, neuropathic injury was produced on the left L5/6 spinal nerves by tightly ligating them with 6.0 silk thread. Behavioral tests for evoked and ongoing neuropathic pain were conducted for one week postoperatively. Rats were perfused on the 7th day after surgery and the L5/6 DRG were removed, vibratome sectioned and immunostained for TH using the ABC method. The morphology and distribution of TH-immunolabeled profiles were examined on the JEOL 100-CX electron microscope.

TH-immunolabeled profiles in the DRG of injured nerve included fine unmyelinated axons and synaptic endings. These synaptic endings contained small spherical and large dense core vesicles. Some TH-immunolabeled profiles, most likely axons, were occasionally found between the soma of the DRG neuron and the satellite cells.

The data demonstrate that sprouting sympathetic fibers form synaptic endings in the DRG after spinal nerve injury. They also suggest that there is a strong possibility that the sympathetic system can modify the activities of the DRG neurons. (Supported by NIH grants NS31680 and NS11255)

356.15

POSSIBLE INVOLVEMENT OF ADRENERGIC RECEPTORS IN THE SKIN TO MODULATE MECHANICAL ALLODYNIA IN A RAT NEUROPATHIC PAIN MODEL. D.E. Moon*, J. Xie, and J.M. Chung. Marine Biomed. Inst., Depts. of Anat. & Neurosci. and of Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX 77555.

The aim of this study was to evaluate a potential contribution of adrenergic receptors in the skin for the mediation of mechanical allodynia in a neuropathic pain state.

Under halothane anesthesia, neuropathic injury was produced by tightly ligating the left L5 and L6 spinal nerves of *Sprague-Dawley* rats. An increase in the frequency of foot withdrawals in response to repeated applications of mechanical stimuli to the plantar surface with von Frey filaments of innocuous bending force was interpreted as a sign of mechanical allodynia.

All tested rats displayed well developed signs of mechanical allodynia. Subcutaneous injection of idazoxan hydrochloride (5 μg in 20 μl), an α2-adrenergic receptor antagonist, produced a significant reduction in signs of mechanical allodynia lasting about 1 hour. Furthermore, the same drug blocked the rekindling of mechanical allodynia which was elicited by subcutaneous injection of norepinephrine in sympathectomized neuropathic rat.

The data suggest that α2-adrenergic receptors modulate the function of mechanoreceptors in the skin for mediation of mechanical allodynia in the neuropathic pain condition. (Supported by NIH grants NS31680 and NS11255)

356.16

CORRELATION BETWEEN PAIN BEHAVIORS AND ECTOPIC DISCHARGES IN A RAT NEUROPATHIC PAIN MODEL. H.C. Han*, D.H. Lee and J.M. Chung. Marine Biomed. Inst., Depts. of Anat. & Neurosci. and of Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX 77555.

The aim of this study was to correlate the characteristics of ectopic discharges arising from injured afferents with behavioral signs of neuropathic pain in a rat model.

The left L5 and L6 spinal nerves of young adult male *Sprague-Dawley* rats were tightly ligated under halothane anesthesia. After surgery in two groups of rats, behavioral tests for neuropathic pain were conducted at regular intervals up to 1 or 10 weeks. Ectopic discharges were sampled in each group by electrophysiological recordings made from the L5 dorsal root, a segment previously injured.

In one week, when the neuropathic pain behaviors peaked, the total number of units showing ectopic discharges in the L5 dorsal root was estimated to be 204 ± 104 (mean ± SD). This reduced to 124 ± 56 at 10 weeks after surgery, a period when signs of neuropathic pain behavior receded considerably. However, the pattern of discharges was similar in both groups.

The data suggest that the amount, not the pattern, of ectopic discharges arising from injured afferents contribute to the severity of neuropathic pain behavior in a rat model. (Supported by NIH grants NS31680 and NS11255)

356.17

GABAPENTIN RELIEVES ABNORMAL PAINS IN A RAT MODEL OF PAINFUL PERIPHERAL NEUROPATHY: W.-H. Xiao and G. J. Bennett*, Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Gabapentin (GP; 1-(aminomethyl) cyclohexaneacetic acid; Neurontin®) is a novel antiepileptic with an unique binding pattern in brain, and an unknown mechanism of action. An open-label clinical trial (Mellick & Mellick, *J. Pain Symptom Mgmt*, 10:1-2, '95) in patients with reflex sympathetic dystrophy suggests that it may have efficacy in the treatment of neuropathic pain. Using rats with the chronic constriction injury (CCI) of Bennett & Xie (*Pain*, 33:87-107, '88) and an observer blinded as to drug condition, we have examined GP's effects following (2, 4 and 24 hr) i.p. and lumbar intrathecal (i.t.) bolus injections on postoperative days 6-16 (at or near the period of peak symptom severity). Prior to drug, rats had significant heat-hyperalgesia (Hargreaves' method), mechano-hyperalgesia (pin-prick test), and mechano-allodynia (v. Frey hair test). None of the drug doses produced any motor side-effects and none had any effect on the responses evoked from the control side. In a dose-response trial of i.p. GP (saline, 10, 25 and 75 mg/kg), heat-hyperalgesia and mechano-allodynia were significantly reduced in a dose-related manner for up to 4 hr. In an i.t. dose-response trial (saline, 7.5, 15, 37.5, and 75 μg; all 10 μl volume), heat-hyperalgesia was significantly reduced in a dose-related manner for up to 4 hr. An i.t. trial using 150 μ g failed to reveal any efficacy against mechano-hyperalgesia; in the same animals this dose produced a significant suppression of heat-hyperalgesia and mechano-allodynia. Our results suggest that GP may be useful in the clinic and that its effects may be mediated by a spinal site of action.

356.18

STRAIN DIFFERENCES IN NEUROPATHIC PAIN BEHAVIOUR IN MICE FOLLOWING CHRONIC CONSTRICTION INJURY (CCI): CORRELATION WITH NGF PRODUCTION IN INJURED NERVE? M.S. Ramer, G.D. French, M. Fahnestock and M.A. Bisby*, Dept. of Physiology, Queen's University, Kingston, Ontario, K7L 3N6 and (MF) Dept. of Biomedical Science, McMaster University, Hamilton, Ontario, L8N 3Z5, Canada.

We compared the effects of CCI on neuropathic pain behaviours in 3 strains of mice: C57BL/Ola [Wld], which have delayed Wallerian degeneration; C57BL/6J, "normals"; and C57BL transgenics expressing the NGF gene driven by a GFAP promoter. We assessed thermal hyperalgesia, using radiant heat, and mechanical allodynia, using Von Frey hairs, before and for 21 days after a 4-ligature CCI made on the sciatic nerve. For both behavioural tests the order of sensitivity was GFAP-NGF transgenics > normals > Olas. ELISA assays of NGF in injured sciatic nerve confirm that Olas produce less NGF than normals: measurements on the transgenics are in progress. Our hypothesis is that, following CCI, NGF is produced by endoneurial cells surrounding adjacent axons which undergo Wallerian degeneration. This NGF may stimulate metabolic changes and altered central synaptic effectiveness in a dose-dependent fashion in the sensory neurons whose axons survive CCI. Supported by the Medical Research Council of Canada (MT-5198).

356.19

EXPERIMENTAL PAINFUL MONONEUROPATHY: CYTOKINES IN C57Bl/6J MICE AND IN MICE WITH DELAYED WALLERIAN DEGENERATION C. Sommer* and M. Schäfers, Neurologische Klinik der RWTH Aachen und Neurologische Universitätsklinik Würzburg, Germany

This study addresses the questions whether delayed Wallerian degeneration alters the development of allodynia in mice with a unilateral sciatic nerve chronic constriction injury (CCI), and if cytokine expression differs accordingly. CCI was induced in C57Bl/6J- and C57Bl/wld-mice with delayed Wallerian degeneration. Allodynia, monitored by von-Frey-hair-testing, was present from day 10 after surgery, with a peak around day 16 to 20 and gradual recovery until week 11. The extent of allodynia was greater in 6J mice than in wld mice. The onset, however, was not delayed in wld mice. Sciatic nerves were harvested on day 4, 8, 12, 28, and 95 after surgery and in controls. Tissue was immunostained for TNF-α, IL-1α, and macrophages. Myelin staining revealed mostly intact myelin sheaths in wld mice up to day 12, whereas in 6J mice, myelin sheath disruption was obvious from day 4. Macrophages were present from day 4 in 6J mice. The number and area occupied by macrophages was significantly greater in 6J mice than in wld mice on days 8 and 12. In control tissue, few Il-1α immunoreactive cells were found in a Schwann cell distribution. In 6J mice, the number of Il-1 immunoreactive cells was increased from day 4, in wld mice from day 12. TNF-immunoreactivity (ir) was present to a variable degree in control tissue. On the experimental side, TNF-ir was significantly increased by day 8 in C57Bl/6J, but not in C57Bl/wld mice. We conclude that cytokines released in the course of Wallerian degeneration influence the degree of allodynia, and possibly other elements of neuropathic pain.

PAIN MODULATION: PHARMACOLOGY—INFLAMMATION AND PROSTAGLANDINS

357.1

EVIDENCE FOR INVOLVEMENT OF SPINAL CORD GLIA IN DIVERSE MODELS OF HYPERALGESIA. L.R. Watkins*, T. Deak, L. Silbert, J. Martinez, L. Goehler, J. Relton[1], D. Martin[1] & S.F. Maier, Dept. Psych., U CO Boulder, Boulder, CO 80309 & Amgen[1], Boulder, CO.

Immune activation by inflammation or infection leads to a variety of centrally mediated illness responses, including hyperalgesia (Watkins, Maier & Goehler, Life Sci., 1995 Minireview, in press). Illness responses have been proposed to result from enhanced glial synthesis & release of central cytokines such as interleukin-1 (IL1). These studies tested if intrathecal (IT) delivery of either a glial metabolic inhibitor (fluorocitrate) or a recombinant human receptor antagonist of IL-1 (rhIL1ra) could block hyperalgesia (tailflick test; TF) induced by either inflammation (500 μl s.c. 5% formalin) or abdominal infection (cecal ligation & puncture; CLP).

For formalin hyperalgesia, rats were tested for baseline TF latencies & then injected IT with equivolume vehicle (1 μl), 1 nmol fluorocitrate, or 50 μg rhIL1ra. Rats received either s.c. formalin or sham injections 10 min later. TF latencies were recorded each 5 min for 55 min. Reliable hyperalgesia was observed in IT vehicle rats receiving s.c. formalin. This hyperalgesia was abolished by either fluorocitrate or rhIL1ra. Neither fluorocitrate nor rhIL1ra affected TF latencies in sham s.c. rats.

To test CLP hyperalgesia, rats were prepared on Day 1 by sham surgery or ligating (1.5 cm segment; 4-0 silk) & puncturing (3x18 g needle; extrusion of a small amount of contents) the cecum. IT catheters & i.p. thermal telemetry probes (Minimitter) were inserted into all rats. CLP resulted in hypothermia on Day 2. TF testing began late afternoon when core body temperatures normalized. Compared to shams, CLP rats showed reliable hyperalgesia which was blocked by IT fluorocitrate (1 nmol at 0900,1100,1300 & 1500 hrs Day 2). The effects of rhIL1ra are currently being tested. Histochemical analysis of potential biotinylated rhIL1ra binding sites in spinal cord is also being examined.

These data suggest that peripheral inflammation & infection induce spinal cord glia to release products such as IL1, resulting in hyperalgesia.

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357.2

INFLAMMATION MODULATES THE CONTRIBUTION OF RECEPTOR-SUBTYPES TO BRADYKININ-INDUCED HYPERALGESIA IN THE RAT. S.G. Khasar, F.J.-P. Miao, H.L. Fields* and J.D. Levine, Departments of Anatomy, Medicine, Oral and Maxillofacial Surgery & Neurology, University of California, San Francisco, CA 94143.

We have evaluated the contribution of the sympathetic nervous system to the hyperalgesia induced by bradykinin (BK), a B_2 agonist, and des-Arg^9-bradykinin (des-Arg-BK), a metabolite of BK and a selective B_1 agonist. Mechanical hyperalgesia was quantified by the Randall-Selitto paw-withdrawal method. Inflammation was induced by injecting Complete Freund's Adjuvant (CFA) into the left hindpaw of the rat and testing mechanical nociception in the right hindpaw after injecting B_1 or B_2 agonists and/or antagonists. Sympathectomy was achieved by surgically removing sympathetic ganglia L_1-L_4 bilaterally. Rats were used 48 h post-CFA injection. In the normal rat, intradermal injection of BK but not des-Arg-BK, into the dorsal surface of the hindpaw, produced a dose-dependent decrease in mechanical nociceptive threshold. NPC 17731 (NPC), a B_2 antagonist, but not des-Arg^9,[Leu^8]-bradykinin (Leu-BK), a B_1 antagonist, inhibited the decrease in mechanical threshold. In rats whose left paws were treated, 48 h earlier, with CFA, intradermal injection of BK or des-Arg-BK, into the right paw produced dose-dependent hyperalgesia. BK hyperalgesia was partially inhibited by NPC, and the residual part by Leu-BK. Des-Arg-BK hyperalgesia was inhibited by Leu-BK but not by NPC. 48 h after injection of CFA in sympathectomized rats, BK or des-Arg-BK failed to produce hyperalgesia.

These results suggest that B_2 receptors mediate BK-induced cutaneous hyperalgesia in the normal rat hindpaw. In the setting of inflammation, BK hyperalgesia is mediated by both B_1 and B_2 receptors. Intact sympathetic postganglionic neurons are required for B_1 and B_2 hyperalgesia in this model.

357.3

Intramuscular Administration of Carrageenan Reduces Grip Force Strength in Rats. L.J. Kehl*, T.M. Trempe and K.M. Hargreaves, Depts. of Restorative Sciences and Pharmacology, University of Minnesota, Minneapolis, MN.

Subcutaneous and intra-articular injection of carrageenan produces a localized hyperalgesia in animals which can be reduced by systemic or peripheral administration of analgesic or anti-inflammatory drugs. We hypothesized that intramuscular administration of carrageenan would likewise produce muscular hyperalgesia. Development of a useful model to study muscle inflammation may have utility in studying nociceptive mechanisms of myalgia.

Male Sprague Dawley rats (100-200g) were briefly anesthetized with halothane and injected with either PBS (n=20) or carrageenan (4mg/50µl; n=20) into the triceps muscles bilaterally. Then each animal was tested at intervals over the next several hours for its capacity to exert grip force with the affected muscles using strain gauges connected via an A-D converter to an online computer. Data were analyzed by two-way ANOVA.

Bilateral injection of carrageenan into the triceps resulted in a time-dependent reduction in forearm grip force over the 8 hr observation period. Peak effects were observed at the 6-8 hour post-injection time points, with carrageenan-injected animals exerting significantly less forelimb force when compared to concurrent control animals. These initial studies are consistent with the development of carrageenan-induced mechanical hyperalgesia following injection into skeletal muscle. This model may be a useful experimental paradigm for studying mechanisms of nociceptive transmission involving muscles. (Supported by DA00240 and DE09737 to LJK and DE9860 to KMH).

357.4

PRE-TREATMENT BUT NOT POST-TREATMENT WITH AN NK-1 RECEPTOR ANTAGONIST ATTENUATES HINDPAW INFLAMMATION-INDUCED HYPERALGESIA IN THE RAT. R.J. Traub* and D. Brozoski, Dept. Pharmacology, University of Iowa, Iowa City, IA 52242

The mechanism(s) of generation and maintenance of hyperalgesia and spinal dorsal horn hyperexcitability subsequent to peripheral injury is not fully understood. Current hypotheses propose a central role for excitatory amino acids, but the role of neuropeptides is still in question. We examined the role of substance P (SP) in the generation of hyperalgesia. The NK-1 receptor antagonist CP-96,345 (100, 50, 25 nmol), the inactive CP-96,344 (100 nmol) or saline was administered into the intrathecal space of rats 10 min before unilateral hindpaw injection of carrageenan (2 or 6 mg) or formalin (1 or 5%). Rats were tested for thermal and mechanical hyperalgesia over 24 hrs (carra) or observed for 1 hr (form). CP-96,345 dose-dependently attenuated thermal and mechanical hyperalgesia (2 mg carra) for up to 5 hrs; 6 mg carra hyperalgesia was unaffected (100 nmol). 300 nmol CP-96,345 caused temporary paralysis. Neither 100 nmol CP-96,344 or saline had any effects, nor did 100 nmol CP-96,345 4 hrs *after* 2 mg carrageenan. 125 nmol CP-96,345 partially attenuated the phase 2 formalin (1% and 5%) response but did not affect the phase 1 response. CP-96,344 had a small effect on the phase 2 response. These results suggest that SP contributes to the generation but not maintenance of hyperalgesia in 2 models of inflammation. However, in some cases the magnitude of the inflammation can override the effects of the antagonist. Further, the inactive enantiomer produced a small effect in the formalin model, suggesting that slightly different mechanisms may produce central sensitization in these models. Supported by NS 30604.

357.5

GLUTAMATE RELEASED INTO PERIPHERAL TISSUE DURING INFLAMMATION CONTRIBUTES TO THE DEVELOPMENT OF THERMAL HYPERALGESIA IN RATS. D.L. Jackson*, N.K. Groves, C.B. Graff & K.M. Hargreaves, Dept. Rest. Sci., Univ. of MN, Mpls., MN 55455

There is increasing evidence supporting the hypothesis that the excitatory amino acid glutamate contributes to the development of hyperalgesia via receptor-mediated mechanisms within peripheral tissue. We have previously demonstrated that local administration of glutamate receptor antagonists attenuates carrageenan-induced thermal hyperalgesia via a peripheral mechanism (Abs. Soc. Neurosci. 20:1390, 1994). To evaluate glutamate release into inflamed tissue, microdialysis probes (BAS; West Lafayette, IN) were inserted into the hindpaws of anesthetized male Sprague-Dawley rats and dialysate was evaluated by reversed-phase HPLC with fluorometric detection. Carrageenan-induced inflammation (2 mg ipl) was evaluated by measuring withdrawal latencies of the hindpaw from a noxious heat source (Hargreaves et al. Pain 1988; 32:77-88). At selected times following the administration of carrageenan and vehicle, groups of rats were anesthetized with halothane (1-2%) for the 30 minute dialysate collection. Data were analyzed by ANOVA and Duncan's post-hoc analysis. Carrageenan evoked a time-dependent release of glutamate relative to the vehicle treated paw, reaching a maximal difference at 180 minutes (baseline: carra: 18.9±6.4 pmol vs. veh: 20.1±1.1 pmol; 180 min: carra: 45.9±10.5 pmol vs. veh: 20.45±2.0 pmol; $p<0.05$). These results correspond to the development of thermal hyperalgesia which also reached a maximum at 180 minutes. By 360 minutes glutamate release from the carrageenan treated hindpaw returned to baseline (27.1±4.5 pmol) and there was a concomitant decrease in thermal hyperalgesia. Collectively, these results support the hypothesis that the local release of glutamate into inflamed tissue corresponds to the development of nociceptive behavior, suggesting that the peripheral release of glutamate participates in the development of thermal hyperalgesia. This research was funded by K16-DE0027, DE9860, and P30DE9737.

357.6

INFLAMMATION-INDUCED CHANGES IN ALPHA-2-ADRENERGIC REGULATION OF PAIN. H.Mansikka and A.Pertovaara*, Depts. of Physiology, Univs. Helsinki and Turku, Finland.

We studied changes in alpha-2-adrenergic regulation of secondary hyperalgesia brought about neurogenic inflammation, following mustard oil treatment of the skin in rats. An alpha-2-adrenoceptor agonist (medetomidine=MED) or antagonist (atipamezole=ATI) were administered systemically (s.c.), intracerebrally or intrathecally (i.t.). Mustard oil induced a marked secondary hyperalgesia to mechanical test stimuli in intact but not in spinalized rats. MED s.c. reversed the hyperalgesia at normally sub-antinociceptive doses. ATI s.c. paradoxically reversed the hyperalgesia in a non-monotonic fashion. MED i.t. produced antinociception at very low doses, whereas in the medullary lateral reticular nucleus (LRN) MED enhanced hyperalgesia. ATI in the LRN, but not in the nucleus raphe magnus nor i.t., produced a paradoxical antinociceptive effect. The results indicate that supraspinal structures are involved in the spinal hyperalgesia induced by neurogenic inflammation. Following inflammation, the enhanced antinociceptive potency of MED is due to a direct spinal action whereas the paradoxical antinociceptive effect of ATI is due to action on the LRN.

357.7

WHAT UNDERLIES WIND-UP IN NORMAL AND CARRAGEENAN ANIMALS? L.C. Stanfa and A.H. Dickenson (SPON: Brain Research Association). Dept. Pharmacology, University College London, London WC1E 6BT, U.K.

Here we investigate the transmitter systems involved in the generation of wind-up in spinal dorsal horn neurones in normal rats and following the induction of peripheral inflammation. Extracellular recordings were made from convergent dorsal horn neurones in halothane anaesthetized rats. Wind-up was induced in the neurones by a train of 16 transcutaneous stimuli (0.5Hz) applied to the hindpaw receptive field and given at 3x the C-fibre threshold. Peripheral inflammation was induced with 100µl of 2% carrageenan. The effects of the NMDA antagonist MK801, the AMPA antagonist NBQX and the nitric oxide synthase inhibitor 7-nitroindazole (7-NI) which lacks vascular effects, all given intrathecally, were tested against wind-up in normal animals and 3 hrs after the induction of carrageenan inflammation.

In normal animals, both MK801 (0.5-50µg) and 7-NI (1-100µg) preferentially inhibited the neuronal responses due to wind-up of dorsal horn neurones whilst having little effect on the constant underlying C-fibre evoked baseline neuronal response. In contrast, NBQX (5-50µg) markedly inhibited the baseline C-fibre response; wind-up was only reduced by 50µg. In the carrageenan animals MK801 was less effective against the wind-up of the neurones although more effective against baseline responses. During inflammation, wind-up remained sensitive to 7-NI which even tended to be more effective at the lowest dose. Interestingly the AMPA receptor antagonist NBQX was more effective against the evoked neuronal responses post-carrageenan, including an enhanced ability to inhibit the wind-up of the neurones.

We conclude that C-fibre evoked release of glutamate activates AMPA receptors to establish baseline responses which are then amplified by NMDA receptor activation and the production of NO in the normal rat. After inflammation wind-up is more sensitive to AMPA blockade, suggesting that this post-synaptic event is now more dependent on pre-synaptic mechanisms, conceivably via retrograde messages which may include NO.

357.8

DIFFERENTIAL ANALGESIC AND STIMULUS PROPERTIES OF SELECTIVE BRADYKININ B_1 AND B_2 ANTAGONISTS IN MODELS OF INFLAMMATORY NOCICEPTION IN RATS. J. T. Roach* and K. J. Sufka, Departments of Psychology and Pharmacology, University of Mississippi, Oxford, MS 38677.

Research has documented the differential role of bradykinin (BK) receptors (B_1 & B_2) in the mediation of temporal aspects inflammatory nociception. This work suggests that selective BK antagonists may have therapeutic potential against chronic inflammatory pain. In addition to the demonstration of differential antinociceptive effects of B_1 and B_2 antagonists on the formalin test (2.5%), the present study sought to further define the stimulus properties (via the conditioned place preference paradigm) of the selective B_1 antagonist des-Arg^9, [Leu^8]-BK (0.0, 0.03, 0.1 & 0.3 mg/kg, sc) and the selective B_2 antagonist HOE-140 (0.0, 0.1, 0.5 & 1.0 umol/kg, sc) in the Freund's adjuvant (0.10 ml, ipl) model of chronic inflammatory nociception. Neither antagonist affected the early phase of the formalin response. In the late phase of the formalin response, Des-Arg^9, [Leu^8]-BK exhibited dose-dependent antinociceptive effects while HOE-140 exhibited only modest antinociceptive effects. In the conditioned place preference paradigm, des-Arg^9, [Leu^8]-BK, but not HOE-140, exhibited negatively reinforcing effects (i.e., analgesia) in adjuvant-inflamed rats and aversive effects in non-inflamed rats. Neither compound exhibited positively reinforcing effects (i.e., abuse potential). These results further define the stimulus properties of these BK antagonists and provide additional evidence of the therapeutic potential of B_1 antagonists against conditions of chronic inflammatory pain.

357.9

THE CONTRIBUTION OF PERIPHERAL BRADYKININ B_2 AND HISTAMINE H_1 RECEPTORS TO CARRAGEENAN EVOKED INFLAMMATION AND SPINAL C-FOS EXPRESSION IN THE RAT J. Buritova, V. Chapman, P. Honoré, M.-C. Lombard* and J.-M. Besson, INSERM U.161, 75014 Paris, France.

The effects of intraplantar HOE140 and thiazinamium, selective antagonists of the bradykinin B_2 and the histamine H_1 receptor respectively, on carrageenan evoked Fos-like immunoreactive (FLI) spinal neurons and peripheral inflammation were studied. Three hours after the intraplantar carrageenan (6mg in 150µl of saline), FLI neurons was observed in the L4-L5 segments of the lumbar spinal cord, with both superficial (I-II) and deep laminae (V-VI) neurons being densely FLI labelled.

Intraplantar HOE140 (0.1, 1 and 10µg in 50µl of saline), co-administered with carrageenan, dose-relatedly (r^2=0.434; p<0.01) reduced the total number of spinal FLI neurons (23±5% reduction of controls, p<0.01, 35±6% and 50±5%, p<0.001 for both, respectively). 1 and 10µg of HOE140 produced similar effects on both the number of superficial (33±6% and 51±6%; p<0.001 for both) and deep (35±8% and 48±7%; p<0.001 for both) laminae FLI neurons.

Intraplantar thiazinamium (25, 50 and 100µg in 50µl of saline), co-administered with carrageenan, dose-relatedly (r^2=0.558; p<0.001) reduced the total number of spinal FLI neurons (32±5%, 41±3% and 55±2%; p<0.001 for all, respectively). 25µg of thiazinamium produced similar effects on the number of superficial (22±7%; p<0.01) and deep (39±6%; p<0.001) FLI neurons. However, 50 and 100µg of thiazinamium had significantly stronger effects on the number of deep FLI neurons (50±4% and 63±3%; p<0.001 for both) as compared to the number of superficial FLI neurons (28±5% and 43±4%; p<0.001 for both).

The HOE140 and thiazinamium mediated reductions of the number of FLI neurons were associated with sequential reductions of the inflamed paw and ankle diameters. These results confirm that peripheral bradykinin and histamine contribute to carrageenan inflammation and that selective antagonism of these inflammatory mediators results in a parallel reduction of the spinal expression of c-Fos.

357.10

PERIPHERAL INFLAMMATION CHANGES THE NMDA RECEPTOR FUNCTION IN SPINAL NOCICEPTIVE SYSTEMS SHOWN WITH DIRECT NERVE STIMULATION. L.J. Rygh, A.Tjølsen* and K. Hole.
Dept. of Physiology, University of Bergen, 5009 Bergen, Norway.

Single unit activity in the dorsal horn of the spinal cord was recorded extracellularly in urethane anesthetized, intact rats by means of tungsten microelectrodes. All cells responded to noxious stimulation of the left hind paw. The posterior tibial nerve was stimulated electrically (16 pulses, 2 ms, 0.5 Hz) at 1.5x threshold for C fibre response (2.7-10.0 mA). The responses were counted separately for Aβ, Aδ and C fibre response and postdischarge according to the response latencies. NMDA was dissolved in saline and applied onto the spinal cord in a volume of 50 µl.

Experiments were done after 16-24 hours of inflammation in the receptive field, induced by s.c. injection of 100 µl of 2% λ-carrageenan in saline, and in controls.

NMDA increased (0.05-5 nmol) and reduced (50 nmol) the Aδ and C fibre response and postdischarge of the dorsal horn cells in a biphasic manner. In addition NMDA seemed to increase wind up (WU) of cells with a low control WU and reduce WU of the cells with a high control WU. Inflammation clearly increased the effect of NMDA on post discharge (0.5 nmol). Generally the cells of the carrageenan injected rats had a greater control WU and the effect of NMDA on WU was reduced. Inflammation clearly increased the receptive field for touch, and raised the threshold for C fibre response.

These results indicate that activation of NMDA receptors affects nociceptive responses in the spinal cord in a biphasic manner. Thus, previous results on NMDA effects are confirmed using stimulation directly on the peripheral nerve, showing the effects to be located in the CNS. Inflammation gave changes in dorsal horn cells in parameters like postdischarge, WU, threshold and receptive field that suggest that NMDA receptor mechanisms are involved in chronic pain states.

357.11

LYSINE ACETYLSALICYLATE INHIBITS COMPOUND ACTION POTENTIALS IN THE RABBIT RETINA. Kenneth I. Maynard*, Volker Limmroth, Pablo M. Arango, Christopher S. Ogilvy.
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Although lysine acetylsalicylate (ASA) is a widely used analgesic agent, it remains unclear whether its action is partially mediated by the central nervous system (CNS), and whether the antinociceptive effect of ASA is entirely due to its anti-inflammatory action. We examined whether ASA affects the CNS using the *in vitro* rabbit retina preparation. Four retinas were stimulated by dim light flashes (1 s), which evoked "ON" AND "OFF" compound action potentials (CAPs) recorded from the optic nerve, and the electroretinogram (ERG), measured transretinally. ASA (6, 60, 180, 670, 1800 µM) inhibited "OFF" CAPs (77±8, 81±30, 62±6*, 0±0* & 0±0* % of control responses respectively, * = P<0.05). Although at low concentrations (6, 60 & 180 µM) ASA tended to increase "ON" CAPs (101±4, 110±8, & 121±26 % of control responses respectively), at higher concentrations (670 & 1800 µM), it reduced these responses (47±25 & 10±1* % of control responses respectively, * = P<0.05). ASA did not affect the light-evoked PIII of the ERG at any of the concentrations examined. In summary, ASA affects neurotransmission (i.e. light-evoked CAPs), but not phototransduction (i.e. light-evoked PIII of the ERG) in a concentration-dependent manner in this *in vitro* CNS preparation. Whether this effect is due to the anti-cyclooxygenase activity of ASA or some other mechanism remains to be elucidated.

357.12

MODULATION OF A TTX-RESISTANT NA^+ CURRENT AS A MECHANISM OF PRIMARY AFFERENT NOCICEPTOR SENSITIZATION. M. S. Gold*, D. B. Reichling, M. J. Shuster and J. D. Levine Depts. of Medicine, and Oral Surgery and Division of Neuroscience, UCSF, Box 0452A, San Francisco CA 94143-0452

Prostaglandin E_2 (PGE_2), an inflammatory mediator released at sites of tissue injury, has been shown to produce hyperalgesia and sensitization of primary afferent nociceptors. While PGE_2 may increase nociceptor excitability in nodose ganglion neurons through the modulation of a specific Ca^{2+}-dependent K^+ current, the apparent absence of that current in dorsal root ganglion (DRG) neurons raises the possibility that sensitization may involve other currents. We tested the hypothesis that a voltage-gated Na^+ current specific to small-diameter primary afferent neurons (a tetrodotoxin-resistant Na^+ current: TTX-R I_{Na}) is a target for PGE_2. We studied the effects of PGE_2 on voltage-gated Na^+ currents in acutely isolated DRG neurons from the adult rat using conventional whole-cell and cell-attached patch clamp techniques. In whole-cell configuration, PGE_2 dose-dependently decreased the activation threshold and increased the magnitude of TTX-R I_{Na} in 52% (16 of 31) of DRG neurons that expressed the TTX-R I_{Na}. These changes were specific for TTX-R I_{Na} as they were not observed for TTX-sensitive I_{Na}. The lowest concentration of PGE_2 that induced changes was approximately 1 nM. The decrease in activation threshold induced by 1 µM PGE_2 was associated with a hyperpolarizing shift in the voltage-conductance relation of TTX-R I_{Na} ($V_{1/2}$ of activation curve decreased 5.2 ± 0.8 mV, n = 8; Mean ± SEM) and the peak current was increased 24 ± 3.2 % (n = 16); no changes were observed in the steady-state inactivation curve of TTX-R I_{Na}. The changes observed with the cell-attached patch configuration were similar to those observed with whole cell recording, except the magnitude of the changes were larger: 1 µM PGE_2 hyperpolarized the $V_{1/2}$ of activation by 14.5 ± 3.6 mV (n = 4) and increased the peak current to 225 ± 38% (n = 8). Since the PGE_2-induced changes in TTX-R I_{Na} should increase nociceptor excitability, we suggest that modulation of TTX-R I_{Na} is an ionic mechanism underlying nociceptor sensitization.

357.13

PARACETAMOL DIFFERENTLY AFFECTS NOCICEPTION AND HYPERALGESIA IN THE RAT. M. Bianchi and A.E. Panerai *. Dept. Pharmacology, Sch. of Med., Univ. of Milano, 20129 Milano, Italy

The classical models usually employed for the study of analgesic drugs in the experimental animal allow to evaluate their ability to enhance nociceptive thresholds. However, it is well known that, differently from nociceptive pain, clinical pain does not only represent a protective response to noxious stimuli, but is characterized by the appearance of an abnormal hypersensitivity. For this reason, experimental models of hyperalgesia seem to be more predictive than classical models of the clinical efficacy of analgesic drugs. We studied the effects of low doses (25, 50, and 100 mg/kg p.o.) of the non-steroidal anti-inflammatory drug paracetamol on "central " inflammatory hyperalgesia (by tail-flick test), on "peripheral" inflammatory hyperalgesia (by Randall-Selitto test), on inflammatory edema (by plethysmometry), and on nociceptive thresholds (by tail-flick test), in rats. Both inflammatory edema and hyperalgesia were induced by the injection of a suspension of 10% brewer's yeast in the plantar part of the left hindpaw. At the lowest dose, paracetamol reduced only "central" hyperalgesia. At the doses of 50 and 100 mg/kg, the drug reduced also "peripheral" hyperalgesia. Moreover, it enhanced nociceptive thresholds to a mechanical stimulus in the non-inflamed paws. Neither paw inflammatory edema , nor tail nociceptive thresholds to a thermal stimulus in the non-inflamed animals have been modified by paracetamol administration. Our results suggest that paracetamol can reduce hyperalgesia without affecting physiological nociception and inflammation.

357.14

EFFICACY OF TWO NON-STEROIDAL ANALGESICS ON RELIEF OF NEUROMUSCULAR TMD PAIN. A. ESHENAUR, J. BUXBAUM, N. MYSLINSKI* AND F. PARENTE. Department of Oral Craniofacial Biological Sciences, Physiology Section, Dental School, University of Maryland, Baltimore, MD 21201

Pain of temporomandibular origin (TMD) is frequently managed by the use of NSAIDS. The purpose of this double-blind study was to compare the effectiveness of Etodolac vs. Ibuprofen in reducing TMD pain, and to examine EMG measures as physiologic correlates to pain. Twenty-four TMD subjects were randomly assigned to 1 of 6 treatment sequences. All subjects received Etodolac, Ibuprofen, and placebo. EMG was used to quantify masseter muscle pain, and a visual analog scale (VAS) was used to quantify perceived pain. Nine additional subjects were treated with an increased dose of Etodolac only. Changes in EMG parameters were analyzed with multivariate analyses. Although both Ibuprofen (F=4.64, p<0.048) and Etodolac (F=4.74, p<0.046) significantly reduced pain, there was no significant difference in efficacy between the 2 agents (F=2.535, p<0.13). EMG measures accounted for 14% of the shared variance of the pain scores. At low pain and drug levels (<"5" on VAS), there was little correlation between change in EMG measures and change in VAS. At high pain and drug levels (>"5" on VAS), band width and power were predictive of change in VAS. The results indicate that this protocol was sensitive enough to segregate the analgesics from placebo, and that Fourier power and band width were reliable predictors of pain. This study was supported by a grant from the Wyeth-Ayerst Corporation.

357.15

PROSTAGLANDIN E_2 SUPPRESSES A POTASSIUM CURRENT IN RAT SENSORY NEURONS. A.R. Evans, M.R. Vasko and G.D. Nicol*, Dept. of Pharmacology and Toxicology, Indiana Univ. School of Medicine, Indianapolis, IN 46202.

Prostaglandins enhance the sensitivity of sensory neurons to excitatory chemical agents by altering the levels of excitability. To investigate the cellular mechanisms mediating this prostaglandin-induced hypersensitivity, we utilized the whole-cell patch-clamp technique to examine the effects of prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on neuronal excitability. Sensory neurons were dissociated from the dorsal root ganglia (DRG) of 15-17 day-old rat embryos and grown in culture for 5-7 days. At the end of each recording the cell was exposed to capsaicin; data from only capsaicin-sensitive neurons are reported here. Treatment with 1 uM PGE_2 for 10 and 20 min increased in the number of action potentials elicited by a current ramp by about two-fold. To further explore this enhanced excitability, addition of 1 uM PGE_2 caused a time-dependent decrease in the outward potassium current (I_K) recorded from neurons bathed in N-methylglucamine Ringer's. After 10 and 20 min treatments, G/G_{max} was reduced to 0.59 $\pm$ 0.05 and 0.47 $\pm$ 0.05 (n=10) of the control value. Measurements obtained from the current-voltage relation between -60 and -40 mV, demonstrated a 1.5- and 1.9-fold increase in membrane resistance at these times. Neither the amplitude of I_K nor the membrane resistance was altered by treatment with 1 uM $PGF_{2\alpha}$ over the 20 min exposure times. Assessment of I_K inactivation indicated two populations of neurons; one having ~40% inactivation for a prepulse to +20 mV and another having only ~15% inactivation. The inactivation profile of either type was unaffected by treatment with PGE_2. These findings suggest that PGE_2 may act, in part, to enhance the excitability of these sensory neurons through modulation of a potassium current(s). Supported by NIH Grant NS30527.

RETINAL SUBCELLULAR MECHANISMS II

358.1

CHANGES IN THE SENSITIVITY AND RECEPTIVE FIELD PROPERTIES OF RETINAL GANGLION CELLS IN RESPONSE TO NITRIC OXIDE DONORS. T.A. Ojala*, R.F. Miller. Graduate Program in Neuroscience and Dept. of Physiology, University of Minnesota, Minneapolis, MN 55455.

The effect of nitric oxide in the retina of the neotenous tiger salamander (Ambystoma tigrinum) was investigated by applying a nitric oxide donor, S-nitrosocysteine (SNC), to perfused whole eyecup preparations while recording either the electroretinogram (ERG) or the extracellular spikes of ganglion cells. Fresh SNC significantly reduced (50-60%) the *b* and *d* waves of the ERG, while day-old solutions had almost no effect. Fresh SNC caused a change in the sensitivity and receptive field size of some ganglion cell subtypes, as determined by spot summation experiments. This latter result suggests that some components of the receptive field may display differential sensitivity to exogenous nitric oxide. Supported by NIH grant GM08471 to TAO and grant EY03014 to RFM.

358.2

SYNAPTIC PROTEINS IN THE OUTER PLEXIFORM LAYER OF THE MAMMALIAN RETINA. J.H. Brandstätter*, C.W. Morgans, H. Betz and H. Wässle. Max-Planck-Institut für Hirnforschung, Frankfurt, Germany.

The two synaptic layers (outer and inner plexiform layer), the clearly defined cell types and the distinct types of synapses (conventional and ribbon synapses) make the retina an attractive model system for correlating structure and function of synapses. We studied by immunocytochemistry the expression of synaptic vesicle-associated proteins (synaptophysin, synaptoporin and synapsin I/II) and a presynaptic plasma membrane protein (syntaxin) in the outer plexiform layer (OPL) of rat, rabbit, cat and monkey.

The synaptic vesicle protein homologs, synaptophysin and synaptoporin, were found to have different distributions in the OPL across species. Synaptophysin was concentrated in the photoreceptor terminals in all species examined. Synaptoporin, on the other hand, was absent from the OPL in rat and cat, but was present in the OPL of rabbit in horizontal cells suggesting the presence of vesicular synapses in these cells.

To confirm this, we examined the distribution of synapsin I/II and syntaxin in the OPL since both proteins are constituents of conventional synapses and are known to be absent from photoreceptor terminals. Consistent with previous reports, we saw very little immunoreactivity for synapsin I/II and no immunoreactivity for syntaxin in the OPL of rat and cat. In contrast, strong labeling for both proteins was observed in the OPL of rabbit and monkey in a pattern possibly reflecting conventional synapses of horizontal cells. These data raise the possibility that vesicular release of neurotransmitter occurs from horizontal cells.

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358.3

GLUTAMATE RELEASE FROM GOLDFISH RETINAL SLICES. K. Dorst & G.S. Ayoub*, Dept of Biology, Westmont College, Santa Barbara, CA 93108

Glutamate is the principle excitatory neurotransmitter in the vertebrate CNS, and is considered the primary transmitter candidate for retinal photoreceptors and bipolar cells. To determine the patterns of release of this amino acid within the goldfish retina, we used an enhanced fluorometric assay for glutamate in conjunction with retinal slices. In this way, glutamate released from retinal neurons could be localized to the immediate location of the specific neurons, thus confirming not only which cells release glutamate, but also where the release sites are localized.

Retinal slices of 125 um thickness were prepared using the procedure described by Wu (1987). The slices were maintained in an oxygenated saline solution and visualized under red light using Nomarski interference optics. Photographs of the slices were made using a SBIG 6I cooled CCD camera, and were saved to the computer disk. An assay solution containing GDH and GPT was introduced to the chamber, in a solution containing D-aspartate to prevent glutamate re-uptake by the cells. In the presence of glutamate, NAD in the solution was catalyzed to NADH, and the fluorescence of NADH was localized within the slice using the CCD camera. We observed several bands of fluorescence in the inner plexiform layer of the retinal slice, as well as a more diffuse band of fluorescence in the outer plexiform layer. Thus, endogenous glutamate is likely released by bipolar cells and by photoreceptors of the goldfish retina.

358.4

LOCALIZATION AND FUNCTION OF DOPAMINE D1 RECEPTORS IN RAT RETINA. M.L. Veruki* and H.Wässle, Max-Planck-Institut für Hirnforschung, Frankfurt, Germany.

Much work has been done to determine the role of dopamine in the outer retina, however, relatively little is known about the action and localization of dopamine receptors in the inner retina, particularly in mammals. Therefore, we have chosen two approaches to study the role of dopamine in the inner retina of the rat. Patch clamp experiments were aimed at determining the effects of dopamine on amacrine cells, and immunocytochemistry was used to identify neurons that express D1 receptors.

Ligand- and voltage-gated currents were monitored in the whole-cell mode from amacrine cells in retinal slices. Test substances were applied via pressure through a puffer pipet. GABA- and glycine-gated currents elicited in amacrine cells were both decreased by application of the D1 receptor antagonist SCH-23390. Additionally, voltage-gated potassium currents in amacrine cells, generated by depolarizing voltage steps, were found to be decreased by SCH-23390. The intracellular mechanisms underlying these actions are currently being studied.

Antibodies to the D1 receptor (the generous gift of S. Grünewald, MPI-Biophysik) labelled membranes of cell bodies and processes of neurons in the inner nuclear layer and most neurons in the ganglion cell layer. Co-localization studies with antibodies against parvalbumin, tyrosine hydroxylase and choline acetyl transferase demonstrated that AII, dopaminergic, and cholinergic amacrine cells display D1 receptor immunoreactivity at the soma and the origin of the primary dendrites.

Our results suggest that dopamine may modulate many, if not all, cells in the inner nuclear and ganglion cell layers of the rat retina.

358.5

RABBIT STARBURST AMACRINE CELLS CONTAIN THE 67kDa ISOFORM OF GLUTAMATE DECARBOXYLASE. C. Brandon*, Department of Cell Biology and Anatomy, Chicago Medical School, North Chicago, IL 60064.

Rabbit retinal starburst amacrine neurons almost certainly release acetylcholine as a neurotransmitter. They may also be GABA neurons: they contain immunoreactive GABA, and they accumulate muscimol, but the presence of glutamate decarboxylase (required for the biosynthesis of GABA) has proven difficult to demonstrate unequivocally. Many GABA-containing displaced amacrine cell bodies (Mosinger '85, '87), as well as the dendrites of displaced cholinergic amacrines (Brandon '87), do not appear to contain immunoreactive GAD.

To resolve this discrepancy, rabbit retinas were examined for the presence of two known molecular-weight isoforms of GAD. Rabbit retinas were labeled with DAPI *in vivo*, then fixed and cut into tangential sections that were stained with antisera specific to GAD65 (Brandon '85) or to GAD67 (Kaufman '91).

The GAD67-specific antiserum labeled a large number of apparent amacrine cell bodies in the ganglion cell layer. The presence of DAB reaction product within these cells blocked the normal DAPI fluorescence, indicating a complete co-localization of DAPI and GAD67. On the basis of DAPI labeling, size (ca. 10-11 microns), cell density, nuclear morphology, and (where visible) radial dendritic morphology, most of the GAD67-immunoreactive cells appeared to be displaced starburst (cholinergic) amacrines. Therefore, rabbit retinal starburst amacrines use the 67kDa isoform of GAD, but not the 65kDa isoform; other (GABAergic) displaced amacrine neurons appear to contain both isoforms.

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358.6

IMMUNOSTAINING FOR GLUTAMATE RECEPTOR SUBUNITS IN MAMMALIAN RETINA. K. Morigiwa*, N. Vardi, and P. Sterling. Dept. Neuroscience, U. Penn, Phila., PA 19104

We studied the immunolocalization of various subunits of the major families of glutamate receptor in retinas of cat, rat, and monkey. Frozen and vibratome sections were labeled with antibodies recognizing GluR2/3, 2/4, 4, 6/7, NMDAR1, mGluR1α and mGluR6. In the outer plexiform layer staining patterns were similar: horizontal cells stained with antibodies against GluR2/3, 2/4, 4, and 6/7 but not NMDAR1 or mGluRs. Bipolar dendrites did not stain for GluR6/7 or NR1 but some did stain for GluR2/3, 2/4, and 4. In rat, but not in cat or monkey, other bipolar dendrites (presumed depolarizing) stained for mGluR6. Most amacrine cells stained for GluR2/3, 2/4, and NMDAR1. A smaller amacrine population stained for GluR4, 6/7, and mGluR1α. Most ganglion cells stained for all of the GluRs, NMDAR1, and mGluR1α, but not mGluR6. Both in outer and inner plexiform layers, processes were most distinctly stained for GluR4 and GluR6/7. Staining for NMDAR1 was more restricted than has been observed by in situ hybridization, suggesting that some cell types expressing its mRNA do not translated it into protein. On the other hand mRNA for GluR6/7 has not been seen in horizontal cells despite their immunostaining. Possibly an edited version of GluR6/7 is expressed that was not probed for by in situ hybridization.

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358.7

DARK- AND LIGHT-INDUCED CHANGES IN GLUTAMATE RELEASE BY THE PHOTORECEPTOR LAYER OF THE XENOPUS RETINA. P. Witkovsky*, Y.Schmitz, J. Ng, Department of Ophthalmology, NYU Medical Center, 550 1st Avenue, New York, NY 10016, USA

Our goal is to investigate the light/dark dependence and the mechanisms of glutamate release by photoreceptors. For this purpose, we use a preparation in which the inner retinal layers are removed by Triton-X and distilled water treatment (Cahill & Besharse 1992, Vis.Neurosci. 8:487). The remaining layer of pigment epithelium, photoreceptors and some adherent horizontal cells is cultured up to 24 hours in a superfusion chamber (1,75 ml/h). The glutamate concentration in superfusate samples is measured by a fluorimetric bacterial luciferase assay (Fosse et al. 1986, J.Neurochem. 47:340). This method is highly specific for glutamate, as we found that aspartate, glutamine and GABA gave no response. First we investigated the viability of the preparation. Electron microscopy revealed normal morphology of photoreceptor terminals and photoreceptors excluded the vital dye trypan blue. Intracellular recordings demonstrated that rods had typical dark potentials and responded to light with normal kinetics. A few light-evoked responses were obtained from horizontal cells indicating intact synaptic transmission. Baseline dark release of glutamate was 7.16±3.31 nMol/retina/min. Exposure to bright white light (ca.30μW/cm^2) attenuated glutamate release by 55%. With the superfusion rate of 1,75 ml/h and 10 min samples, the lowest glutamate values were obtained 30 min after light onset. Thereafter release in the light increased by about 10% over the next 90 min. With these baseline values, we are now able to investigate prolonged adaptational changes, calcium-dependence and modulation of glutamate release from photoreceptors. Supported by a fellowship from the DFG to Y.S. and NIH grant EY 03570 to P.W.

358.8

AMPA-PREFERRING RECEPTORS MEDIATE EXCITATORY SYNAPTIC INPUTS TO RETINAL GANGLION CELLS. P.D. Lukasiewicz*, J. Wilson, J.E. Lawrence. Depts. of Ophthalmology and Anatomy & Neurobiology, Washington Univ. Sch. of Med., St.Louis, MO 63110.

Pharmacological studies were performed to determine whether AMPA- and/or kainate (KA)-preferring receptors mediate excitatory synaptic inputs to tiger salamander retinal ganglion cells. Excitatory postsynaptic currents (EPSCs), evoked by light or by stimulating bipolar cells with puffs of K$^+$, were measured using whole cell recording techniques in the retinal slice. The AMPA/KA component of the EPSCs was isolated by including strychnine (2μM), picrotoxin (150μM), TTX (0.5μM) and AP7 (50μM) in the bath.

The AMPA receptor antagonist, GYKI-52466, (100μM) reduced the ON, light-evoked EPSCs and puff-evoked EPSCs to 2±1% and 6±0.3% of control. The mean IC50 values were 3.6μM and 4.2μM for the light- and puff-evoked responses, respectively. GYKI also reduced KA-evoked currents (1mM) in a concentration-dependent manner (IC50=12μM). Concanavalin A (300μg/ml), a compound that preferentially potentiates responses mediated by KA-preferring receptors, did not enhance either EPSCs or KA responses. By contrast, cyclothiazide, which selectively enhances AMPA-preferring receptor mediated responses, was found to enhance both EPSCs and KA-evoked currents (Lukasiewicz *et al.*, 1995, Invest Ophthal & Vis Sci, 36:S406). Our results indicate that the non-NMDA component of ganglion cell EPSCs is mediated predominately by AMPA-preferring receptors. Supported by NIH Grants EY08922, EY02687 and RPB, Inc.

358.9

(RS)-3,4,5-TRIHYDROXYPHENYLGLYCINE: A POTENTIAL ANTAGONIST AT L-AP4 RECEPTORS IN AMPHIBIAN ON BIPOLAR CELLS J. Gottesman[1*], W.B. Thoreson[2], D.E. Jane[3], J.C. Watkins[3], and R.F. Miller[1]. [1]Physiology Dept. & Graduate Program in Neuroscience, Univ. of Minn. Minneapolis, MN 55455; [2]Ophthalmology Dept., Univ. of Nebraska Med. Cent. Omaha, NE 68198; [3]Pharmacology Dept, Sch. of Med. Sci., Univ. of Bristol, Bristol, UK.

Antagonists to the L-2-amino-4-phosphonobutyric acid (L-AP4) receptor of ON bipolar cells, believed to be mGluR6, have yet to be identified. However, since phenylglycine derivatives can act as antagonists at a variety of other metabotropic glutamate receptors, the actions of an new phenylglycine derivative, (RS)-3,4,5-trihydroxyphenylglycine (THPG), were tested at L-AP4 receptors in ON bipolar cells in the mudpuppy and tiger salamander retinae. Actions of THPG were assessed via whole cell recording in an amphibian retinal slice preparation and field potential recordings of the ERG in the eyecup.

Consistent with L-AP4 receptor antagonism, in whole cell recordings from ON bipolar cells, THPG (1mM) reduced the outward current evoked by L-AP4 (5μM). THPG applied alone also evoked inward currents which reversed near 0 mV. Amphibian ON bipolar cells do not possess ionotropic glutamate receptors nor does AMPA (50μM) mimic the effect of THPG. THPG inward currents when applied alone may therefore reflect antagonism of endogenous glutamate. Concentration/response data were obtained using the ERG b-wave as an assay for ON bipolar cell activity. IC_{50} for THPG was ≈700 μM.

This is the first report of a compound whose action is consistent with that of an antagonist for the ON bipolar cell receptor. Further study will be required to verify the mechanism of action and the specificity of THPG for the ON bipolar versus other metabotropic glutamate receptors.

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358.10

EFFECTS OF IONOTROPIC GLUTAMATE RECEPTOR AGONISTS ON RAT RETINAL BIPOLAR CELLS. E. Hartveit* Dept. Neurophysiol., Univ. Oslo, Norway.

Several lines of evidence suggest that in the mammalian retina, the glutamatergic photoreceptor input to rod bipolar cells and ON-cone bipolar cells is mediated by an APB-sensitive metabotropic receptor, whereas the input to OFF-cone bipolar cells is mediated by an ionotropic receptor. Direct physiological evidence for this scheme in the intact mammalian retina is still lacking. From experiments on isolated rod bipolar cells (*in situ* hybridization, electrophysiology), there is also evidence that these cells might express ionotropic glutamate receptors. This raises the question whether ON-bipolar cells receive visual input through ionotropic as well as metabotropic receptors. I have therefore compared the effects of application of AMPA, kainate and NMDA on rod and cone bipolar cells in the rat retina. For reliable identification of bipolar cells in electrophysiological experiments, a database of different bipolar cells was first established by injection of Lucifer Yellow in bipolar cells in lightly fixed retina slices, largely confirming the recent findings of Euler & Wässle (1994). Whole-cell voltage clamp recordings (int: cesium gluconate, TEA; ext: 100μM picrotoxin, 10 μM strychnine, 2.5-5 mM Co^{2+}) were obtained under visual control (IR-DIC videomicroscopy) from all 3 classes of bipolar cells in vertical slices of the rat retina. Responses to glutamate agonists were obtained by pressure application from a multibarrel pipette. No cells responded to NMDA. Responses mediated directly by non-NMDA-type ionotropic glutamate receptors (E_{rev} ~ 0 mV) have only been obtained from cone bipolar cells with axonal terminals stratifying in the distal part of the IPL (presumed OFF-cone bipolars). For rod bipolar cells and cone bipolar cells with axon terminals stratifying in the proximal part of the IPL (presumed ON-cone bipolar cells), no evidence for responses mediated directly by conventional ionotropic glutamate receptors was observed. Where responses to non-NMDA agonists could be obtained, they reversed close to the equilibrium potential for chloride ions, suggesting that they were indirectly mediated by $GABA_C$ receptors. The results suggest that ionotropic glutamate receptors are involved only in mediating photoreceptor input to OFF-cone bipolar cells, not to rod bipolar cells or to ON-cone bipolar cells.

358.11

5HT7 RECEPTOR ISOLATED FROM RABBIT RETINA.

K. Pootanakit[1], S.R. Wheeler[1], D.D. Hunter[2] and W.J. Brunken*[1].
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Previous studies have shown that the serotonincrgic system is associated with the rod pathway; for example, radioligand binding and electrophysiological studies implicate 5HT1, 5HT2 and 5HT3 receptor subtypes in retinal proccssing. Recently, a new receptor subtype -5HT7- with a high affinity for serotonin has been cloned from rat and mouse brain. We are particularly interested in this receptor because it has a pharmacological profile similar to the 5HT1a receptor. In contrast to 5HT1a, 5HT7 is positively coupled to adenylyl cyclase. Such a receptor would be more consistent with the effects of serotonin and selected agonists on adenylyl cyclase activity in rabbit retina.

First, we determined whether the 5HT7 transcript is present; RT-PCR was performed on RNA isolated from rabbit retina using oligonucleotide primers made from a highly conserved region of mouse and rat sequences. After PCR, a single distinct band of the expected length was obtained; the product was subcloned into a plasmid vector and the sequence determined. The sequence shows >90% sequence identity with both rat and mouse 5HT7. We are currently performing *in situ* hybridizations using this probe; our preliminary data suggest that a population of bipolar cells express these transcripts. Since the presence of a transcript does not necessarily mean that the cell will express the protein, we plan to make antibodies against 5HT7 receptor using the deduced amino acid sequence of the clone we have obtained.

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358.12

NEUROGENESIS OF NICOTINIC ACETYLCHOLINE RECEPTORS IN THE CHICK RETINA. P.F. Gardino*[1], K.C. Calaza[1], D.E. Hamassaki-Britto[2], J.M. Lindstrom[3], L.R.G. Britto[4] and J.N. Hokoç[1]. [1] Lab. Neurobiologia da Retina, 21949-900, Brazil; [2]Dept. of Hystol. and Embryol., ICB/USP-SP, Brazil; [3] Dept. of Neurosci., Univ. of Pennsylvania, USA; [4]Dept. of Physiol. and Biophys., ICB/USP-SP-Brazil.

The development of cells containing neuronal nicotinic acetylcholine receptors (nAChRs) in the chick retina revealed different ontogenetic patterns for $\alpha 3$ and $\alpha 8$ nAChR subunits (Hamassaki-Britto et al., J. Comp. Neurol., 347:161, 1994). In this study, the neurogenesis of these cells was evaluated by combining the autoradiographic technique of [^{3}H]-thymidine incorporated into dividing cells with the immunohistochemical technique of using antibodies directed against $\alpha 3$ and $\alpha 8$ nAChR subunits. Alpha3-like immunoreactive cells (α3-LI) in the ganglion cell layer (GCL) are generated from embryonic day 2 (E2) to E6, while α8-LI in the GCL is generated from E1 to E6. In the GCL, the results show that the neurogenesis period of α8-LI begins one day before the generation of α3-LI. The neurogenesis of both $\alpha 3$ and α8-LI amacrine cells (AC) extend from E2 to E9, whereas α3-LI displaced ganglion cells (DGC) are generated from E3 to E9. The time course of generation of each α3-LI populations in the inner nuclear layer has a different rate. The majority of DGCs leave the cell cycle earlier than ACs. Alpha8-LI bipolar cells begin to be generated late, from E5 to E10, indicating that these cells are the last ones to enter and leave the mitotic cycle. These results suggest that the neurogenesis of $\alpha 3$ and $\alpha 8$ cells follow the general pattern of cell generation during development of the chick retina.

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358.13

IS IP_3 A TERMINAL TRANSMITTER IN *LIMULUS* PHOTORECEPTORS? M. Dorlöchter* G. Stommel, K. Kosfeld, M. Klemeit, C. DellaCorte, H. Stieve. Inst. für Biologie II, RWTH Aachen, 52074 Aachen, FRG, and Monell Chemical Senses Center, Univ. Pennsylvania, Philadelphia, PA 19104, U.S.A.

IP_3-gated cation channels in the plasma membrane have been found in olfactory cells in vertebrate (Restrepo et al., Science 249:1166, 1990) and invertebrate species (Fadool & Ache, Neuron 6:907,1992). In *Limulus* ventral photoreceptors IP_3 causes calcium release from internal stores. In addition, influx of external Ca through plasma membrane channels has been postulated (Stommel et al., Proc. Göttingen Neurobiol. Conf., 1994). Are these channels gated by IP_3? We injected antibodies against the plasma membrane form of the IP_3 receptor in vertebrates (courtesy of L. Kalinoski; Kalinoski et al., Soc. Neurosci.,1993) into ventral photoreceptors and measured light-induced receptor currents (ReC) and cytosolic Ca increase using arsenazo. In 3 cells, ReC were reduced in amplitude and current time integral by up to 40%. The latency of arsenazo signals was longer by up to 50%, and the rising phase was slowed down. Controls with anti tetanus toxin antibodies showed no effects. Anti IP_3 antibodies detected protein bands of about 60-80 kDa in Western blots of *Limulus* ventral nerve and lateral eye preparations. The specificity of this binding is currently being tested. These results suggest that IP_3 may directly mediate the opening of plasma membrane channels and thus be one of the terminal transmitters in the tranduction cascades in *Limulus* photoreceptors.

358.14

DEVELOPMENT OF SUBSTANCE P (SP)-CONTAINING CELL POPULATIONS IN THE POSTNATAL RABBIT RETINA

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In the mammalian retina, SP is likely to be involved in regulatory and/or modulatory actions in the plexiform layers. To contribute to the knowledge of the maturation of transmitter-identified retinal cell types, we used immunohistochemical methods to visualize SP-immunoreactive (IR) cells in rabbit retinas from birth (postnatal day -P- 0) to adulthood. During the first postnatal week, SP immunoreactivity is confined to large and small cell bodies in the ganglion cell layer (GCL) and to a few somata in the inner nuclear layer (INL). At P6, many SP-IR processes run obliquely or vertically oriented in the inner plexiform layer (IPL), and they are characterized by the presence of a growth cone at their distal end. At P11 (eye opening), SP-IR somata in the INL are more numerous than at P6. SP-IR processes are longer and confined to laminae 1, 3 and 5 of the IPL. Growth cones are still observed. Some immunolabeled fibers originating from SP-IR somata in the INL reach the outer plexiform layer, where they form sparse arborizations, suggesting the presence of SP in interplexiform cells. Long bundles of SP-IR processes (likely ganglion cell axons) are also seen in the ganglion cell axon layer. At P14, no growth cones are present on SP-IR processes and no further changes are observed up to the adult age. Our results demonstrate the presence of SP in amacrine, displaced amacrine, interplexiform and ganglion cells. In addition, consistent with other studies on developing retinal cell populations, mature characteristics of SP-IR neurons are established by eye opening, which represents a critical period in retinal maturation.

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358.15

ROLE OF PRESYNAPTIC CALCIUM CHANNEL GATING IN DIVALENT BLOCK OF TRANSMISSION AT THE PHOTORECEPTOR SYNAPSE. D. Kurenny[1], M. Wilkinson[1], G. Thurlow*[1], A. Pignatelli[2], F. Sappia[2], M. Piccolino[2,3], A. Byzov[4] and S. Barnes[1]. [1]Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1; [2]Dipartimento di Biologia, Universita di Ferrara, 44100 Ferrara, Italy; [3]Istituto di Neurofisiologia del C.N.R., 56100, Pisa, Italy; [4]Institute for Problems of Information Transmission, Moscow, Russia.

Divalent cation-induced block of synaptic transmission from photoreceptors to horizontal cells was reversed when [Ca^{2+}] was reduced, a paradoxical result since the blocking divalent was still present. We studied the presumed presynaptic mechanisms of these effects by voltage-clamping isolated cone photoreceptors. Cells were bathed in standard solutions with 2 or 3 mM $CaCl_2$. Ca current activation curves were obtained by dividing leak-subtracted current-voltage relations by the driving force and normalizing. In 2 mM Ca^{2+}, 5 μM Zn^{2+} produced a positive shift of the Boltzmann-fitted activation curve midpoint of 3.7 $\pm$ 0.3 mV and decreased conductance to 91% $\pm$ 7% (mean $\pm$ s.d., n=5). Lowering [Ca^{2+}] to 0.5 mM then produced a negative activation shift of -5.4 $\pm$ 0.8 mV and further decreased conductance to 48% $\pm$ 4% of control. Similar shifts were seen with 10 μM Zn^{2+}, 5 μM Cd^{2+}, 50 μM Ni^{2+} or 10 μM Co^{2+} added to 2 or 3 mM Ca^{2+}, and during reduction of divalent strength to 0.5 mM Ca^{2+} or 0.75 mM Ca^{2+} (n=14). The negative shift of Ca channel activation brought about by lowering [Ca^{2+}], restores Ca^{2+} influx in the "synaptic window" (below -35 mV), in spite of the reduced Ca^{2+} conductance, and thus restores glutamate release and transmission of the light response.

358.16

POTENTIOMETRIC DYE REVEALS PICROTOXIN- AND BICUCULLINE-INSENSITIVE GABA RESPONSES IN DISSOCIATED RAT RETINAL NEURONS. R. Nelson*[1], A.E. Schaffner[1], Y.-X. Li[1], and M.K. Walton[2] Lab. of Neurophysiology, NINDS, NIH, Bethesda, MD 20892[1]; and Div. of Clinical Trial Design and Analysis, OTRR, CBER, FDA, Rockville, MD 20852[2].

Retinal neurons express GABA receptors with diverse pharmacological properties. With a potentiometric probe, such properties can be localized to specific cell types. Oxonol, a negatively charged lipophilic dye, slowly partitions (1-5 min) into depolarized, and out of, hyperpolarized neurons (Walton et al, 1993[a]), and signals membrane potential changes in rod bipolar cells (RB's) and other retinal neurons (round cells) dissociated with papain from adult Sprague-Dawley rats. GABA (25-100 μM) evoked fluorescence changes in 23% of RB's and 29% of round cells. Fluorescence decreases (-.03 to -.3 log units), reflecting membrane hyperpolarizations, predominated (80%). Bicuculline methyl bromide (BCC, 100-200 μM) blocked GABA responses in only 20% of responding cells. Picrotoxin (PTX, 50 μM) was rarely effective, and failed to block GABA responses in cells sensitive to BCC. Muscimol (50 μM) was less effective than GABA, evoking predominantly fluorescence decreases in 16% of RB's and 19% of round cells. In a given cell, responses to muscimol were smaller than to GABA. CACA (50 μM) was nearly ineffective. This pharmacology resembles descriptions of GABA-gated Cl^- currents in rat RB's (Feigenspan et al, 1993[b]). Thus both RB's and round cells in rat retina exhibit unique GABA physiologies: 1) BCC-insensitive and conforming neither to $GABA_A$, nor to $GABA_C$ patterns, and 2) BCC-sensitive and $GABA_A$-*like*, but not blocked by PTX. [a]J. Neurosci. *13:* 2068; [b]Nature *361:* 159.

358.17

DOPAMINE ACTS AS A CIRCADIAN CLOCK EFFECTOR BY ACTIVATING D4 RECEPTORS IN FISH RETINA. S. C. Mangel* and Y. Wang. Univ. of Alabama Sch. of Med., Birmingham, AL 35294.

In fish retina, cone horizontal cells (HCs) receive synaptic contact from cones and not from rods. A circadian clock regulates cone HC light responses so that cone input predominates during the subjective day and rod input predominates during the subjective night (Mangel and Wang, 1994). To determine whether dopamine acts as a circadian clock effector, the effects of dopamine and dopamine drugs on L-type cone HC light responses were studied during the subjective day and night. Following 14 days of a 12/12 hr light/dark cycle, goldfish were maintained in constant darkness for 24-48 hrs. Surgery was performed under dim red or infrared light. HCs were impaled without the aid of any light flashes. Superfusion of dopamine (.01-1 μM) or quinpirole (1 μM), a D2-like agonist, during the subjective night increased cone input and eliminated rod input to the cells, a state typically observed during the subjective day. In contrast, application of spiperone (1-10 μM), a D2-like antagonist, clozapine (1-10 μM), a preferential D4 antagonist, or forskolin (10 μM), an activator of adenylate cyclase, during the subjective day reduced cone input and increased rod input. Eticlopride (50 μM), a D2 antagonist, and SCH23390 (10 μM), a D1 antagonist, were without effect. Destruction of dopaminergic cells following 6-hydroxydopamine treatment increased rod input and decreased cone input during the subjective day. Because D4 receptors are found on photoreceptor cells, but not on HCs (Cohen et al., 1992), these results suggest that a circadian clock regulates rod and cone input to cone HCs by modulating rod-cone coupling. The clock increases dopamine levels during the day so that D4 receptors on photoreceptor cells are activated. This in turn decreases rod-cone coupling via a decrease in cAMP. Supported by grants from the NIH and NSF.

358.18

CIRCADIAN RHYTHMICITY AND ADRENERGIC STIMULATION ARE INVOLVED IN THE GENETIC REGULATION OF MELATONIN SYNTHESIZING ENZYME (HIOMT) AT THE TRANSCRIPTIONAL LEVEL IN RAT PINEAL AND RETINA. Cheryl M. Craft* and François Gauer. Mary D. Allen Laboratory for Vision Research, Doheny Eye Inst., Dept. Cell & Neurobiol., Univ. Southern Calif. Sch. of Med., Los Angeles, CA 90033.

Hydroxyindole-*O*-methyltransferase (HIOMT) is the final enzyme in the melatonin synthesis pathway and is highly expressed in rat pineal and retina. To address the environmental and the circadian regulation of melatonin, we investigated the molecular expression and localization of rat HIOMT in pineal and compared it to the retina, utilizing a cDNA encoding the rat HIOMT. Because rat HIOMT mRNA expression was not detectable in retina by Northern analysis, we devised a sensitive Ribonuclease Protection Assay (RPA). The analysis revealed a RPA protected fragment of HIOMT mRNA with 40 fold lower expression per μg total RNA isolated from retina compared to pineal. Transcription of an HIOMT mRNA at 6 timepoints throughout a 24-hr period was determined and was significantly higher in expression levels during nighttime in pineal and retina. Pineal HIOMT mRNA transcriptional levels persist in constant darkness (mid-day vs. mid-dark) and with exogenous adrenergic stimulation (isoproterenol 4mg/kg, 30-150 min after injection). The tissue localization and fluctuations of these mRNAs was confirmed by *in situ* hybridization. In conclusion, the mRNA for rat HIOMT is expressed on a daily basis in the rat retina and pineal. Although only modest changes occur in rat HIOMT enzyme and protein levels, the mRNA has a distinct, circadian rhythm and is under adrenergic regulation, reflecting the synthesis of melatonin. The physiological and genetic relevance of this HIOMT transcriptional regulation is now under investigation.

Supported by NIH grant NS28126, EY00395, EY03040, and Mary D. Allen.

VISUAL CORTEX: EXTRASTRIATE—FUNCTIONAL ORGANIZATION I

359.1

ORGANIZATION OF VISUAL CORTEX IN THE MONGOLIAN GERBIL *(Meriones unguiculatus)*: EVIDENCE FOR MULTIPLE VISUAL AREAS. L.I. Cudmore* and C.G. Ellard. Department of Psychology, University of Waterloo, Waterloo, Ontario, CANADA N2L 3G1.

The visual cortex has been shown to have distinct architectonics as well as a unique pattern of connections with other visual structures. However, the question of the number of visual areas that exist in posterior neocortex and whether different mammalian species differ in the number of visual areas remains in doubt. This study attempted to address the issue of visual cortex organization by investigating this area in the Mongolian gerbil *(Meriones unguiculatus)*. Small quantities (0.2-0.3 μl) of three different fluorescent tracers (RITC, Fluorogold, Fast Blue) were pressure injected into the right occipital area of the brain. Following a survival period of 7 days, gerbils were perfused. Brains were removed and prepared for flatmount sectioning. Sections were cut at 40μ and were mounted unstained for fluorescent microscopy. Results demonstrated that the injection of multiple tracers labeled some distinct areas in lateral and medial extrastriate cortex and these areas appeared to be retinotopically organized. These distinct regions were consistently labeled from animal to animal. Despite this consistency, these was also a noticeable amount of variability between brains in the extent of extrastriate cortex containing labeled cells. It is thought that the gerbil visual cortex is organized as multiple visual areas but that individual differences in the connections of these areas are a feature of this organization.

This research was funded by a grant to CGE from the Natural Sciences and Engineering Research Council of Canada.

359.2

VISUAL AREAS IN THE EXTRASTRIATE CORTEX OF THE MARMOSET. M.G.P. Rosa, L.M. Schmid, J.C. Clarey*. Vision, Touch & Hearing Research Centre, The University of Queensland, Brisbane, QLD 4072, Australia

The visuotopy of extrastriate cortex was studied by electrophysiological recordings in ten marmosets anaesthetised with sufentanil and nitrous oxide. Large portions of cortex were studied in each animal with penetrations 300-800 μm apart. In the dorsomedial cortex, we identified four visuotopic representations between the second visual area,V2, and posterior parietal cortex. The zone of densest myelination coincided precisely with the dorsomedial area (DM). Within DM, the lower quadrant representation is continuous, with central vision represented laterally, peripheral vision medially, the horizontal meridian caudally and the vertical meridian rostrally. In contrast, the upper quadrant representation is split, with the central portion represented at the lateral edge of DM on the dorsal surface, and the periphery along the midline. Three other visual field representations, including both quadrants, surrounded DM. These were named the dorsointermediate (DI) and medial (M) areas, based on similar areas in the owl monkey, and the dorsoanterior area (DA), rostral to DM. In caudal temporal cortex, the middle temporal area (MT) contained a precisely organised visuotopic map, and ventral to MT there was another systematic representation of both quadrants, perhaps corresponding to the dorsal subdivision of FST (FSTd). Lateral cortex, between foveal V2 and MT, contained multiple representations of the fovea and lower visual field. Our results reveal a highly complex visuotopy in primate cortex, with local discontinuities in representation, and borders between areas that are often not coincident with either the horizontal or vertical meridians. In these aspects, the visual areas of the marmoset resemble those of cats. However, there was no evidence of visual areas with partial representations of the visual field, such as those described in cats and macaques.

359.3

DIFFERENTIAL INPUT FROM AREA V2 TO AREAS V4, MT AND PO IN CEBUS. S. Nascimento-Silva, R. Gattass, M. Fiorani Jr., A. P.B. Souza*. Lab. Neurobiologia III, IBCCF, UFRJ, Ilha do Fundão. Bl G. 21949- 900. Rio de Janeiro, RJ, Brasil.

To investigate the differential inputs from area V2 to areas V4, MT and PO in **Cebus** monkeys we injected multiple retrograde tracers in nine hemispheres and analysed the distributions of labeled neurons in V2. We sectioned one hemisphere in the parassagittal plane, two in the coronal plane and in the remaining ones we used flattened preparations of cortex. After injections in V4, MT and PO we found labeled neurons located in stripes running orthogonal to the V1/V2 border in flattened preparations. As in the macaque MT-projecting neurons were located in the cytochrome oxidase-rich thick stripes, and V4-projecting neurons were located both in thin stripes and interstripes regions. The PO-projecting neurons were found both in thick stripes and in the interstripe regions. The laminar distribution of the neurons in V2 that project to V4 and MT are different from those that project to PO. V4- and MT-projecting neurons are located in upper layers (2, 3), while PO-projecting neurons are located both in upper (2, 3) and lower (5, 6) layers. These results indicate that three distinct visual pathways arise from different sets of modules in V2. They also support the hypothesis of a subdivision of the dorsal stream of visual information processing into a dorsolateral projecting to MT and a dorsomedial projecting to PO, as suggested by Gattass et al., 1990, Brazilian J Med Biol Res, 23:375-393.

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359.4

CALLOSAL CONNECTIONS INTERLINK ANATOMICALLY CORRESPONDING REGIONS IN VISUAL AREA V2 OF MACAQUE MONKEY. P.L. Abel*, B.J. O'Brien, and J.F. Olavarria. Department of Psychology, University of Washington, Seattle, WA 98195.

In visual area V2 of the macaque monkey accumulations of callosal projecting neurons are found along the V1/V2 border and within finger-like regions extending 7-8 mm into area V2. The extent of this distribution raises the question of whether or not callosal fibers interlink anatomically corresponding regions in V2. We addressed this issue by analyzing the distribution of retrogradely labeled cells in V2 following tracer injections that were positioned at different locations from the V1/V2 border in contralateral V2.

Small injections of different fluorescent tracers were made into dorsal area V2 of adult monkeys (*Macaca fascicularis*). In order to reach regions of area V2 buried within the lunate sulcus, a portion of the prelunate gyrus was removed. Data were analyzed in sections cut parasagittally or tangentially to the cortical surface, and the position of labeled cells and injection sites was measured relative to the V1/V2 border revealed by Nissl staining or cytochrome oxidase histochemistry.

We found accumulations of labeled callosal cells predominantly in supragranular layers. Analysis of these labeling patterns demonstrated that the distances from the fields of labeled cells to the V1/V2 border were similar to the distances from the corresponding injection sites to the V1/V2 border. However, small numbers of labeled cells were also observed near the V1/V2 border after some injections placed distant from the V1/V2 border. Thus, our results suggest that callosal projecting neurons in area V2 predominantly interlink anatomically corresponding regions. This pattern of connections may facilitate interactions between cortical regions representing visual fields located on opposite sides of the vertical meridian.

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359.5

CORTICOTHALAMIC CONNECTIONS OF EXTRASTRIATE REGIONS IN RHESUS MONKEYS. E.H. Yeterian* and D.N. Pandya. Dept. of Psychology, Colby College, Waterville, ME 04901, E.N.R.M. V.A.M.C., Bedford, MA 01730, and Boston University School of Medicine.

Corticothalamic connections of extrastriate visual areas were studied using the autoradiographic anterograde tracing technique. The results show that the medial extrastriate region above the calcarine sulcus projects mainly to the lateral pulvinar (PL), medial pulvinar (PM), and lateral posterior (LP) nuclei. In addition, the dorsal portion of the medial region has connections to the lateral dorsal (LD) as well as to intralaminar nuclei. The dorsolateral extrastriate region projects strongly to the PL and LP nuclei, to the PM and inferior pulvinar (PI) nuclei, and to the LD and intralaminar nuclei. The lateral extrastriate region above the inferior occipital sulcus (IOS) has strong connections to both the PL and PI nuclei, and has minor projections to the PM and oral pulvinar (PO) nuclei. The ventrolateral extrastriate region below the IOS projects mainly to the PI nucleus, to the caudal portion of the PL nucleus, and has some projections to the PM nucleus. The ventromedial extrastriate region, medial to the occipitotemporal sulcus, has strong connections with the ventral and medial sectors of the PI nucleus. This region also projects to the caudal portion of the PL nucleus, and has minor connections to the LP nucleus. Finally, the annectant gyrus projects to the PL nucleus, to the rostral portion of the PI nucleus, and has minor connections to the PM nucleus. Thus, the medial and dorsolateral extrastriate regions are related mainly to the PL and LP nuclei, as well as to intralaminar nuclei. In contrast, the ventrolateral and ventromedial regions are connected strongly with the PI nucleus. This connectional organization appears to reflect functional differentiation at the cortical level (Supported by the Dept. of Veterans Affairs, NIH grant 16841, and Colby College Social Science grant 01 2239.)

359.6

SYNCHRONOUS FIRING LEADS TO EFFECTIVE NEURONAL INTERACTIONS IN A MODEL OF THE THALAMOCORTICAL SYSTEM.
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Many efforts at understanding cortical functioning assume the existence of codes based on either mean firing rate or temporal coincidences. In this report, we describe a model based on the anatomy and physiology of the thalamocortical system, the behavior of which cannot be accounted for purely in terms of either firing rates or temporal coincidences. This conclusion is derived from the responses of the model both before and after perturbations affecting either one of these variables. The model contains a lower and a higher visual area, two corresponding specific thalamic sectors, and a thalamic reticular nucleus. Cortical neurons, both excitatory and inhibitory, are organized in three laminae corresponding to supragranular layer, infragranular layer, and layer 4. The connectivity includes various sets of intra- and interlaminar, interareal, thalamocortical, and corticothalamic connections. Different cell types are modeled as integrate-and-fire elements with membrane time constants τ of 8-20msec, each equipped with appropriate sets of synaptic channels. When presented with visual patterns, the simulated model showed transient episodes of population oscillations as well as synchronous firing. These episodes were highly dependent on the mean firing rate of the activity transmitted accross multiple intra- and interareal reentrant loops. To address the role of temporal coincidences, a random jitter in the timing of action potentials was introduced, with a spread that was varied systematically from 2msec to several times τ. A jitter with a spread on the order of the membrane time constant led to a decrease of mean firing rates, an impaired detection of specific features of visual stimuli, and a disappearence of episodes of coherent activity. These experiments demonstrate that firing rate and synchrony of firing both contribute to causally effective interactions within the thalamocortical model in an interdependent way.

359.7

INPUTS TO VISUAL AREAS V1, V2, AND MT FROM THE MACAQUE PULVINAR AND THEIR RELATIONSHIPS TO CHEMOARCHITECTONIC SUBDIVISIONS. M.M. Adams[1], M.J. Webster[1], R. Gattass[2], P.R. Hof[1] and L.G. Ungerleider*[1]. [1]Lab. Neuropsych., NIMH, Bethesda, MD 20892; [2]Inst. Carlos Chagas Filho, Univ. Fed., Rio de Janeiro, Brazil; [3]Dept. Neurobiol., Mount Sinai Sch. Med., New York, NY 10029.

Previously, we defined three fields in the pulvinar based on projections from MT (Ungerleider et al., JCN 223:368,1984): P1, which is located primarily in the inferior pulvinar (PI) but extends into a portion of the adjacent lateral pulvinar (PL); P2, which surrounds P1 and is entirely in PL; and P3, which is found posteromedially in PI but also includes small portions of PL and the medial pulvinar that lie dorsal to the brachium of the superior colliculus. Both P1 and P2, but not P3, contain clear retinotopic maps of the visual field. In the present study, we investigated the inputs from these fields to V1, V2, and MT and related this information to chemoarchitectonic subdivisions based on calbindin (CB) immunostaining. V1 and V2 were injected with retrograde tracers in retinotopically matched visual field representations in 6 monkeys, and MT was injected in an additional 3. Retrogradely labeled cells resulting from V1 and V2 injections were completely overlapping and organized into two separate fields, one in P1 and another in P2. The locations of these cells, which were rarely double-labeled, were in register with the visual field maps of P1 and P2. The P1 field, but not other parts of PI, was characterized by moderate CB staining and seems to correspond to the lateral subdivision of PI described by Gutierrez et al. (Soc. Neurosci. Abstr., 20:770,1994). Cells projecting to MT were also found in P1 and P2 but were mainly concentrated in the most medial portion of P3, which was largely devoid of CB staining and may correspond to the medial subdivision of PI described by Gutierrez et al. The lateral portion of the P3 field was characterized by dense CB staining, devoid of MT- and V1-projecting cells, and contained only a few projecting to V2. The cortical connections of this subdivision of P3, which appears to correspond to the central subdivision of PI described by Gutierrez et al., remain to be determined.

359.8

FEEDFORWARD AND FEEDBACK CORTICOCORTICAL PROJECTIONS IN THE MONKEY VISUAL SYSTEM DISPLAY DIFFERENTIAL NEUROCHEMICAL PHENOTYPE. P.R. Hof*[1], L.G. Ungerleider[2], M.J. Webster[2], R. Gattass[3], M.M. Adams[2], C.A. Sailstad[1], W.G.M. Janssen[1] and J.H. Morrison[1]. [1]Dept of Neurobiol., Mt Sinai Sch. Med., New York, NY 10029; [2]Lab. of Neuropsychol., NIMH, Bethesda, MD 20892; [3]Inst. Carlos Chagas, Rio de Janeiro, Brazil.

Previous studies of the primate cerebral cortex have shown that neurofilament protein triplet (NFP), and glutamate receptor subunit proteins (GluRs) are present in pyramidal neuron subpopulations displaying specific regional and laminar distribution patterns. To define the neurochemical phenotype of the neurons of origin of feedforward and feedback corticocortical projections linking areas V1, V2, V3, V4 and MT of the monkey visual system, we performed a quantitative analysis of the distribution of NFP and various GluRs using tract-tracing combined with immunohistochemistry and computer-assisted morphometry. Injections of Fast Blue and Diamidino Yellow were placed either within areas V4 and MT or areas V1 and V2 in 13 adult rhesus monkeys. The brains were cut in a series of 40 µm-thick coronal sections that were incubated with specific antibodies to NFP, or to the GluR5-7 (kainate), GluR2(4) (AMPA), or NMDAR1 subunits. There was a higher proportion of neurons projecting from layers III and V/VI of V1, V2, and V3 that were NFP-positive projecting forward to MT (58-100%), than to V4 (25-36%). Comparable results were obtained with GluR5-7 (68-100% vs 48-56%). Contrasting with these differences, the feedback projections from MT, V4, and V3 to V1 and V2 originated mostly from layer VI neurons, and contained consistently high proportions of NFP and GluR5-7 (80-100% independently of the projection). In all projections analyzed, AMPA and NMDAR1 subunits appeared to be present in nearly all retrogradely labeled neurons. These results indicate that NFP and GluR5-7 are present in select neuronal populations that are differentially represented among feedforward and feedback projections in the monkey visual system. These specific distribution patterns across corticocortical projections also suggest the existence of differential neurochemical coding of the magno- and parvocellular visual streams. Supported by NIH AG06647 and MH52154.

359.9

FUNCTION OF FEEDBACK FROM EXTRASTRIATE TO STRIATE CORTEX IN THE CAT: COMPARISON OF COOLING AND LESION EFFECTS. Y.P. Danilov, R.J. Moore, V.R. King, and P.D. Spear*. Dept. of Psychology and Ctr. for Neuroscience, Univ. of Wisconsin, Madison, WI 53706.

Stimulation of the far surround (outside the classic receptive field) alters the responses of striate cortex cells to receptive-field center stimulation. We examined the effects of reversibly cooling lateral suprasylvian (LS) extrastriate cortex on this far-surround effect. Prior to cooling, 47% of the cells (N=45) showed an inhibitory far-surround influence and 6% showed a facilitory influence on responses to gratings of optimal orientation and spatial frequency. Cooling LS cortex had no significant effect on the far-surround influence. We were concerned that this was because the cooling device may not have inactivated a large enough area of visual-field topography deep in the LS sulcus. Therefore, we tested additional striate cortex cells (N=43) after a large LS cortex lesion. Only 7% of these cells showed an inhibitory far-surround effect, and 26% showed a facilitory effect. In normal controls (N=33), 45% of the cells showed an inhibitory effect and 15% showed facilitation.

These results led us to compare the three groups in terms of spatial-frequency (SF) tuning, which had been determined prior to testing the far-surround influence. In cats with an LS lesion, responses to low SFs were decreased and responses to high SFs were increased compared to normal. Conversely, among cells tested prior to cooling, responses to low SFs were increased and responses to high SFs were decreased. There also were opposite effects on maximal response and contrast sensitivity.

These results suggest that LS cortex inhibits far-surround influences and modifies the spatial-frequency tuning of striate cortex neurons. In addition, ablation and cooling of LS cortex can have different effects, perhaps because of post-cooling rebound. (Supported by R01 EY01916)

359.10

FEEDBACK CONNECTIONS CONTRIBUTE TO CENTER-SURROUND INTERACTIONS IN NEURONS OF MONKEY AREAS V1 AND V2. A.C. James, J.-M. Hupé, S.L. Lomber, B.Payne, P. Girard and J. Bullier* Cerveau et Vision INSERM U371 18 avenue du Doyen Lépine 69500 Bron/Lyon, France.

Corticocortical connections can be classified as feedforward and feedback. Nothing is known concerning the role of feedback cortical connections. We have tested the role of feedback connections in shaping the receptive field properties of visual cortical neurons by inactivating reversibly higher-order areas. Two preparations were used. In one case, we inactivated the entire extent of area MT and the surrounding areas of the superior temporal sulcus by cooling and we recorded neurons in area V2. In the other, we inactivated a limited extent of area V2 by small GABA injections and recorded in area V1. In both cases we assessed the center-surround interaction by presenting a variety of center-only and center-surround stimulus configurations and comparing the neuron responses to these stimuli.

The data show changes in the interactions between center and surround. In some neurons, inactivation of the higher-order areas produced a disfacilitation. In other cases, we observed a desuppression, which in some cases was total.

These results suggest that feedback connections act upon the integration of information concerning neighbouring regions of the visual field.

359.11

COMPUTER SIMULATIONS OF THE ROLE OF REENTRANT INTERACTIONS IN MEDIATING CONTEXTUAL ASPECTS OF COLOUR VISION.

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It is known that the perceived colour of an object depends both on the context in which it is viewed and on its physical properties. What is not known, however, is how this perceptual construction is achieved. The hypothesis underlying the present study is that a process of reentry mediated by long-range, reciprocal connections within and among visual cortical areas results in the integration of context into the colour percept. To test this hypothesis, a computer model was constructed based on known anatomical and physiological properties of visual cortex, in particular those of areas V2 and V4. Both areas were modelled as topographic maps containing excitatory and inhibitory units tuned to respond maximally to red, yellow, green and blue. An extensive pattern of reciprocal connections was also modelled. The architecture of the model took account of physiological data, such as the nonclassical receptive field properties of visual cortical neurones, and anatomical data, such as the spread of intracortical horizontal connections and the extent of intercortical forward and backward connections. The model was evaluated using stimuli, obtained via a video camera, that are analogous to those used in psychophysical colour induction and colour constancy experiments. Simulation experiments revealed that the modelled system gave rise to population firing patterns that varied continuously when the hue and saturation of a stimulus was varied continuously. Interactions among units in V2 and V4 produced response patterns that were influenced by visual context in a fashion compatible with human psychophysical data on colour induction and colour constancy. Interruption of intrinsic paths within V4 severely compromised the model's properties of colour constancy and colour induction. Disruption of V2 intrinsic connections and V4 → V2 backward connections showed a smaller but measurable effect on contextual integration. These simulations illustrate a potential role in colour vision of reentrant signalling within V4 and V2 and between V4 and V2.

359.12

SELECTIVE INTEGRATION: A MODEL FOR DISPARITY ESTIMATION.

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Because local disparity information is often sparse and noisy, there are two conflicting demands when estimating disparity in an image region: the need to spatially average to get an accurate estimate, and the problem of not averaging over discontinuities. We have developed a network model of disparity estimation based on disparity-selective neurons, such as those found in the early stages of processing in visual cortex. The model can accurately estimate multiple disparities in a region, which may be caused by transparency or occlusion.

The model consists of several stages and computes its output using only feedforward processing. One-dimensional binocular retinal input is preprocessed with disparity energy filters at a range of spatial frequencies and phases. The output of these disparity energy filters forms the input to two separate pathways: the local disparity pathway, and the selection pathway. The local disparity pathway computes an estimate of the disparity in a local region of the image. Because these local disparity measurements may be unreliable, a process is needed to determine which signals to integrate. The selection pathway fulfills this role by selectively gating those disparity signals that reliably indicate the true disparity of the object. The output of this stereo model is a distributed representation of disparity.

This selective integration of reliable local estimates enables the network to demonstrate stereo hyperacuity (i.e., sub-pixel disparity estimation from pixel-based inputs) on normal and transparent random-dot stereograms. Analysis of the model suggests that the selection units appear to respond to disparity contrast — that is, edges in depth. We predict that neurons in visual area MT will demonstrate a similar selectivity to disparity contrast.

VISUAL CORTEX: EXTRASTRIATE—FUNCTIONAL ORGANIZATION II

360.1

CORRELATED ACTIVITY BETWEEN NEURONS IN AREA 17 AND LATERAL SUPRASYLVIAN AREA STUDIED BY CROSS-CORRELATION ANALYSIS IN THE CAT.

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The posterior medial lateral suprasylvian area (PMLS) of cerebral cortex is one of extrastriate visual areas in the cat and thought to be involved in motion perception, stereopsis and eye movements. Previous anatomical studies have revealed that area 17 and PMLS are connected through corticocortical projection fibers. PMLS also receives thalamocortical inputs from visual thalamic nuclei such as the lateral posterior nucleus (LP), lamina C of the lateral geniculate nucleus (LGN) and the medial interlaminar nucleus (MIN). To assess functional connectivity between area 17 and PMLS physiologically, we recorded single unit activities simultaneously from both areas and carried out cross-correlation analysis of neuronal spike trains. Adult cats were anesthetized with a mixture of 70 % N_2O, 30 % O_2 and 0.3-0.5 % halothane. In PMLS, recordings were done from the posterior part of suprasylvian sulcus so that the center of receptive field of each cell was located in foveal and parafoveal areas. We studied a total of more than two hundred and fifty neuron pairs, of which about 10 % had significant peaks in their cross-correlograms. Significantly correlated activities were often observed in cell pairs with following features: 1) their receptive fields were overlapped, 2) their preferred direction of motion of visual stimuli differed by less than 90 degrees from each other, and 3) they were located mostly in supragranular or granular layers of the cortices. In some cell pairs we observed asymmetric peaks which indicated that PMLS neurons fired earlier than area 17 cells. These results suggest that cell populations with similar properties in area 17 and PMLS were functionally connected and they process visual information cooperatively.

360.2

DYNAMIC PATTERNS OF SYNCHRONIZATION BETWEEN NEURONS IN AREA MT OF AWAKE MACAQUE MONKEYS

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Recent models of visual information processing suggest that neuronal assemblies, representing sensory stimuli, are defined by the synchronous spiking of their constituting neurons. To investigate the dynamic properties of neuronal synchronization and the conditions for their occurrence, multi-unit responses to moving bar stimuli were recorded with multiple electrodes in area MT of awake fixating macaque monkeys. We analysed the relation between the temporal patterning of individual spike trains and the probability of occurrence of cross-correlations between two recording sites. It was found that cross-correlations are rare and weak if one or both of the spike trains exhibited a flat auto-correlogram which suggests a poisson-like firing pattern of the corresponding multi-unit activity. In contrast, the auto-correlograms of correlated spike trains showed a 5 to 10 ms broad centre peak, indicating synchronization between discharges of different simultaneously recorded neurons. To assess the influence of response strength on synchronization, the cross-correlation was computed as a function of time and compared with the time course of the PSTHs. In periods, where activity rises in one recording site and decays in the other, fast systematic shifts of up to 12 ms of the correlation peak position are found. If, on the contrary, the response strength changes in parallel at both sites, then the correlation peak position stays constant. In almost all cases these shifts follow the rule that the more strongly activated neurons lead over those responding more weakly.

These results suggest that local synchronisation is a prerequisite for synchrony between spatially segregated neurons and that fast graded changes of the temporal phase of synchrony could be exploited to represent graded relations among members of the same assembly.

360.3

TEMPORAL CORRELATION OF FMRI SIGNALS MAY REVEAL BRAIN CONNECTIVITY

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In this study, we mapped the temporal correlation of FMRI signals between pairs of voxels in the visual and motor areas in 12 alert human subjects under resting and task conditions. Series of 512 sequential gradient recalled echo-planar images were obtained under 3 conditions: 1) Rest: The subjects were specifically asked not to perform or imagine any motor, sensory or cognitive tasks. 2) Visual stimulation: Subjects viewed a flashing checkerboard at 8 Hz for 10 sec alternating with 10 sec of a fixation point. 3) Motor task: Subjects were asked to sequentially tap their fingers in both hands for 20 sec alternating with 20 sec of rest. For conditions 2) and 3) a synthetic time course representing the stimulus or task alteration frequency was used in a cross-correlation analysis to identify task-activated voxels. For each activated voxel, the time course of the rest state signal was temporally cross-correlated with the rest state signals from all other voxels in order to identify pairs of foci having inherent temporal correlation. Maps of voxels activated in the task conditions were highly similar to maps of correlated voxels in the rest state (0.73 spatial correlation in visual cortex, 0.87 correlation in motor cortex). In both visual and motor cortices, statistically significant resting correlation was found between patches of activated voxels in roughly corresponding positions in each hemisphere, but not among non-activated voxels. The mean correlations in functionally related regions during task activation and rest state were 0.59 and 0.37, respectively. This was substantially higher than in non-activated regions ($r<0.1$). In addition, voxels in extrastriate visual area, MT, in lateral occipitotemporal cortex correlated with voxels in calcarine V1. Similarly, it was found that, in primary motor cortex, voxels not only correlated with corresponding foci in contralateral motor cortex but also with foci in the supplementary motor area. Although known anatomical connectivity (callosal and ipsilateral cortico-cortical) suggests a possible anatomical substrate, it is not clear if the resting correlations reflect causal connectivity between foci or some common input such as arousal or attentional factors or common vascular control signals.

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360.4

MULTIPLE SINGLE UNIT RECORDING USING TETRODES IN MACAQUE VISUAL CORTEX: ELECTRODE DESIGN AND SPIKE IDENTIFICATION

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We have adapted the McNaughton-Wilson tetrode for use in the awake behaving monkey preparation. Our design combines a standard guide tube for puncturing dura with a much finer carrier tube to hold the tetrode bundle. The assembly is manipulated using standard hydraulic microdrives, assuring good positional accuracy.

Eleven penetrations were made into parietal cortex. Tetrodes were made with 15 and 25 micron insulated commercial nichrome wire running in oil-filled 33 gauge stainless steel carrier tubes. Two control penetrations were made with standard tungsten wire electrodes. Direct digital recordings were made of the four-channel waveforms.

Histology shows that the tetrode wire runs straight up to our maximum extension of 12 mm, and that the carrier tube does not cause excessive neural trauma. Tracks left by the three electrode types are nearly identical.

Coarse analysis of recorded data shows the tetrode wires compare favorably against traditional coated tungsten electrodes in terms of impedance (0.5–0.7 MΩ vs. 0.8 MΩ), signal pickup, and signal to noise ratio (15:1 vs. 23:1).

Finer analysis of recorded data shows clearly separable multi-unit activity. Initial analysis was done by clustering four-channel peak height. We observed that false clusters were created by simultaneous firing of distinct units. Additional analysis was done using M. S. Lewicki's Bayesian waveform classification tools. This resulted in classification consistent with the peak height analysis, while resolving simultaneous activity.

360.5

CLUSTERS IN RESPONSES OF VISUAL NEURONS. O. Ruksenas, R. Satinskas and P. Kaufmann.* Dept. of Biochemistry and Biophysics, Vilnius University, Ciurlionio 21, 2009 Vilnius, Lithuania.

The time course of neuronal response to presentation of a static and a moving light slit was investigated in the Clare-Bishop area of the cat cortex. Five parameters were used to estimate neuronal response: the total number of impulses, the number of clustered impulses, the number of isolated impulses, the number of clusters, and the maximum frequency of the poststimulus histogram.

Our experimental data demonstrate that: (1) only two neurons of the 56 units tested had no inherent clustered activity; (2) the number of clusters is the best parameter of neuronal response for estimating the orientation and direction selectivity of neurons; (3) clustered impulses contribute up to 44% of the total response, whereas in the case of spontaneous activity they contribute only up to 25%; (4) variability of clustered constituents is as much as twice that of other parameters; (5) a decrease of stimulus orientation/direction optimality causes a quantitative decrease of neuronal response parameters which is most reliably expressed in terms of the number of clusters; (6) increasing the level of stimulus illumination causes an increase in the number of clusters, but the number of impulses forming a cluster does not depend on the level of illumination - this increase is accompanied by a decrease in the latency of the first cluster and in the average interval between clusters; (7) increasing the speed of stimulus movement causes changes in the structure of neuronal response similar to the changes caused by increasing the level of stimulus illumination.

360.6

TEMPORAL COMPETITION MECHANISM PRODUCING NEURAL RESPONSES OF THE SUPERIOR TEMPORAL SULCUS. Kiyohiko Nakamura*. Grad. Sch. of Info. Syst., Univ. of Electro-Communications, Chofu, Tokyo 182, Japan.

Neurons of the superior temporal sulcus are capable of discriminating visual stimuli in about a hundred milliseconds (Oram & Perrett 1992). This study tests the hypothesis that cortical processing of that time range is performed by competition of the first spikes delivered by neuronal populations. MODEL: The visual pathway of form recognition, cortices V1-V2-V4-PIT-CIT-AIT-STPa, was modeled by a sequece of 7 arrays of 5×5 neuronal popolations. Each population included three types of neurons; pyramidal, stellate, and inhibitory neurons. There were100 neurons for each type in every population. Behavior of single neurons was given by a Hodgkin-Huxley type of model. PROCEDURE: Model parameters were given according to physiological data. The experiment by Oram & Perrett was simulated. RESULTS: (1) The empirical data on neural responses of visual discrimination were quantitatively reproduced by the model cortex. (2) The model showed that their analysis on firing latencies was inadequate and predicted that firing latencies should decrease with selectivity of stimuli for each neuron. The empirical data partially support the prediction. (3) Cell responses of other experiments such as oscillatory activity were explained by the model. CONCLUSION: These support the present hypothesis of competitive cortical processing.

360.7

INACTIVATION OF THE STRIATE-RECIPIENT ZONE OF LP INFLUENCES THE RESPONSES OF ONLY A FEW CELLS IN PMLS CORTEX. K. Minville*, T. Savard, and C. Casanova. Departments of Ophthalmology and of Physiology and Biophysics, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Quebec, Canada.

Removal of the primary visual cortex has little effect on the spatial frequency (SF) sensitivity of cells in the posteromedial lateral suprasylvian (PMLS) cortex (Guido *et al., J. Neurophysiol.* 5: 1636, 1990). Neuroanatomical studies has shown that neurons in the striate-recipient zone (LPI) of the lateralis posterior-pulvinar complex projects to layer IV in PMLS. Since cells in LPI and PMLS share similar spatio-temporal properties, we have investigated the possibility that the LPI could contribute to the spatial frequency sensitivity of cells in the PMLS area. Experiments were performed with anesthetized normal adult cats. Receptive fields of 110 PMLS units were characterized with drifting sine-wave gratings presented at saturation and half-maximum contrast. An injecting-recording microelectrode filled with GABA (200μM) or Xylocaine (2%) was placed in the LPI. A total of 45 cells was successfully tested. Results indicate that 1) only a few cortical cells (22%) were affected by the inactivation of the LPI. 2) in almost all cases, the responses were reduced (up to 80%) at all SF tested. 3) we did not observe any change in tuning function, nor in the cells' spatial resolution (except for one unit). These results suggest that, in normal cats, the LPI is not strongly involved in spatial frequency processing of most PMLS cells. It is possible however that afferents from the two other main parts of the LP-pulvinar complex (LPm and pulvinar) may contribute to the spatial frequency properties in the PMLS area. *Supp. by Grant MT-10962 of MRC of Canada, FRSQ and FCAR.*

360.8

INCREASED OXIDATIVE METABOLISM IN MIDDLE SUPRASYLVIAN CORTEX FOLLOWING REMOVAL OF AREAS 17 & 18 IN NEWBORN CATS. K.D. Long*, S.G. Lomber and B.R. Payne. Laboratory for Visual Perception and Cognition. Department of Anatomy and Neurobiology, Boston Univ. School of Medicine, Boston, MA 02118.

Based on electrophysiological and behavioral data, middle suprasylvian (MS) cortex has been implicated in the sparing of visually guided behaviors following early ablation of areas 17 & 18. In this study, we sought to measure changes in metabolic activity that could be related to changes in inputs to MS cortex following early area 17 & 18 damage. We measured changes in cytochrome oxidase (CO) activity in MS cortex in intact cats and cats with areas 17 and 18 removed in adulthood (P180), postnatal day 28 (P28) or postnatal day one (P1). After at least a nine month interval to allow for any potential reorganization to occur, brain sections were prepared and reacted for the presence of CO. The density of CO reactivity in each of the 6 cortical layers in MS cortex was measured and standardized against densities from ventral periaqueductal gray on the same section. Significant increases in CO activity occurred in deep layer III and in layer IV of cats which incurred lesions on P1. In contrast, there were no significant differences in the level of CO activity among intact, P28 or P180 cats for any of the cortical layers, and all were lower than the P1 levels. In the P1 ablated group, the increase in activity was restricted to the medial bank of the MS sulcus, including all of area PMLS and part of AMLS. The metabolic change in the P1 ablated group can be linked to a number of rewired pathways and further implicates the region in the sparing of certain visual functions after early brain damage. Supported by NS07152 and MH44647.

360.9

MORPHOLOGY OF REWIRED LGN NEURONS PROJECTING TO MIDDLE SUPRASYLVIAN CORTEX FOLLOWING REMOVAL OF IMMATURE AREAS 17 AND 18 IN CATS. M. A. MacNeil*, G. Einstein, & B.R. Payne. Department of Anatomy & Neurobiology, Boston University School of Medicine, Boston, MA 02118.

Removal of cats areas 17 and 18 early, but not late, in postnatal development results in the sparing of reflexive and non-reflexive visually-guided behaviors. These spared behaviors are accompanied by a massive compensatory rewiring of geniculocortical projections to middle suprasylvian (MS) cortex. However, little is known about the types of visual signals relayed along these pathways. The purpose of our study was to reveal the morphologies of the neurons participating in the rewired circuits and relate them to the morphologies of functionally characterized neurons described by others. In lightly fixed tissue slices, LGN neurons retrogradely labeled from MS cortex with fluorescent microspheres, were intracellularly filled with Lucifer Yellow. Reconstructed neurons were classified according to a battery of parameters including soma size and shape, and dendritic field-form and specializations. In normal cats, thalamic neurons projecting to MS cortex comprised types I-IV of Guillery (1966). However, in adult animals that had incurred removal of areas 17 and 18 on the day of birth, only class I and IV neurons were identified. Moreover, these neurons had changes to their dendrites that resulted in broader and more profuse dendritic arbors. In normal animals, type I and IV neurons transmit Y and W visual signals, and these are the same types of signals we suspect are transmitted to MS cortex along the rewired pathways. These signals are likely to influence the types of behaviors spared by early lesions of areas 17 and 18. (Supported by NIMH, NINDS and R29EY07840).

360.10

PHYSIOLOGICAL DEGRADATION IN VISUAL NEURONS OF MULTIMODAL AND NONPRIMARY CORTICAL AREAS IN BINOCULARLY DEPRIVED CATS. I. Gershon and U. Yinon* *Physiol. Lab., Goldschleger Eye Res. Inst., Sheba Med. CTR., Tel-Aviv Univ. Fac. Med., Tel-Aviv., 69978, Israel.*

The activity of cortical cells was physiologically studied in visual primary (VI,17), nonprimary (SSS: AMLS, PMLS) and multimodal (MS: AMSA, PMSA) cortical areas. Extracellular unit recordings were performed in 12 cats binocularly deprived (BD) at age of 1 week , in 7 cats monocularly deprived (MD) at ages of 1-11 weeks, and in 31 normal (NOR) controls. The cats were anesthetized and paralysed, the receptive fields (RFs) were mapped and classified (incomplete, diffuse, habituation) and PSTHs were computed.

Cortical area	Total no. of cells			Responsive cells, %			Irregular RFs, %		
	BD	MD	NOR	BD	MD	NOR	BD	MD	NOR
VI	189	64	429	33.7	81.2	85.6	32.5	6.2	3.5
SSS	311	122	398	25	50.4	55.7	70.8	23.4	20.4
MS	191	164	362	25.1	50.7	52.2	85.8	11.3	13.5

While a remarkable reduction was found in the responsiveness of the VI, MS and SSS areas in the BD cats, it was similar in the MD and NOR cats (see table). Accordingly, the percentage of cells with irregular RFs increases in all areas of BD cats, compared with the MD and NOR cats. There is also a consistent degradation of function in the multimodal and nonprimary areas compared with the primary areas. This is also consistent with previous results (Soc. Neurosci. Abstr., 20:1742, 1994), showing reduction in selectivity both for orientation and direction in multimodal and nonprimary areas compared with the primary area. In conclusion, the degradation of function in the multimodal and nonprimary areas is facilitated by the early absence of vision.

360.11

COMPARISON OF LATERAL EXCITATORY AND INHIBITORY CONNECTIONS IN CORTICAL ORIENTATION MAPS OF THE CAT. Z.F. Kisvárday*, É. Tóth, M. Rausch, U.T. Eysel, Ruhr-Universität Bochum, Institut für Physiologie, 44780 Bochum, Germany

We asked whether the existing controversy in the literature concerning the orientational topography of corticocortical connections (Gilbert and Wiesel, 1989; Matsubara et al,1985) could be explained by the difference in the topography of lateral excitatory and/or lateral inhibitory connections. Accordingly, orientation maps were obtained with multiunit recordings and corticocortical connections were revealed with biocytin injections and analyzed in horizontal sections. In order to distinguish putative excitatory boutons from putative inhibitory boutons a battery of morphological features were taken into account: the pattern and fine morphology of parent axons of the labeled boutons, bouton size and shape and for inhibitory boutons of basket cells the presence of perisomatic contacts onto neighboring neurons. All labeled boutons were classified as either excitatory or inhibitory and reconstructed from the entire cortical thickness with the help of a 3-dimensional reconstruction system (Neurolucida).The resulting anatomical maps were compared with smoothed orientation maps using an interpolation procedure and dissected into iso- (±30°), oblique- (±[30-60]°) and cross- (±[60-90]°) orientational zones with respect to the orientation preference at the injection site.

The results for areas 17 and 18, respectively, showed that: (a) of the excitatory boutons an average of 53 and 59% occupied iso-zones, 30 and 30% oblique-zones and 17 and 11% cross-zones, (b) of the inhibitory boutons an average of 48 and 46% occupied iso-zones, 28 and 39% oblique-zones and 24 and 15% cross-zones, (c) no significant differences in the corresponding proportions between the two areas were found. The distribution of boutons as a function of lateral distance from the center of the injection site - we used this as the measure of functional impact - revealed that lateral iso-excitation and iso-inhibition in both areas are strongest in the close vicinity of the injection site, lateral oblique-excitation and oblique-inhibition follow a broad spectrum and lateral cross-excitation and cross-inhibition are strongest at half a hypercolumn distance. The ratio between the absolute number of excitatory and inhibitory boutons in each orientation zone at different distances did not vary up to the hypercolumn distance beyond which the number of inhibitory boutons rapidly declined. The only significant difference between the two areas was that the hyper-column distance in area 18 was ~1.4 times larger than that of area 17.

We conclude that corticocortical connections in areas 17 and 18 follow the same topographical principles, excitatory connections are biased in connecting similar orientations and inhibitory connections are biased in connecting dissimilar orientations.

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360.12

CORTICOCORTICAL CONNECTIONS SHOW LOW ORIENTATIONAL SPECIFICITY IN AREA 18 OF CAT VISUAL CORTEX. É. Tóth*, T. Bonhoeffer§, U.T. Eysel, Z.F. Kisvárday, Ruhr-Universität Bochum, Institut für Physiologie, 44780 Bochum, Germany, §Max-Planck Institut für Psychiatry, Am Klopferspitz 18A, 82152 München, Germany

In area 17, corticocortical connections were shown to link similar orientation preferences (Gilbert and Wiesel, 1989) while in area 18 were shown to link dissimilar orientation preferences (Matsubara et al, 1985).We revisited this conundrum by examining the orientational specificity of excitatory and inhibitory connections in an orientation map obtained with optical imaging of intrinsic signals. In the center of an orientation domain biocytin was iontophoretically injected into layer III. All labeled structures were reconstructed in a horizontal plane from the entire depth of the cortex with the help of a 3-dimensional reconstruction system (Neurolucida). Labeled excitatory boutons and somata were distinguished from labeled inhibitory boutons and somata on the basis of morphological features (Kisvárday et al., in this volume). Additionally, parvalbumin (PV) immunohistochemistry was applied to determine the distribution of GABAergic somata contacted by labeled inhibitory basket axons (Kisvárday et al, 1993).The resulting anatomical maps were aligned and quantitatively compared with the corresponding orientation map. The orientation map was dissected into iso-(±30°),oblique-(±[30-60]°) and cross-(±[60-90]°) orientational zones with respect to the average orientation preference at the injection site. The results showed that: (a) of the excitatory boutons an average of 48.1% occupied iso-, 30.3% oblique- and 21.6% cross-zones, (b) of the inhibitory boutons an average of 35.1% occupied iso-, 36.8% oblique- and 28.1% cross-zones. Retrogradely labeled somata showed the following distribution at iso-, oblique and cross-orientation sites, respectively: of the pyramidal type 63%, 22.3% and 14.5%, of the inhibitory type (large basket cell) 31.3%, 25% and 43.7%, of the unknown type 87.7%, 5.8% and 6.5%, in total (all types included) 72.3%, 15.1% and 12.6%. Immunopositive PV somata receiving basket cell input showed almost equal distribution regarding iso- (33.1%), oblique- (33.1%) and cross-orientation (33.8%).

In summary:(i) although the remote patches of excitatory corticocortical connections are centered on similar orientations (Kisvárday et al, 1993) on the global scale they are less specific showing only a bias to similar orientations, (ii) inhibitory corticocortical connections by large basket cells also target regions of all orientations, however, with a bias to dissimilar orientations. Interestingly, the network of PV-positive cells contacted by basket cells showed no specificity at all orientational zones.

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360.13

ORIENTATION CENTERS REVEALED WITH OPTICAL IMAGING AND THEIR CONNECTIONS IN AREA 18 OF THE CAT. T. Yousef, U.T. Eysel, M. Rausch, M. Lappe*§, Z.F. Kisvárday, Ruhr-Universität Bochum, Institut für Physiologie, 44780 Bochum, Deutschland, §Ruhr-Universität Bochum, Institut für Allg.Zool. und Neurobiologie, 44780 Bochum, Deutschland

Orientation centers are intriguing locations in the orientation map where a broad spectrum of orientation columns meet in a small volume of cortex. We studied the orientational topography of corticocortical connections at orientation centers. Orientation maps were obtained with optical recording of intrinsic signals evoked by the optimal visual stimulus and the exact topographic location of orientation centers was determined with respect to the vascular pattern of the imaged cortex. Under microscope control the tip (12-16 µm) of an injection glass pipette was guided into an orientation center and fluorescent latex-beads were pressure injected (40-70 nl) into layer III over a period of 10 min. After 1-2 days, the animals were perfused with 4% paraformaldehyde and horizontal sections (30 µm) were prepared. The topography of retrogradely labelled somata were reconstructed from the entire cortical depth using an image reconstruction system (Neurolucida). The results showed that the overall distribution of the labeling was patchy and 12-15 distinct patches (400-600 µm in diameter) could be distinguished. Patch-to-patch distance was 1.2-1.4 mm. The labeling spanned an overall distance of 8-10 mm in AP and 5-6 mm in LM direction. Qualitative comparison between the orientation map and the anatomical map showed that the patches largely avoided orientation centers although a few labeled neurons were found in orientation centers. In order to make a quantitative comparison between the topography of the orientation map and the topography of the labeled somata the orientation was dissected into iso-(±30°),oblique-(±[30-60]°) and cross-(±[60-90]°) orientational zones with respect to the average orientation preference at the injection site. The proportion of labeled somata within each patch was then calculated according to iso-, oblique- and cross-orientations. It was found that different patches occupied regions possessing different orientations, respectively. The proportional variations of the orientational distribution of the labeling between different patches were the following: iso-(19-75%), oblique-(12-30%), cross-(9-60%).

These results indicate that the functional topography of corticocortical connections at orientation centers might be radically different from that of orientation domains (Tóth E, Bonhoeffer T, Eysel UT, Kisvárday ZF, in this volume). It would be interesting to know whether a similar difference is also present between the axonal topography of individual cells of orientation domains and of orientation centers.

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360.14

CUE-INVARIANT ORIENTATION AND DIRECTION SENSITIVITY OF CELLS IN EXTRASTRIATE VISUAL CORTEX. D. Liu, Y.-C. Wang, S.J. Ault, and A.G. Leventhal*, Dept. of Neurobiology and Anatomy, Univ. of Utah Sch. of Med., Salt Lake City, UT 84132.

The purpose of this study was to determine if cortical cells have the computational power to analyze complex stimuli that give rise to the perception of a boundary in the absence of a luminance boundary. The stimuli we employed included boundaries defined by relative motion (motion-defined boundaries), differences in textures (texture-defined boundaries), illusory contours, and luminance-defined boundaries (bars and gratings).

We find that many complex cells in area 18 of cat visual cortex respond to boundaries defined by motion, texture, illusory contours and luminance. Individual cells responded to boundaries regardless of the cue defining the boundary. Cue invariant (CI) cells exhibited clear orientation and direction selectivity when tested with all types of boundaries. The degree of selectivity did not vary significantly when individual CI cells were tested with boundaries defined by different cues. In addition, when boundaries were made more salient by varying the texture, motion, illusory contour or luminance defining them, the responses of CI cells increased accordingly.

Our results indicate that cells responding selectively to boundaries in a cue invariant fashion occur at relatively early stages in visual cortex. Supported by EY04951 to A.G.L.

360.15

NEURAL CORRELATES OF CUE-INVARIANT BOUNDARY PERCEPTION. Y.-C. Wang*, D. Liu, S.J. Ault, and A.G. Leventhal, Dept. of Neurobiology and Anatomy, Univ. of Utah Sch. of Med., Salt Lake City, UT 84132.

The purpose of this study was to determine if cue invariant (CI) cortical cells can integrate multiple cues that simultaneously define a boundary. We tested the responses of CI cells to boundaries defined by texture, relative motion, illusory contours and luminance in various combinations. The salience of the boundaries was varied by altering the texture, motion, illusory contour generating stimuli and luminance levels defining the boundaries. CI cells in area 18 of cat visual cortex responded more strongly to more salient stimuli. This was true when the increased salience resulted because a more salient cue was used (i.e. a more salient texture versus a less salient texture) as well as when two or more less salient cues (i.e. a less salient texture combined with a less salient motion or a less salient luminance) defined the boundary. Clear orientation and direction selective responses could be evoked when a texture boundary that was not salient enough to elicit a response (subthreshold texture) was combined with a motion or luminance boundary that was not salient enough to elicit a response (subthreshold motion or subthreshold luminance).

Our results indicate that CI cortical cells can extract and integrate linear and directional information about boundaries using multiple cues simultaneously. These cells may provide the neural substrate for cue invariant boundary perception. Supported by EY04951 to A.G.L.

361.1

PARALLEL TIME COURSES OF AGE-RELATED ANATOMICAL CHANGES IN THE ANTEROVENTRAL COCHLEAR NUCLEUS AND PROGRESSIVE HEARING LOSS IN MICE. J. F. Willott* and L. S. Bross. Dept. Psychology, Northern Illinois Univ., DeKalb, IL 60115.

This study tests the hypothesis that cochlear pathology modulates age-related neuronal changes in the anteroventral cochlear nucleus (AVCN). Measurements of AVCN neuronal number, size, and neuropil volume were made on mice of the DBA/2J (DBA) and C57BL/6J (C57) strains at ages ranging from 15 days to 2 years and on CBA/J (CBA) mice aged 1 month to 2 years. DBA and C57 mice possess genes that cause progressive cochlear pathology and hearing loss. DBA mice exhibit some threshold elevations by 4 weeks of age and this becomes severe by 2 months. C57 mice hear well through 1-2 months of age then exhibit hearing loss progressing gradually over the next 12 months. CBA mice hear well through 2 years of age. The hypothesis predicts that the time course of AVCN changes would parallel that of cochlear dysfunction, and this was supported by the results. The period between 2 and 3 weeks of age appeared to be one of growth in both DBA and C57 mice, as AVCN volume increased. In DBA mice, AVCN volume decreased after 23 days and significant neuronal loss occurred by 31 days, coinciding with the early onset of progressive hearing loss. In C57 mice, changes were not significant until 7 months of age. In CBA mice, AVCN volume continued to increase for the entire first year of life, and no loss of neurons was observed. Another observation was that the age-related loss of volume and neurons were greater in DBA mice than in C57 mice.

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361.2

SPEECH RECOGNITION PERFORMANCE IN NOISE BY PRESBYCUSIC SUBJECTS. D.R. Frisina, D.G. Sims and J.R. Ison*, Dept. of Communications Sciences, Rochester Institute of Technology, Rochester, NY 14623-0887, USA.

Previous speech recognition experiments with presbycusic subjects have suggested changes in the cochlea and central auditory pathways. This study is part of a larger multi-disciplinary program-project effort to determine the role of the central auditory system in presbycusis. We explored speech recognition aging-effects with three measures used under two intensity levels and two types of background noise. Age-effects on speech recognition in noise performance were evaluated in two groups of young and old normal-hearers (N=40), matched for absolute hearing sensitivity. In addition, the effect of hearing loss on speech recognition in noise performance was examined by comparing the performance of old subjects with mild hearing impairment (N=30) to that of the old normal-hearing group. Speech recognition in noise performance results revealed reduced performance of aging subjects with otherwise normal absolute sensitivity thresholds relative to the young subjects. In addition, the effect of mild hearing loss was explored in old subjects. This study begins to examine functional assessment of aging changes in the peripheral and central components of the human auditory system.

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361.3

TEMPORAL ACUITY FOR ACOUSTIC TRANSIENTS FOLLOWING AUDITORY CORTEX LESIONS IN RODENTS, IN COMPARISON TO AGE EFFECTS. D. Y. Lin, J. R. Ison, M. K. Taylor, G. P. Bowen, & W. E. O'Neill*, Departments of Psychology and Physiology, University of Rochester, Rochester NY 14627.

Age-related decrements in speech perception ("presbycusis") can be attributed to peripheral changes in audibility or to distortion, perhaps with a central neural component. Temporal lobe damage also diminishes speech recognition ("word deafness"), which has been attributed to a high-level deficit in language processing. Recent reviews (Phillips & Farmer, 1990, Beh Brain Res) suggest, however, that word deafness lies in the central processing of acoustic transients, as is seen, e.g., in temporal acuity for gaps in noise. Using startle reflex modulation audiometry, we find that rats with temporal lobe lesions and substantial medial geniculate degeneration lose noise-gap detection, with no change in light-gap detection or peripheral function. (These data capture a case report in Buchtel & Stewart, 1989, Brain & Lang). Noise increment deficits were apparent at first but recovered in 3 months, while gap detection only partly recovered. Old CBA mice (> 24 vs 3 mo) also showed deficits in noise gaps with near normal responses to noise increments, and differed from brain damaged rats largely in their having ABR and noise offset deficits. The data reveal that gap detection is not solely provided by the increment in noise that ends the gap, but depends further on an active inhibition of neural activity within the gap to provide contrast at noise onset. The overall outcome supports the idea that inhibitory deficits at central auditory sites are responsible for a loss of temporal acuity and the resulting distortion of complex acoustic input in brain damaged and aged rodents and humans.

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361.4

PARADOXICAL GAP DETECTION THRESHOLDS OF MOUSE INFERIOR COLLICULUS NEURONS IN BACKGROUND NOISE. W.W. Wilson*, J.P. Walton and R.D. Frisina. Div. of Otolaryngology, Univ. of Rochester, Rochester, NY 14642-8629, USA.

A common psychophysical measure of auditory temporal acuity is the detectability of a silent gap in an ongoing signal. Gap detection thresholds measured physchophysically decline when background noise is added to the silent interval (Forrest and Green, 1987; JASA 82(6)). We recorded the response of IC neurons in 2 month old C57Bl/6 mice to a gap-detection paradigm in a search for physiological correlates to this psychophysical degradation of temporal processing in background noise.

Neurons were characterized by their best frequency, threshold for tones and wideband noise, rate/intensity functions, and minimal gap threshold (MGT). "Gap series" typically consisted of two wideband noise bursts at 65 dB SPL separated by a silent gap ranging from 0 ms (control) to 96 ms long. MGT was defined as the shortest gap duration capable of eliciting a discrete neural response evident in PST histograms. After determining MGT in quiet, gap series were run in continuous background noise at 6 dB intervals below the signal level. Recording sites were verified by HRP histochemistry.

Phasic and *tonic* units typically had MGTs under 10 ms and background noise tended to increase MGT. *Inhibitory* units had much higher MGTs, corroborating findings in the avian forebrain (Buchfellner et al., 1989; J Comp Physiol A:164). Surprisingly, MGTs improved in poor signal/noise conditions for *inhibitory* units. MGT was shortest for the highest background noise conditions and gradually became longer with increasing signal-noise ratio. Inhibitory units may therefore play an important role in auditory temporal processing under poor signal/noise conditions. *NIH-NIA Grant P01 AG09524 and the International Center for Hearing and Speech Research, Rochester, NY*

361.5

AGING-RELATED CHANGES IN CONNECTIONS TO A FUNCTIONALLY-CHARACTERIZED REGION OF THE INFERIOR COLLICULUS OF THE CBA MOUSE MODEL OF PRESBYCUSIS. R.D. Frisina*, J.P. Walton, M.A. Lynch-Armour and J.D. Byrd. Otolaryngology Div., Univ. Rochester School of Medicine, Rochester, NY 14642-8629, USA.

Explorations of the neural basis of presbycusis have implicated changes in the cochlea and brain. We previously discovered that the proportion of neurons capable of encoding short noise gaps (1-2 ms) in central nucleus of the inferior colliculus (ICC) of old CBA mice declines relative to young adults (Walton *et al., Assoc. Res. Otolaryngol. Abstr., 17*:83, 1994). To start to ascertain why this occurs, injections of HRP were made following these recordings in dorsomedial ICC in 18-24 kHz region of CBA mice, 3 ages: Young adult, 2-4 mon (N=5); Mid age, 14-16 mon (N=4); Old, 22-27 mon (N=5). Retrogradely labeled perikarya were found in the brainstem. Analyses revealed: 1) The density of HRP in retrogradely stained perikarya declined with age; 2) The number of labeled cells in some nuclei such as the superior olivary complex (SOC) remained stable, whereas others decreased with age. The latter included statistically significant reductions in all 3 contralateral cochlear nucleus (CN) divisions and the ipsilateral anterolateral periolivary nucleus of the SOC. Cell counts and output analyses of other regions are currently being analyzed. Declines in inputs from the CN and other areas may underlie physiological and psychophysical degradations in temporal and speech processing, characteristic of presbycusis. *Supported by NIH Grant P01 AG09524 and the International Center for Hearing & Speech Research - Rochester, NY.*

361.6

CALCIUM BINDING PROTEIN AND GABA IMMUNOREACTIVITY IN THE INFERIOR COLLICULUS OF THE AGED CBA/J MOUSE MODEL OF PRESBYCUSIS. M.L. Zettel,* W.E. O'Neill,[1] R.D. Frisina. Depts of Surgery (ENT Div.) & Physiology[1], University of Rochester School of Medicine, Rochester, N.Y. 14642-8629, USA.

As part of ongoing research to examine the auditory system of the aged mouse model of presbycusis we have analyzed the inferior colliculus (IC) using antibodies against the inhibitory neurotransmitter GABA and the calcium binding proteins calretinin (CR), calbindin (CB), and parvalbumin (PV). Following measurement of ABR audiograms and gap detection thresholds in a startle response inhibition paradigm, the brains of young (4-6 mon. old) and old (>24 mon. old) CBA/J mice were immunoreacted with an antibody against GABA (5 mice each age group) or with antibodies against CR, CB and PV (5 mice each age group). In the old mice ABRs showed an average flat loss of 20-30 dB from 4 to 80 kHz and gap detection showed deficits consistent with a loss of central inhibitory mechanisms involved in temporal processing.

GABA is abundant in the IC and may be important for temporal acuity. GABA(+) cells were numerous and evenly scattered throughout the IC in both age groups. However, four of the five old mice showed an average 24% decrease in the number of GABA(+) cells in the central nucleus of the IC (ICC); the fifth mouse showed an increase. Calcium binding proteins are abundant in the auditory system, and are a possible protective or regulatory mechanism against calcium induced neurotoxicity. CR(+) cells were numerous in the the dorsal cortex, external cortex, commissural nucleus, and the brachium of the IC. They were rare and widely scattered in the ICC. A 26% increase in the number of CR(+) cells was observed in the old IC . An analysis of CR(+) cell types is underway. PV staining was intense in both cell bodies and neuropil in all divisions of the IC while CB antibody stained only a few scattered cells. Comparative counts and densitometry are in progress. *Supported by NIA P01-AG09524 and the International Center for Hearing and Speech Research.*

361.7

AUDITORY PATHWAYS OF A SONIC FISH (*Pollimyrus*). James Kozloski, John D. Crawford.* Inst Neurological Sci & Psychology Dept, U of Pennsylvania, Philadelphia, PA 19104.

Pollimyrus (Mormyridae) has been of interest in neuroethological studies of hearing and communication because of its specialized ears and simple acoustic repertoire. Neurophysiological studies have focused on the response properties of the midbrain nucleus MD, and have revealed complex specificity which likely plays a role in processing communication signals. In an effort to understand both the emergence of these properties and the fate of information extracted in the midbrain, we have investigated anatomically both inputs from the medulla and outputs to the diencephalon and optic tectum. Using double barrel electrodes, we have physiologically characterized auditory areas then injected biotin-based pathway tracers at recording sites within the auditory pathway. Biocytin injections into MD revealed multiple areas in the medulla which project to MD via an organized fiber sheet within the lateral lemniscus (LL). Groups of cell bodies were labeled contralateral to the injection site near the LL and MD (ie. nLL), further posterior on the midline at the ventral margin of the cerebellar crest cell layer (cc), and bilateral and slightly ventral relative to cc (Bell's nucleus octavius). In addition to these three groups of cell bodies presumably afferent to MD, bilateral terminal fields were labeled more ventral, possibly a nucleus receiving descending inputs from the midbrain. Physiological recordings from these medullary sites revealed clear transformations along this pathway, from the medulla to the midbrain. [NIDCD R01 1252; PAL HRF GA1205; U of P Res F]

361.8

THREE-DIMENSIONAL MORPHOLOGY OF SPECIALIZED SYNAPSES IN COCHLEAR NUCLEI OF THE NEWLY HATCHED CHICKEN. E.G. Dobbins* & G. Laurent, Division of Biology, 139-74, California Institute of Technology, Pasadena, CA 91125.

The cochlear branch of the eighth cranial nerve (VIIIn) innervates both nucleus angularis (NA) and magnocellularis (NM) in the owl (Carr & Boudreau, J. Comp. Neurol. 314('91):306-318; Köppl, J. Comp. Neurol. 339('94):438-446) and chicken (Jhaveri & Morest, Neurosci. 7('82):809-836). Although single axons have collaterals to both nuclei in owl, this has not been established in chicken. Biotinylated dextran amines (m.w. 10,000) were injected into the cochlea of newly hatched chickens. After survival times of two weeks, the chickens were perfused and brainstems processed with avidin-biotin and cobalt-intensified 3'3'5'5 Diaminobenzidine. The afferent and synaptic morphology was analyzed with light and laser scanning confocal microscopy. The afferents enter the rostral medulla in a dorsolateral position, ascend dorsomedially between the two nuclei, and branch, entering each nucleus from its dorsal aspect. Single axons send collaterals to NA and NM in chicken. Each of these collaterals supports synapses with different morphology in the two nuclei: boutons in NA and end-bulbs in NM. Three-dimensional reconstructions of a single synapse in NM, from confocal microscopy, show its highly specialized character. Supported by NIMH and NSF-PFF (GL).

361.9

CELL DEATH IN THE NUCLEUS LAMINARIS AND NUCLEUS MAGNOCELLULARIS OF THE BARN OWL (*TYTO ALBA*). D. P. Massoglia, C. E. Carr*. Dept. of Zoology, Univ. of Maryland, College Park, MD 20742.

During embryonic development of the chicken auditory system there is approximately 80% cell death in the Nucleus Laminaris (NL) and 30% cell death in the Nucleus Magnocellularis (NM) (Rubel, Smith & Miller, 1975). The Barn Owl (*Tyto alba*) has a specialized auditory system, and substantially more cells in NL and NM than in the adult chicken (Winter & Schartzkopff, 1961). At least two mechanisms may underlie this hypertrophy: an increase in cell proliferation and/or a decrease in cell death. Since cell birth in the owl NL and NM is complete by E8 (Carr & Cohen, unpublished observations), owl embryos were examined after E15, when cells were considered post-mitotic and migration to the nuclei complete. Embryo ages were also chosen to correspond with the onset of cell death observed in the chicken (E9, or 40% of incubation time). Owl embryos of age E8,17,18,20,22,23 and 35 post fertilization and an adult brain were either embedded in paraffin (10µm), or plastic (8µm; E18, E20) and counterstained. Nuclear profiles in both NL and NM were counted in every other section, and corrected total counts were obtained for each series (Coggeshall, 1992). At E18, the owl NL and NM contain a larger number of neurons than the E9 chicken. Substantial cell death was also observed. Thus the hypertrophied auditory brainstem of the adult Barn Owl appears to be regulated by increased cell proliferation and by cell death. At present we do not know whether the rate of cell death is less than that observed in the chicken.
Supported by NIH DC00436

361.10

CALCITONIN GENE-RELATED PEPTIDE IMMUNOREACTIVITY IN THE CHICK BRAINSTEM. R.A. Code*[1], M.S. Dart and C.E. Carr
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In the mammalian brainstem, calcitonin gene-related peptide-immunoreactivity (CGRP-I) is found within neurons of cranial motor nuclei, preganglionic parasympathetic motoneurons, in vestibular efferent neurons and in some olivocochlear (OC) neurons. Within the OC system, CGRP I is localized primarily within cell bodies, nerve fibers and terminals of the lateral OC (LOC) division which terminate on auditory nerve endings under inner hair cells in the ipsilateral cochlea. In contrast, most neurons of the medial OC (MOC) division, which project predominantly to the base of outer hair cells in the contralateral cochlea, lack, or have much lower levels of, CGRP-I. Both LOC and MOC neurons are immunoreactive for choline acetyltransferase, (ChAT), the biosynthetic enzyme for acetylcholine. In the chick, ChAT-immunoreactive (ChAT-I) neurons projecting to the inner ear are also found in two groups in the brainstem. We wished to determine whether chick cochlear efferent neurons could be divided into two neurochemically distinct groups based on their CGRP-immunoreactivity. We combined immunohistochemistry using antisera to ChAT and CGRP with the retrograde transport of rhodamine dextran amine from the inner ear. CGRP-I was found in neurons of cranial motor nuclei and scattered around the superior olive. Unlike mammals, however, chick cochlear efferent neurons do not appear to contain CGRP-I, or they have CGRP levels that are too low to be detected immunohistochemically.

The ChAT antiserum was kindly provided by Dr. Miles Epstein, U. Wisconsin Madison; the CGRP antibody was donated by Dr. Catia Sternini, UCLA. This work was supported by NIDCD grants DC01867 (RAC), DC00436 (CEC) and a Howard Hughes Fellowship (MSD).

361.11

THE DEVELOPMENT OF POSTNATAL OLIVOCOCHLEAR NEURONS: IS IT COMPETITION OR A WAITING PERIOD? D.D. Simmons* J.H. Kim, and N. Moore, Dept. of Physiological Science and Brain Research Institute, UCLA, Los Angeles, CA 90095-1527.

As neurons grow toward their target cells, they synthesize specific neurotransmitters that are important for mature function. Previous studies have suggested that periolivary (PO) olivocochlear (OC) neurons wait for a defined period below inner hair cells (IHCs) in the cochlea before growing towards the outer hair cells. The OC system is the major efferent pathway to the cochlea and is responsible for modulating activity of the cochlear nerve. Choline acetyltransferase (ChAT), calcitonin gene-related peptide (CGRP), and glutamate decarboxylase (GAD) serve as neurochemical markers for OC neurons. Standard immunocytochemical techniques were used to study the development of neurotransmitter expression in OC neurons that were defined on the basis of tracer injections into the crossed OC bundle.

ChAT-immunoreactive cells were first detected in the superior olive around postnatal days (P) 5-6. ChAT-positive cells in PO regions had larger somal sizes and had multipolar shapes with an average of 3 primary dendrites while ChAT-positive cells in the lateral superior olive (LSO) were smaller and had more fusiform shapes with an average of 2 primary dendrites. CGRP-positive cells co-labeled with ChAT-positive cells in the LSO. GAD-positive cells in the LSO were identified at P12.

ChAT immunoreactive terminals in the cochlea were first detected at P4 in basal regions below IHCs. CGRP-positive terminals did not label until P6. GAD-positive terminals were not present below IHCs at P6 but were found below IHCs at P12.

Thus, ChAT expression occurs just prior to CGRP expression, at a time when GAD expression is absent. This raises the possibility that cholinergic OC neurons may competitively interact.
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361.12

DEVELOPMENTAL CHANGES IN GLUTAMATE RECEPTOR SUBUNIT IMMUNOCYTOCHEMISTRY IN THE NEONATAL GERBIL COCHLEAR NUCLEUS. I.R.Schwartz' and P.R.Eager, Dept. of Surgery/Otolaryngology, Yale Univ. Sch. of Med., New Haven, CT 06520

Cryostat sections of aldehyde fixed brains from neonatal gerbils (at postnatal day (P) 7,14,21,28) & adult animals were incubated with antibodies (Ab) recognizing the glutamate receptor subunits GluR1, 2/3, or 4 (Chemicon) and examined light microscopically.

In the youngest animals there was a high level of diffuse staining throughout the cochlear nucleus (CN) and the entire brainstem. With increasing age, the brain showed a decrease in this diffuse stain more marked in some areas. With GluR1 and 2/3, lightly stained somata were present close to the ependymal surface of the dorsal CN (DCN) at P7 and there was a thin region of slightly darker diffuse staining in the region of the molecular (ML) & cellular (CL) layers. At P14 the ML & CL of the DCN were notably darker than the deep layer (DL). Diffuse staining of DL decreased progressively from P7-P28. Diffuse staining of ML & CL was most intense at P14 & P21 with some decrease at P28. GluR4 produced little if any differential diffuse staining of ML & CL compared to DL at all ages.

Medium sized somata scattered throughout the ML & CL, including those with the size and distribution of cartwheel cells, were progressively more darkly stained by GluR1 & 2/3 from P7-P21, with a slight decrease at P28. More somata and their dendrites were darkly stained by GluR2/3 than GluR1 in the ML & CL, their size and location suggesting that they were both cartwheel and fusiform cells. With GluR4 cellular staining in DCN was relatively light. GluR1 & 2/3 produced little staining of cells in the octopus cell area (OCA) compared to GluR4. OCA cells stained progressively more darkly with GluR4 from P7-P21, and slightly less darkly at P28. Diffuse staining of the OCA neuropil declined from P7 to 28 with all Abs.

While GluR1,2/3 & 4 may all be present in DCN cartwheel cells, comparison of sections from a single animal indicate that at P14 GluR1 is preferentially present in cartwheel cells, GluR2/3 in fusiform cells & dendrites and GluR4 in octopus cells. The increase in staining at P14 & P21 is consistent with the development & functional maturation of synapses over this time. The decreased somatic and neuropil staining at P21 & P28 probably reflect the incorporation of subunits into functional receptors at the synapses and their removal from the cytoplasm. Supported by NIDCD DC00132.

361.13

DIFFERENTIAL CELL BIRTH OF THE PERIOLIVARY NEURONS IN THE RAT. M. Kudo[1], Y. Kitao [1] and S. Higo[2]*. [1]Dept. of Anatomy, Sch. of Med., Kanazawa Univ., Kanazawa 920, Japan and [2]Dept. of Morphological Neural. Sci., Kumamoto Univ. Med. Sch., Kumamoto 860, Japan.

In a recent study (Kudo et al. 1992) we provided evidence that neurons having different laterality of projections have different birthdays in the lateral (LSO) and the medial (MSO) superior olive. The present study is aimed at examining the birth dates of the periolivary nuclei neurons which exhibit various morphology and different laterality of projections to the inferior colliculus (IC). Rat fetuses were exposed to 5-bromodeoxyuridine (BrdU), the thymidine analogue, in utero to label neurons proliferated at different embryonic days (E). Upon reaching adulthood, rats were given injections of Fluoro-Gold (FG) into the IC unilaterally to identify the laterality of the projection and the cell morphology by the retrograde labeling. The results indicate that neurogenesis in the periolivary nuclei are between E12 and E16. The superior paraolivary nucleus (SPN) neurons were generated on E12 and E14 with a strong peak on E13. The medial nucleus of the trapezoid body (MTB) neurons were generated mainly on E14, although they did not project to the IC (BrdU+ and FG-). In the ventral nucleus of the trapezoid body (VTB), many of the IC projecting neurons (BrdU+ and FG+) were produced on E14, while non-IC projecting neurons (BrdU+ and FG-) were on E15.

361.14

GROWING AUDITORY AFFERENTS IN THE TRAPEZOID BODY RECOGNIZE THE MIDLINE. L.S. Ross*, Neurobiology Program, Dept. of Biological Sciences, Ohio University, Athens, OH 45701.

During development of the auditory system, axons from the cochlear nucleus must make connections with a number of specific auditory nuclei in the superior olivary complex of the brainstem. Many of these axons bypass ipsilateral olivary nuclei, cross the midline, and innervate contralateral olivary nuclei. The cues that guide these outgrowing auditory axons are poorly understood. Using DiI as a neuronal tracer, I have investigated the morphology of axonal growth cones at various points along the trapezoid body in the developing mouse embryo. During embryonic days 12-18, labeled growth cones were found throughout the trapezoid body and auditory nuclei. Growth cones within auditory nuclei were larger and had more filopodia than growth cones in fiber tracts. Growth cones also displayed a more complex morphology at the midline and appeared to defasciculate from other axons at this point. As axons reached the midline, they did not cross directly; rather they made a dorsal turn, crossing the midline at an angle, and then refasciculated and returned to a more ventral trajectory. Acetylcholinesterase histochemistry revealed a population of cells residing at the midline that differentiate just prior to the arrival of these auditory afferents. These results suggest that outgrowing auditory afferents may recognize and use the midline as a guidance cue.

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361.15

AGE-DEPENDANT DEGENERATION OF SPIRAL GANGLION CELLS IN WILD TYPE MICE. S. Dazert, M.L. Feldman and E.M. Keithley*. Division of Otolaryngology, UCSD Sch. of Med., La Jolla, CA 92093-0666.

Age-related hearing loss in humans typically shows a bilateral sensorineural hearing impairment, predominantly with high-frequency sounds. In coincidence with the audiometrical findings histological examinations of temporal bones show pathological changes at the basal end of the cochlea. Investigations of pathological changes in rodent cochleas as a function of age revealed varying results in different species. In aging rats and gerbils a decreased number of spiral ganglion cells (SGCs) was mainly detected in both the basal and apical turns of the cochlea. In contrast to these findings other investigators described genetically inbred C57BL/6 aged mice with the main loss of SGCs in the basal turn.

The aim of this study was to investigate the cochlea and especially the SGC density as a function of age in wild type pigmented mice to determine the pattern of degeneration. Three groups of different ages ranging from 2 to 31 month were studied. Each group contained 3 or 4 animals. Either the left or right ear was chosen randomly and prepared for histological examination of plastic sections using light microscopy. In middle aged wild type mice (18-19 month old) the main loss of SGCs occured in the basal turn (49% loss compared to 2-3 month old animals) followed by the apical turn (31%). The greatest SGC losses in 28-31 month old wild type mice were present at the apical region of the cochlea (76%) followed by the basal turn (74%). The mid-region showed a 45% decrease of SGCs. The oldest animals also had degenerative changes in the stria vascularis in the apical turn.

These findings lead to the assumption that age-dependent degeneration of the spiral ganglion in mammals including humans occurs over the entire length of the cochlea with emphasis on the both ends of the cochlea.

361.16

4-METHYLTHIOBENZOICACID PROTECTION AGAINST CISPLATIN OTOTOXICITY: CORRELATION OF AUDITORY BRAINSTEM RESPONSES AND THE ANTIOXIDANT SYSTEM. C. Morris, K. Husain, C. Whitworth, L. Evenson, S.M. Somani, and L.P. Rybak*. Department of Pharmacology and Surgery, Southern Illinois University School of Medicine, Springfield, Illinois 62794-9230.

This study investigates the auditory brainstem responses and antioxidant system in cisplatin-induced ototoxicity and the otoprotection with 4-methylthiobenzoic acid (MTBA). Male Wistar rats were injected with 1) saline for control animals, 2) cisplatin (16 mg/kg, i.p.), 3) MTBA (250 mg/kg/i.p.), 4) cisplatin plus MTBA. Rats were subjected to auditory brainstem response (ABR) recording prior to sacrifice (three days post-treatment) and cochlae were isolated and were analyzed for lipid peroxidation (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase (GR). Glutathione (GSH) and oxidized glutathione (GSSG) were also analyzed in the cochleae. Cisplatin elevated the ABR thresholds and increased the cochlear MDA level (20%) whereas reduced glutathione (GSH) level, SOD, CAT, GSH-Px and GR activities decreased 23, 43, 15, 22, and 48% respectively. Pretreatment with MTBA demonstrated smaller ABR threshold shifts and normalized the activities of antioxidant enzymes, GSH, and MDA levels. These results suggest that MTBA provides otoprotection against cisplatin-induced ototoxicity by enhancing antioxidant system. Supported by NIH RO1 DC 0239601.

361.17

P_1 PURINOCEPTOR AGONIST STIMULATES ANTIOXIDANT ENZYMES IN THE COCHLEA OF CHINCHILLA. M.S. Ford, S.B. Maggirwar, C. Whitworth, L.P. Rybak, D.M. Caspary* and V. Ramkumar. Dept. of Pharmacology, Southern Illinois Univ. Sch. of Med., Springfield, IL 62704

In an initial step towards assessing the role of purinergic receptors in the peripheral auditory system, we have begun to characterize the subtypes of P_1 purinoceptors in the cochlea. Both the A_1 and A_3, adenosine receptor (AR) were detectable in membranes prepared from the cochlea, using [^{3}H]CPDPX and [^{125}I]APNEA, respectively. The estimated number of A_1 and A_3AR (B_{max}) were 368 and 175 fmol/mg protein with equilibrium dissociation constants (K_d) averaging 5.6 and 9.9 nM, respectively. In membrane preparations, R-phenylisopropyladenosine (R-PIA, 1 μM) elicited a small degree of inhibition of adenylyl cyclase activity, which was only partially blocked by theophylline (100 μM). Activation of the cochlear ARs by round window application of R-PIA had no effects on auditory measures such as endocochlear potential and compound action potential. However, treatment of R-PIA for 1 h elicited significant increases in the activities of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase which were reversed by theophylline (1 mM). These studies suggest that while adenosine may not contribute directly to the propagation of an auditory signal, it might provide cytoprotection against oxidative damage to structures in the cochlea intimately involved in the processing of auditory signals.

362.1

GLUTAMATERGIC AND GABAERGIC POSTSYNAPTIC RESPONSES OF STRIATAL SPINY NEURONS TO INTRASTRIATAL AND CORTICAL STIMULATION. H. Kita,* Dept. Anatomy, and Neurobiol, Coll. of Med., Univ. of Tennessee, Memphis, TN 38163.

Glutamatergic and GABAergic responses of the neostriatal (Str) spiny neurons to Str and cortical (Cx) stimulation were characterized by intracellular recording in slice preparations. Single Str or Cx stimulation induced a fast AMPA/kainate-receptor mediated PSP followed by a slow NMDA-receptor mediated PSP. The NMDA-response was small in neurons at resting membrane potential but became a significant component when the neurons were depolarized to subthreshold membrane potential. Repetitive Str or Cx stimulation induced a large, long duration depolarization with spikes in Str spiny neurons. Application of CPP reduced the amplitude of the response to the subthreshold level of spike generation. BMI, on the other hand, greatly increased the response amplitude and the number of spikes. The AMPA/kainate-response, which was isolated by BMI and CPP, alone can not induce sustained depolarization in spiny neurons to repetitive stimulation. Application of NBQX abolished $GABA_A$-response of spiny neurons to Cx stimulation, indicating that, in order for Str spiny neurons to respond with $GABA_A$-response after Cx stimulation, the AMPA/kainate-response must be induced in the GABAergic secondary neurons in the Str.

This study indicates the main synaptic driving forces of Str neurons include AMPA/kainate-, NMDA- and $GABA_A$-responses. Although AMPA/kainate-response is the main synaptic input, the generation of action potentials in Str neurons is greatly influenced by both $GABA_A$- and NMDA-responses. Supported by NS-25783 and NS-26473.

362.2

PARVALBUMIN-IMMUNOREACTIVE NEURONS IN RAT GLOBUS PALLIDUS: A LIGHT AND ELECTRON MICROSCOPE STUDY. J. Q. Ren* and H. Kita Dept. Anatomy, and Neurobiol, Coll. of Med., Univ. of Tennessee, Memphis, TN 38163.

Light and electron microscopic analysis of parvalbumin immunoreactive neurons (PV-neurons) in the GP was performed. Approximately two-thirds of the GP neurons were PV-positive. The somata of PV-neurons were, on average, larger than PV-negative ones. The proximal dendrites of PV-neurons were smooth and often lay parallel to the border between the GP and the neostriatum. Distal dendrites of PV-neurons were varicose. Thin PV-positive fibers with large boutons were observed in the neuropil of the GP.

Electron microscopic observations revealed that PV-neurons contained deeply indented nuclei and a large volume of cytoplasm. The somata of PV-negative neurons were occupied by deeply indented nuclei and a small volume of cytoplasm. Both PV-positive and negative neurons received synaptic boutons identical to the known striato-pallidal, subthalamo-GP, and local collateral boutons. The PV-positive boutons contained small round or elongated vesicles and often more than one mitochondrion. Most of the boutons (i.e., 86 %) formed symmetric synapses with somata and large dendrites, and the others (14%) formed asymmetric synapses with small dendrites.

The study indicated that GP projection neurons can be divided into two subgroups according to their PV-immunoreactivity. PV-positive and negative neurons received similar extrinsic synaptic inputs and both types of neurons were connected through their local collateral axons. SuppOrted by NS-25783 and NS-26473.

362.3

ADENOSINE A1 RECEPTOR MODULATION OF CORTICO-STRIATAL SYNAPTIC POTENTIALS. D.R.Stevens*, J. Flagmeyer and H.L. Haas Physiologisches Institut II, Heinrich-Heine-Universität, Postfach 101007, Düsseldorf, D-40001 Germany

The striatum contains relatively high levels of adenosine receptors. We have therefore examined the role of adenosine in the striatum using intracellular recording in a rat corticostriatal slice.

Adenosine reversibly reduced corticostriatal EPSPs in most neurons. This action was mimicked by the A1 agonist N6-cyclopentyl adenosine (CPA) but not by the A2 agonist 5-'(N-cyclopropyl) carboxyamido-adenosine. Adenosine did not decrease input resistance. DPCPX (an A1 antagonist) blocked the action of adenosine and enhanced EPSPs. Inhibition by CPA, but not by adenosine, was accompanied by enhanced paired-pulse facilitation. The lack of enhancement during adenosine treatment may indicate a competing action at an A2 receptor. These results are consistent with the reported inhibition of corticostriatal EPSPs by adenosine and suggest that endogenous adenosine acting at A1 receptors modulates activity and may be protective against excitotoxicity in the striatum.

In neurons not exhibiting the properties of principal neurons, adenosine did not depress evoked EPSPs. Corticostriatal activation of cholinergic interneurons is spared under these conditions and may result in increased vulnerability of interneurons to excitotoxicity.

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362.4

THE SPACING OF SYNAPSES ALONG CORTICOSTRIATAL AXONS. A.E. Kincaid* T. Zheng, and C.J. Wilson, Dept. Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, TN 38163.

Convergence of excitatory synaptic activity of corticostriatal (CS) neurons is a major determinant of the firing of neostriatal (NS) projection neurons. Each NS cell receives several thousand corticostriatal synapses. The number of CS axons that contribute synaptic input to each NS neurons is not known. To obtain an upper limit to the number of synpases made by one axon onto single NS neurons, we measured the number of varicosities along the course of single CS axons as they traverse the dendritic fields of neostriatal neurons.

CS axons were labeled by intracellular injection of biocytin or by small extracellular injections of biocytin or BDA. Selected tissue sections from these same animals were processed for electron microscopy, and the diameters of labeled CS axons were measured at synaptic and nonsynaptic regions for comparison to the diameters of varicosities and non varicose segments seen in the light microscope.

The average diameter of labeled CS axons containing vesicle accumulations and synaptic specializations was 0.53 µm and for CS axons without vesicles or synaptic specializations was 0.18 µm. Thus the axonal varicosities identified with the light microscope represented synapses. The average intervaricose distance for CS axons was the same in the patch and matrix compartment (10.6 µm and 11.6 µm respectively). Individual branches of single axons generally were separated by more than the diameter of a spiny cell's dendritic tree. Thus, if all possible contacts were made onto one spiny cell, from a single CS axon would be 30-40, and the minimum number of CS axons contacting a single NS neuron would be at least 250-300. If connections were made upon spiny cells at random, the average number of connections would be about 0.04, and each NS cell would receive inputs from about 10,000 different CS axons. Supported by NS20743.

362.5

PERIODIC AND STOCHASTIC COMPONENTS OF THE SUBTHRESHOLD MEMBRANE POTENTIAL OF CORTICOSTRIATAL AND STRIATAL NEURONS RECORDED *IN VIVO*. E. A. Stern* and C. J. Wilson, Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, TN.

In vivo recordings of identified corticostriatal projection neurons and striatal medium spiny neurons in rats anaesthetized with urethane show that these neurons exhibit spontaneous, subthreshold membrane potential shifts. The depolarized (Up) state of the neurons results from convergent synaptic activity interacting with the membrane rectification properties.

Fourier analysis of the membrane potential in the ≤100 Hz range reveals three constituent parts of the spectrum: A periodic component of ~0.1-1 Hz contributed to the timing of the spontaneous subthreshold shifts. The presence of a stochastic component was indicated by low-frequency noise with approximately $1/f$ decay, which was present primarily in the Up state. An additional white noise component was present in both the Up and Down (hyperpolarized) states. In corticostriatal neurons, the $1/f$ component often showed a significant peak at ~40 Hz .

The frequency components of the membrane potential state transitions are within the range described for cortical slow oscillations. However, the pattern of state transitions could not be approximated by a single periodic function. The Up and Down state durations are stationary within each neuron, enabling classification of the two-state activity as renewal processes. The intensity functions of the Up state transitions are modeled as doubly stochastic point processes, with one stochastic variable being the state of the neuron (Up or Down) and the other being the strength of the synaptic input. *Supported by NS20743.*

362.6

PREDICTED RESPONSES OF NEOSTRIATAL SPINY NEURONS TO CURRENT STEPS BASED ON PROPERTIES OF THEIR IONIC CONDUCTANCES. Charles J. Wilson*. Department of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, TN 38163.

Voltage clamp studies of striatal spiny neurons have provided accurate measurements of the voltage dependence and kinetics of several ionic conductances that are believed to be important for these neurons. However, the best descriptions of these conductances have been obtained using dissociated striatal neurons, while most conventional intracellular recording has been done on striatal tissue slices. To determine the degree to which available quantitative descriptions of ionic conductances can predict the responses of striatal spiny neurons, computer simulations of standard intracellular recording experiments were performed using quantitative descriptions of ionic conductances obtained from dissociated neurons. Application of ion channel blockers was simulated by reducing channel density according to dose-response curves obtained in voltage clamp experiments,. The results were directly compared with intracellular recording data from slices.

All 4 potassium conductances known to be active at subthreshold membrane potentials (Ih, IAs, IAf, and IK) were required to reproduce the control responses of spiny neurons to subthreshold current pulses. These were also sufficient to account for the alterations in subthreshold responses seen after partial K channel blockade with 4-AP, TEA, or cesium. These channels, in combination with somatic Na channels were sufficient to account for some, but not all of the repetitive firing properties of the cells.

362.7

DOPAMINE D_1 RECEPTOR STIMULATION SELECTIVELY DEPRESSES A SLOWLY INACTIVATING K^+ CURRENT IN STRIATAL NEURONS.

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Evidence indicates that D_1 receptor activation can modulate the spike discharge properties of striatal neurons. Although this modulation may result from effects on several different ionic conductances, outward K^+ currents are likely candidates due to their critical role in regulating spike discharge. Striatal neurons possess three types of depolarization-activated K^+ currents, including fast (I_{Af}) and slowly (I_{As}) inactivating A-currents and a noninactivating current. The present experiments investigated which of these currents were modulated by D_1 receptor stimulation using whole-cell voltage-clamp recording from acutely isolated rat striatal neurons.

Depolarizing voltage steps (0.5-2 s in duration) from -80 mV to +35 mV evoked I_{Af}, I_{As}, and the noninactivating current. Application of the D_1 receptor agonist, 6-chloro-APB (APB, 10 μM) reduced the amplitude of the total outward K^+ current by approximately 30%. The effect of APB application was rapid and completely reversible having onset and offset time constants of 1.1 ± 0.3 s and 1.7 ± 0.3 s (n=6), respectively. Subtraction of the currents evoked before and after application of APB yielded the APB-sensitive current which activated rapidly (τ~4 ms) and inactivated relatively slowly (τ~625 ms). These kinetics of activation and inactivation are very similar to those previously described for I_{As} in striatal neurons.

These results suggest that D_1 receptor stimulation preferentially depresses I_{As} in striatal neurons. In light of our previous studies, a D_1 receptor-mediated decrease in the availability of I_{As} would be expected to shorten that latency to discharge and increase the frequency of firing of striatal neurons in response to long (e.g., ≥ 500 ms) periods of depolarization.

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362.8

Long-term synaptic plasticity in the striatum: Distribution of response patterns and modulation.

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The present study examined the distribution of long-term synaptic plasticity response patterns under conditions of differing stimulation intensity, elimination of dopaminergic innervation with 6-OHDA and block of metabotropic glutamate receptors. A set of aged animals (>24 mo.) were also examined.

When tetanus stimulation intensity equalled the test stimulus intensity (no action potentials were evoked), 60% of cells from young animals showed LTD, 35% showed LTP and 5% showed no change (n=17). In aged animals, 83% showed LTD, 17% LTP and 0% no change (n=6). Increasing the stimulation intensity during the tetanus to twice the test intensity increased the synaptic depolarization and reliably generated action potentials. Under these conditions, 90% of young cells showed LTD, 10% LTP and 0% no change (n=10). In aged animals, 80% showed LTD, 0% LTP and 20% no change (n=5). Data reflects 20 min posttetanus sampling, ±5% of control was categorized as no change.

Elimination of DAergic input to the striatum by nigral 6-OHDA injections shifted the distribution toward LTP, with 29% of cells showing LTD, 71% LTP and 0% no change (n=7) in response to same intensity stimulation. Block of met-GLU receptors with AP-3 did not affect the distribution of responses, with 67% of cells showing LTD, 33% LTP and 0% no change (n=3). These data indicate that: (1) LTP can be generated in some cells under "normal" conditions, (2) postsynaptic action potentials and activation of met-GLUr are not absolute requirements for LTD induction, and (3) DAergic lesions increase the probability of expressing LTP even though some cells still show LTD.

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362.9

DOPAMINE-ACETYLCHOLINE INTERACTIONS IN A BIOPHYSICALLY-BASED MODEL OF THE NEOSTRIATUM.

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Although it is generally accepted that the actions of dopamine and acetylcholine in the neostriatum are somehow complementary, the details of their interaction remain unclear. Based on voltage-clamp studies, Surmeier suggested specific actions of these neuromodulators on ion currents which could account for the nature of this interaction. Surmeier's results were incorporated into a computational simulation using the GENESIS simulator. Striatal medium spiny cells and giant aspiny cholinergic cells were both simulated at a moderate level of detail, with degenerate morphology, but with a fairly complete complement of conductances.

The model neurons have firing patterns which closely resemble the real cells. The model medium spiny cells, in particular, exhibit the characteristic bistable membrane potential pattern seen in real spiny neurons.

As suggested by Surmeier, acetylcholine tends to stabilize the model spiny neurons at either the "up" or "down" membrane potential levels, while dopamine facilitates shifting from one state to the other.

When placed into a network based on the cytoarchitecture of the neostriatum, the medium spiny cells and cholinergic interact in a way that allows the structure to function as a simple working memory. It is suggested that this could be the basis for the role of the striatum in encoding "set" in action sequences. A deficit in establishment and maintenance of set is one of the signal characteristics of Parkinson's disease, a phenomenon which maps naturally to the characteristics of the model presented here.

362.10

NMDA-INDUCED EXCITOTOXICITY IN VISUALLY IDENTIFIED NEOSTRIATAL NEURONS IN SLICES. MODULATION BY DOPAMINE.

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This study examined dopamine's (DA) ability to modulate excitotoxicity induced by activation of N-methyl-d-aspartate (NMDA) receptors. Nomarski optics and infrared videomicroscopy were used to visualize individual neostriatal neurons in thick slices (350 μm) obtained from rat pups (12-18 days of age). Bath application of NMDA (25, 50, and 100 μM) induced cell swelling in a dose-dependent manner. This effect was blocked by 2-amino-5-phosphonovalerate (AP5) (10-20 μM), an antagonist of NMDA receptors. DA (20-50 μM) or its receptor agonists, SKF 38393 (20-30 μM) (D_1 agonist) and quinpirole (20 μM) (D_2 agonist), had no effect on neostriatal neurons when applied alone. However they modified NMDA-induced swelling. DA (50 μM) produced a 20% enhancement of NMDA-induced cell swelling. This effect was mimicked by SKF 38393 suggesting the enhancement is mediated by activation of D_1 receptors. Furthermore, SCH 23390, a D_1 receptor antagonist, reduced cell swelling (15% instead of 30%) alone or in combination with DA. In contrast, quinpirole conferred some protection against the swelling (20% instead of 30% increase). These results indicate that DA and its receptor agonists and antagonists can effectively modulate NMDA-induced cell swelling. DA, via D_1 receptors, enhances NMDA's effects. In contrast, activation of D_2 receptors reduces NMDA-induced swelling. These observations agree with our previous electrophysiological findings in which selective activation of D_1 receptors potentiates electrophysiological responses induced by activation of NMDA receptors while activation of D_2 receptors tends to attenuate NMDA-induced responses. Supported by USPHS Grant HD 05958.

362.11

ALTERATIONS IN NEOSTRIATAL NEURONAL RESPONSES TO DOPAMINE RECEPTOR AGONISTS IN A MUTANT MOUSE LACKING D_{1A} DOPAMINE RECEPTORS.

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Previously, we have shown that dopamine (DA) enhances N-methyl-d-aspartate (NMDA)-induced responses in neostriatum. This effect is mimicked by the D_1 agonist, SKF 38393 and blocked by the D_1 antagonist, SCH 23390. This study uses a genetic approach to further examine this role of D_1 receptors. Homologous recombination was used to generate mutant mice lacking functional D_{1A} DA receptors (Drago et al., 1994, PNAS, 91:12564-12568). Intracellular recordings were made from neostriatal neurons in brain slices from mutant and wild-type control mice. In one set of experiments, we used iontophoresis of NMDA and DA combined with bath application of SKF 38393. NMDA induced depolarizations accompanied by action potentials in all neurons. In wild-types, DA or SKF 38393 markedly potentiated NMDA-induced responses. In mutants, DA produced only a small enhancement and SKF 38393 had no effect. In a second set of experiments, we assessed the effect of SKF 38393 on depolarizing synaptic responses (DPSPs) induced by activation of cortical afferents. In standard artificial cerebrospinal fluid (ACSF), this response is mediated by non-NMDA receptors. When cells are exposed to Mg^{2+}-free ACSF and 6-cyano-7-nitroquinoxaline-2,3-dione, a non-NMDA receptor antagonist, the DPSP is primarily mediated by NMDA receptors. SKF 38393 (5-20 μM) had little effect on the amplitude of the DPSP (3% increase) in mutants but increased the amplitude in wild-types (34%). Taken together, these results support the hypothesis that DA's ability to potentiate NMDA-induced responses is mediated by D_1 receptors. Supported by USPHS Grants HD 05958 and AG 10252.

362.12

THE EFFECTS OF SEROTONIN AND THE SELECTIVE SEROTONIN REUPTAKE INHIBITOR FLUOXETINE ON STRIATAL NEURONS RECORDED IN VITRO.

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The neostriatum is known to receive a substantial serotonergic innervation from the raphe. The physiological role of this neurotransmitter in the regulation of striatal cell activity and its relationship to other striatal afferents is presently not well understood. In the present study, the physiological and pharmacological effects of the selective serotonin reuptake inhibitor fluoxetine was investigated using current clamp techniques in striatal slices. Intracellular recordings were made *in vitro* from neostriatal neurons. Perfusion of fluoxetine (1 μM) in the bath resulted in a modest reduction in input resistance (8 %) and hyperpolarization of the membrane (-6.5 mV). Fluoxetine administration also resulted in a shortened spike latency (15%) and an increase in the number of spikes evoked by constant current depolarizing pulses. This latter effect may possibly be due to an observed decrease (39%) in the afterhyperpolarization of neostriatal neurons. Following the administration of fluoxetine, serotonin (50 μM) was added through the bathing medium. Administration of serotonin following fluoxetine augmented the response observed after fluoxetine in neostriatal neurons, suggesting that the effects of fluoxetine may be due to potentiation of the effects of endogenous serotonin within the slice. Supported by MH42217, MH45156, MH01055.

362.13

ION MECHANISMS OF DOPAMINERGIC INHIBITION IN NEOSTRIATAL NEURONS. S. Hernández, J. Bargas, A. Reyes and E. Galarraga*
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Intracellular recordings in a brain slice preparation of the rat brain were performed to explore the actions of dopamine and dopaminergic agonists on the firing response of neostriatal neurons. The most reproducible effect of dopamine or D1-agonists upon the firing response of neostriatal neurons is a decrease in firing rate (100% of cases, n > 50 neurons) together with an increase in the current needed to achieve threshold for action potential firing. This effect was accompanied by a decrease in slope input resistance in the subthreshold voltage range with a reversal potential between -80 and -90 mV. These actions persisted in the presence of 10mM TEA or TEA+TTX (10mM, 1 μM) suggesting that: 1) the effects are direct on the post-synaptic cell, and 2) not due to TTX- or TEA-sensitive conductances. However, here we report that, 5 mM Cs$^+$ completely abolished most inhibitory action of dopamine or D1-agonists upon the firing response. This suggests that dopamine actions mainly depend on a Cs$^+$-sensitive conductance, which once blocked, occludes the effect on the firing rate. In fact, some cells showed an increase in firing rate after dopamine agonists and Cs$^+$. Other D1-agonists effects which may in part explain an inhibitory action upon firing response were: 1) an increase in the amplitude and duration of the afterhyperpolarizing potential (AHP) which follows an action potential, and 2) an enhancement of the calcium plateau potential induced by 10-20 mM TEA which correlates with an increase in inward calcium current. This last effect was probably mediated by channels of the L-type as it was occluded by BayK8644. Both effects may be related because part of the AHP is activated by Ca^{2+} entry.
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362.14

POTASSIUM CHANNEL BLOCK INDUCES DYE COUPLING IN NEOSTRIATAL NEURONS. A. Reyes, E. Galarraga, D. Tapia and J. Bargas*
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According to the "equivalent cylinder" (**EC**) model (Rall, 1977), the charge function of dendritic neurons can be approximated to a sum of exponential functions. The "electrotonic length" (**L**) is a function of the proportion between the longest time constant (τ_0) and the equalizing ones (τ_n).The "equalizing time constants" represent dendritic charge redistribution. In our case: L_{peel} ($\mu \pm SD$)= 1.72± 0.02 (n=46)(including R_m heterogeneity, e.g., Durand, 1984). Thus, if only one exponential can be extracted from a charge function, it cannot be differentiated from the isopotencial case (**L**= 0); meaning that charge transfer from soma to dendrites occurs virtually instantaneously. Here we report that (in mM): 5Cs$^+$, 10TEA, or 5Ba^{2+} increase R_N and induce **L**= 0 in most neurons. However: 1) Not all blockers increased R_N in the same proportion, suggesting that just some resting K$^+$-channels need to be blocked in order to have **L**= 0, 2) Different classes of K$^+$-channels may produce the same effect, 3) The neurons that did not become "isopotential" during blockers appeared to be dye coupled with neighboring neurons after anatomical reconstruction (intracellular biocytin)(42%). As the probability of dye coupling in control slices is very low (2%), it appeared to be significantly increased after K$^+$ blockade, 4) $C_N \cong A_N \cong (\tau_0/R_N)/(1\mu F/cm^2)$ determined after isopotential charge functions during Ba^{2+}, coincided with anatomical measurements (Wilson, 1993), 5) A_N together with somatic resistance and capacitance calculated from anatomical methods and whole-cell recordings of isolated somas coincided to give and independent measure of ρ (≅ 20). Supported by DGAPA/PADEP-UNAM and CONACyT (México).

362.15

ACTIONS OF NEUROPEPTIDES ON LARGE ASPINY NEURONS OF RAT NEOSTRIATUM IN VITRO. Toshihiko Aosaki* and Yasuo Kawaguchi Lab. Neural Circuits, Inst. Phys. & Chem. Res. (RIKEN), Nagoya, Aichi 456, Japan.

Whole-cell patch clamp recordings were performed in a brain slice preparation to study the actions of substance P (SP) and opioids on large aspiny neurons of the rat neostriatum. All of the neurons fit well with physiological properties of large aspiny neurons described by Kawaguchi (1992) and some of them were further identified morphologically. Intrastriatal stimulation (20 Hz, 0.5 ms pulses for 500 ms) evoked spike discharges followed by a slow excitatory, or inhibitory postsynaptic response lasting for about 1 min (11/14 cells; 10 excitatory, 1 inhibitory). Antagonist of SP, [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]-SP, suppressed the slow e.p.s.c.(p.) (4/5 cells) and, in 2 cells (2/4), unmasked the i.p.s.c. In one cell, i.p.s.c. was evoked and the antagonist enhanced its amplitude, due to blockade of masked e.p.s.c. by the antagonist. Bath-application of SP transiently evoked an inward current (24/25, 100-200 pA, ED50, 0.1 μM) in a dose-dependent manner. The excitatory effect of SP was mostly accompanied by a decrease (23/24) in input resistance. At holding potential of -50 to -70 mV, SP current was unchanged even in the presence of K-channel blockers (Cs+, TEA, Ba^{2+}, 4-AP) and a Ca-channel blocker (Co^{2+}). However, replacement of extracellular Na$^+$ with choline substantially suppressed it and that of extracellular Cl$^-$ with Na isethionate had no effect. The effect of SP on Ih was variable and its blockade by Cs+ had no effect on SP current. It is therefore suggested that the SP current was mainly elilcited by opening of non-selective cation channels at the resting membrane potential. On the other hand, bath-application of [Met]-enkephalin (10 μM) reversilby evoked an outward current in 2 cells (2/2), which also showed an inward current evoked by SP. DAMGO (1 μM, 4/4), μ-receptor antagonist, and κ-selective antagonist, U-50488 (1 μM, 2/2), had no effect on the outward current, whereas δ-selective antagonist, DPDPE (10 μM, 2/2), suppressed it. These results suggest that SP, released from striatonigral neurons, and enkephalin, from striatopallidal neurons, may reciprocally regulate the activity of large aspiny neurons of the striatum.

362.16

THREE CLASSES OF GABAERGIC INTERNEURONS IN RAT NEOSTRIATUM Y. Kubota* and Y. Kawaguchi, Lab for Neural Circuits, Bio-Mimetic Control Research Center, RIKEN, Atsuta, Nagoya, Aichi 456, Japan

In the neostriatum aspiny interneurons containing a calcium binding protein, parvalbumin, had been known as GABAergic. The purpose of the present study is to show other striatal interneurons are also GABAergic. Male Wistar rats (weight 100-200g) were used. Using mirror image immunohistochemical methods with antiserum against GAD67 (one of the two forms of synthetic enzyme for GABA) revealed that two other classes of interneurons were also GABAergic in the neostriatum of colchicine treated rat. Those were nitric oxide synthase-(NOS), which also contained somatostatin, and calretinin- (another calcium binding protein) positive cells. We also investigated GABA immunoreactivity in the terminals of striatal interneurons of untreated rats. Aspiny interneurons were identified in isolated slices of striatum from young rats (18-22 days postnatal) by whole cell, current-clamp recording, followed by intracellular injection of biocytin. Three types of neostriatal interneurons were identified, which were long-lasting afterhyperpolarization (LA) cells, fast spiking (FS) cells, and low-threshold spike (LTS) cells and those corresponded to cholinergic large aspiny cells, parvalbumin-containing cells, and NOS-containing cells, respectively. After overnight fixation, the slices were histochemically processed and embedded in Epon. Electron microscopic observations of postembedding GABA staining revealed that the terminals of FS cells and LTS cells were immunoreactive for GABA, however, the terminals of LA cells did not show GABA immunoreactivity. These results suggest that GABAergic interneurons are heterogeneous in neostriatum.

362.17

INCREASED SUBTHALAMIC NEURONAL ACTIVITY IN DOPAMINERGIC LESIONED RATS NOT RELATED TO A DECREASED PALLIDAL INHIBITION. J.Féger*, M.Mouroux and O-K.Hassani Lab. de Pharmacologie, Fac.Pharmacie, Paris, France.

There is a general agrement to describe an increased activity of the subthalamic neurons in the animal models of Parkinson's disease: MPTP-treated monkeys or 6-OHDA-induced dopaminergic lesion in rats. In this last species, this lesion induces an increase of neurochemical index in the inhibitory striatopallidal neurons. Inhibition of the pallidal neurons is believed to release the activity of the subthalamic neurons. Following this proposal, a pallidal or a dopaminergic lesion should induce the same quantitative and qualitative changes in subthalamic neurons. Unit activity were recorded in the subthalamic nucleus in three groups of anæsthetized (urethane) rats: control, rats with a pallidal or midbrain dopaminergic lesion. The lesion of the globus pallidus was induced by a microinjection of ibotenic acid (20 nmol in 400 nl). The dopaminergic midbrain lesion was obtained with a microinjection of 6-OHDA (8 μg in 2 μl) in the substantia nigra. In the control rats, the pattern of firing was slightly irregular with a mean value of 14.7 ± 0.4 spikes per second. In the rats with a pallidal lesion performed 8 to 10 days before, the activity was more regular than in the control rats and the discharge rate was between 13.7 and 22.6 sp/sec with a mean value of 17.6 ± 0.6 sp/sec ($p < 0.05$). In the 6-OHDA-lesioned rats three weeks before, the pattern of activity was clearly irregular with bursts and pauses. The discharge rate was between 22.3 and 36.7 sp/sec with a mean value of 30.3 ± 1 sp/sec ($p < 0.001$). The activity of the pallidal neurons was recorded in control and 6-OHDA-lesioned rats. The mean value were 29.5 ± 1 in control rats and 25.2 ± 2.8 with an irregular and bursting activity in the dopaminergic lesioned rats. The differences in the increase of the discharge rate of the subthalamic neurons, 19.5% versus 105.7% and in their pattern of unit activity dispute the importance of an inhibition/disinhibition processus in the relation between the striatum and the subthalamic nucleus in midbrain dopaminergic lesioned animals. These results fit well with anatomical and neurochemical data suggesting that the globus pallidus is more than a simple relay in the striato-pallido-subthalamic circuit.

362.18

SUBTHALAMIC ABLATION REVERSES CHANGES IN BASAL GANGLIA OXIDATIVE METABOLISM AND MOTOR RESPONSE TO APOMORPHINE INDUCED BY NIGROSTRIATAL LESION

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In Parkinson's disease (PD), dopaminergic denervation of the striatum leads to a cascade of functional alterations in basal ganglia circuitry which causes overactivity of the output nuclei of the circuit, substantia nigra pars reticulata (SNr) and medial globus pallidus (MGP). Increased firing of the subthalamic nucleus (STN), which sends excitatory projections to both SNr and MGP, seems to be mainly responsible for this phenomenon. The pivotal role of STN is further suggested by the fact that reduction in STN activity is associated with amelioration of PD symptoms. The aim of this study was to investigate the effect of selective STN ablation on basal ganglia oxidative metabolism, as well as on the motor response to apomorphine, in rats with prior nigrostriatal lesion. Sprague-Dawley rats received a unilateral sterotaxic injection of 6-hydroxydopamine in the substantia nigra pars compacta (SNc) and medial forebrain bundle. Eight months later, the same animals underwent an ipsilateral STN lesion by means of an N-methyl-D-aspartate injection (25 nmoles). Histochemical staining for succinate dehydrogenase and cytochrome oxidase were performed on slide-mounted brain sections. Side-to-side differences in staining intensity were analyzed by means of computerized densitometry. Rotational response to i.p. apomorphine was tested before and after STN ablation.

The nigrostriatal lesion alone induced ipsilateral increases in enzymatic activity of both SNr and entopeduncular nucleus (the rodent homologue of human MGP). This phenomenon was completely abolished, or reversed, by STN ablation. Complete STN lesion also abolished apomorphine-induced rotational behaviour. These results confirm the pivotal role of STN in the modulation of basal ganglia output and shed further light on the neural basis of the beneficial effect of STN manipulation on PD symptomatology.

362.19

SUBTHALAMIC NUCLEUS NEURONAL ACTIVITY IS INCREASED BY DOPAMINE D_1 RECEPTOR AGONISTS. D.S. Kreiss*, L.A. Anderson, J.R. Walters. NIH, NINDS, ETB, Bethesda, MD 20892.

By providing links from the cortex and globus pallidus to the substantia nigra and entopeduncular nucleus, the subthalamic nucleus (STN) plays an integral role in the transmission of information through the basal ganglia. The regulation of STN neuronal firing rates by dopamine (DA) D_1 receptors was investigated using *in vivo* extracellular single unit recording techniques in locally-anesthetized, paralyzed male rats. STN neuronal firing rates were dose-dependently increased by the selective D_1 receptor agonist SKF 38393 (1.25-20 mg/kg, iv); 20 mg/kg increased firing rates to 269% of basal rates. A similar increase was produced by the D_1 agonist SKF 82958 (0.43 mg/kg, iv). SKF 38393- and SKF 82958-induced increases in firing rates were reversed by the D_1 antagonist, SCH 23390 (0.5 mg/kg iv). STN neuronal firing rates were also increased by the nonselective DA agonist apomorphine (0.32 mg/kg, iv), but not by the indirect acting DA agonist amphetamine (1.6, 5.0 mg/kg, iv). Apomorphine-induced firing rate increases could be prevented by SCH 23390 or chloral hydrate or ketamine anesthesia. Local administration of SKF 82958 (3.0 μg/0.3 μl) into the STN increased STN neuronal firing rates 2 to 3-fold; these increases could be reversed by systemic SCH 23390 (0.5 mg/kg, iv). Local administration of SCH 23390 (0.3 μg/0.3 μl) into the STN prevented systemic SKF 38393 (20 mg/kg, iv) from increasing STN firing rates. These results support an excitatory role of D_1 agonists on STN neuronal activity, which is mediated, at least in part, by D_1 receptors located within the STN itself. These findings contradict current models of basal ganglia function which predict an indirect inhibitory effect of DA on STN neuronal activity resulting from disinhibition of globus pallidus neuronal activity.

CEREBELLUM: BEHAVIOR, DEVELOPMENT, MODELS

363.1

VISUALLY GUIDED REACHING WITH THE FORELIMB CONTRALATERAL TO A "BLIND" HEMISPHERE: CONTRIBUTIONS OF THE CEREBELLUM. H.E. Savaki*, C. Kennedy[1], L. Sokoloff[1] and M. Mishkin[1]. Dept. Basic Sciences, Div. Medicine, Univ. of Crete, Iraklion, Crete, Greece 71110, and [1]NIMH, Bethesda, MD 20892.

The 2-[^{14}C]deoxyglucose method was used to map local metabolic activity in the cerebellar cortex as well as its major inputs and targets in monkeys performing visually guided reaching with the left forelimb. The study was carried out both in normal (N) monkeys and in monkeys that had the right cerebral hemisphere deprived of visual input by right optic tract section combined in some cases with forebrain commissurotomy (TC). The metabolic mapping revealed significant activations of the left cerebellar hemispheric extensions of vermian lobules V, VI, and VIII in all monkeys . In the N monkeys, however, the activations were somewhat smaller in the lateral ("visual") than in the paravermal ("motor") zones of these hemispheric extensions, whereas in the TC monkeys the activations in these two zones were equally large. The findings suggest that the activated loci in the left cerebellum combine (i) visual information about the target relayed by either cerebral hemisphere and (ii) sensorimotor information concerning intended and actual movements of the left forelimb relayed by the right cerebral hemisphere and the limb, and then send this integrated information back to the motor system of the right cerebral hemisphere, which is thus enabled to guide the left forelimb to the visual target whether it is visually intact or "blind" (Savaki et al., J.Neurosci. 13:2772-2789, 1993).

363.2

IMPAIRMENTS IN PLANNING, COORDINATION, AND ADAPTATIVE ADJUSTMENTS OF REACHING PRODUCED BY ANTERIOR AND POSTERIOR INTERPOSITUS AND RED NUCLEUS INACTIVATION. J.H.Martin*, A.Hacking, S.Cooper, C. Ghez. Ctr. Neurobiol & Behav, Columbia Univ. and NYS Psychiatric Institute, New York, NY 10032

To investigate cerebello-rubral control of limb movement we studied the effects of muscimol inactivation of AIP, PIP and RN on performance of a reaching task in the cat. Animals reached into a narrow food well at various heights, distances and inclinations to grasp a small piece of beef.

AIP inactivation produced under-reaching, wrist and tip paths that were more curved and variable, and increased movement time. Animals were unable to reprogram paw kinematics to reach over obstacles. While elbow scaling to target height was preserved, associated motions of shoulder, wrist and digits, needed to linearize paw path and to adapt the paw to the food well, were reduced or eliminated. PIP inactivation produced over-reaching but did not impair obstacle avoidance. While paths were more variable, like AIP, path linearity, limb deceleration, and movement time were unaffected. As noted previously for AIP, (Cooper, et al; 1993) impairments in interjoint coordination and end-point biases during PIP inactivation also resulted, in part, from failure to take account of interjoint interaction torques.

Trajectory variability was increased during RN inactivation but without biases in movement end-points or increases in movement time. Rubral inactivation also produced deficits in interjoint coordination and reaching over obstacles that resembled those during AIP inactivation.

Our results support the idea that the intermediate cerebellum and the red nucleus, play a critical role in several aspects of forelimb control needed to assure spatially accurate paw paths, especially in specifying muscle contractions and joint torques. We have previously reported (Martin and Ghez, 1993) that impaired obstacle avoidance and over-reaching occur during rostral motor cortex inactivation, suggesting a role for the corticospinal system in expressing some of these deficits. (NS31391)

363.3

CEREBELLAR INACTIVATION ABOLISHES THE CAPABILITY OF CATS TO COMPENSATE FOR UNEXPECTED BUT NOT EXPECTED PERTURBATIONS OF A REACH MOVEMENT. Yu. Shimansky*, J.-J. Wang, V. Bracha, J.R. Bloedel. Barrow Neurological Institute, Phoenix, AZ 85013.

The purpose of this study was to determine the effects of inactivating concurrently the interposed and dentate nuclei on the capacity of cats to compensate for the perturbation of a forelimb reaching movement. Cats were trained to reach for a vertical bar and move it to a food reward zone through a straight groove in a horizontal template. During the reach a perturbation was applied consisting of an external force that was engaged at a certain point during the reach and transiently modified the trajectory of the limb. Once engaged, the force increased linearly as the paw moved towards the bar. Perturbations were applied either on successive trials (predictable) or randomly (unpredictable). Normal cats compensated for predictable perturbations in a few trials and for unpredictable perturbations over 1 - 2 days. The animals with the interposed and dentate nuclei inactivated by focal 1μl injections of muscimol (800 ng/μl) required significantly more trials to adapt to the predictable perturbation, and they were unable to compensate for unpredictable perturbations applied at low enough frequencies to be unexpected. However, when the perturbation probability was higher than 50%, compensatory behavior was present in *every* trial independent of whether a perturbation actually was applied. After considerable practice, the compensation consisted of a motor pattern that was effective in both perturbation and non perturbation trials. Furthermore, the the strategy employed to compensate for unpredictable perturbations during cerebellar nuclear inactivation was qualitatively different than the strategy employed by control animals. The data suggest that the cerebellum is necessary for implementing adaptive motor strategies requiring short-latency modifications of ongoing movements on a trial by trial basis. Supported by NIH grants NS30013 and NS21958.

363.4

EFFECT OF CEREBELLAR INACTIVATION ON THE ACQUISITION AND RETENTION OF PRISM ADAPTATION DURING GOAL-DIRECTED REACHING IN CATS. M.S. Milak*, M. Kozicki, V. Bracha, J.R. Bloedel. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

Experiments were performed to test the hypothesis that adaptation to optical prisms could occur during the inactivation of the interposed and dentate nuclei in cats and that inactivation performed after acquisition would not eliminate retention. After cats were trained to reach for a vertical bar under normal vision and pull it through a straight groove to a reward zone, they were adapted to 20 degree Fresnel prisms which displaced the image to the right. Throughout the experiment the cat could visualize its paw during the terminal phase of the trajectory. While recording the time course and kinematics of the reach, the effects of injecting 1 μl buffered saline simultaneously in the interposed and dentate nuclei on the adapted behavior, extinction, and readaptation to a novel prism (40 degrees in the opposite direction) were determined. Using the same protocol, nuclear inactivation (1 μl of a 800 ng/μl muscimol solution) produced a very brief (2-4 trials) decrease in the animals' capacity to reach the bar, after which they resumed the performance of accurate reaches despite the ataxia. Furthermore, during nuclear inactivation the cats were able to adapt quickly to the novel 40° prism. Thus, in this paradigm the inactivation of the intermediate and lateral cerebellar nuclei in cats did not eliminate the previously acquired adaptation, nor did it block their adaptation to novel prisms. Supported by NIH grants NS 30013 and NS 21958.

363.5

INVOLVEMENT OF THE CEREBELLAR INTERPOSED NUCLEUS IN REFLEXIVE AND VOLUNTARY FORELIMB MOVEMENTS. N.K. Winters, K.B. Irwin, F.P. Kolb, J.R. Bloedel*, V. Bracha. Barrow Neurological Institute, Phoenix, AZ 85013.

Our previous studies in the cat demonstrated that the cerebellar nucleus interpositus (NIP) is involved in the expression of classically conditioned and unconditioned withdrawal reflexes (WR) in several skeleto-muscular effector systems. Recently, we suggested that WR circuits are implemented by the nervous system in the execution of more complex voluntary limb movements. This hypothesis predicts that inactivation of the NIP should result in qualitatively similar performance deficits within the same effector system regardless of the nature of the movement.

To test this notion, we compared the NIP inactivation-induced deficits in forelimb WR to the deficits in forelimb movements during different modes of locomotion and reaching. The effects of muscimol inactivations of the NIP on the forelimb WR were: a) reduced amplitude of flexion; b) changes in the limb's trajectory; c) impaired precision placement of the limb during transition from withdrawal to a quadruped stance; and d) hypermetria of associated corrective postural responses. Qualitatively similar deficits were observed in forelimb movements during reaching and locomotor tasks. The similarity of NIP inactivation-induced deficits in a variety of forelimb movements could be at least partly attributed to a dysfunction of shared reflex substrates.

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363.6

BUFFER KINETICS INFLUENCE THE FIRING PROPERTIES OF A PURKINJE CELL MODEL WITH REALISTIC CALCIUM DYNAMICS P. Smolen* and E. De Schutter. Born Bunge Foundation, University of Antwerp, 2610 Antwerp, Belgium.

Previously used compartmental modeling to characterize the electrophysiology of cerebellar Purkinje cells (De Schutter and Bower, J. of Neurophysiology: 71, 375-400, 1994), had primitive (single-pool) calcium dynamics. We have now incorporated more realistic calcium handling, and updated channel kinetics, into this Purkinje cell model. Calcium diffuses radially between cylindrical shells (but not longitudinally between compartments), with diffusible buffers incorporated, and with channels and pumps in the outer shell. K-Ca channels are coupled to the calcium in the outer shell. We simulate responses to somatic current injection.

At moderate current injections, we find that a typical pattern of slow dendritic Ca bursting can only be obtained if most of the calcium buffer has slow kinetics (relaxation time on the order of 100 ms). The bursting pattern becomes unphysiological upon increasing the proportion of fast buffers, which are however known to dominate Ca buffering in the cytoplasm of some other cell types. Also, K-Ca channels must be sensitive to calcium on the order of 1 uM to drive dendritic bursting. Given these conditions, typical dendritic bursting is obtained for 2-3 seconds. In this model, large gradients of calcium do not develop within spiny or smooth dendritic compartments due to their narrow diameter. We are exploring an alternative hypothesis that K-Ca channels and Ca channels are clustered such that K-Ca channels are exposed to localized peaks of high "domain" calcium.

Regarding channel properties, a very high density of somatic NaF channels and a low density of somatic CaP channels are both required to properly reproduce somatic spiking.

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363.7

CEREBELLAR COMMUNICATIONS WITH THE PREFRONTAL CORTEX: EFFECT ON HUMAN COGNITIVE SKILLS. A.L.Leiner, H.C.Leiner, and C.R.Noback*. Channing House, 850 Webster Street (635), Palo Alto, CA 94301; & Dept. of Anatomy and Cell Biology, Columbia University, College of P&S, 630 West 168 Street, New York, NY 10032.

Recent discoveries about the structural and functional connections of the cerebellum to cognitive areas of the cerebral prefrontal cortex have led us to analyze how the neural projections from the neocerebellum can convey complex messages to the cerebral cortex. These neural projections are organized into small bundles of fibers, called fascicles, which can convey not merely a sequence of signals over each individual fiber but also various combinations of signals over each individual fascicle. In information-processing terms, such combinations of signals are termed "symbols"; and the various "symbols" comprise the neural "alphabet" by which the fascicle can communicate what to do and when to do it. The cerebellum, by learning to project such messages to the proper areas of the cerebral cortex at the proper times, can help the cerebro-cerebellar system to automatize some human motor, cognitive, and language skills.

363.8

A QUALITATIVE MODEL OF CEREBELLAR PLASTICITY THAT LEARNS TEMPORAL PATTERNS. J. L. Krichmar*. PMI Incorporated, 11744 Parklawn Dr., Rockville, MD 20852.

A qualitative computer model of the cerebellar cortex is presented to investigate the ability of the cerebellum to learn sequences. The cerebellum provides control to motor areas of the nervous system by issuing sequences of commands. The cerebellum must decide which motor command is appropriate based on sensory afferents. The vast array of sensory information that converges on a Purkinje cell (PC) makes the cerebellum suitable for pattern recognition. This study proposes that: 1) associative learning occurs by correlating mossy fiber (MF) sensory afferents with MF motor efference copy, 2) climbing fiber input signifies a learning phase by gating in sensory input for evaluation, and 3) the cerebellum learns a motor program that is made up of sequences of motor commands. A biologically plausible model of a PC population is developed using the artificial intelligence technique "Qualitative Reasoning". The qualitative reasoning neuronal model (QRN) is significantly more computationally efficient than differential equation models of neurons, yet unlike artificial neural networks, QRN does not abstract important aspects of neural function. QRN qualitatively describes the dynamics and kinematics of the channels existing in a PC. The simulated PC population employs a learning rule based on current evidence of Long-Term Depression at the parallel fiber-PC synapse. QRN models calcium spikes in the PC dendritic tree as well as sodium spikes in the PC soma. The output, membrane potential as a function of time, is derived from the qualitative interaction between the model's parameters. QRN demonstrates the ability to learn multiple sequences by making associations from its inputs. The model suggests that the role of the climbing fiber is to divide cerebellar motor control into a motor phase and an evaluation phase. The QRN modeling technique may be a useful tool for addressing other open questions in neuroscience.

363.9

A MODEL FOR HOW THE CEREBELLUM ANTICIPATES SENSORY INPUTS AND MODULATES THE VESTIBULO-OCULAR REFLEX (VOR). Olivier J.M.D. Coenen* and T.J. Sejnowski. UC San Diego, Howard Hughes Medical Institute, CNL, The Salk Institute, PO Box 85800, San Diego, CA 92186-5800.

The gain of the VOR in monkeys is modulated by many inputs including eye position, target distance and otolith signals. We have developed a kinematic equation for the VOR that describes how the signals from the vestibular canals and otoliths can be combined with eye position and vergence angle signals to give the ideal VOR response for any head rotation and translation in three dimensions. By simulating head rotations for different locations of the axis of rotation, and by including delayed otolith and canal inputs, we obtain results which quantitatively match the VOR dynamics observed in monkeys (Snyder & King; J.Neurophys. 67:4,1992).

We have also developed a dynamic model for how the nonlinear modulation of the VOR could be learned. Snyder & King (Vision Res.32:3,1992) have shown that the gain of the VOR changes in anticipation of vergence movements and have suggested that a central command signal could drive the vergence system and the VOR response via the cerebellum. Our adaptive cerebellar model describes how such a central command signal can 1) become associated with the modulation of the VOR and 2) produce anticipatory modulation of the VOR to compensate for retinal slips which may occur hundreds of milliseconds later. The model is a dynamical neural network represented by a set of differential equations. Computer simulations of the model show how the cerebellum *may* construct predictive representations of the retinal slip signals which can modulate the gain of the VOR in anticipation of new behavioral conditions.

363.10

THE EFFECT OF CORRELATED INPUTS ON SPIKE TRAINS IN A PURKINJE CELL MODEL: D. Jaeger*, E. De Schutter^ and J.M. Bower. Div. of Biology 216-76, Caltech, Pasadena, CA 91125 and U. of Antwerp - UIA^, Belgium

Electrophysiologists frequently use the cross-correlation method to examine functional connectivity between two simultaneously recorded cells. Classical analysis suggests that the shape and amplitude of peaks in cross-correlograms is indicative of reciprocal or shared input. In the present study we use a previously constructed realistic Purkinje cell model (De Schutter and Bower, J. Neurophysiol. 71: 375-419) to examine how single cell properties may influence the expression of input correlations in cross-correlograms.

Cross-correlograms were constructed between the spike trains of two simulation runs with controlled correlations in synaptic input superimposed on random background synaptic activation. As expected from classical analysis, the presence of simultaneous excitatory inputs was associated with a narrow peak in the cross-correlogram, while simultaneous inhibitory inputs lead to a much broader peak. However, the size of peaks in our cross-correlograms was dependent on multiple variables and therefore was not a good indicator of the strength of input correlations. In addition, the same set of shared inputs could lead to peaks of different amplitude and shape depending on the pattern of uncorrelated input. For example, if one simulation had a slower baseline spike rate than the other, the peak in the cross-correlogram due to simultaneous excitation had an offset of several msec from the center bin due to dendritic properties of the cell. Such a delayed peak is frequently interpreted as indicating an excitatory connection from one cell onto the other, which would be erroneous in the present case. Overall, the present study indicates that observed cross-correlations in spike trains can be strongly influenced by single cell properties, thus confounding the interpretation with respect to synaptic input.

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363.11

A NETWORK MODEL OF THE SAGITTAL OLIVO CEREBELLAR COMPLEX. J.L. Contreras-Vidal*, J.R. Bloedel, and G.E. Stelmach. Lab. of Motor Control, Arizona State University, Tempe, AZ 85287 - 0404 and Barrow Neurological Institute, Phoenix, AZ.

The goal of this study is to assess the operational role of the cerebellum during on-going motor behavior by developing a network model of this system. The model explores the spatiotemporal interactions involving the sagittal organization of the cerebellar cortex, cerebellar nuclei, and inferior olive. Non-linear ordinary differential equations are used to model the membrane potential for each cell type (short-term activations), and their physiological action (inhibitory or excitatory). The network 1) differentiates between granule cell ascending axons and parallel fibers; 2) characterizes the Purkinje cell dendritic tree as a single summing node at the soma; 3) accounts for the short-term enhancement of Purkinje cell response to inhibitory and excitatory inputs; and 4) models electrotonic coupling at the inferior olive.

The dynamic response generated by the model was compared with actual physiological results. The model was able to produce spatiotemporal patterns of activity similar to those recorded physiologically in response to brief natural stimulation, and provides theoretical support for the Dynamic Selection Hypothesis regarding the functional basis for the sagittal organization of the cerebellum.

Supported by RS Flinn Foundation and NIH grant NS21958. Contreras - Vidal is on leave from Monterrey Institute of Technology, México.

363.12

SIGNAL TRANSDUCTION IN A CEREBELLAR GRANULE CELL: A MODELING APPROACH. Huo Lu*, F.W. Prior[1] and L.J. Larson-Prior. Dept. of Neuroscience & Anatomy, Dept. of Radiology[1], Penn State Univ. College of Med., Hershey, PA 17033.

The cerebellar granule cell (GC) receives sensory data from incoming mossy fibers which synapse at the tip of the GC dendrite within the glomerulus. Cerebellar granule cell axons may take their origin from the soma, but more frequently arise from one of the dendrites (Palay and Chan-Palay (1974) *Cerebellar Cortex Cytology and Organization*). This architecture may preferentially weight signals on the dendrite from which the axon arises. Thus, differences in the location of the axon hillock region may have a significant impact upon the transduction of synaptic input.

A fifteen compartment model of a cerebellar granule cell, modified from Gabbiani *et al.* (*J. Neurophysiol* (1994) 72:999) was developed and accurately predicts GC behavior. The model was validated by simulation of voltage clamp responses that matched those experimentally derived from recordings of rat cerebellar GCs (Cull-Candy *et al.*, *J. Physiol.* (1989) 414:179; De Waard *et al.*, *Eur. J. Neurosci.* (1991) 3:771), and by current clamp simulations which matched reported GC firing patterns.

The model explicitly includes compartments for the axon hillock and proximal axon. Four dendrites are modeled, each consisting of two compartments and a terminal bulb on which all synaptic contacts are made. One proximal dendritic compartment contains active conductances. The axonal compartments were placed either at the soma or on the active dendritic compartment and tested for their responses to synaptic activation at each terminal bulb. Inputs varied in their number, frequency and phase relationships and were transduced via activation of ionotropic (AMPA and NMDA type) and metabotropic glutamate receptor subtypes.

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363.13

THE POSTEMBRYONIC DEVELOPMENT OF THE CEREBELLUM IN GYMNOTIFORM FISH. G.K.H. Zupanc*, I. Horschke, R. Ott and G.B. Rascher. Department of Physical Biology, Max Planck Institute for Developmental Biology, D-72011 Tübingen, Federal Republic of Germany.

In contrast to mammals, in teleost fish the capability for the production of new neurons and glial cells during adulthood is very pronounced in many brain regions. A previous quantitative mapping of the proliferation zones in the brain of adult *Apteronotus leptorhynchus* (Teleostei, Gymnotiformes) has shown that 75% of all mitotic active cells are situated in the cerebellum [Zupanc and Horschke, *J. Comp. Neurol.* **353**, 213-233, 1995]. By employing the thymidine analogue 5-bromo-2'-deoxyuridine, we have, in the present study, investigated the postembryonic development of this brain region in detail.

In the corpus cerebelli and the valvula cerebelli, the vast majority of newborn cells originates in the respective molecular layers. Within the first few days of their life, these cells migrate towards specific target areas, namely the respective granule cell layers. Ten days after birth, more than 80% of them have reached the granule cell layers of the corpus cerebelli, valvula cerebelli pars lateralis, and valvula cerebelli pars medialis. In the caudal part of the cerebellum, the granule cell layer of the eminentia granularis pars medialis displays the highest mitotic activity. From there, the cells migrate through the adjacent molecular layer to the granule cell layer of the eminentia granularis pars posterior, and within 10 days 70% of the newborn cells have reached this target area. Time-lapse experiments employing survival times between 1 hour and 161 days after the administration of 5-bromo-2'-deoxyuridine (n = 48 fish) demonstrated that a high percentage of the newly generated cells survives for long periods of time. Volumetric measurements of the various cerebellar subdivisions in fish of different sizes (n = 12 fish) indicate that a large fraction of these cells is added to the population of already existing cells, thus resulting in a permanent growth of the target areas and their associated structures.

363.14

AN EARLY CRITICAL PERIOD IN OLIVOCEREBELLAR INTERACTIONS DEFINED BY TARGETED ABLATION OF PURKINJE CELLS. T. Chu*[1,2] and J. Oberdick[2,3]. [1]Neuroscience Program, [2]The Neurobiotechnology Center, and [3]The Department of Cell Biology, Neurobiology and Anatomy, The Ohio State University, Columbus, OH 43210.

In last year's abstract we reported that olivary neurons (ONs) begin to degenerate at P4 in the novel mouse mutant L7ADT as observed in Nissl preparations, and that by P15 few ONs are detectable (T. Chu et al. 1994, Soc. Neurosci. Abstr.). However, using an olive-specific probe (Brn-3), we now show by *in situ* hybridization that there are clearly Brn-3 positive ONs in P41 mutants. The size of the mutant olive at this relatively late age is reduced along the rostrocaudal, the mediolateral and the dorsoventral axes, with uniformly distributed cell loss. In contrast, at P4 the most severe cell loss occurs at the medial accessory olive (MAO). We further performed TUNEL assay, an assay that detects "apoptotic" cell death, on P4 brain sections and found specific nuclear labeling in only the caudal olive, especially the MAO. The caudal olive has been shown to project to the medial cerebellum (M. Paradies et al. 1993, Dev. Dynam. 197:125-145), which is the part of the cerebellum which first shows Purkinje cell (PC) degeneration in our mutant. Thus these data suggest an early target-dependent and topographically oriented interaction between ONs and PCs. TUNEL assay using P7 or older brain sections, however, fails to detect any specific nuclear labeling above that in wild-type mice, even though the data from Nissl stain and *in situ* hybridization demonstrate that ON loss continues as the animal approaches adulthood. Therefore, we propose that ONs are most vulnerable to PC loss during the first four or five postnatal days in the mutant and that the severity of this dependence tapers off as the animal ages. This is consistent with the observation of a period of olive cell loss in normal development that extends from P3 to P5 in the rat (N. Delhaye-Bouchaud et al. 1985, J. Comp. Neurol. 232:299-308.).

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CEREBELLUM: CLINICAL STUDIES

364.1

KINESTHETIC CONTROL OF MOVEMENT IN CEREBELLAR DISEASE. Stephen E. Grill[1]*, Mark Hallett[1], and Lisa M. McShane[2], Human Motor Control Sect.[1], Biometry and Field Studies Branch[2], NINDS, NIH, Bethesda, MD 20892.

Normal subjects use kinesthetic information to control movement (Cordo et al 1993). Cerebellar dysfunction may be in part due to improper use of kinesthetic information. We evaluated the ability to use kinesthetic information during movement in 14 patients with cerebellar degenerations and 14 normal controls.

Subjects were seated with the right index finger connected to a rotary torque motor and the left hand holding a switch triggered by the thumb. They viewed an oscilloscope on which one beam represented the right index finger angle and the other corresponded to a joint angle at which they were to trigger the switch. Initial finger position was 18 deg extended relative to the trigger angle. In training, 500 ms after a tone the index finger was moved at 20 deg/s in the flexor direction. After each trial, subjects received feedback on the oscilloscope showing how much they undershot or overshot. Further training used the same velocity but the beams vanished at trial onset, and reappeared upon completion to give feedback. For the experimental session 200 flexor movements ranging from 10 to 88 deg/s were imposed on the index finger in pseudorandom order. Subjects adjusted the timing of triggering depending on the velocity. An additional 200 similar trials were run in which subjects reacted as quickly as possible to perception of movement. EMG was recorded from the flexor pollicis brevis muscle in a subset of the subjects.

Cerebellar patients performed much worse compared to controls. Although able to trigger accurately when the movements were slow, they triggered too late for faster movements. The difference between the minimum reaction time and the minimum time to produce coordinated triggering reflects kinesthetic processing time and was not different in patients and controls. Poor patient performance was instead due to prolonged reaction times which the EMG recordings indicate was partly due to longer time from onset of EMG to switch press.

364.2

PERFORMANCE OF CEREBELLAR PATIENTS IN A MULTI-JOINT, VISUOMOTOR ADAPTATION TASK. S. J. Shanbhag*, J. Matsumoto, E. Ahlskog, R. F. Poppele, and T. J. Ebner. Departments of Neurosurgery and Physiology and Graduate Program in Neuroscience, University of Minnesota, Minneapolis, MN 55455, and Department of Neurology, Mayo Clinic, Rochester, MN 55905.

The role of the cerebellum in motor learning and adaptation remains a controversial issue. We have examined the influence of the cerebellum in motor learning through the use of a visuomotor adaptation task to assess the motor learning deficits in human patients with acute cerebellar damage. Subjects were seated in front of a video display and controlled the movement of a cursor on the display using a planar, 2-joint manipulandum. A sling supported the elbow to restrict movement to the horizontal plane. Subjects were required to hold the cursor in a central "start box" until one of eight equidistant "target" boxes appeared; the cursor then had to be moved to and held inside the target box within a movement time window. After a practice period on a normal hand-cursor movement relationship, the X and Y cursor gains were reversed. At the end of this learning period, the initial gains were restored. Our previous study with this task in normal subjects (Shanbhag & Ebner, *Soc Neurosci. Abstr.* 1994) showed that learning may be characterized by two phases of movement kinematics. The first, feedforward phase is rapidly reorganized during the learning of the visuomotor transformation while the second phase, which utilizes visual feedback, is modified more gradually. Patients with isolated, stable cerebellar lesions did not display these characteristics, but instead showed increased movement times and multiple corrective movements as a result of dysmetria. However, quantification of the initial direction of movement under the different gain conditions suggests that the patients are able to adapt to the changes in the hand-cursor relationship. Supported by NIH grants NS-18338 and NS-28633.

364.3

LOCALIZATION OF SPECIFIC REGIONS OF THE CEREBELLAR SYSTEM INVOLVED IN PRISM ADAPTATION. T.A. Martin*, J.G. Keating, H.P. Goodkin, A.J. Bastian, and W.T. Thach. Dept. of Anatomy and Neurobiology and IWJ Rehab. Res. Inst., Washington University School of Medicine, St. Louis, MO, 63110.

To test prior assertions that the cerebellum is involved in wedge prism adaptation of limb movements directed to visual targets (Baizer and Glickstein, 1974; Weiner et al., 1983), we studied patients with cerebellar damage. Our preliminary results (Thach et al., Soc. Neurosci. Abs. 551.2, 1991) supported the hypothesis that the olivocerebellar system plays an important role in this visuomotor adaptation. Further studies of patients with specific lesions of the cerebellum or its inputs or outputs have been done to localize the regions of the olivocerebellar system that are necessary for normal prism adaptation.

Patients with generalized cerebellar atrophy, lesions of the superior vermis, damage of the inferior olive, infarcts in the distribution of the posterior inferior cerebellar artery (PICA; possibly with inferior cerebellar peduncle involvement), and focal infarcts in the contralateral basal pons or ipsilateral middle cerebellar peduncle had absent or impaired ability for prism adaptation. This group often showed mild or no limb ataxia. By contrast, subjects with infarcts in the distribution of the superior cerebellar artery territory (SCA; involving the anterior superior surface of the cortex and the dentate nucleus) or in the cerebellar receiving zones of the thalamus usually adapted normally. This group often had marked limb ataxia.

These results implicate climbing fibers from the contralateral inferior olive via the ipsilateral inferior cerebellar peduncle, mossy fibers from the contralateral pontocerebellar nuclei via the ipsilateral middle cerebellar peduncle, and superior vermal cerebellar cortex as being critical for this adaptation. Not necessary are lateral hemisphere cortex and the dentato-thalamic projection. (Supported by ONR N00014-92-J-1827 and NIH Grant NS12777)

364.4

SCHEMATIC MODEL OF SHORT- AND LONG-TERM ADJUSTMENTS OF EYE-HAND COORDINATION IN THROWING W.T. Thach*, T.A. Martin, J.G. Keating, H.P. Goodkin, and A.J. Bastian. Dept. of Anatomy and IWJ Rehab. Res. Inst., Washington University School of Medicine, St. Louis, MO, 63110.

In throwing at a visual target while wearing laterally displacing wedge prisms, subjects may undergo two types of adjustment of the gaze-throw angle. Over a short term (10-50 throws), the subject changes this angle to a new calibration value (Kane and Thach, 1989; Thach et al., 1992). The new calibration is retained for a day or longer. Over a longer term (6000 throws, 6 weeks) of alternate prism/no-prism throws, the subject acquires a second prism calibration in addition to the no-prism calibration. He hits the target upon each first throw, prism or no-prism. The two calibrations are retained without practice for 18 months or longer.

To determine the anatomic components of the gaze-throw recalibration in two long term prism/no-prism trained subjects (Martin et al., 1993), we video-recorded positions of head-in-space and shoulders-in-space while throwing with and without prisms. Knowing that eyes foveated the target and that throws hit the target, we computed the angular positions of eyes-in-head, head-on-trunk, and trunk-on-arm. We found that for both subjects, the gaze adjustment was not confined to any one set of 2 members (e.g. eyes-in-head), but instead was distributed across all 3 sets of coupled body parts. Moreover, each subject had a different distribution of coupling changes across the 3 sets of coupled body parts.

We have created a schematic model based on the knowledge that the cerebellum plays roles both in the coordinated movement of multiple body parts and in motor learning and the assumption that the inferior olive climbing fiber discharge is sensitive to the direction of visually-detected error of movement and can adjust movement performance through LTD of pf-Pc contacts. The model uses these features to account for (1) the distribution of the gaze-throw short- and long-term adjustments across multiple body parts, (2) the privacy of adaptation to the trained body parts or task, (3) the differences between short-term adaptation and long-term acquisition of skills. (Supported by ONR N00014-92-J-1827 and NIH Grant NS12777)

364.5

RESPONSES TO REPEATED LOCOMOTOR PERTURBATIONS IN CEREBELLAR PATIENTS.

M.K. Rand*, D.A. Wunderlich, P.E. Martin, G.E. Stelmach, J.R. Bloedel. Arizona State Univ., Tempe, AZ, 85287; Barrow Neurological Institute, Phoenix, AZ, 85013.

The cerebellar patients' locomotor behavior in response to treadmill perturbations was studied to test whether they can establish strategies for responding to unexpected perturbations that minimize alternations of gait. The perturbation, which lasted about 1.5 sec and consisted of a sudden slowing of the treadmill followed by a sudden increase in speed up to the original speed, was given repeatedly at random intervals during treadmill walking. The cerebellar patients participating in this study had recovered from mass lesions affecting the cerebellum extensively enough so that no clinical locomotor deficit was apparent in normal over-ground walking.

Age matched control subjects could modify their gait in a manner that permitted them to locomote with near normal stepping and EMG pattern in the gastrocnemius and anterior tibial muscles after adaptation to the perturbation. In contrast, cerebellar patients attempted to compensate for the perturbation with a variety of strategies, none resulting in the re-establishment of a relatively normal gait pattern with minimal variability in EMG amplitude over the time period of the perturbation. The results suggest that, although the cerebellar patients can adapt their behavior in response to the perturbation, they cannot establish a consistent, efficient strategy for overcoming the imposed modification in treadmill movement.

Supported by the Flinn Foundation and NIH grant PO1 NS30013.

364.6

VERBAL FLUENCY IMPAIRMENT IN PATIENTS WITH CEREBELLAR LESIONS.

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Emerging evidence suggests that Cerebellar computational properties may be important for cognitive functions. Recently we have reported a single case of agrammatism after focal cerebellar damage. Aim of the present study was to investigate verbal output in cerebellar patients by means of a timed verbal task requiring naming production under forced (phonemic or semantic) conditions. Cerebellar patients (n=25) affected by right (n=6) or left (n=13) focal lesions or with cerebellar atrophy (n=6) have been compared to age and education matched controls (n=30). Cerebellar patients produced significantly fewer words in both tasks although with poorer performances under phonemic conditions. To better define the production patterns semantic and phonemic clusters analysis was performed. Cerebellar patients displayed clear difficulties in phonemic clustering. Although right lesioned patients presented the most affected performances, comparison between right and left lesioned groups fail to reach statistical significance. Statistically significance difference were observed between atrophic and focal lesioned groups, with poorer performances for the focal group. The present findings indicate that cerebellar patients are impaired in verbal production with specific difficulties to utilize phonemic retrieval strategies. This defect may represent a specific impairment of verbal skills or a disturbance of correct timing and planning for the access to information.

364.7

CEREBELLAR INVOLVEMENT IN MOTOR PREPARATION: A PET-REACTION TIME (RT) STUDY.

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Regional cerebral blood flow (rCBF), measured by [O-15]water and PET, and RTs were obtained during performance of two tasks in which 13 right-handed subjects responded with one of two right finger movements in one of two dimensions after receiving either complete (full condition) or no advanced (none condition) visual information as to finger and dimension (Deiber et al., Soc. Neurosci. Abstr. 1994, 20: 443). RT was significantly longer during the none condition than during the full condition. Correlations between RT and normalized rCBF were obtained using a pixel-based method (Horwitz et al., Beh. Brain Res. 1995, 66: 187-193). Significant negative correlations between cerebellar rCBF and RT were found for both conditions, but not for other motor cortical structures (primary motor, supplementary motor, premotor). Comparing the none vs. full conditions, the right cerebellum (ipsilateral to the movements) was the only brain structure for which the change in RT signficantly correlated with the change in rCBF ($r=-0.686$, $p<.01$). These results demonstrate that faster RT during tasks primarily concerned with movement preparation is related to higher cerebellar rCBF, but not to rCBF in cortical motor structures. They further suggest that the cerebellum plays a major role in allowing subjects to react quickly in the absence of advanced information needed for preparation of specific movements.

365.1

DISTRIBUTION OF SPONTANEOUSLY ACTIVE NEURONS IN MEDIAL AND LATERAL VESTIBULAR NUCLEI OF RAT BRAINSTEM SLICES. Y. Sun[1], H. J. Waller*[2], D. A. Godfrey[1], and A. M. Rubin[1]. Depts. of Otolaryngology[1] and Neurological Surgery[2], Medical College of Ohio, Toledo, OH 43699.

Spontaneous neuronal firing is prominent in some brainstem structures *in vitro*, but uncommon in others. However, the functional significance of spontaneous activity and its relationships to membrane, interneuronal, and neurochemical variables are largely unknown. In this study, we have explored the medial (MVN) and lateral (LVN) vestibular nuclei with extracellular microelectrodes to determine the locations of spontaneously active neurons.

Transverse or horizontal slices of rat brainstem were cut with a Vibratome at 550 μm, and the location and firing pattern of each spontaneously active neuron were recorded. 266 neurons were found in 1166 penetrations of MVN, 15 neurons in 433 penetrations of LVN. In horizontal sections, relatively more neurons were found in the rostral half (0.46 neurons/penetration) than the caudal half (0.18 neurons/penetration) of MVN. In transverse sections, relatively more neurons were found in the medial half (0.26 neurons/penetration) then the lateral half (0.10 neurons/penetration) of MVN.

Most of the neurons showed regular patterns (97% in MVN, 73% in LVN). Mean firing rates were similar in MVN and LVN (22.5 ±12.0/s and 19.4 ± 10.7/s respectively), but MVN neurons showed greater regularity (coefficients of variation of intervals: 0.11±0.27 in MVN, 0.25±0.17 in LVN, $P<0.05$). In contrast with dorsal cochlear nucleus (Waller and Godfrey, 1994), no neurons showed bursting patterns. We conclude that there are marked regional differences in the relative numbers of spontaneously active vestibular neurons, but only small differences in the characteristics of their discharge.

365.2

NMDA RECEPTOR-MEDIATED INHIBITION OF THE BRAINSTEM VESTIBULAR SYSTEM IN VESTIBULAR COMPENSATION IN RATS. T.Kitahara, N.Takeda*, T.Kubo and H.Kiyama. Depts. of Neuroanatomy & ORL, Osaka Univ. Med. Sch., Suita, Osaka 565, Japan.

After unilateral labyrinthectomy (UL), Fos-like immunoreactive (-LIR) neurons appeared in the ipsi-MVe, contra-PrH and contra-IO beta, and then gradually disappeared in accordance with the development of vestibular compensation. This finding means that the activation of these nuclei is the initial event of vestibular compensation. By means of retrograde tracing, it was revealed that a part of the Fos-LIR neurons project their axons to the vestibulocerebellum.

Before vestibular compensation is accomplished, injection of MK801, an antagonist of NMDA receptor, caused reappearance of UL-induced behavioral deficits (decompensation) and Fos expression in the contra-MVe, ipsi-PrH and bilateral-IO beta. These findings indicate that the neurons in which Fos was expressed by MK801 had been inhibited after UL by glutamatergic synapses driving inhibitory neurons via NMDA receptors and that disinhibition of these neurons induced by MK801 caused decompensation. Accordingly, it is suggested that the NMDA receptor-mediated inhibition of the brainstem vestibular system plays an important role for the initial processes of vestibular compensation. Cerebellar afferents are mediated by NMDA receptors and cerebellar efferents are GABAergic. Therefore, it may be further suggested that the vestibulocerebellum is a component of the NMDA receptor-mediated inhibition.

365.3

NITRIC OXIDE SYNTHASE LOCALIZED IN A SUBPOPULATION OF VESTIBULAR EFFERENTS WITH NADPH DIAPHORASE HISTOCHEMISTRY. M. Singer and A. Lysakowski*. Dept. of Anatomy and Cell Biology, Univ. of Illinois College of Medicine, Chicago, IL, 60612.

Nitric oxide (NO) has recently been discovered to be both a second messenger and a neurotransmitter in a wide variety of physiological systems. Recent reports indicate it may play an important role in the vestibular periphery. One report (Chen and Eatock, *Biophys. J.*, A430, 1994) has shown that NO-producing agents inhibit a low-voltage-activated potassium current ($I_{K,L}$) specific to type I hair cells. Other reports claim nitric oxide synthase (NOS) is present in rat vestibular ganglion cells and in boutons throughout the sensory epithelium (Lyon et al., *Neurosci. Abst.*, 20:969, 1994; Harper et al., *OHNS* 111: 430, 1994). Vestibular bouton afferents have a restricted distribution, namely the peripheral zone of the crista (Fernández et al., *J. Neurophysiol.* 60:167, 1988; 73:1253, 1995). We decided to investigate the distribution of NOS to clarify the role of NO in the vestibular periphery.

No known single neurotransmitter colocalizes with NOS. NADPH diaphorase histochemistry has been used to selectively label NOS neurons because fixation of tissue with paraformaldehyde appears to inactivate all NADPH-dependent oxidative enzymes, except for NOS (Dawson et al., *PNAS*, 88: 7797, 1991; Matsumoto et al., *Neurosci. Lett.*, 155: 61, 1993).

The vestibular endorgans and brainstem of adult chinchillas (*C. laniger*) and Long-Evans rats were reacted using NADPH diaphorase histochemistry. Diaphorase-positive staining was present in hair cells of both types (mainly at the apex of the crista), in boutons at the basal end of hair cells, and in a subpopulation (20%) of the lateral vestibular efferent brainstem group. Label was markedly absent from calyces and ganglion cells. In ultrastructural material, the reaction product was seen in efferent boutons.

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365.4

CALRETININ IS NOT SPECIFIC FOR CALYCEAL AFFERENTS IN THE SEMICIRCULAR CANALS OF *PSEUDEMYS SCRIPTA*. G. Monk and E. H. Peterson*, Dept. Biological Sciences and Neurobiology Program, Ohio University, Athens, OH 45701.

The calcium-binding protein calretinin has been reported to stain calyx afferents selectively in the vestibular endorgans of rodents. The reason for this selectivity is unknown. Calyx afferents in mammals typically have larger axon diameters than other afferent types. Thus, two interpretations of calretinin staining patterns are possible: calretinin may be selective for (a) calyx afferents or (b) large diameter afferents.

To distinguish between these possibilities, we assayed calretinin-like activity in the posterior canal of the turtle, *P. scripta*. *Pseudemys* is unusual among amniotes in possessing a population of bouton afferents with axons as large or larger than calyx afferents (β-bouton afferents; Brichta and Peterson, 1994, J. Comp. Neurol., 344:481-507); they are located preferentially toward the canal center.

Two types of afferents were labeled following immunocytochemical demonstration of calretinin-like activity. Calyx afferents were stained throughout the central zones of each hemicrista; their distribution and structure were consistent with our earlier description of these neurons (Brichta and Peterson, 1994). In addition, a small number of very large diameter bouton afferents were labeled near the canal center; their numbers, structure, and distribution suggests that they correspond to β-bouton afferents.

Our data demonstrate that calretinin-like activity is not restricted to calyx afferents in *Pseudemys*. Thus, calretinin may be selective for very large diameter afferents, independent of type.

Supported by NIDCD 00618.

365.5

CALCIUM-BINDING PROTEIN STAINING REVEALS DIFFERENCES IN THE DISTRIBUTION OF PRIMARY AFFERENT AND PURKINJE CELL TERMINALS IN THE VESTIBULAR NUCLEI. Golda Anne Kevetter* and Robert B. Leonard. Depts. Otolaryngology, Anat. and Neurosciences, Physiology and Biophysics, Univ. TX Med. Br., Galveston, TX 77555

Previously we have shown staining for calcium-binding proteins in the vestibular nuclei. The distribution of staining suggests these proteins stain at least the primary afferents, cerebellar Purkinje cells, or both. To distinguish these possibilities we made lesions either in the vestibular nerve central to Scarpa's ganglion or the cortex of the cerebellum. Immunohistochemistry with antibodies for calretinin or calbindin was performed. Analyses concentrated on the medial vestibular nucleus (MVN). Calretinin-staining was substantially reduced after lesion of the nerve. Staining was most reduced over the magnocellular portion of MVN. Changes in labeling adjacent to the ventricle could not be determined since a population of neurons in this location, possibly intrinsic, stain with calretinin histochemistry. No reduction of staining was seen after lesion of the cerebellum. This was not surprising since neither Purkinje cells nor cells in the cerebellar nuclei stain with calretinin. Staining with calbindin was different. After lesion of the nerve, calbindin staining was also significantly reduced in the magnocellular MVN. However, a dense area of terminal staining was apparent in the medial MVN, especially along the ventricular border and the ventral border with the nucleus prepositus hypoglossi. After lesion of the cerebellum, staining was reduced in those areas. Remaining fibers and terminals that stained positive for calbindin were concentrated in the lateral and magnocellular MVN. These preliminary experiments indicate that select populations of afferents to the vestibular nuclei may possess different chemical properties.(Supported in part by NIH grant DC0052 and Deafness Research Foundation)

365.6

QUANTITATIVE DISTRIBUTION OF CHOLINE ACETYLTRANSFERASE ACTIVITY IN RAT VESTIBULAR NUCLEAR COMPLEX. H.Li*, A.B. Squire, D.A.Godfrey, and A.M.Rubin. Dept. of Otolaryngology-Head and Neck Surgery, Med. Col. of Ohio, Toledo, OH 43699-0008.

Cholinergic structures in and near the rat vestibular nuclear complex (VNC) probably include secondary projections from the VNC to cerebellar cortex (Barmack et al., 1992), centrifugal fibers to the vestibular labyrinth (Schwarz et al., 1986), and innervation from unidentified sources (Burke and Fahn, 1985). In order to estimate the prominence of these cholinergic structures, choline acetyltransferase (ChAT) activity was mapped at 3 rostral-caudal levels through the VNC, by microdissection of freeze-dried sections and radiochemical assay. The overall ChAT activity in the VNC was only about 1/4 of that of the cochlear nucleus, about 1/6 of that of whole rat brain, and about 1/50 of that of the cholinergic facial motor nucleus. Among VNC regions, ChAT activity (see Table) was higher in the medial (MVN) than in the lateral (LVN), superior (SuVN), or spinal (SpVN) nuclei. Activity tended to be highest in the most superficial part of MVN, but no rostral-caudal gradient was found. ChAT activity among the main group of vestibular efferent neurons was about twice that in the nuclei containing olivocochlear neurons. It is suggested that: 1) The activity of ChAT in rat VNC is lower than previously estimated. 2) Cholinergic structures are more prominent in MVN than in other VNC regions. 3) Cholinergic mechanisms are important in vestibular centrifugal control. *(NIH grant RO1-DC2550)*

μmol/kg dry wt/min (mean±SE, n=4 rats)	
MVN dorsal	145±11
MVN ventral	133±20
LVN dorsal	64±13
LVN ventral	86±12
SuVN	67±10
SpVN	68± 4

365.7

CONSTITUTIVE AND INDUCED NITRIC OXIDE SYNTHASE IN THE VESTIBULAR COMPLEX AND GANGLION. Dale W. Saxon* and Alvin J. Beitz. Dept. of Vet/Pathobio., Univ. of Minn., St. Paul, MN, 55108. The enzyme nitric oxide synthase (NOS) is responsible for the production of nitric oxide, a gaseous molecule now widely believed to be involved in neurotransmission in the CNS and PNS. In addition to its widespread distribution in the nervous system under normal circumstances NOS has recently been shown to be induced in neurons of precerebellar nuclei (Saxon and Beitz, '94). Using NADPH-diaphorase as a marker, the distribution of neurons containing constitutive (cNOS) and induced NOS (iNOS) was investigated in the vestibular complex and vestibular (Scarpas) ganglion. Neurons containing cNOS in the vestibular complex were largely confined to the medial vestibular nucleus (MVN) with a small number of fainty stained neurons occasionally encounterd in nucleus X. c NOS neurons were distributed along the entire length of the MVN although the rostral part of the nucleus contained only a sparce population. Except for the presence of a network of positively stained fibers which permeate the neuropil of the entire vestibular complex, vestibular nuclei other than the MVN lacked perikaryal staining. The vestibular ganglion contained very little constitutive NOS. However, following lesions to the cerebellar cortex and survival times of 7 days or greater, neurons containing induced NOS were found in the inferior, lateral and superior vestibular nuclei. In addition, induced neurons were found in the nucleus of Roller, nucleus X, nucleus linearis, lateral retcular nucleus and external cuneate nucleus. Lesion/Fluoro-Gold injections into the midline cortex resulted in double-labeled neurons in several precerbellar nuclei including the inferior vestibular nucleus. Although the MVN contained numerous retrogradely labeled neurons, particularly in the caudal half of the nucleus none of these neurons were found to contain NOS. Following cerebellar lesions that included damage to the vestibular complex induced NOS was evident in cell bodies of the vestibular ganglion and in axonal profiles in the root of the VIII nerve ipsilateral to the lesion. The impact of induced NOS on the function of the vestibular system remains to be elucidated, but the present study provides the groundwork for the undertaking of such future studies. This work was supported by NIH Grant #NS31318.

365.8

IMMUNOCYTOCHEMICAL STUDY OF GLIAL REACTION IN THE IPSI AND CONTRALATERAL VESTIBULAR NUCLEI OF HEMILABYRINTHECTOMIZED RATS. C. de Waele*, A. Campos Torres, R. Pochet, P. Josset, P.P. Vidal. L.P.P.A., CNRS-Collège de France, Paris. Lab. Anatomy and cytology, Hôpital Trousseau,Paris, France and Lab. Histologie, ULB, B 1070, Bruxelles, Belgique.

Vestibular compensation is an attractive model for investigations of cellular mechanisms underlying post-lesional plasticity in the adult central nervous system. Immediately after hemilabyrinthectomy, the spontaneous activity in the deafferented second-order vestibular neurons falls to zero, resulting in a strong asymmetry between the resting discharge of the vestibular complexes on the lesioned and intact sides. After about fifty hours, the deafferented vestibular neurons recover a quasi normal resting activity, which is thought to be the key of the compensation of the static vestibular syndromes observed at the acute stage. In this study, we investigated by means of different monoclonal and polyclonal antibodies whether a glial reaction is involved during the vestibular compensation process. Potential degenerating axons were also studied using a silver impregnation method. Adult rats were hemilabyrinthectomized and the appearance of reactive astrocytes was studied at 1, 2, 4, 8 and 21 days post-lesion by means of selective monoclonal GFAP and Vimentin antibodies. Specific antibody binding sites were visualized using the avidin biotin method and the 3-3'-diaminobenzidine as a peroxydase substrate. Double labelling experiments were performed using secondary fluorescent antibodies. Finally, GFA and Vimentin protein levels were assessed by dot immunoblot techniques. In the normal rats, the vestibular nuclei were devoid of vimentin-positive glial cells and GFAP-positive astroglial cells were uniformly distributed within all the vestibular nuclei. In the hemilabyrinthectomized rats, although very few degenerating axon terminals could be detected, numerous vimentin-positive astroglial cells appeared within the deafferented vestibular nuclei. This reaction became evident between 1 and 2 days following the lesion, peaked at about three days and then declined during the following days. In conclusion, the decrease of the resting discharge of the deafferented central vestibular neurons most probably induced this glial reaction. It remains to be determined the exact role of these reactive astrocytes in the vestibular compensation process.

365.9

SYNAPTIC INTERACTIONS IN THE VESTIBULO-OCULAR PATHWAYS OF GUINEA PIG STUDIED IN AN IN VITRO WHOLE BRAIN PREPARATION. [1]A. Babalian, [1]N. Vibert, [2]M. Serafin, [2]M. Mühlethaler and [1]P.-P. Vidal*. [1] L.P.P.A., CNRS-Collège de France, 15 rue Ecole de Médecine, 75006 Paris, France; [2] Dpt. Physiologie, CMU, 1 rue Michel-Servet, 1211 Geneva 4, Switzerland.

The isolated and perfused whole brain of guinea-pig is a powerful tool to tackle various neurophysiological problems. It combines the advantages of in vitro preparations (stable recording conditions, control of the extracellular medium, etc...) with the preserved connectivity of an intact brain. We studied on this preparation synaptic transmission between vestibular afferents and second-order vestibular neurones. Both extracellular field potentials and synaptic responses of single neurones were recorded within the vestibular nuclei (mainly the medial one) following stimulations of vestibular nerves. Using a low Ca^{2+}/high Mg^{2+} solution which blocks chemical transmission, we assessed the pre- and postsynaptic components of field potentials. They were similar to those observed in vivo, with delayed latencies (by 30 to 40%) due to lower recording temperature (29°C). Stimulation of the ipsilateral vestibular nerve produced monosynaptic EPSPs (latency 1,1-1,8 ms) in all three main cell types previously identified in slices (A, B and B+LTS neurones). Type A and B neurones revealed regular and irregular spontaneous discharges, respectively, implying that they might correspond to the tonic and phasic vestibular neurones described in vivo. Both synaptic field and EPSPs were largely blocked by CNQX, indicating AMPA receptor-mediated synaptic transmission. The remaining component was generally blocked by APV, suggesting involvement of NMDA receptors. In some cases however, a small synaptic component resistant to both antagonists was observed. We have furthermore studied the vestibulo-oculomotor interactions by recording: a) discharges of abducens and oculomotor nerves; b) synaptic field potentials in the antidromically-identified abducens and oculomotor nuclei; c) postsynaptic potentials in abducens motoneurones following stimulation of vestibular nerves. It confirmed the existence of strong bilateral, disynaptic inputs (latencies of synaptic events 2.1-3.1 ms) from vestibular afferents to abducens and oculomotor nuclei.

365.10

IMMUNOHISTOCHEMICAL STAINING FOR CALCIUM BINDING PROTEINS: POTENTIAL CORRELATIONS WITH ANATOMICAL AND/OR PHYSIOLOGICALLY DEFINED VESTIBULAR PRIMARY AFFERENTS. R.B. Leonard* and G.A. Kevetter. Departments of Anat. & Neurosci., Physiol. & Biophysics, and Otolaryn., Univ. of TX Medical Branch, Galveston, TX 77555

Vestibular primary afferents in several mammalian species have been subdivided using a combination of physiological and morphological properties of the endings within the sensory neuroepithelium (e.g., Lysakowski et al., '95; Fernandez et al., '95). Other than intracellular labeling of single axons, anatomical techniques used to examine the central projections label all types of afferents. We have been exploring the possibility that staining for various calcium binding proteins can be used to identify types of afferents in gerbils. In initial experiments, we have examined staining for calretinin and calbindin in both the cristae and maculae.

Currently, the results for calretinin are the clearest. Stained fibers progress through the middle of the cristae to reach the sensory epithelium of the central zone. There the fibers form clear, well-stained calyx endings. The stained fibers appear to correspond to the largest fibers seen in plastic embedded sections. No boutons were observed. The same statements apply to the maculae. It is hard to escape the conclusion that these afferents correspond to the calyx-only, highly irregular fibers, originating from the apex of the cristae. Calbindin stains afferents occupying a wider region of the crista. Axonal staining is less intense, but the axon diameters appear to be more variable than seen with calretinin. Calyx endings are readily apparent in the central and intermediate zones and can be found in the peripheral zone. Structures that appear to be boutons can be seen, although continuity with the parent axon is difficult to detect. These statements also apply to the maculae. This pattern suggests that calbindin stains primary afferents with dimorphic endings. (Support by Deafness Res. Fnd)

365.11

DEVELOPMENT OF A NOVEL, REVERSIBLE LABYRINTHECTOMY MODEL: BEHAVIORAL AND ANATOMICAL CORRELATES. A. J. Beitz*, D. W. Saxon and J. H. Anderson. Dept. of Vet. Pathobiology and Dept. of Otolaryngology, Univ. of Minnesota, St. Paul, MN 55108.

Previous studies of the effects of unilateral labyrinthectomy (UL) have used models in which the labyrinth is destroyed surgically or by injection of toxic chemicals into the middle or inner ear. In the present study we have developed a reversible UL procedure in which 25 µl (0.3 mM) of tetrodotoxin (TTX), a voltage sensitive sodium channel blocker, is injected into the middle ear of adult Long-Evans rats. This procedure is adapted from that described by Rubel and colleagues for blockage of cochlear nerve activity. Compared to saline injected controls, the effects of TTX were evident within 10-20 min following injection, as evidenced by the typical behavior signs: nystagmus, tonic head tilt, and ataxic gait combined with circling of the animal toward the side of injection. These vestibular signs lasted for up to 24 hr. Two groups of rats were perfused with fixative at 3 hr and 24 hr postinjection, respectively, and the brains were processed for immunocytochemical localization of the immediate early gene, Fos. In TTX-treated rats sacrificed at 3 hr Fos expression was prominent bilaterally in the prepositus hypoglossi and the rostral portion of the SVN, contralaterally in the rostral portion of the medial vestibular nucleus (MVN), and ipsilaterally in the IVN, the Y-group and the caudal portion of the MVN. By 24 hr post-injection Fos immunolabeling decreased throughout the vestibular complex, except in the caudal MVN and contralateral prepositus hypoglossi. In addition to the vestibular complex, there was Fos expression in various subnuclei of the ipsilateral inferior olivary complex and in the fastigial nucleus and the granule cell and molecular layers of lobules 9 and 10 of the cerebellum. These data support and extend previous studies examining Fos expression following UL and indicate that this new TTX model may be useful for future studies of UL. Supported by NIH grants NS31318, DCD 01086 and DCD 00110.

365.12

ONTOGENY OF VESTIBULAR COMPOUND ACTION POTENTIALS IN THE CHICKEN. T.A. Jones*, and S.M. Jones. ENT Vestibulo-cochlear Research Lab, University of Missouri, School of Medicine, Columbia, Missouri 65212.

Compound action potentials of the vestibular nerve were measured from the surface of the scalp in 72 chickens (Gallus domesticus). Ages ranged from approximately -48 hours (traditional E19) to 336-hours-old (14-days-post-hatch). Zero-hours-old was defined as the time of hatch. Responses were elicited using 2ms duration cranial linear acceleration pulses. Stimuli were expressed in units of jerk (g/ms) or in dB relative to 1.0g/ms. Traditional signal averaging was used to resolve vestibular responses out of the background activity.

Response thresholds decreased systematically up to approximately 200 hours of age. Amplitudes of early peaks (P1, N1, P2) increased linearly as a function of age presenting a rate of maturation of 0.11 ± 0.02 µV/day (mean ± sd). Response latencies decreased with increasing age up to 350-hours-old (rate = -28.1 ± 8.5 µs/day). Responses were not resolved at ages younger than -52-hours-old (late E18). These data suggest that functional development continues after all major morphological structures are in place. Functional improvement may correspond to neural myelination and synaptic maturation, two of many possible explanations for the findings. (Supported by NASA NAGW 1275, 3910)

365.13

CONTRIBUTION OF TONIC INPUT FROM THE LABYRINTH TO THE POSTURAL CONTROL IN THE RAT. T. Deliagina*, L. Popova, G. Grant, G. Orlovsky. Department of Neuroscience, Karolinska Institute, S-171 77, Stockholm, Sweden.

Removal of a vestibular organ (unilateral labyrinthectomy, UL) results in severe motor disorders. Two principally different primary causes are responsible for them: a considerable loss of the sensory (vestibular) information and a loss of tonic excitatory inflow from the labyrinth. In the present study, a role of the tonic inflow was investigated. For this purpose, the effect of electrical stimulation of the transected vestibular nerve on different motor disorders, evoked by UL was studied in freely behaving rats. This stimulation was used to "substitute" the lost tonic vestibular input. Six UL-evoked motor disorders were investigated. They appeared in a fixed order after surgery: (1) body twisting, (2) rolling, (3) extension of limbs contralateral to UL, (4) circling, (5) head roll tilt, (6) spontaneous ocular nystagmus. Stimulation of the vestibular nerve in UL-rats completely abolished the symptoms (1-5), and reduced considerably the symptom (6). Thus, an artificial tonic input from the vestibular nerve, not depending on movements of the animal and on its position in space, can abolish most motor disorders evoked by UL. In addition, postural disorders evoked by electrical stimulation of the vestibular nerve in the rats subjected to bilateral labyrinthectomy, were studied. By increasing gradually the strength of current stimulating the vestibular nerve, we could evoke all the UL-symptoms, but in a reversed order. Thus, a loss of tonic input from the labyrinth, rather than a loss of specific vestibular information, is the cause for most motor disorders evoked by UL in the rat.

365.14

ENDOLYMPHATIC POLARIZATION AND MECHANICAL STIMULI REVEAL DIVERSITY IN THE ACTIVATION OF SEMICIRCULAR CANAL HAIR CELLS IN THE TOADFISH, *OPSANUS TAU*. R. D. Rabbitt*[1,4], S. M. Highstein[3,4] and R. Boyle[2,4] [1]Dept. Bioeng. Univ. Utah, Salt Lake City, UT; [2] Dept. of Otolar. and Physiol., OHSU, Portland, Oregon; [3]Dept Otolar., Anat. and Neurobiol., Wash. Univ., St. Louis, MO; [4]Marine Biological Laboratory, Woods Hole, MA.

Extracellular responses of individual afferents were recorded for electrical polarization of the endolymphatic space and for micro-mechanical indentation of the limb of the horizontal semicircular canal in the oyster toadfish, *Opsanus tau*. Results delineate the relative contributions of mechanical and post-transduction-current (PTC) mechanisms to the sensory process. The indentational stimulus was designed to mimic physiological head rotation, and the electrical stimulus was designed to bypass the mechanics and drive the transduction current via the Nernst-Planck potential. Overall transfer functions reflect the entire cascade of events from the stimuli to the neural spike train. PTC processing is equivalent for the electrical and mechanical stimuli and hence differences between the afferent responses reflect mechanical events taking place prior to the PTC segment. The mechanical segment leading to gating of hair-cell transduction currents was thereby isolated from the overall process. Results for the mechanical activation of hair cells confirm exsitence of a purely mechanical lower-corner frequency <0.1Hz. Above ~0.5Hz, results show a systematic inter-afferent diversity in both the gain and phase of mechanical activation of hair cells that acts in concert with PTC processing to determine the overall afferent response. For afferents typed as low gain (see Boyle and Highstein, J. Neurosci. 10, 1990), a phase lag in the mechanical activation of hair cells is canceled by a phase lead in PTC to generate responses that align with angular head velocity. In contrast, acceleration type afferents combine a phase lead in the mechanical activation with additional phase lead in PTC to achieve responses that align with angular head acceleration. High-gain type afferents fall in between these two extremes. Commensurate differences in the relative contributions of mechanical and PTC processing are observed in the gain. [sponsored by NIH NIDCD DC01837]

365.15

PROGRESSIVE FUNCTIONAL DECLINE OF VESTIBULO-OCULAR REFLEX AFTER INTRA-OTIC ADMINISTRATION OF GENTAMICIN IN THE CHINCHILLA. Christian Head, Karl Beykirch, Alan Greenfield, Ivan Lopez, Larry Hoffman* UCLA School of Medicine, Los Angeles, CA 90095

Vestibulo-ocular reflexes were measured in the chinchilla to document the functional decline after intraotic gentamicin (GM) administration. The time course of functional loss was correlated with the histological evidence of hair cell loss from the sensory epithelium. Eye movements induced by rotation about the yaw axis in the dark (VOR) were measured in adult animals pre-and post-GM treatment. Sinusoidal rotational stimuli at .0125, .05, .2 and .8 Hz. and 120 °/sec peak velocity were used to characterize the VOR pre- and post- GM administration. Following the collection of normative data, the animal subjects received bilateral intra-otic administration of GM. VOR testing was resumed one day post GM treatment (PT). These results were compared to findings from a parallel histologic evaluation of the crista sensory epithelium. Data obtained over a period of six days following GM administration were fit to the standard first order high pass VOR model. The gain obtained from these fits declined exponentially during this period. At one day PT the mean gain declined to 78% of the normal value. The histological data on day one indicated initial damage in hair cells with signs of cytoplasmic vacuolization, nuclear pyknosis and swelling. By day four the mean gain had decreased further to 12% of the normal value. The morphology on day 4 showed a significant increase in destroyed HCs. By day 6 the VOR gain in all three animals at the tested frequencies was 0.003±0.001. This paralleled histologic findings in animal subjects sacrificed at 7 days post-administration demonstrating HC loss in the sensory epithelium. Our study demonstrates that there is a progressive decline in vestibular function as measured by VOR, after local administration of GM. This progressive loss of VOR also correlates well with the histological time course of HC loss and cellular damage to the sensory neuroepithelium. (Supported by NIDCD grant number DC 01404 and DC01404-03)

OCULOMOTOR SYSTEM: SACCADES—BEHAVIOR AND IMAGING

366.1

FIXATION AND THE GENERATION OF EXPRESS SACCADES IN HUMANS. M. Biscaldi, D. Cavegn, B. Fischer*. Brain Research Unit, Institute of Biophysics, University of Freiburg, 79104 Freiburg, Germany

A large incidence of express saccades (ES; latency about 100 ms) without training paralleled by an inability to suppress ES to suddenly appearing stimuli has been observed in a subgroup of dyslexics, but only very rarely in normally reading subjects (Biscaldi et al., 1994 Invest Ophthal & Vis Sci. 35:1951). We studied the latency and the metrical properties of saccades in an adult, non-dyslexic subject. The subject produced 65-95% ES in the gap (fixation point removed 200 ms before target onset) as well as in the overlap (fixation point remains on) paradigm with the target randomly at 4° to the left or right. The number of ES increased when the fixation foreperiod, the gap duration, and the possible target locations varied from trial to trial as compared when they remained fixed. In the gap paradigm, the gain of saccade amplitude was normal. In the overlap paradigm, we observed in some ES an amplitude transition function with the smallest gain at the longest latency. In the antisaccade and memory-guided saccade paradigms, the subject often erroneously reacted with ES to the target, the gain of these ES also systematically decreased with increasing latency. Our findings can be interpreted by a selective dysfunction of the collicular fixation system. A deactivation of the collicular fixation neurons in monkeys results in a strong increase of ES and a loss of fixation control; saccades executed during electrical stimulation of the fixation neurons are strongly hypometric (Munoz & Wurtz, 1993 J Neurophysiol. 70:576).

366.2

GAP-EFFECTS IN DUAL TASK EXPERIMENTS WITH ISO/ANTI TASK CONDITIONS. A. Roll[1] W. Wolf[1], M. M. Wierzbicka*[2]

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Saccadic and manual reaction times were measured in single and dual task experiments. The target (LED), presented 8° left or right from the fixation point, appeared at the instant when the fixation point was switched off (gap 0) or 200 ms later (gap 200). In the single task condition, subjects responded by either horizontal saccades or forefinger isometric movements as fast as possible, where movement direction requested was to (iso) or opposite to (anti) the target direction in separated sessions. In the dual task condition both movements were required, in which the direction of eye and finger movements could be the same or opposite. We tested all possible combinations of iso and anti direction of the two movements.

The aim of this study was to compare the gap-effect (i.e. shorter reaction times in the gap 200 condition) of saccadic and manual reaction times in single and in dual task experiments in view of a dependence between both movements. We were interested in the relation between target and movement directions, and also in the relation between the directions of the two movements in dual task trials.

In addition, we compared the gap-effects in elderly people (60 years or older) to those in subjects aged below 40 to see if there was the same amount of prolongation of the reaction time as it was seen before in single task studies (Tedeschi et al., Functional Neurology 4(4), 1989).

366.3

Brain oscillations: the origin of express saccades?

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The time that elapses between the appearance of a target toward which the eye is supposed to move and the beginning of the eye movement is rather long: 200 ms. According to Carpenter (in Eye Movements: Cognition and Visual Perception, ed. D. F. Fischer; Hillsdale, New Jersey: Erlbaum 1981) the appearance of the target generates a signal that loads an integrator, and once the integrator value reaches a certain threshold, a saccade is triggered. Under a specific experimental paradigm ("gap conditions"), the latency of the *(slow) regular saccades* is shortened, and the latency-histogram can become multimodal. Saccades with latencies in the region of the first peak are called *express saccades*, those associated with the second peak *fast regular saccades*. The different peaks have been ascribed to different anatomical pathways, operating with individual time delays (Fischer and Weber, Behavioral and Brain Sciences **16**, 553-610, 1993). We present a different model based on the idea that shortening of saccadic latency results from a threshold reduction and multimodality from threshold oscillations in the saccade-generating pathway. To oscillate in response to a stimulus is a general property of the CNS. It can be ascribed to feedback loops, the gain in which is high - on the verge to instability - in order to make the system fast.

366.4

PERCEIVED VISUAL DIRECTION AFFECTS SACCADIC GAIN. D.R.Lucas* and W.A.Hershberger, Psychology Department, Northern Illinois University, DeKalb, IL 60115.

The saccadic eye movement system responds to unusual re-fixation errors by modifying its gain (saccade amplitude/target eccentricity). This adaptive repair mechanism can be invoked in the laboratory by physically displacing the visual target during saccades. We reported in a preliminary study (Lucas & Hershberger, IOVS 35:2033, 1994) that saccadic gain can also be invoked without physical target displacement. Here, we extend our findings to a sample of subjects increased eightfold. On 2-target trials, subjects made horizontal saccades from a central visual fixation target (FT) to a saccade target (ST) displaced 8° rightward, and flashed for 1 ms as soon as FT was extinguished. On 3-target trials, we presented a perisaccadic target (PST) after ST. The PST was displaced 10°, 8°, or 6° rightward from FT and was flashed near the beginning or end of the initial saccade, for a short (1 ms) or long (1 s) duration. Each subject participated in 2 blocks of 30 3-target trials. Before and after each 3-target trial block, subjects participated in blocks of 10 2-target trials (to measure gain changes caused by the 3-target trials). Sixteen subjects served in each of 4 groups defined by factorial combination of PST onset (begin or end) and duration (short or long). PST-10° trials caused gain increases for all groups; PST-6° trials caused gain decreases except for the begin-short group; PST-8° trials caused no gain changes except for the begin-short group. In both exceptional conditions, the begin-short group had gain increases. We propose these increases were caused by the perceptual displacement of the PST known as the perisaccadic illusion of visual direction: Visual targets briefly presented near the beginning of a saccade are seen as displaced in the direction of a saccade. Our results imply that the perisaccadic illusion of visual direction can alter the size of the very saccades that occasion the illusion.

366.5

OCCURRENCE OF ANTICIPATORY SACCADES AS A FUNCTION OF EXPECTANCY FOR INFORMATION. L. Wang* and J. A. Stern. Dept. of Psychology, Washington Univ. St. Louis, MO 63130.

Expectancy for information in this study refers to attentionally anticipating information imparted in forthcoming visual events. Timing and frequency of anticipatory saccadic gaze shift was examined as a function of such expectancy.

Subjects participated in a Continuous Performance Task (CPT). A visual stimulus sequence consisted of 1440 randomly permuted digits. Fifteen target-sequences of three unequal digits with isoparity (target-parity), odd or even, were randomly imbedded in each quarter of the stimulus sequence. The stimuli were presented one digit at a time for 300 ms at a stimulus onset asynchrony (SOA) of 2500 ms. The stimulus locations jumped across the display at the spatial interval of about eight degrees of visual angle from left to right and then reversed, and so on. The participant's task was to press a button when they had detected a target-sequence. They were also informed that the target-sequence would be followed by a digit with non-target-parity.

It was assumed that expectancy for the occurrence of a target-sequence would gradually build up following presentation of relevant stimuli. For example, one's expectancy for the next unequal target-parity digit would be higher after seeing two preceding unequal target-parity digits than after seeing one.

RESULTS Anticipatory saccades occurred earlier on trials immediately following two preceding unequal target-parity digits than on other trials. For trials following the target-sequences, anticipatory saccades occurred later, and the frequency of the anticipatory saccades was lower than on the other trials.

CONCLUSIONS Expectancy for information affects visual strategies of information acquisition. Gaze shift is facilitated by the expectation to acquiring information, while inhibited by the expectation that the next stimulus will be one not requiring processing.

366.6

SACCADES TO REMEMBERED LOCATIONS DURING TRACKING OF ILLUSORY TARGET MOTION A.Z. Zivotofsky, K.G. Rottach, A.A. Kori, L. Averbuch-Heller, V.E. Das, C.W. Thomas, S. Kamran and R.J. Leigh*
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Using the magnetic search coil technique, we measured the accuracy of saccades to remembered target locations that were flashed at eccentricities of up to 10.5 deg on a 20 X 30 deg random dot display that was either stationary or moving horizontally sinusoidally at 0.3 Hz. Saccades that were made to targets flashed on a stationary background were accurate (average RMS error of 1.7 deg). When targets were flashed as subjects smoothly pursued a laser spot that moved vertically across the stationary background, saccadic gain was also good (average RMS error of 2.1 deg). The vertical saccade size was closely correlated to spatial, not retinal, error. When saccades were made to targets flashed during attempted fixation of a stationary spot while the background was moving horizontally, the saccades were noticeably inaccurate horizontally for 3 of the 4 subjects (average RMS error for the 3 was 4.6 deg). When targets were flashed as subjects smoothly pursued a laser spot that moved vertically across the horizontally moving background (a condition that induces a strong illusion of diagonal target motion), saccades were strikingly inaccurate horizontally (average RMS error of 5.3 deg), but quite accurate vertically. The horizontal saccadic mislocalization of targets flashed during illusory, diagonal target motion was correlated with both the position of the background at flash and the distance the background traveled subsequent to the flash. In general, the error decreased significantly from the first saccade in darkness to the final position reached by the eye in darkness (an average 18%). Supported by NIH EY06717, VA, Armington Fund, Dt. Forschungsgemeinsch.

366.7

SACCADIC LATENCY DEPENDS ON TARGET DIRECTION, NOT ECCENTRICITY. E. Leslie Cameron and Peter Lennie*. Center for Visual Science, University of Rochester, Rochester, NY 14627.

Measurements of saccadic latency are generally used to characterize the performance of the oculomotor system. Here we explore some of the decision processes underlying saccadic latency. The main goal was to assess how the amount and form of target uncertainty affect the latency of saccades. To do this we varied the number and distribution of possible target locations. A gap between fixation offset and target onset is known to reduce latency (the gap effect). A second goal was to establish whether spatial uncertainty influences the gap effect.

We monitored eye position with an SRI dual-Purkinje eye tracker. The amount of target uncertainty was varied by setting the number of possible targets between one and twenty. The form of uncertainty was varied by specifying the placement of potential targets in three different ways: (1) variable direction and eccentricity, (2) variable direction, fixed eccentricity and (3) fixed direction, variable eccentricity. We measured the latency of saccades under gap, no gap and overlap conditions.

Saccadic latency increases when the number of possible targets increases from one to two, but shows little increase in latency when the number of possible targets increases beyond two. Latency is longest when direction is variable, regardless of whether eccentricity is variable. When direction is fixed, latency is short, regardless of whether eccentricity is variable. We conclude that direction is more important than eccentricity in determining latency. Our results can be understood in the context of a model of saccade initiation in which the direction and amplitude are programmed in parallel (Becker and Jürgens, *Vision Res.*, 1979, 19, 967). Spatial uncertainty has no influence on the gap effect. Supported by NIH grants EY04440 and EY01319.

366.8

CONTEXT-SPECIFICITY OF HUMAN SACCADIC ADAPTATION. M. FUJITA[1,2]*, A. AMAGAI[2], F. MINAKAWA[1] Communications Research Laboratory, Koganei, Tokyo 184, Japan[1], Grad. Sch. Inf. Sys., Univ. Electro-Communications, Chohu, Tokyo 182, Japan[2].

Response of the visually guided saccade (V-sacc) is well known to be under adaptive control in metrical aspects such as gain and direction. Adaptive modifications are specific to higher-level context aspects as well as to spatial parameters such as to the direction of the response (Deubel, 1993). Present study shows how widely context-specificity of saccadic adaptation works.

There are at least three types of saccades, a memory-guided saccade (M-sacc) made to a remembered target position, and by classifying V-sacc into two types, a visually guided and externally triggered saccade (VE-sacc) which pursues a jump-like target displacement, and a visually guided and internally initiated saccade (VI-sacc) which is initiated to a stationary visible target.

Firstly, adaptive modifications in either M-sacc or VI-sacc could be induced by intrasaccadic target shifts as in Deubel's paradigm. Each session comprised of adaptive phase, and pre- and post-adaptive phase. For adaptation, subjects had to follow displacements of a target of LED (light-emission diode) on a panel. Step-like intrasaccadic target shifts were systematically elicited at the saccade offset and in the opposite direction with respect to the saccade. Saccadic gain decreased fast during the first 100 trials and seemed to reach plateau after 500 trials in individual sessions.

Secondly, adaptive modification in one type of saccdes , M-sacc, VI-sacc, or VE-sacc was transferred little to another type of saccade.

Thirdly, delay was introduced up to 400 ms between the offset of a primary saccade and the presentation of a shift target. Although being late in modification, even at 400 ms latency, still appeared adaptation in VE-sacc or M-sacc we investigated.

These findings have important implications for oculomotor learning. They suggest that VE-sacc, VI-sacc and M-sacc may have different motor pathways, and adaptation must have taken place more likely during primary saccades than during corrective saccades, since corrective saccades were led by visual target shifts in every type of saccade.

366.9

OPPOSITE GAIN ADAPTATION IN EQUAL-DIRECTION REFIXATIONS WITH DIFFERENT DEPTH COMPONENTS. J.A.M. Van Gisbergen* and V. Chaturvedi. Medical Physics & Biophysics, KUN, Nijmegen, The Netherlands.

Primates show direction-specific plastic gain adaptation of saccades when the target is repeatedly displaced intra-saccadically. A different paradigm, wearing glasses with unequal magnification, has been used to show adaptation of the depth component in binocular refixations. In order to gain more insight into the adaptive capacity of gaze shifts in 3D space, we investigated whether it is possible to adapt the depth component of binocular refixations with the target displacement paradigm. In addition, we tested whether refixations with equal version but different vergence components can be adapted in gain independently.

Binocular eye movements to LEDs were recorded in three human volunteers using the coil technique. In the depth-adaptation experiment, the target was first presented in the frontal plane at the left and then switched intra-saccadically to a more nearby LED at the same eccentricity. In the differential saccade-gain adaptation experiment, we first adapted saccades to a far target on the left using the gain-shortening version of the target-shifting paradigm. When gain reduction was clearly established, we continued these gain-shortening trials and alternated them with gain-increase trials to a nearby target at the same eccentricity.

The depth adaptation experiment yielded a gradual change in the depth component of the refixation that subsided gradually when the intra-saccadic depth displacement of the target was discontinued. The second experiment showed that the system can manifest opposite gain changes in saccades to different depths. We conclude that the effectiveness of the intrasaccadic displacement paradigm is not limited to the frontal plane and that it can reveal not only directional specificity of gain adaptation in the frontal plane, but also depth specificity.

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366.10

MODEL-BASED ALGORITHMS FOR TRACKING EYE ORIENTATION USING A VIDEO BASED APPROACH D. Zhu, T. Raphan*. Institute of Neural & Intelligent Systems, Dept. of CIS, Brooklyn College of CUNY.

To bypass the invasive nature of the coil system in measuring eye movements in three dimensions, there have been a number of investigations on the use of cameras and video-based techniques (Parker et al, 1985; Hatamian et al, 1983; Vieville et al, 1987; Clarke et al, 1991; Moore et al, 1994). The methodology developed by Moore et al (1994) uses a three dimensional rotation model to find the eye orientations in three dimensions. It is based on finding the orientation of the optic axis by computing the center of mass of the pupil and then computing the rotation about the optic axis utilizing variation in intensity along an iral arc. This methodology is dependent on maintaining a fixed pupil size in order not to have changes in the iral striation patterns which would cause errors in computing torsional eye position. In addition, the algorithm for computing the orientation of the optic axis is based on finding the center of mass of the pupil. This too is dependent on pupil size variation and orientation of the eye. The purpose of this study was to derive an algorithm based on a more global model of how circular contours which enscribe the pupil and iris are mapped to elliptical contours under a rotation and projection mapping, i.e., affine transformations. We have implemented an edge detection algorithm that determines the contour at each given eye orientation. The orientation of the optic axis relative to the axis of the image plane is computed by finding the best fitting ellipse for this contour. The shape of the ellipse can then be related to the orientation of the optic axis. Torsion about the optic axis is computed by using a minimum distance pattern matching algorithm on the totality of the iral signature. This more global approach to computing the torsional component of eye orientation tends to be immune to artifacts which arise when the eye moves into positions where there is partial lid occlusion. The algorithms developed are also robust with regard to pupil size and illumination conditions.

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366.11

DOES THE SHORT TERM ADAPTATION IN SACCADIC EYE MOVEMENTS INFLUENCE IMAGINED HEAD POINTING ?
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When during ongoing eye saccades the visual target is displaced from one position to another, the oculomotor system adapts to the changed situation after several trials and performs a single saccade to the second target position. To study whether this adaptation influences head movements 10 experiments were done in humans. Each experiment consisted of a pre- and a post-adaptation test and the adaptation period. During the pre- and post-tests the subjects were asked to perform head rotations according to verbal instructions starting always from a LED in front of them (the center target). During the adaptation phase the subjects were requested to perform saccades from the center target to LEDs in the periphery on both sides, while only the targets on the right side changed their position during the saccades. The position-shifts were directed toward the center LED. The subjects were instructed to not move the head during adaptation. Head position and EOG were monitored on-line. This insured that a sufficient degree of adaptation was achieved before the post-adaptation test was commenced.

The results show that head pointing to both sides, the perturbed and the unperturbed one, do not differ significantly. Compared to the pre-test in the post-test some subjects exhibited smaller head movements while others increased the amplitude of head rotations. We could not find clear asymmetries in this 'expansion/shrinkage' pattern. This indicates that, in all likelihood, remembered head pointings are not affected by adaptation of saccadic eye movements. We conclude that the internal representation of the visual surround and the performance of non-targeting head movements are processed outside brain structures which are involved in saccadic short term gaze adaptation.

366.12

BINOCULAR COORDINATION OF EYE MOVEMENTS DURING WAKEFULNESS, NON-REM and REM SLEEP IN MONKEYS. Wu Zhou* & W.M. King. Neuroscience Program,U. Rochester, Rochester, NY 14642 and Depts. of Neurology and Anatomy, U. Mississippi Med. Ctr., Jackson, MS 39216

Binocular coordination of eye movements is important for normal fusion and stereopsis in animals with frontal eyes. Hering's Law of Equal Innervation has been interpreted to imply that binocular coordination is anatomically hard wired (Westheimer,1977). Alternatively, Helmholtz argued that binocular coordination is not "an obligatory anatomical mechanism" (Helmholtz, 1910). To address this issue, we have examined binocular coordination in monkeys during three states of consciousness: wakefulness (in light and dark), non-REM sleep and REM sleep. We found that the visual axes of the eyes intersected at a single point in space (the fixation point) whenever monkeys were awake. During sleep, however, the eyes were often (80% of the time) diverged horizontally and vertically beyond infinity so that there was no single fixation point. During non-REM sleep, drifting eye movements and occasionally saccadic eye movements were observed in one eye but not in the other eye. During REM sleep, both eyes moved synchronously during saccades. Rapid eye movements during REM sleep, however, were often disjunctive rather than conjugate as described by classical studies (Aserinsky &Kleitman, 1955). It has been suggested that REMs during sleep are related to "watching" dream content (Roffwarg et al, 1962). Since the eyes of our subjects were often diverged beyond infinity during sleep, the lines of sight did not intersect at a single fixation point. Thus, each eye was often directed at different parts of visual field during REM sleep. These observations suggest that REMs may be an epiphenomena related to the dream state, but not to the visual content of the dreams themselves. Finally, these results suggest that binocular coordination is not necessarily hardwired, but rather is a distinguishing characteristic of the awake state. (Supported by EY04045 to Dr. W.M.King)

OCULOMOTOR SYSTEM: CLINICAL STUDIES

367.1

SCHIZOPHRENIC PATIENTS WITH DEFICIT SYNDROME HAVE ABNORMAL SMOOTH PURSUIT EYE MOVEMENTS. D.E. Ross*, G.T. Thaker, R. Buchanan, A. Lahti, M. Moran, D. Medoff. Maryland Psychiatric Research Center, P.O. Box 21247, Baltimore, MD 21228

Previous studies in patients with schizophrenia and related phenomena have found an association between abnormal smooth pursuit eye movements and psychopathology which may be related to the deficit syndrome of schizophrenia, which is characterized by primary and enduring negative symptoms. Objective: The purpose of this study was to examine the potential relationship between abnormal smooth pursuit and the deficit syndrome of schizophrenia. Methods: 16 normal controls, 11 schizophrenic patients with deficit syndrome and 22 non-deficit patients were assessed with infrared oculography. The target consisted of a series of foveapetal and foveafugal step-ramps. Measures of smooth pursuit function included initial acceleration, velocity during pursuit maintenance and, in response to the foveafugal target, initial saccadic response. Results: MANOVA revealed that the three groups differed significantly with respect to the dependent variables, Wilk's lambda = 0.69, $F(2,20) = 4.4$, $p = .025$. Post-hoc Tukey's tests revealed that the patients with deficit syndrome had abnormally decreased initial acceleration, velocity maintenance, and, in response to the foveafugal target, increased initial saccadic latency (all p's $< .05$). The oculomotor performance of the non-deficit patients was generally intermediate between that of normal controls and deficit patients. Conclusion: These findings are consitent with and extend previous ones by showing that abnormal smooth pursuit eye movements in schizophrenic patients are strongly associated with the deficit syndrome. Eye tracking disorder and deficit syndrome in schizophrenia may share a common pathophysiology and/or etiology, probably involving cortical-subcortical circuits.

367.2

DEFICITS OF SMOOTH PURSUIT INITIATION IN SCHIZOPHRENIC PATIENTS. **D. Schoepf, W. Heide[2], V. Arolt, K. Junghanns and D. Kömpf[2]. Dep. of Psychiatry and Neurology[2], University of Lübeck, FRG. (SPON: European Brain and Behaviour Society).**

Abnormalities of smooth pursuit (SP) eye movements have frequently been reported in schizophrenic patients. To evaluate, if this could be due to deficits of SP initiation or of cortical visual motion processing we recorded ocular motor responses to unpredictable horizontal step-ramp movements of a foveal laser target, using infrared reflection oculography. Step-ramps with initial steps of 3° and 8° succeeded by constant foveofugal (FF) and foveopetal (FP) constant velocity-ramps of 10, 15 or 30°/s were administered. We assessed position errors (PE) and latencies of the primary and secondary saccades in reponse to a step-ramp target, postsaccadic SP velocity (PV) and in case of presaccadic pursuit initiation pursuit latency and initial eye acceleration. The maximum velocity of pursuit maintenance (PM) was determined 300 to 500 msecs after the target step. The control condition was the PE for saccades to stationary visual targets and the pursuit responses to predictable (periodic) stimuli of comparable velocities. Data from 15 subacute schizophrenic patients and 15 age-matched normals were analysed. Statistically significant differences between patients and normal subjects in the step-ramp paradigm were: (1) a reduced gain of PM at all velocities of step-ramps but a comparably higher gain of predictive pursuit; (2) a reduced PV with fast FF ramps (30°/s); (3) an increased number of catch-up saccades; (4) an elevated PE of the primary and secondary saccades at most ramp velocities in contrast to a normal amplitude of saccades to stationary targets. The latency of the first saccade, the latency of pursuit initiation, the PV at 10 and 15°/s and the initial eye acceleration were within normal limits. The present data demonstrate that schizophrenia affects the pursuit response to unpredictable targets more than to predictable targets, but leaves the parameters of pursuit initiation largely intact. In addition, saccadic dysmetria to moving targets implies a defective use of visual motion information by the saccadic system.

367.3

EYE TRACKING DYSFUNCTIONS IN SCHIZOPHRENIC AND DEPRESSED PATIENTS

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Although eye tracking dysfunctions (ETD) have received much attention as a biological marker for vulnerability to schizophrenia, some studies, however, have failed to find a significant difference between ETD in patients with schizophrenia and affektiv disorder.

We examined tracking performance of 43 schizophrenics and compared them with 33 depressed patients and 42 healthy volunteers. Subjects were asked to follow a red laser light point moving sinusoidally with a frequency of .4 Hz and an amplitude of ±10°. Eye movements were recorded with a high resolution infrared scleral reflection technique. We counted the number and amplitudes of saccades during SPEM and determined the average gain (ratio stimulus velocity to eye velocity). Compared to normals, mean number of saccades was significantly increased and mean gain was reduced in schizophrenic patients. However, depressed patients also showed ETD in a minor degree.

Our results strengthen the hypothesis that ETD may not be specific to schizophrenic disorders in psychiatric populations.

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367.4

EFFECTS OF GABAPENTIN ON PENDULAR OCULAR OSCILLATIONS. J.S. Stahl*, K.G. Rottach, L. Averbuch-Heller, R.D. von Maydell, S.D. Collins, R.J. Leigh Ocular Motor Lab. VA Medical Center and Case Western Reserve University, Cleveland OH 44106

Spontaneous, quasi-sinusoidal oscillations of the eyes (acquired pendular nystagmus) that occur in humans may represent instability in feedback loops between the cerebellum and brainstem. The neurotransmitter gamma-aminobutyric acid (GABA) might be involved in the control of such feedback, since 1) $GABA_A$ is important for normal functioning of the neural integrator for horizontal eye movements; 2) $GABA_B$ is involved in the inhibitory control of "velocity storage" by the nodulus and uvula; 3) the $GABA_B$ agonist baclofen is able to abolish periodic alternating nystagmus and may also improve downbeat nystagmus. We measured the effects of the drug gabapentin, which is structurally similar to GABA, on vision and eye movements in three patients with acquired pendular nystagmus. A single oral 600 mg dose of gabapentin reduced the ocular oscillations and improved visual acuity in all three patients by a factor of ≥ 2. The effect was sustained after five weeks of treatment in two patients who elected to continue taking it. Two normal subjects who took a single 600 mg dose developed gaze-evoked nystagmus in darkness. The drug effect might be exerted via modification of GABA metabolism in the cerebellar-brainstem circuits. Alternatively, the drug could act similarly on the optic nerve, affecting visual feedback. These preliminary results suggest that a systematic, blinded evaluation of the effects of gabapentin on normal human eye movements and pathological oscillations should be carried out. (Support: NIH EY06717, Veterans Affairs, Dt. Forschungsgemeinschaft, Ernst Jung Stiftung & Evenor Armington Fund)

367.5

SACCADIC EYE MOVEMENTS IN HUMANS WITH ATTENTION-DEFICIT HYPERACTIVITY DISORDER. J.E. Goldring, K.A. Hampton, D.P. Munoz*, MRC Group in Sensory-Motor Physiology, Queen's University, Kingston, Ontario, Canada.

Attention-deficit hyperactivity disorder (ADHD) is a common disease characterized by abnormal patterns of attention, impulsivity, and hyperactivity. Normally, when fixating a visual stimulus, visuospatial attention is actively engaged upon the point of fixation. This active visual fixation must be disengaged before the generation of a saccade, and this process influences saccadic reaction time (SRT). Because ADHD children have difficulty keeping their attention engaged on a task, we hypothesized that they would have shorter SRTs. We studied the saccadic eye movements in children with ADHD as well as control children and adults. Subjects initially fixated a central visual stimulus (fixation point-FP). A target stimulus then appeared to the right or left of the FP. Subjects were told to look to (pro-saccade task) or away from (anti-saccade task) the target. The target was presented while the FP was on (overlap condition), or 200 ms after it was turned off (gap condition). ADHD subjects had considerable difficulty maintaining steady fixation on the FP, and tended to generate many extraneous saccades. These movements were reduced when taking their prescribed drug, methylphenidate (ritalin). There was no significant difference in mean SRT between the control and ADHD children, however there was an increase in the variability of SRTs in ADHD children. We predicted that they would make more mistakes in the anti-task (difficulty suppressing pro-saccades), however once again, they did not differ from the control children. Both control and ADHD children triggered many directional errors in the anti-task whereas control adults did not. Surprisingly, although ritalin decreases impulsivity and increases the ability to concentrate, it had no significant effect on the number of mistakes made on the anti-task. Rather, the ability to perform the anti-task with few mistakes was dependent upon subject age.

367.6

BEHAVIORAL INHIBITION DEFICITS IN MEDICATION-NAIVE CHILDHOOD OBSESSIVE COMPULSIVE DISORDER PATIENTS

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Obsessive Compulsive Disorder (OCD) is characterized by recurrent intrusive thoughts and repetitive ritualistic behaviors. Approximately 50% of all OCD cases have onset in childhood, and its lifetime prevalence is two to three times greater than schizophrenia and bipolar disorder. Neuroimaging studies implicating orbital prefrontal cortical and striatal region involvement in OCD, together with the apparent inhibitory deficit suggested by the symptomatology of OCD, suggest that a deficit in response suppression may be a central neurocognitive deficit in these patients. Seventeen medication-naive, non-depressed OCD patients 8-20 years of age, and 17 age and sex case-matched healthy comparison subjects performed an oculomotor delayed response task, an antisaccade task as well as a control visually guided saccade task. While no differences were observed between childhood OCD patients and controls in the latency, accuracy or peak velocity of reflexive visually guided saccades, a higher percentage of response suppression failures was observed in childhood OCD patients on both the oculomotor delayed response task and antisaccade task. On the oculomotor delayed response task, pediatric and adult controls performed equally well on all parts of the task regardless of the delay period duration, while on the antisaccade task, pediatric controls performed more "poorly" on the most difficult part of the task. Detection of such deficits depends on age-related ability to inhibit responses, as well as task difficulty and characteristics. The findings in this study suggest that there is a basic disturbance of neurobehavioral inhibition in OCD that may underlie the repetitive symptomatic behavior that characterizes the illness.

367.7

UNEQUAL SACCADES IN STRABISMICS AFTER EXPOSURE TO ANISEIKONIA. Z. Kapoula* M.P. Bucci, T. Eggert and L. Garraud[1]. LPPA, CNRS-Collège de France, Paris. [1]Douarnenez Hospital, Brittany, France.

In normals, saccade inequality is induced immediately when the image is made larger for one eye. Such disconjugacy reduces disparity at the point of fixation; it persists under monocular viewing which suggests a fast learning mechanism. We examined this mechanism in 8 subjects with long-standing convergent strabismus. Four of them had a small squint (4 to 18 pd) and maintained peripheral fusion and gross stereopsis. The other four had larger squint (22 to 30 pd), no fusion and no stereopsis. No subject had amblyopia. All subjects wore an afocal magnifier (8%) in front of their preferred eye, and a prism in front of the other eye that reduced the squint. They viewed a random dot pattern and made saccades (typically 8°) for 20 minutes. Binocular recordings of horizontal saccades were made with an IR device.

In subjects with partial binocular vision saccades became larger in the eye wearing the magnifier immediately (0.88°); this disconjugacy persisted under monocular viewing (0.67°), suggesting that fast learning is preserved. Subjects with no binocular vision also made large changes in saccade disconjugacy (1.52°) that persisted under monocular viewing (1.09°). These changes, however, were not correlated with the change required by the magnifier. Most likely, the use of the prism caused the non-preferred eye to take up fixation thereby modifying the baseline disconjugacy.

We conclude that fast disconjugate changes in saccade amplitude are possible in all strabismics, with or without partial binocular vision. In the former, saccade disconjugacy responds to the disparity and aims to maintain peripheral binocular vision. By contrast, in subjects without binocular vision, disconjugate changes seem to be driven by monocular visual input.

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367.8

THE ROLE OF VISUAL STIMULI IN PREDICTIVE SACCADES ELICITED AT VARIOUS RATES. C. Kennard*, E. O'Sullivan, S. Shaunak, M. Hawken, T. Crawford and L. Henderson Academic Unit of Neuroscience, Charing Cross and Westminster Medical School, London W6 8RF, U.K.

Saccades of patients with mild Parkinsonian disease (PD) are said to be abnormal in the absence of a concurrently visible target or when they are part of a rapid sequence of eye movements. We tested this hypothesis by using a predictive saccade paradigm in which target visibility is withdrawn for a period, but the subjects are requested to continue tracking as if the target had remained visible. Three rates of target alternation were used (0.25 Hz, 0.5 Hz and 1.0 Hz). The PD patients rapidly developed predictive behaviour at all three frequencies of target presentation. Withdrawal of target visibility brought out the extremes of primary saccade gain, most undershoot (hypometria) being displayed at the lowest frequency, whereas the gain was greatest - actually overshooting the target location - at the highest frequency. The control group showed similar but greater overshoot resulting in a markedly hypermetric final eye position which was independent of frequency. These results demonstrate that the spatial error of Parkinsonian saccades does not invariably take the form of hypometria when part of a rapid sequence of eye movements and that they can be hypermetric relative to the target location.

367.9

SPONTANEOUS EYE-BLINKING AS A FUNCTION OF VISUAL ATTENTION IN SCHIZOPHRENIA. S. Windmann, I. Curio and I. Jurna*. Medical and Clinical Psychology, University of the Saarland, 66421 Homburg/Saar, Germany.

Elevated dopaminergic transmission in mesolimbic and mesocortical pathways have long been discussed as a direct or indirect cause for schizophrenic symptoms, such as attention deficits. Pharmacological studies in animals as well as human clinical studies point to a positive link between dopaminergic transmission and eye-blink rate. However, the neurophysiological mechanisms of spontaneous eye-blinking are not yet fully understood. Psychophysiologically, eye-blinking can be described as a function of the perceptual demands of the situation. We examined eye-blinking in 14 schizophrenic subjects and 17 normal controls under three experimental conditions, in which the subject's visual attention was i) externally controlled (by means of an intermittent flash light); ii) completely internally controlled (free talk); and iii) alternating (filling in a form) in an intrasubject replication design. Among all three conditions, MANOVA-results showed consistent and stable increased blink-rates in schizophrenic patients (no interaction effects and no effects for repeated measurements were found). Effects were even marked in several descriptors of the blink-interval-distribution. The findings are interpreted in terms of the blink-alpha-neuro-circuit (BANC) postulated by Karson (1990, Schizophrenia Bulletin, 16, 345-354). Applicability of parameters of spontaneous eye-blinking as diagnostic and prognostic indicators or even as biological markers of psychopathology is discussed.

SPINAL CORD AND BRAINSTEM: PHARMACOLOGY AND TRANSMITTERS

368.1

GABAERGIC ORIGIN OF THE MONOSYNAPTIC PAD EVOKED IN SINGLE GROUP I MUSCLE AFFERENTS BY INTRASPINAL MICROSTIMULATION. J.N. Quevedo*, J. Lomelí, P. Linares and P. Rudomin. CINVESTAV-IPN, México, D.F. 07000.

We have shown previously that in the anesthetized cat, intraspinal microstimulation (IS-μSt) with strengths below threshold for spike generation, produces a monosynaptic depolarization of single afferent fibers (MS-PAD). This PAD has been attributed to direct activation of last-order GABAergic interneurons (*Exp. Brain Res.* 91:29, 1992). Simultaneous measurement of the intraspinal threshold of two collaterals of the *same* afferent fiber has shown that IS-μSt with low strengths, may produce a MS-PAD that is restricted to one collateral only, while higher stimulus strengths produce a MS-PAD in both collaterals (*Brain Res.* 643:328, 1994). This has raised the question on whether the MS-PAD that is restricted to one of the two collaterals is due to monosynaptic activation of GABAergic interneurons, or, instead, to local responses produced by the electrical pulse. The MS-PAD produced by IS-μSt that was restricted to *one* collateral only, had an onset latency shorter than 1.5 ms and lasted up to 100 ms. In two cases this PAD was depressed 20-40 min after PTX (1 mg/kg i.v.) by 50 and 100% of control, respectively. In one case, low strength IS-μSt produced a MS-PAD in *both* collaterals that was depressed 20 min after PTX by 73% and 100% of control, respectively. These observations suggest that the MS-PAD evoked by IS-μSt either in one or in both collaterals of the same afferent fiber has a GABAergic component. They further indicate that the excitability increase produced by IS-μSt in one collateral may not necessarily spread to nearby collaterals of the same afferent fiber, suggesting that presynaptic inhibition due to activation of GABAa receptors can be rather selective. *Partly supported by NIH NS09196 and CONACyT 039-N9107.*

368.2

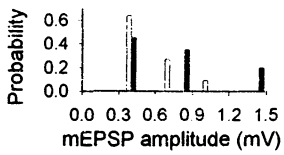
TONIC INHIBITION OF SPINAL PRESYNAPTIC INHIBITION BY DESCENDING NORADRENERGIC NEURON Y Hasegawa, H. Araki* and H. Ono. Dept. of Pharmacy, Branch Hosp., Faculty of Med., Univ. of Tokyo, Tokyo 112, Japan. *Dept. of Pharmacol., Res. Cent., Taisho Pharmaceutical Co., Ltd., Saitama 330, Japan.

Presynaptic inhibition was induced by conditioning stimulation of the L4 dorsal root (DR) was measured in terms of the reduction in the amplitude of the monosynaptic reflex (MSR) in the L5 ventral root which was evoked by test stimulation of the L5 DR in anesthetized rats. Depression of the MSR was evoked within a conditioning-test stimulation interval of 5 - 30 msec. This depressant response was inhibited by the direct application of bicuculline (100 μM), but not strychnine (100 μM), to the spinal cord surface. Thus, this depressant response most likely represents presynaptic inhibition. A significant difference was recognized between the percent presynaptic inhibition in intact (26%) and spinalized (69%) rats. In intact rats in which noradrenaline had been depleted by DSP-4 (50 mg/kg, i.p.), presynaptic inhibition was significantly enhanced to 67 %. In spinalized rats, L-DOPA (5 mg/kg i.v.) with prazosin-HCl (0.5 mg/kg, i.v.) inhibited presynaptic inhibition. The α_2-adrenoceptor agonist clonidine-HCl (0.1 mg/kg, i.v.) significantly inhibited presynaptic inhibition, and this effect was antagonized by the α_2-adrenoceptor antagonists idazoxan (0.5 mg/kg, i.v.) and yohimbine-HCl (0.5 mg/kg, i.v.). These results suggest that descending noradrenergic neurons inhibit presynaptic inhibition, and this action is mediated via α_2-adrenoceptor.

368.3

FACILITATION OF MEPSPS IN RAT TRIGEMINAL MOTONEURONES IN-VITRO. Ming-Yuan Min, John C. Curtis and Kwabena Appenteng. (SPON: Brain Research Association) Dept. of Physiology, Univ. of Leeds, Leeds, UK.

Plots of the amplitude distribution of mEPSPs (i.e. EPSPs recorded in the presence of tetrodotoxin) reveal clear, equidistant, peaks with small coefficients of variation. Changes in the mean spacing and probability of the peaks can thus be used to infer changes in postsynaptic and presynaptic function. Transmission at excitatory synapses on trigeminal motoneurones has been reported to be subject to a tonic inhibition which has been suggested to be mediated by GABA. We have found that bath application of the $GABA_B$ receptor antagonist 2-OH-saclofen (50-100 μM) produces increases in quantal amplitude and release probability, indicating the presence of postsynaptic and presynaptic $GABA_B$ receptors (Fig.1: open bars; peaks, in a motoneurone, in absence of drug: filled bars; peaks in presence of drug). The changes are transient when (whole-cell) patch electrodes not containing the sodium salts of GTP or ATP are used, but are sustained when electrodes containing both salts are used, with the activity returning to control levels after washout of drug. However, use of electrodes containing GTP alone can result in increases in mEPSP amplitude which are sustained for over 4 hours after washout of saclofen.



368.4

ASSAYS OF GLYCINE RECEPTOR FUNCTION IN THE ISOLATED SPINAL CORD OF *OSCILLATOR* MUTANT MICE. E. S. Simon* and R. E. Burke, Lab. Neural Control, NINDS, NIH, Bethesda, MD 20892.

As part of an investigation of the function of inhibitory glycine receptors (GlyRs) in spinal networks, we examined **oscillator** (*spd^{ot}*) mice, a mutant with a deletion in the gene encoding the α1 GlyR subunit (Buckwalter et.al. Hum. Mol. Genet. 3:2025,1994). During the 2nd postnatal week, oscillator homozygotes, identified by molecular genotyping (ibid), exhibit progressive tremor, rigidity, gait abnormalities, and myoclonus that worsen until death at P18-P21. *In vitro* preparations of lumbar spinal cords were examined in P11-P13 normal (wild type) mice and oscillator homozygotes. In normals, strychnine-sensitive (glycinergic) **recurrent inhibitory postsynaptic potentials** (RIPSPs; approx. reversal potential -60 mV) were evoked by ventral root (VR) stimulation and recorded in motoneurons (MNs) with sharp intracellular electrodes. In 15/16 MNs from the oscillator mice, RIPSPs were undetectable even during long de- and hyperpolarizing current pulses. **Synchronized 5-15 Hz sinusoidal oscillations** in VR and MN records were found in normal spinal cords after bath application of strychnine (Simon, Neurology, in press) but were present in oscillator spinal cords without drug treatment. In both normal and mutant cords, these network-generated sinusoidal oscillations were markedly and reversibly attenuated by bath-applied glycine (1-2 mM). In oscillator, the absence of glycinergic RIPSPs in MNs contrasts with the potent effects of glycine on network oscillations. Network oscillations appear to represent a more sensitive assay for the presence of functional GlyRs at P11-P13.

368.5

RECIPROCAL INHIBITION OF EXPIRATORY NEURONS IS GLYCINERGIC AND NOT REQUIRED FOR RHYTHM GENERATION.
J.L. Feldman* & X.M Shao Systems Neurobiology Laboratory, Dept. of Physiological Science, UCLA, Los Angeles, CA, 90095-1527

Does respiratory rhythm generation *require* phasic inhibitory interactions between inspiratory and expiratory neurons? In *en bloc in vitro* neonatal rat brainstem, rhythmic motor outflow is relatively unaffected by global perturbation of Cl^- or K^+ channels, or $GABA_{(A\&B)}$ and glycine receptors (Feldman & Smith, NY Acad. Sci., '89; Onimaru, et al. Pflug. Arch '90). To extend these studies to single neurons, we whole cell patch clamped expiratory neurons in rhythmically active medullary slices from neonatal rats (P0-P4) (Smith et al. Science, '91). We recorded from 6 neurons in the preBötzinger Complex (preBötC) which generated impulses during expiration and were silent –with IPSPs– during inspiration. Application of bicuculline (bath: 10 μM; local injection into preBötC: 0.2-0.4 mM) had no effect on inspiratory inhibition. The phasic inhibitory activity disappeared following strychnine application (bath: 5 μM; local injection: 0.1 mM) or using a bathing solution with Cl^- replaced by isothionate. Following local injection of strychnine (0.4 mM) or bicuculline (0.2-0.4 mM) in preBötC or XII nucleus, changes in XII nerve respiratory pattern –irregular seizure-like discharge on top of the relatively unchanged rhythm– were observed. These results suggest that: i) the inspiratory-modulated inhibitory synaptic input to preBötC expiratory neurons is glycinergic, but not required for rhythm generation, and, ii) *in vitro*, endogenous GABA and glycine affect respiratory pattern within preBötC and XII nucleus. Supported by NIH Grant HL 40959.

368.6

EXCITATION OF UPPER AIRWAY MOTONEURONS BY IONTOPHORETIC APPLICATION OF SEROTONIN. V. Fenik, L. Kubin, S. Okabe, A.I. Pack, and R.O. Davies*. Dept. of Animal Biology and Center for Sleep and Respiratory Neurobiology, University of Pennsylvania, Philadelphia, PA 19104

Our earlier studies led us to propose that the hypotonia of upper airway muscles observed during sleep, especially REM sleep, is dependent on the serotonergic innervation of motoneurons (mns) to the upper airway muscles. Serotonin (5HT) excites upper airway mns and this excitation is withdrawn during REM sleep, when the firing of medullary serotonergic neurons is reduced. We hypothesized that the sensitivity of different groups of upper airway mns to 5HT could be related to the magnitude of their depression during REM sleep, e.g., pharyngeal dilator mns would be more sensitive than laryngeal mns. Experiments were done on decerebrate, vagotomized, paralyzed and artificially ventilated cats. Hypoglossal (XII) and vagal (X) nerves were prepared for recording and the medulla exposed. Five-barrel pipettes (7 μm tip diameter) were filled with: 3 M NaCl for recording, 3 M NaCl for current balancing, 10 or 20 mM 5HT, 10-75 mM glutamate, and 5 mM methysergide. Mns were identified by spike-triggered averaging the corresponding whole nerve activity, and characterized by their activity pattern with respect to phrenic nerve activity. To date, 59 XII mns have been studied (40 inspiratory, 9 expiratory, 10 tonic) and 13 X mns (3 inspiratory, 10 expiratory). In all mn types, 5HT produced a gradually developing and long-lasting excitation that could be blocked by methysergide. In an attempt to quantify the sensitivity to 5HT, 14 XII inspiratory mns and 5 X expiratory mns having similar conrol firing rates were tested with small 5HT currents (15-25 nA, mean: 18 nA ± 4.5 (SD)) applied for 3 min from a barrel containing 10 mM 5HT and 150 mM NaCl. Under these standardized conditions, the maximal firing rate increase was 10 Hz ± 5 (SD) in XII mns and 2.5 Hz ± 1.3 (SD) in X mns ($p < 0.004$). Thus, the sensitivity to the excitatory effects of 5HT varies among functionally different airway mns. This may be related to different receptor densities, receptor location, or local differences in 5HT reuptake mechanisms. (Supported by HL-42236 and HL-47600)

368.7

ACTIVATION OF ADENOSINE A_1 AND A_2 RECEPTORS DIFFERENTIALLY MODULATES CALCIUM CHANNELS AND GLYCINERGIC SYNAPTIC TRANSMISSION IN RAT BRAINSTEM. M. Umemiya* and A.J. Berger. Dept. Physiology and Biophysics, Univ. of Washington, Seattle, WA 98195-7290

Multiple types of calcium channels are responsible for evoked transmitter release at the presynaptic terminal in the mammalian CNS. To test the contribution of each calcium channel type to synaptic modulation, we recorded unitary glycinergic postsynaptic currents in rat hypoglossal motoneurons. Activation of A_1 adenosine receptors inhibited synaptic transmission. The data suggested that synaptic inhibition was mediated by inhibition of N-type calcium channels at the presynaptic terminal because 1) ω-conotoxin GVIA sensitive synaptic transmission was more sensitive to A_1 receptor activation than ω-conotoxin GVIA resistant synaptic transmission, 2) when recorded from the soma of presynaptic interneurons, N-type calcium channels were more sensitive to A_1 receptor activation than were ω-conotoxin insensitive calcium channels. In contrast, activation of A_2 adenosine receptors predominantly potentiated ω-conotoxin GVIA resistant synaptic transmission. As ω-conotoxin GVIA resistant synaptic transmission was more sensitive to the activation of A_2 receptors, we conclude that A_2 receptor activation facilitates synaptic transmission through potentiating P-/Q-type (ω-Agatoxin IVA sensitive) calcium channels. Thus, it is likely that N-type and P-/Q-type calcium channels play different roles in synaptic modulation by neurotransmitters in the CNS. (Supported by NS 14857)

368.8

PRESYNAPTIC DEPRESSION OF GLUTAMATERGIC EPSCs IN RAT HYPOGLOSSAL MOTONEURONS (HMs) BY MUSCARINIC RECEPTORS IS NOT DUE TO INHIBITION OF N- OR P-TYPE CALCIUM CURRENTS. M.C. Bellingham* and A.J. Berger. Department of Physiology and Biophysics, University of Washington, Seattle, WA 98195-7290.

Cholinergic modulation of synaptic inputs is significant in regulation of motor responses. Whole-cell patch-clamp recordings were made from young adult rat HMs (n = 17), visually identified using Nomarkski optics and infrared transluminations of transverse brainstem slices. Electrical stimulation lateral to the hypoglossal motor nucleus evoked short-latency excitatory postsynaptic currents (EPSCs), which were markedly suppressed or abolished by bath application of kynurenic acid (2 mM; n = 4), showing that they were glutamatergic. Bath application of carbachol (10 μM), a cholinergic agonist, reversibly reduced EPSC amplitude to 37 ± 17% (mean ± SD, n = 11) of control, caused an inward holding current (-65 ± 59 pA) and a small decrease (14 ± 13%) in input resistance. The muscarinic receptor antagonist atropine (1 μM) blocked EPSC reduction (n = 4). Postsynaptic currents evoked in HMs by focal pressure injection of L-glutamate (500 μM, n = 3) were not significantly reduced by carbachol, while spontaneous glutamatergic miniature EPSCs were significantly ($P < 0.01$, n = 3) reduced in frequency without reduction in amplitude, indicating that excitatory glutamatergic inputs to rat HMs are modulated by muscarinic receptors at a presynaptic site. Bath application of ω-conotoxin (ω-CgTx, 2 μM, n = 4), a blocker of N-type calcium channels, then ω-agatoxin-IVa (ω-AgaTx, 200 nM, n = 8), a blocker of P-type calcium channels, irreversibly reduced EPSC amplitude to 62 ± 12% and 37 ± 23% of control, respectively. Sequential blockade of N- and then P-type calcium channels did not significantly alter the reduction of the remaining EPSC by carbachol, which reduced EPSCs to 33 ± 9% (n = 4) after ω-CgTx (*cf.* reduction to 32 ± 8% of control before ω-CgTx) or to 32 ± 10% (n = 4) after ω-AgaTx (*cf.* reduction to 32 ± 8% of control before ω-AgaTx). We conclude that muscarinic modulation of presynaptic N- or P-type calcium channels is not essential for the mechanism(s) by which activation of presynaptic muscarinic receptors depresses excitatory synaptic transmission to HMs. Supported by Parker B. Francis Foundation, NS 14857, HL 49657.

368.9

CLONIDINE REDUCES I_h IN HYPOGLOSSAL MOTONEURONS (HMs). M.A. Parkis* and A.J. Berger, Dept. of Physiology and Biophysics, University of Washington, Seattle, WA 98195-7290

We used intracellular recording techniques in 400 μm-thick brainstem slices from juvenile rats to investigate the effects of clonidine, an α_2-adrenoceptor and non-adrenergic imidazoline receptor agonist, on HMs in vitro. In current clamp, clonidine (10-100 μM) elicited a slow hyperpolarization and increased input resistance (R_N) in all 14 HMs tested (R_N in 10 μM clonidine = 142 ± 23% of control, n=4). Concurrently, the time course of the depolarizing "sag" of the voltage response to hyperpolarizing 250-msec current steps was slowed. This effect on the sag was not altered by 0.5 mM TTX (n=2), indicating that it was direct.

Since this sag represents the activation of a hyperpolarization-activated inward current, I_h, (Bayliss et al., J. Neurophys. 71:119, 1994), we investigated clonidine's effect on I_h, using single electrode voltage clamp (SEVC). In SEVC, I_h was activated by applying a series of hyperpolarizing steps from a holding potential of -60 mV. Clonidine decreased the amount of I_h activated at any given potential, thus shifting the I_h activation curve more negative. A first order exponential fit of the activation time course of I_h revealed that clonidine increased the activation time constant, τ, in all cells (n=6). In steps to -90 mV, average τ increased from 160 ms in control to 253 ms in clonidine.

Yohimbine (10-100 μM), an α_2-antagonist, did not block clonidine's effect on the depolarizing sag (n=4) or, in SEVC, on I_h (n=1). This suggests that the effect of clonidine on I_h is not mediated by α_2-adrenoceptors, but may involve imidazoline receptor activation. Supported by HL 49657.

368.10

MODULATION OF CAUDAL RAPHE NEURONS BY SEROTONIN: 5-HT_{1A} RECEPTOR-MEDIATED INHIBITION OF CALCIUM CURRENT AND ACTIVATION OF AN INWARDLY-RECTIFYING K^+ CURRENT. Douglas A. Bayliss*. Department of Pharmacology, University of Virginia, Charlottesville, VA 22908.

Raphe neurons of the caudal medulla oblongata provide a major descending influence onto spinal motoneurons and sympathetic preganglionic neurons, yet little is known regarding intrinsic ionic conductances or neuromodulation in those raphe cells. Whole-cell recordings were made from visually identified neurons in a rat medullary thin-slice preparation in order to determine effects of serotonin (5-HT) on voltage-activated calcium currents and inwardly-rectifying K^+ currents in caudal raphe cells. Currents through calcium channels were evoked by steps to 0 mV from a holding potential of -70 mV. In the majority of caudal raphe neurons, 5-HT decreased peak current dose-dependently; inhibition at a maximally effective concentration of 5-HT (1 μM) averaged 49.4 ± 3.1%. Calcium current inhibition was apparently mediated by a 5-HT_{1A} receptor, as it was mimicked by the 5-HT_{1A} receptor agonist 8-OH-DPAT and blocked by the 5-HT_{1A} antagonist NAN-190, but unaffected by ketanserin, a 5-HT_2 antagonist. Caudal raphe neurons expressed N-type (ω-Conotoxin GVIA-sensitive) and P-type (ω-Agatoxin IVA-sensitive) calcium current, both of which were inhibited by 5-HT. In addition to its effects on calcium currents, 5-HT also caused a membrane hyperpolarization and/or outward current. The current activated by 5-HT ($I_{5\text{-}HT}$), measured during hyperpolarizing ramp potentials from -40 mV, was strongly inwardly-rectifying. In elevated external K^+, the reversal potential of $I_{5\text{-}HT}$ was shifted in the depolarizing direction and the conductance induced by 5-HT was enhanced, peak $I_{5\text{-}HT}$ in 6-mM external K^+ averaged 16.7 ± 2.9 pA with an attendant 6-fold increase in conductance. Effects of 5-HT were mimicked by 8-OH-DPAT, suggesting that they were also mediated by a 5-HT_{1A} receptor. These results indicate that 5-HT_{1A} receptors inhibit N- and P-type calcium currents and activate inwardly-rectifying K^+ currents in caudal raphe neurons; such effects may underlie 5-HT-mediated autoinhibition of caudal raphe cells. (Supported by NS33583).

368.11

PONTINE MICROINJECTION OF THE M2 MUSCARINIC ANTAGONIST METHOCTRAMINE BLOCKS BETHANECHOL-INDUCED REM SLEEP (REM-BETH). H.A. Baghdoyan* and J.L. DiVittore. Department of Anesthesia, The Pennsylvania State University, College of Medicine, Hershey PA 17033

Activation of muscarinic cholinergic receptors (mAChRs) in the medial pontine reticular formation (mPRF) is known to be important for generating rapid eye movement (REM) sleep. Currently, there is much interest in specifying the precise roles of individual mAChR subtypes in REM sleep generation. Recent *in vitro* receptor autoradiographic studies have demonstrated the presence of M2 binding sites in feline mPRF (*NeuroReport* 5:1631-1634, 1994). Therefore, the present study is testing the hypothesis that mPRF administration of methoctramine (METH) will block cholinergic REM sleep generation. To date, 11 control recordings (no injection) and 57 microinjections of bethanechol alone (BETH, 4.3 µg/.25 µl, 87 mM) and bethanechol following METH pretreatment (6 doses of METH ranging from 10^{-12}M to 10^{-2}M) have been made in 3 conscious cats. All injections were made during quiet wakefulness, and polygraphic recordings were obtained for 2 hrs post-injection to quantify sleep and wakefulness. Following mPRF microinjection of BETH alone, cats spent an average of 74% of the total recording time in the REM sleep-like state (REM-BETH), representing a 466% increase over control REM sleep levels. Microinjection of METH 15 min prior to BETH significantly reduced REM-BETH ($F=20.09$; $df=7,67$; $p<0.001$), and the ability of METH to block REM-BETH was dose-dependent. A 22% decrease in REM-BETH was obtained following pretreatment with 10^{-10} M METH, but a concentration of 10^{-4} M METH was required to significantly ($p<0.05$) block REM-BETH and return REM sleep to control levels (13%). The present data are consistent with previous anatomical studies localizing M2 receptors to the mPRF (*NeuroReport* 5:1631-1634, 1994), and provide functional evidence that M2 receptors in the mPRF contribute to generating the BETH-induced REM sleep-like state. *Support: Department of Anesthesia and MH45361.*

SPINAL CORD AND BRAINSTEM: ANATOMY

369.1

IDENTIFICATION OF GLUTAMATE, GABA AND PARVALBUMIN-CONTAINING TRIGEMINAL PREMOTOR NEURONS IN THE RABBIT. A. Kolta*, K-G. Westberg, P. Clavelou, D. Veilleux and J.P. Lund. RIKEN institute Wako, Japan and Univ. of Montreal, CRSN, Mtl, P.Q., Canada, H3C 3J7.

This study was designed to histochemically identify excitatory and inhibitory trigeminal premotor neurons in the rabbit. Last-order interneurons were retrogradely-labeled with rhodamine conjugated dextran-amines injected into the Vth motor nucleus (NVmt). As in other species, many premotor neurons were found throughout the rostro-caudal extent of the reticular formation (RF). By far the largest projection originated from the parvocellular region (Rpc) including pars α (Rpcα) (64% of all reticular projections). Within the trigeminal system proper, last-order interneurons were found mainly in the regio h of Meessen and Olzchewski (comprising the supra- (NsV), juxta- (JuxtV) and inter-trigeminal (NintV) areas) and the subnucleus oralis of the spinal V tract (NVspo) (respectively 46 and 23% of trigeminal projections). Fewer projections arose from the main sensory nucleus, the subnucleus caudalis of the spinal V tract and the contralateral NVmt. All of the above groups were bilateral with the exception of NVmt. The contralateral olivary complex also provided a massive innervation of trigeminal motoneurons. Glutamate-containing premotor neurons were found across all areas of the RF, with again the highest proportion in Rpc and Rpcα. Some of the retrogradely-labeled neurons in medial NsV, NVspo and the Olive were also glutamate immuno-reactive. GABA immuno-positive premotor interneurons were localized in lateral NsV, NintV, Rpc, Rpcα and the gigantocellular and lateral RF. The distribution of parvalbumin-containing neurons paralleled closely that of GABA immunoreactive neurons in the RF but not in regio h, suggesting that these may be physiologically different populations of GABAergic interneurons. These results provide anatomical evidence for the existence of several populations of excitatory and inhibitory interneurons innervating the trigeminal motoneurons of the rabbit.

This work was supported by the human frontier research program, the Swedish MRC (K94-10133, K93-10529), a group grant from the Canadian MRC and Glaxo-France.

369.2

EFFECTS OF A COMPLETE SPINAL TRANSECTION ON NMDA RECEPTORS IN CAT SPINAL CORD. R.S. Aloyz, N. Giroux, S. Rossignol, T.A. Reader and H.H. Jasper*
Centre de recherche en sciences neurologiques, Département de physiologie, Faculté de médecine, Université de Montréal, Montréal (Québec) Canada.

Excitatory amino acids have been shown to mediate locomotion in paralyzed decerebrate cats, in *in vitro* neonatal rats and in lampreys. In chronic spinal cats, the *N*-methyl-D-aspartate (NMDA) receptor antagonist AP5 can block locomotion and NMDA can restore locomotion after AP5, suggesting that NMDA receptor activation may be important for the expression of locomotion. The distribution of amino acids and glutamate receptors of the NMDA subtype was studied in the lumbar spinal cord in four control cats and in four animals with different survival time (13, 33, 70 and 279 days) after spinalization at T13. The localization and changes of glutamate NMDA receptors were examined in the different cytoarchitectonic *laminae* of the lumbar spinal cord, by quantitative autoradiography using [^{3}H]MK-801. In control cats, the highest densities of NMDA receptors were in laminae II-III, followed by lamina I and X, and with lower densities in the rest of the gray matter. After spinalization, there was an up-regulation at 13 and 33 days after spinalization in the dorsal horns (laminae I-IV) with no variation in the other laminae. At longer survival times, i.e. 70 and 279 days post spinalization, there was a return to normal values in the spinalized cats. Samples of spinal cord from the same cats were also used to measure amino acids by HPLC; one of the most abundant amino acids found in the lumbar spinal cord of control cats was glutamic acid (GLU) with endogenous levels of 14.1 µmoles/mg protein. On day 13 following complete spinalization, there was a decrease in GLU (8.7 µmoles/mg protein), but at longer survival times, i.e. 33 days post spinalization, the levels had returned to control values (13.5 µmoles/mg protein). The receptor changes reported could eventually be correlated to the alterations in the levels of amino acids, and to the effects of administration of NMDA agonists and antagonists in this animal model of functional recovery. *[Supported by the MRC(C) and the NeuroScience Network]*

369.3

SEROTONIN$_{1A}$ AND α_1- AND α_2-NORADRENERGIC RECEPTORS IN THE SPINAL CORD OF SPINALIZED CATS. N. Giroux, R.S. Aloyz, S. Rossignol and T.A. Reader* *Centre de recherche en sciences neurologiques. Département de physiologie, Faculté de médecine, Université de Montréal, Montréal (Québec) Canada.*

The serotonin (5-HT) antagonist cyproheptadine reduces spasticity and improves locomotor function of spinal cord injured patients. In chronic spinalized cats, the 5-HT agonists quipazine and 5-MeODMT increase the amplitude of activity in flexor and extensor muscles during locomotion. Also, in spinalized cats as well as in spinal cord injured patients, the α_2-noradrenergic agonist clonidine improves recovery of locomotion. To establish a basis for locomotor pharmacotherapy the fate of monoamine receptors was examined after a complete spinal transection in cats. The distribution of 5-HT$_{1A}$, α_1 and α_2-noradrenergic receptors was studied in the lumbar spinal cord of six control cats and in animals with different survival times (13, 33, 70, 165, 279 and 409 days) after spinalization at thoracic 13 level. The 5-HT$_{1A}$ receptors were localized with [^{3}H]8-OH-DPAT in the gray matter; the highest densities were in laminae III and IV, and around the central gray (lamina X). After spinalization, [^{3}H]8-OH-DPAT binding increased, mainly in laminae III and IV at 33 days. For the other laminae, there were no differences when compared to control cats. The α_1- and α_2-noradrenergic receptors were labelled with [^{3}H]prazosin and [^{3}H]idazoxan, respectively. Both receptors showed a similar pattern of distribution; the highest levels were in the dorsal horn (laminae II-III) but also in laminae IX and X, with moderate labelling in the remaining laminae. [^{3}H]prazosin binding increased after 33 and 70 days following spinalization in laminae I, II-III, and after days 70 and 165 in laminae II-III and IX. At longer survival times, *i.e.*: 279 days or more, [^{3}H]prazosin binding was lower than in control cats for all laminae. There was also an up-regulation of α_2 receptors, especially at 13 and 33 days of survival, followed by a decrease at longer survival periods. The pronounced and acute up-regulations observed in the lumbar region suggests that mechanisms involving 5-HT$_{1A}$, α_1- and α_2-noradrenergic receptors may be important after spinalization. *[Supported by the MRC(C) and the NeuroScience Network]*

369.4

LOCALIZATION OF CALCIUM-DEPENDENT PROTEIN PHOSPHATASE (CALCINEURIN) IN THE BRAIN STEM AND SPINAL CORD OF THE RAT. F.F. Ebner*+, B.E. Wadzinski$ and S. Strack+ +Institute for Developmental Neuroscience and $Dept. of Pharmacology, Vanderbilt University, Nashville, TN 37203.

Calcineurin (CaN) is a brain enriched protein phosphatase that has been implicated in synaptic transmission, cytoskeletal dynamics and other calcium-regulated events. We have studied the distribution of the major, α–isoform of CaN in the rat spinal cord and brainstem with a polyclonal antibody raised against a peptide sequence present in αCaN. Quantitative immunoblot analysis showed that αCaN is present in the spinal cord at 3 to 4 fold lower concentration than in the forebrain. Light microscopic immunohistochemistry revealed a strikingly restricted pattern of αCaN staining. In the brain stem, high αCaN immunoreactivity was observed in a subset of large neurons in several motor nuclei: the trigeminal (V), facial (VII), and the perihypoglossal nucleus of Roller. Axons, dendrites and the soma excluding the nucleus were immunoreactive. Some axons in cranial nerves were also stained. Neurons in the hypoglossal nucleus and throughout the reticular formation were faintly labeled. Terminal field-like staining in the nucleus trigeminalis interpolaris was present in clusters which likely correspond to the "barrelettes" of whisker pad afferents. In the spinal cord, αCaN was found in a subset of large motoneurons in the lateral aspects of the ventral horn and diffusely in the superficial laminae of the dorsal horn. Dorsal root ganglion neurons stained faintly; axons in the dorsal and ventral roots were largely unlabeled. In the white matter, the corticospinal tract is faintly stained. A small fiber tract running ventrolateral of the dorsal horn was strongly immunoreactive and was identified as the spinocervical tract, an ascending tract that carries somatosensory information from dorsal horn lamina IV neurons to the cervical nucleus. The staining pattern was qualitatively similar in cervical, thoracic and lumbar segments of the spinal cord. (Supported by NS13031 to F.F.E. and GM51366 to B.E.W)

369.5

GAP JUNCTIONS ARE ABUNDANT ON NEURONS THROUGHOUT ADULT RAT SPINAL CORD

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Using confocal "grid-mapped" freeze-fracture, we have visualized and mapped 81 gap junctions (GJs) on motor neurons and interneurons in Laminae IV-IX from lumbosacral, thoracic, and cervical regions of adult rat spinal cord. GJs were found in 29 clusters on 27 neurons, primarily on somata and proximal dendrites, forming "mixed" (chemical and electrical) synapses. Most neuronal GJs were very small (mean = 87 connexons; median = 52; range = 6-704). GJs were present in neuronal plasma membranes at an average density of 1/34 μm^2 in the lumbosacral enlargement, and at 1/158 μm^2 in the cervical enlargement. Thus, we calculate that the average neuron in the ventral horn of rat spinal cord contains 300-1000 GJs. Since most neuronal GJs are less than 0.06 μm in diameter, they are too small to have been detected previously by thin-section TEM methods or by conventional immunocytochemical staining techniques. Most neuronal GJs in the spinal cord are too small for direct intercellular propagation of action potentials. However, they may provide pathways for metabolic coupling at mixed synapses or for electrical modulation of chemical synaptic transmission. Contrary to published reports, GJ are a general component of neuronal plasma membranes in adult rat spinal cord -- they were present in all levels examined, they were in all laminae examined, and they were present on interneurons and motor neurons. New electrophysiological monitoring strategies may be required to ascertain the role of neuronal GJ in spinal cord function.

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369.6

GROWTH OF DENDRITIC TREES OF NECK MOTONEURONS IN ADULT CATS FOLLOWING AXOTOMY. P.K. Rose* and M. Odlozinski, MRC Group in Sensory-Motor Physiology, Queen's University, Kingston, ON Canada K7L 3N6

Long term axotomy leads to several major structural alterations in spinal motoneurons, including loss of synapses on the cell body. Since most synapses on motoneurons are located on the dendritic tree, the initial goal of the present study was to determine the rearrangement of synapses located on different regions of the dendritic trees. Following a 12 to 16 week permanent axotomy, motoneurons supplying the cat neck muscles, biventer cervicis and complexus, were antidromically identified and intracellularly stained with horseradish peroxidase. In preparation for the electron microscopic studies, dendrites were systematically reconstructed at the light microscopic level. Three of the 9 motoneurons examined appeared normal, but, unexpectedly, the other motoneurons showed evidence of growth. Some distal dendrites gave rise to complex appendages that consisted of tangled branches, some fine, some swollen. Other distal dendrites had long segments that were unusually swollen (3 - 4 μm) and displayed a nonuniform staining pattern similar to that seen in myelinated axon collaterals. A quantitative comparison of the dendritic trees of three axotomized and three control motoneurons demonstrated that the total number of dendritic branches increased by 60 to 350%. Some dendrites from the axotomized cells terminated more than 3200 μm from the soma, compared to a maximum dendritic length of 2,050 μm in the control cells. Two of the axotomized motoneurons had surface areas more than 50% larger than the control cells.

These results indicate that the dendritic trees of some neck motoneurons expand following long-term axotomy. The results contrast with comparable studies of axotomized hindlimb motoneurons whose dendritic trees shrink by 35%. The additional membrane may provide a site for additional synaptic contacts or may represent aberrant axonal growth. (Supported by MRC and MDAC)

369.7

PHRENIC MOTONEURONS ADAPT TO INACTIVITY. G.C. Sieck*, W.Z. Zhan, H. Miyata, Y.S. Prakash & K.G. Smithson. Depts. of Anesthesiology, and Physiology & Biophysics. Mayo Foundation, Rochester, MN 55905.

Motoneuron (MN) plasticity may be influenced by the level of activation and/or by interactions between MN and muscle fibers (MF). The goal of this study was to examine phrenic MN adaptations to diaphragm (DIA) muscle inactivity, and to determine the effect of mismatched MN and MF activities on such adaptations. Two models of DIA inactivity were compared in the rat: 1) spinal isolation (SI) by hemisection at C2 where MN and MF inactivities were matched; and 2) tetrodotoxin (TTX) nerve blockade where MN activity increased and was mismatched with MF inactivity. Fluorescent dextrans (3000 MW) or cholcratoxin B-fragment (CTB) were injected into the DIA to retrogradely label phrenic MNs. Following 2 weeks of paralysis, cervical spinal cords were excised. Fluorescently-labelled MNs were optically sectioned using confocal microscopy and MN somal volumes were measured. CTB was detected using a peroxidase reaction and dendritic architecture was assessed using computerized camera-lucida analysis. Compared to controls, phrenic MN somal volumes decreased with SI, but increased with TTX. Dendritic arborization increased with both SI and TTX, but to a greater extent with TTX. These results suggest that morphological adaptations of phrenic MNs are dependent on a match between MN and muscle activities. Supported by NIH grants HL34817 and HL37680.

369.8

LIGHT AND ELECTRON MICROSCOPE STUDY ON SYNAPTIC CONTACTS MADE BETWEEN MUSCLE-SPINDLE AFFERENT TERMINALS AND α-MOTONEURONS IN THE CAT TRIGEMINAL MOTOR NUCLEUS. N.H.Yabuta, Y.C.Bae#, A.Yoshida, M.Takemura* & Y.Shigenaga. Dept. of Oral Anatomy II, Osaka Univ. Faculty of Dentistry, Suita, Osaka 565, Japan; #School of Dentistry, Kyungpook University, Taegu, Korea.

We have previously reported that jaw-closing spindle afferents can be classified as type I and type II which may represent Ia and secondary afferents. This study aimed to investigate the spatial distribution of contacts made by spindle afferents upon motoneurons light microscopically, and to examine ultrastructures of their terminals in the central and peripheral Vmo.dl. HRP was injected into single spindle afferent(s) and a motoneuron in the same preparation. The type I terminals made contacts on the more proximal dendrites than the type II terminals did. The average number of contacts made by a spindle afferent upon a motoneuron was about two. For electron microscope study, 76 boutons in peripheral Vmo.dl and 105 in central Vmo.dl were analyzed. Two characteristics were commons to labeled boutons in the two regions. First, all the labeled boutons were densely filled with clear, spherical, synaptic vesicles. Second, neither the part of the collateral branch proximal to the bouton, or the intermediate fiber connecting the boutons, were seen to participate in a synaptic specialization. However, following differences were observed. (1) the total number of contacts per bouton was significantly fewer ($P<.05$) in central Vmo.dl neuropile than in peripheral one, (2) labeled boutons made significantly higher numbers of synaptic contacts on proximal dendrites in the former than in the latter region, and (3) the number of labeled boutons postsynaptic to axon terminals containing pleomorphic synaptic vesicles was higher in the latter than in the former regions. These data demonstrate that the spatial distribution of synaptic contacts made by spindle afferent fibers upon motoneurons is different between primary and secondary spindles.

369.9

ULTRASTRUCTURE OF PHYSIOLOGICALLY IDENTIFIED JAW-MUSCLE SPINDLE AFFERENT TERMINATIONS ONTO RETROGRADELY LABELED JAW-ELEVATOR MOTONEURONS. P. Luo* and D. Dessem. Dept. of Physiology, University of Maryland Dental School, Baltimore, MD 21201.

Synaptic relationships between rat jaw-muscle spindle afferents and jaw-elevator motoneurons were studied with combined retrograde and intracellular labeling. Initially motoneurons were retrogradely labeled by injecting horseradish peroxidase (HRP) into the jaw-elevator muscles. After 2 days, jaw-muscle spindle afferent axons were physiologically identified by their response to stretching of the jaw muscles and intracellularly stained with biotinamide. 243 intracellularly stained jaw-muscle spindle afferent boutons were examined which synapsed either with retrogradely-labeled motoneurons (n=85) or unlabeled structures (n=158) in the jaw-elevator motoneuron pool. 68% of stained spindle boutons synapsed with large or medium sized, retrogradely-labeled motoneuron dendrites. 86% of these synapses were asymmetric; 14% were symmetric. 32% of identified spindle boutons formed axosomatic synapses of which 78% were symmetric and 22% were asymmetric. Approximately 24% of the stained spindle boutons comprised the intermediate element of a synaptic triad sandwiched between an unlabeled presynaptic P-type bouton and a postsynaptic HRP-labeled motoneuron dendrite or soma. Numerous unlabeled S-, F-type, and dense-cored vesicle containing boutons (n=831) converged onto the same motoneuron elements which received spindle afferent synapses. 53% of these unlabeled boutons were F-type (n=444), 28% were S-type (n=230) and 19% (n=157) contained dense-cored vesicles. Most dense-cored vesicle containing boutons terminated on motoneuron somata (81%, n=129) rather than dendrites (19%, n=28). These data indicate that jaw-muscle spindle afferent synapses onto motoneurons are both morphologically and spatially diverse and that a smaller percentage of these synapses may be under presynaptic control than in the spinal cord. These data also imply that premotor neurons containing inhibitory neurotransmitters, catecholamines and neuropeptides may modulate trigeminal motoneuron excitability. Supported by NIH DE10132.

369.10

COMPARATIVE SYNAPTIC ULTRASTRUCTURE OF CAT AND MACAQUE TRIGEMINAL MOTONEURONS. N.E. Capra*, J.M. Bernanke and P.J. May. DOCBS., U. of Maryland, Baltimore, MD 21201; Dept. of Anatomy, U. of Mississippi Med. Ctr., Jackson,MS 39216.

The central morphological correlates for the species specific coordination of jaw muscle activity indicated by behavioral and electrophysiological data have not been fully elucidated. This study compares the synaptic ultrastructure of trigeminal motoneurons in cats and monkeys. A mixture of WGA-HRP/HRP was injected into either the masseter, temporalis, or anterior digastric muscle of 4 cats and 5 macaque monkeys. Within the monkey trigeminal motor nucleus (Vmot), temporalis and masseter motoneurons were broadly distributed, but a small injection into posterior temporalis produced a discrete focus of label. In cat, these two motoneuron pools were located in the dorsolateral Vmot. In both species, anterior digastric motoneurons were more ventromedial. The somata and proximal dendrites of neurons, labeled from each muscle injection, were examined with the EM. Boutons on all the motoneurons studied contained either small clear spherical vesicles (Type 1), small-flattened vesicles (Type 2) or a mixture of clear vesicles and large dense-cored vesicles (Type 3). The largest terminals were generally associated with subsynaptic cisterns. Descriptions of bouton types are remarkably consistent regardless of species or motoneuron population. Thus, bouton morphology and motoneuron somatotopy do not seem to provide sufficient bases for structure-function correlation. However, in cat, specific differences were identified in the relative synaptic density and in the distribution of bouton types on motoneurons supplying different muscles. For example,Type 2 terminals were more numerous on digastric neurons, while Types 1 and 3 were more common on masseter neurons. Ongoing analysis is directed at determining how the synaptic distribution on identified macaque motoneurons compares with that found in the cat. Supported by NIH grants DE06027 & EY09762.

369.11

ULTRASTRUCTURAL CHARACTERIZATION OF ESOPHAGO-MOTOR AND PHARYNGO-MOTOR NEURONS IN THE NUCLEUS AMBIGUUS OF THE RAT. T. HAYAKAWA*[1], Y. YAJIMA[2] and K. ZYO[1]. Dept. of Anatomy[1] and Physiology[2], Hyogo Coll. of Med., Nishinomiya, Hyogo 663, JAPAN.

The viscerotopic organization of the upper alimentary tract in the nucleus ambiguus has been well studied, but there is little information about the morphology of each neuron innervating the pharynx or the esophagus. We studied the ultrastructure of pharyngo- (PH), cervical esophago- (CE) and subdiaphragmatic esophago-motor (SDE) neurons labelled by retrogradely transported WGA-HRP. The WGA-HRP was injected into the lower pharynx, the cervical or subdiaphragmatic esophagus of male S-D rats. The PH neurons were large-sized, polygonal, and containing well-developed cell organella with a round nucleus. Both the CE and the SDE neurons were medium-sized, oval, and containing well-developed cell organella, but the SDE neuron was larger than the CE neuron. The average number of axosomatic terminals in a sectional plane was 30.8 in the PH, 7.9 in the CE, and 4.2 in the SDE neurons. The number of Gray's type I and type II terminals in the axosomatic terminals was almost equal in the PH and the CE neurons, but there were few Gray's type II terminals in the SDE neurons. Desmosome-like junctions between dendro-dendritic or somato-dendritic appositions were often present in the area surrounding the SDE neurons. There were also unlabeled small interneurons in the compact formation of the nucleus ambiguus.

369.12

QUANTITATIVE ANALYSIS OF SYNAPSES FORMED BY INDIVIDUAL RETICULOSPINAL FIBERS ON LUMBAR MOTONEURONS OF FROG A.E.Dityatev*[1], N.M.Chmykhova[2], A. L. Babalian[2], G.V.Dityateva[1] and H.P.Clamann[1], Department of Physiology, University of Bern, Switzerland[1] and Institute of Evolutionary Physiology and Biochemistry, St.Petersburg, Russia[2].

Although much is known about the effects and connections of descending tracts on spinal neurons, little is known about the spatial pattern of interaction between individual elements. Such knowledge is needed for an understanding of complex information transfer. In this work, several pairs formed by a reticulospinal fiber contacting a lumbar motoneuron were intracellularly stained after identification of the contacting elements. Pairs were reconstructed using a computer-assisted camera lucida (Eutectic NTS) with high resolution (10x100x, N.A.=1.4). The simplest contact system (5 contacts) was formed by 2 collaterals with total path lengths of 7045 and 4490 μm resp. on a rather simple motoneuron (membrane surface 136880 μm^2). The collaterals had similar average densities of boutons (62/mm). Boutons were mostly found on fine terminal branches (density = 220 bouton/mm). The diameter of terminal boutons (28 % of all boutons) was smaller than that of en passant boutons (1.1±0.33 μm vs. 1.3±0.35 μm). The most complicated connection had 19 contacts formed by 4 collaterals (total path length 16404 μm) on a very complex motoneuron (275560 μm^2). Sizes and distribution of boutons were similar to the first connection. Mean distance from collateral origin to any bouton was 566±215 μm. Action potentials must pass 11 branch point on average to get there. Boutons contacting motoneurons were 558±212 μm from the origin of the collateral and 311±154 μm from the motoneuron soma. Over half the dendritic surface was more than 500 μm from the soma. The collaterals of an axon stained with neurobiotin formed 6 contacts on the proximal dendrites of the motoneuron (surface 192050 μm^2). The axon had a relatively high density of boutons (84/mm), which were 1.48±0.45 μm thick. Only 18% were end boutons. These data allow quantitative comparison of reticulospinal connections with the synapses formed by fibers having other supra- and intraspinal origin. Supported by the Swiss NF.

369.13

PONTINE AND MEDULLARY AFFERENT PROJECTIONS TO THE NUCLEUS RETROAMBIGUUS: A WGA-HRP AND AUTORADIOGRAPHIC TRACING STUDY IN THE CAT. Peter O. Gerrits* and Gert Holstege. Department of Anatomy and Embryology, University of Groningen, Oostersingel 69, Medical School, 9713 EZ Groningen, The Netherlands.

The nucleus retroambiguus (NRA) is a premotor interneuronal cell group in the lateral part of the most caudal medulla. It projects to a set of motoneuronal cell groups which take part in respiration, vocalization, vomiting, and lordosis. The NRA receives strong afferent input from the periaqueductal gray (PAG), and the PAG-NRA pathway has been shown to be crucial for vocalization. Structures rostral to the PAG seem not to project to the NRA. To determine whether there exist NRA afferent projections other than those originating in the PAG, a combined retrograde and anterograde transport study was carried out in the cat. Injections of WGA-HRP were centered on the NRA, and [^{3}H]-leucine injections were placed in various brainstem sites. The results demonstrated that NRA afferents not only originate in the PAG but also in the lateral tegmental field, i.e. the ventrolateral parabrachial and Kölliker-Fuse nuclei, the retrotrapezoid nucleus, the solitary nucleus, and the ventrolateral part caudal to the facial nucleus (VII). Afferents also originate in two cell groups in the ventral part of the medullary **medial** tegmental field, one at the level of VII and one just rostral to the hypoglossal nucleus. The results indicate that virtually all respiratory related cell groups have strong access to the NRA. The cell groups in the **medial** tegmental field might be involved in eating and drinking related behaviors during which respiratory control is necessary.

369.14

FUNCTIONAL ORGANIZATION OF THE BULBAR RETICULAR FORMATION IN THE MALLARD, ANAS PLATYRHYNCHOS. J.L.Dubbeldam,A.J.Tellegen,R.G.Bout (Spon: European Brain and Behaviour Society). Institute of Evolutionary and Ecological Sciences, Leiden University, 2300 RA Leiden, The Netherlands.

The bulbar reticular formation contains part of the premotor systems for jaw, tongue and neck muscle motor nuclei. It is also recipient of input from various sensory systems. We analysed the pattern of afferent and efferent connections using a combination of anterograde and retrograde tracing techniques. Roughly,the reticular formation can be subdivided in three longitudinal zones: a dorsolateral and a ventromedial parvocellular zone (RPcdl and RPcvm) and a medioventral gigantocellular zone (RGc). A plexus of Horsley cannot bedistinguished in normal material of the mallard brainstem. RPcdl and RPcvm receive trigeminal input fromthe nuclei of the descending trigeminal system. The mesencephalic trigeminal nucleus projects to the rostral part and the caudal part of RPcdl leaving an intermediate compartment free of input. The rostral and caudal compartments of RPcdl send projections to ipsilateral motor nuclei innervating jaw closing and jaw opening muscles respectively. The ventromedial zone of RPcvm and the adjoining laterodorsal zone of RGc receive vestibular input, the medial zone of RGc receives visual input through the decussating tectobulbar tract. There seems to be no overlap of these two zones of sensory input. RPcvm projects bilaterally to motornuclei of the jaw muscles; RPcdl and RPcvm both project to (mainly ipsilateral) motor nuclei of the tongue muscles, whereas RPcvm and RGc both project to motor centers innervating neck muscles. This pattern of afferent and efferent reticular connections strongly suggests the existence of functionally defined zones in the bulbar reticular formation.

369.15

CENTRAL AUDITORY AND TRIGEMINAL NERVE PROJECTIONS REVEALED BY IN VITRO TRANSPORT OF NEUROBIOTIN IN TURTLE. J. Keifer* and J. L. Herrick. Dept. of Anatomy and Structural Biology, Univ. of South Dakota School of Medicine, Vermillion, SD 57069.

One aim of our laboratory is to examine the feasibility of developing an *in vitro* model of the classically conditioned eye-blink reflex to be used for cellular studies of conditioning. Recent studies in the turtle have demonstrated that a neural correlate of the eye-blink reflex can be generated in the *in vitro* brainstem-cerebellum by stimulation of the trigeminal nerve while recording responses in the abducens nerve (Exp. Brain Res. 97: 239, 1993). Evidence also suggests that paired stimulation of the auditory and trigeminal nerves results in classically conditioned activity in the abducens nerve (J. Neurosci., 1995). The aim of the present study was to examine the central projections of auditory and trigeminal nerve inputs in this species in order to reveal neural pathways that might underlie conditioning.

Neurobiotin (4%, 0.1-0.5 μl) was pressure injected into the cut end of either the trigeminal or posterior eighth nerve of the *in vitro* brainstem-cerebellum preparation from the turtle *Chrysemys picta*. Transport times ranged from 3-17 hr and tissue was processed using standard methods. The results of the trigeminal nerve injections showed retrogradely labeled motor V neurons, axons in spinal V and terminal label in the trigeminal nucleus following 3 hr transport time. After 6 hr, these regions labeled in addition to neurons in the principal and accessory abducens nucleus. Longer transport times resulted in projections to the cerebellum and labeled neurons in contralateral red nucleus. Injections of the eighth nerve showed terminal label in the ipsilateral cochlear nucleus and the cerebellum in 6 hr. Longer transport times resulted in labeled neurons in the ipsilateral cochlear nucleus and fibers that crossed the midline to the superior olivary and cochlear nucleus. Terminal label was observed in the abducens nuclei and extensive label was observed in the region of the trigeminal nucleus. These data suggest that Neurobiotin was transported transneuronally with times >6 hr. Furthermore, the results suggest that brainstem as well as cerebellar sites receive direct input from the auditory (CS) and trigeminal (US) nerves during conditioning. Supported by a USD Minigrant and USDSM Parson's Research Fund.

370.1

ENTORHINAL FIBERS TO THE INNER MOLECULAR LAYER AND HILUS OF THE DENTATE GYRUS: A PHAL TRACING STUDY.

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Entorhinal fibers to the molecular layer of the dentate gyrus are believed to be strictly confined to the outer two thirds. These fibers arise from layer II stellate neurons in the entorhinal cortex (EC) and curve upwards to more rostrally located portions of the hippocampal formation. Another entorhinal projection terminates in the hilus of the dentate gyrus (Köhler, C. (1985) Neurosci. Lett. 56:13-19). These axons originate from neurons located in layers IV-VI and are believed to project horizontally to the hippocampal formation. It has been suggested that these fibers reach the hilus of the dentate gyrus via CA3. Using anterograde tracing with PHAL we unraveled another trajectory of entorhinal fibers that originate from neurons located in layers IV and VI and reach the hilar area via the inner molecular layer of the dentate gyrus. These fibers enter the outer two-thirds of the molecular layer, traverse the inner molecular layer and the granule cell layer, and finally arborize subjacent to the granule cell layer. This projection is strongest in the temporal portion of the ipsilateral hippocampus and almost absent septally. Electron microscopy revealed that these fibers form asymmetric synapses on dendrites in the inner molecular layer, somatic spines of granule cells, and spineless dendrites beneath the granule cell layer. Postembedding immunogold staining indicates that entorhino-hilar fibers do not contain the inhibitory neurotransmitter GABA. These data demonstrate that not all entorhino-dentate fibers follow the classical laminar organization of afferents in the rat fascia dentata. While entorhinal fibers from the superficial layers terminate exclusively in the outer zone of the dentate gyrus, entorhinal fibers arising from deeper layers are not confined to laminar boundaries (SFB 528, Ni 322/1-2, SFB 1523, Fr 620/4-2, Leibniz Program).

370.2

POSTNATAL DEVELOPMENT OF GRANULE CELLS AND THEIR AFFERENT SYNAPSES IN THE DENTATE GYRUS OF RHESUS MONKEYS. L. Seress*, B.J. Baumgartner and C.E. Ribak. Dept. of Physiol., Univ. Med. Sch., Pécs, Hungary and Dept. of Anat. & Neurobiol., Univ. of Calif., Irvine, CA 92717.

Granule cells, the molecular layer and subgranular zone of the hilus were examined in both developing and adult rhesus monkeys. Total dendritic length and the properties of the dendrites were measured by a computer assisted 3-D tracing system. Spine density was counted for dendrites under oil immersion (100X obj.). Vertical probes through the molecular layer and horizontal probes in the hilus were obtained with electron microscopy to count the number of synapses per unit area in the developing neuropil. The total dendritic length of granule cells displayed a significant increase from birth until the 6th postnatal month. It was followed by a smaller increase until 1 year. No decrease was observed in adults. The spine density on dendrites of granule cells increased from birth to 3 months of age where it then remained at a level observed at all older examined ages. The number of asymmetric synapses per unit area in the molecular layer was highest between 3 and 7 months. A similar peak in synapse number per unit area was observed in the hilus. Between 7 months and 1 year of age, the number of asymmetric synapses per unit area decreased to a level that was found in newborn monkeys. There was no significant change in the number of both axosomatic symmetric synapses for granule cells and axodendritic symmetric synapses in the molecular layer between birth and 1 year. These data suggest that the inhibitory innervation of granule cells develops prenatally whereas the excitatory innervation undergoes significant postnatal changes. Both dendritic length and spine density of granule cells steadily increase from birth until they reach the mature stage. The transient peak in the number of asymmetric synapses per unit area in the dentate gyrus may be explained by a "dilution" caused by the increased volume of the dentate gyrus and the increase in the number and size of glial processes.

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370.3

EFFERENT PROJECTIONS OF THE CLAUSTRUM TO THE AMYGDALA: AUTORADIOGRAPHIC ANALYSIS IN THE MACAQUE MONKEY. **K. Coleman-Mesches* and D. G. Amaral.** The Center for Behavioral Neuroscience, SUNY at Stony Brook, Stony Brook, NY 11794-2575.

The mammalian claustrum is a large, narrow sheet of gray matter with dorsal and ventral enlargements. The dorsal claustrum is extensively interconnected with the neocortex bilaterally, whereas the ventral claustrum has reciprocal projections with a variety of subcortical structures and limbic cortical regions. We have examined the projections from the claustrum to the amygdaloid complex after injections of a mixture of 3H-leucine and 3H-proline into the claustrum in Macaca fascicularis monkeys. Injection into the ventral portion of the claustrum demonstrated a heavy ipsilateral projection to the magnocellular division of the basal nucleus throughout the rostral-caudal extent, and light-to-moderate projections to the intermediate division of the basal nucleus. Moderate labeling was also noted over the dorsal portion of the lateral nucleus. The ventral-most injection also demonstrated light-to-moderate projections to the lateral division of the central nucleus. There were no significant projections to any other nucleus within the amygdaloid complex. Nor was there any labeling of the contralateral amygdala. Injections into the dorsal portion of the claustrum revealed no projections to any nucleus within the amygdaloid complex at any rostral-caudal level. Injections of the anterograde tracers Phaseolus vulgaris leucoagglutinin or 3H-amino acids into the magnocellular division of the basal nucleus revealed a heavy return projection to the ventral claustrum. This projection innervated mainly the ventral enlargement of the claustrum. These findings will be discussed in relation to published information on claustrocortical connections and the implications for the nature of the amygdalocortical interactions.

370.4

SYNAPTIC INPUT FROM THE RAT ENTORHINAL CORTEX, AMYGDALA, MIDLINE THALAMUS AND PREFRONTAL CORTEX ONTO STRIATAL NEURONS. D.M. Finch.* Department of Neurology and Brain Research Institute, University of California, Los Angeles, CA 90095-1761.

Tracing studies have shown that projections from the entorhinal cortex, amygdala, midline thalamus and infralimbic cortex all terminate in the same general region of the striatum, particularly the nucleus accumbens (Acb). Physiological responses of striatal neurons to stimulation of these areas were examined. Sprague-Dawley albino rats were anesthetized with chloral hydrate (400 mg/kg i.p.) and extra- and intracellular recordings from Acb and caudate putamen neurons were obtained. A retrograde tracer (WGA-apoHRP-Gold) was deposited in some animals in the vicinity of recording sites to confirm that stimulating electrodes were located near cells that projected to the striatum. Excitatory responses to stimulation of all of the areas were obtained. In many cases, excitatory responses were intermittent and intracellular recordings showed that even large EPSPs could be insufficiently large to trigger action potentials except in the presence of "spontaneous" depolarizations. Some cells showed short term frequency potentiation, in which 5/sec stimulation increased the probability of spike firing. Recordings from spontaneously active cells showed a period of spike suppression lasting hundreds of msec after stimulation. A number of cells showed responses to more than one stimulating locus and in a subset of these cases it was possible to show synaptic interactions. These results indicate that functional influences from diverse areas can converge onto single Acb neurons to effect synaptic integration. Supported by NIH Grant DA09543.

370.5

ORGANIZATION OF PROJECTIONS FROM THE BASOMEDIAL NUCLEUS OF THE AMYGDALA: A *PHASEOLUS VULGARIS*-LEUCOAGGLUTININ STUDY IN THE RAT. G. D. Petrovich*, P. Y. Risold and L. W. Swanson. NIBS Program and Dept. of Biol. Sci., USC, Los Angeles, CA 90089-2520.

PHAL was injected stereotaxically into 30 rats to describe the overall pattern of efferent projections from the basomedial nucleus of the amygdala (BMA). The anterior and posterior parts of the BMA, recognized on cytoarchitectonic grounds, were shown to display significantly different patterns of projections.

Within the amygdala, the anterior basomedial nucleus (BMAa) heavily innervates the central, medial, and anterior cortical nuclei. In contrast, the posterior basomedial nucleus (BMAp), sends a dense input to the lateral nucleus, and to restricted parts of the central and medial nuclei.

Extraamygdalar projections from the BMA may be divided into ascending and descending components. The former end in the cerebral cortex, striatum, and septum. The BMAa mainly innervates primary olfactory cortical areas (piriform, transitional) while the BMAp innervates association cortical areas (perirhinal, ectorhinal) and the medial prefrontal cortex (infralimbic, prelimbic areas). Within the striatum, the BMAa densely innervates the striatal fundus and olfactory tubercle, while the nucleus accumbens receives a heavy input from the BMAp. Both parts of the BMA send massive inputs to distinct regions of the bed nuclei of the stria terminalis.

Descending projections from the BMA end primarily in the hypothalamus, where the BMAa mainly innervates the lateral hypothalamic area, while the BMAp projects especially heavily to the ventromedial nucleus.

In conclusion, the cytoarchitectonically distinct anterior and posterior parts of the BMA are also hodologically distinct. Thus, the BMAa and BMAp form parts of anatomical circuits that probably mediate different functions.

370.6

INTRINSIC CONNECTIONS OF THE RAT AMYGDALOID COMPLEX: PROJECTIONS ORIGINATING IN THE ACCESSORY BASAL NUCLEUS. V. Savander[1]*, J.E. LeDoux[2], C.-G. Go[2], A. Pitkänen[1, 3]. [1]Dept. of Neurology and [3]A.I. Virtanen Institute, Univ. of Kuopio, P.O.Box 1627, FIN-70211 Kuopio, Finland and [2]The Center for Neural Science, New York University, NY 10003, USA.

The aim of present study was to determine the distribution and topography of the intra-amygdaloid projections originating in the accessory basal (basomedial) nucleus of the rat amygdala. The anterograde tracer, *Phaseolus vulgaris* leucoagglutinin was iontophoretically injected into different rostrocaudal levels of the accessory basal nucleus. The distribution of labelled terminals was analyzed from immunohistochemically stained sections. On the basis of the cytoarchitectonics and the connectional data arising from the present study, we divided the accessory basal nucleus into two divisions, the magnocellular and the parvicellular division. The main projections originating in *the magnocellular division* terminated in the medial division of the lateral nucleus, in the periamygdaloid cortex and in the capsular division of the central nucleus. *The parvicellular division* projected to the medial division of the lateral nucleus, the central division of the medial nucleus, the capsular and medial divisions of the central nucleus, the medial division of the amygdalohippocampal area and to the posterior cortical nucleus. The present study shows that the projections originating in the accessory basal nucleus, like those originating in the lateral and basal nuclei (Pitkänen et al., 1995; Savander et al., 1995) are topographically organized to a greater extent than previously thought.

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370.7

THE LATERAL NUCLEUS OF THE RAT AMYGDALA IS RECIPROCALLY CONNECTED WITH ITS MAIN INTRA-AMYGDALOID TARGET NUCLEI. A. Pitkänen[1, 3*], J. E. LeDoux[2], C. G. Go[2], V. Savander[1]. [1]Dept. of Neurology and [3]A. I. Virtanen Institute, Univ. of Kuopio, P.O.Box 1627, FIN-70211 Kuopio, Finland and [2]The Center for Neural Science, New York University, New York, NY 10003, USA.

Phaseolus vulgaris leucoagglutinin (PHA-L), an anterograde tracer, was used to investigate the intra-amygdaloid afferent connections of the lateral nucleus of the rat amygdala. PHA-L was iontophoretically injected into the basal nucleus, the accessory basal nucleus and the periamygdaloid cortex of the rat amygdaloid complex. The results obtained from the present study revealed that the lateral nucleus received substantial projections from its main intra-amygdaloid target areas: the basal nucleus, the accessory basal nucleus and the periamygdaloid cortex. We also observed that the afferent projections were organized in a manner that supports the idea of dividing the lateral nucleus into three divisions, namely, the medial, dorsolateral and the ventrolateral divisions. The medial division received projections from the accessory basal nucleus and the periamygdaloid cortex. The ventrolateral division received projections from the parvicellular division of the basal nucleus and the periamygdaloid cortex. The dorsolateral division, instead, did not receive any projections from the nuclei investigated. The present study demonstrates that the organization of the information flow within the amygdaloid complex is not as unidirectional as previously thought.

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370.8

INTRACELLULARLY LABELED HIPPOCAMPAL HILAR INTERNEURONS IN VIVO. A. Sik, M. Penttonen, A. Ylinen* and G. Buzsaki. CMBN, Rutgers University, Newark, NJ 07102

Interneurons in the dentate area were characterized physiologically and filled with biocytin in urethane anesthetized rats. The neurons were double stained for various peptides and calcium binding proteins. In some cases the PV target cells were investigated. The labeled interneurons were divided into subgroups. (1) NPY-containing interneuron, with very spiny dendrites in the hilar region. The axon collaterals covered the 2/3 of the dentate gyrus (septo-temporally) innervating the outer half of the entire extent of the molecular layer. In addition, boutons contacted more than 1000 PV positive targets. (2) Calbindin positive cell, with axon collaterals mostly in the stratum oriens and radiatum of the entire dorsal CA3. A secondary axon penetrated into the hippocampal fissure, giving rise several collaterals in the CA1 radiatum and the subicular area. (3) Basket-like cell in the granule cell layer with axon collaterals in the granule cell layer, and the inner molecular layer. (4) Interneuron in zone 3 innervating the granule cell layer and inner/middle molecular layer of both upper and the lower blades. (5) Bistratified interneuron in CA3 pyramidal layer innervating the stratum oriens and radiatum. The subgroups had characteristic and different physiological response properties.

370.9

PROJECTIONS FROM THE PARAVENTRICULAR NUCLEUS OF THE THALAMUS TO THE LIMBIC CORTEX. T. van Groen*, I. Kadish and J.M. Wyss. Dept. of Cell Biology, University of Alabama, Birmingham, AL 35294-0019

Previous studies indicate that the paraventricular (PV) nucleus of the thalamus projects primarily to the prelimbic cortex (area infraradiata; IRbα), and to the ventral striatum. However, our anterograde tracing studies suggest that many axons extend beyond these areas to synapse in more caudal limbic cortical areas. The present study used anterogradely and retrogradely transported tracers to characterize the projections of PV. Injections into PV label axons and terminals in the frontal polar, agranular insular, medial orbital and IRbα cortices, in the anterior olfactory nucleus and in the basolateral, central and lateral amygdaloid nuclei. In the caudal limbic cortex, labeled axons extend to the caudal piriform, and to perirhinal and entorhinal cortices, and the ventral subiculum. In addition to the cortical projections, PV projects to a number of subcortical areas; PV injections label a dense terminal field in the ventral striatum (nucleus accumbens) and a sparse terminal field in the olfactory tubercle and in the lateral part of the lateral septal nucleus. Further, labeled axons and terminals are in the rostroventral reticular nucleus of the thalamus and sparsely to the dorsal periaqueductal gray. Neurons in different parts of PV have distinct projections, e.g., only rostral parts of PV project to the subicular and entorhinal cortices. Axons of PV terminate primarily in layers I and V in most cortical areas, but the projections to the entorhinal and perirhinal cortices terminate in deep layers (i.e., layers V-VI). These results demonstrate that PV projects to functionally distinct limbic areas, e.g., regions implicated in learning and memory versus regions involved in cardiovascular regulation and control of emotions.

370.10

HYPOTHALAMO-THALAMO-CORTICAL CIRCUITS INFLUENCED BY PHEROMONAL INFORMATION, AND INVOLVED IN REPRODUCTIVE AND DEFENSE BEHAVIORS. P.Y. Risold*, L.W. Swanson. NIBS Program, Dep. of Biol. Sci., USC, Los Angeles, CA 90089-2520.

Recently, dense projections from the highly interconnected anterior (AHN), ventromedial (VMH), and dorsal premammillary (PMd) nuclei of the hypothalamic medial zone were identified to the anterior nucleus reuniens (RE) and the ventral anteromedial nucleus (AMv) of the rat thalamus. In this study, simultaneous injections of PHAL and fluorogold (FG) were placed in the RE. The distribution of retrograde labeling confirmed afferents to this nucleus from the AHN, VMH, and PMd, while an analysis of the distribution of PHAL-labeled axons showed that through this pathway the same hypothalamic nuclei may influence ventral regions of the hippocampal formation (SUBv and field CA1v). However, projections from the RE were also found to the cingulate cortex and, in particular, a small terminal field was identified in the lateralmost part of the agranular retrosplenial area (RSPagl). Connections of the RSPagl were subsequently analyzed by the PHAL-FG procedure. Moderate afferents from the RE were confirmed, but interestingly, the RSPagl was found to be connected densely with the AMv as well as with the rostromedial part of the lateral posterior nucleus. In addition, this area was found to innervate brainstem centers involved in head and eye movements (including the superior colliculus), and the periaqueductal gray, which receives dense descending inputs from the AHN, VMH, and PMd.

The AHN and VMH are known to be targeted by very dense medial amygdalar projections. The AHN is also bidirectionally connected with the ventral lateral septal nucleus, which is a major terminal field for SUBv and CA1v projections. Thus, via these thalamocortical pathways, the AHN, VMH, and PMd may participate in attentional responses, probably initiated in part by pheromonal information. These circuits may also play an important role in the initiation and coordination of goal-oriented behaviors in which the AHN, VMH, and PMd are suspected to be involved (e.g., reproductive and defensive).

370.11

ANATOMICAL EVIDENCE FOR A POSSIBLE VISCERALLY-RELATED PREMOTOR AREA IN THE LATERAL FRONTAL CORTEX OF THE RAT M.D. Cassell* & C-J. Shi. Dept. of Anatomy, University of Iowa, Iowa City, IA 52242

The rostral medial agranular frontal cortex (AgM) in the rat is connected with somatosensory, posterior parietal insular and motor cortices and the ventrolateral part of the mediodorsal thalamic nucleus (MD). It is usually considered to function as a premotor area mediating sensory-guided voluntary movements. Corticocortical projections arising from gustatory/viscerosensory areas of the anterior parietal insular cortex terminate heavily in a band of dysgranular insular cortex on the lateral frontal surface (dorsolateral orbital and lateral frontal polar cortices) that is continuous with AgM rostrally. To determine if this cortical area has similarly organized connections as AgM, microinjections of biocytin were made at all levels of its rostrocaudal extent. Projections to the thalamus targeted the ventromedial parts of caudal MD, and the centromedian and parafascicular nuclei. Cortical projections were directed at head and forelimb regions of motor cortex, as well as viscerosensory parts of the parietal insular cortex. Strong projections to the rostral caudate-putamen and lighter projections to the anterior basolateral amygdaloid nucleus were also observed. The organization and extent of these projections parallel those arising from AgM, with projections targeting motor-related cortical and subcortical areas, but indicate a strong relationship with the anterior parietal insular cortex. As behavioral studies have consistently shown deficits in appetitively-guided motor activity following lesions of this area, we suggest this area of prefrontal insular cortex may function as a viscerally-related premotor zone. Supported in part by NS25139.

370.12

HODOLOGICAL FRONTO-HIPPOCAMPAL INTERACTION: RE-DISCOVERING THE CINGULUM BUNDLE. R. Morris*, M. Petrides and D.N. Pandya. Montreal Neurological Institute, McGill University, Montreal, CANADA and Edith Nourse Rogers Memorial Veterans Hospital, Bedford, MA, USA

Evidence from the clinical literature has shown that certain aspects of memory depend on the integrity of both the frontal cortex (FC) and the hippocampal system (HS) as well as on their functional interaction. Small surgical removal of the anterior portion of the temporal region including the entorhinal cortex (EC) is not sufficient to disrupt that fronto-hippocampal relationship. More radical temporal removal, however, including a considerable extent of the HS disrupts that reciprocal communication. These clinical data strongly suggest that the interaction between the FC and the HS is not exclusively dependent upon the integrity of the EC. Thus, the source of frontal inputs to the HS that might bypass the EC was investigated by means of autoradiography in the macaque monkey. The origin and course of the cingulum bundle have been carefully examined to see if this fiber system could sustain such fronto-hippocampal interaction. The results have revealed that the cingulum bundle links the FC with the HS. This hodological affiliation originates in the mid-dorsolateral portion of the FC that lies above the sulcus principalis. The presubicular component of the HS is the final recipient of the cingular projection originating in the mid-dorsolateral FC. The results are discussed in the context of the cognitive deficits observed after surgical removals from frontal and temporal lobes for the treatment of pharmacologically intractable epilepsy.

370.13

DIFFERENTIAL PROJECTION FROM THE FRONTAL CORTEX TO THE ROSTRAL TEMPORAL LOBE IN THE FASCICULARIS MONKEY. A RETROGRADE TRACING STUDY. R. Insausti*, E. Sanz, A. Insausti and L.M. Gonzalo. Dept. Anatomy, University of Navarra, Apdo. 273, 31080 Pamplona, SPAIN.

The rostral temporal lobe of the monkey is made up of several cytoarchitectonic areas (tip, area 36p or temporopolar cortex; ventromedial aspect, perirhinal cortex; ventrolateral aspect, rostral area TE and dorsal aspect area TS1), as shown in cytoarchitectonic studies. Given the importance of the rostral temporal lobe in visual (rostral area TE), memory (perirhinal cortex, temporopolar cortex), and emotional (temporopolar cortex) processing, we have attempted to individualize differences in connectivity of the various components of the rostral temporal lobe. We report here the differences found in the frontal cortex input. Retrograde tracers (1% WGA-HRP, 3% Fast Blue or 2% Diamidino Yellow) were injected after exposure of the temporal pole (two cases), rostral perirhinal cortex (three cases) and rostral area TE (one case). After appropriate survival, animals were perfused transcardially and the brain serially cut at 50 µm. One section every 500 µm was analyzed and the location of neurons recorded with a digitizing system. Labeling was charted onto two-dimensional reconstructions of the frontal cortex in each case. Our results can be summarized as follows: The orbitofrontal cortex is the only cortical area projecting to all the components of the rostral temporal lobe, being the temporal pole the region recipient of the largest projection both in extent and density. The rostral portion of area TE is the only one receiving input from the ventral lateral frontal cortex (Area 46 and area 12). Perirhinal cortex receives the least projection and only from the orbitofrontal cortex. Thus, the anatomical and functional heterogeneity of the rostral temporal lobe is enhanced by the different patterns of frontal cortical projection, what suggests that both the frontal and the rostral temporal lobe are connected in such a way that favors the functional segregation of the frontal lobe. Supported by grant DGICYT PM92-0166

370.14

LAMINAR ORIGIN OF THE SUBICULAR CORTEX PROJECTION TO THE BED NUCLEUS OF THE STRIA TERMINALIS IN THE RAT. K. Sripanidkulchai and J.W. Brown *. Department of Anatomy, Khon Kaen University, Khon Kaen Thailand and Department of Cell Biology, University of Alabama at Birmingham, Birmingham, AL 35294.

Previous studies have demonstrated that the hippocampal formation projects to the bed nucleus of the stria terminalis in the rat. Whereas the projection from the entorhinal cortex to nuclei near the bed nucleus (e.g., nucleus accumbens) has been shown to originate in layer V, the origin of the projection to the bed nucleus from the hippocampal formation is less well documented. This study characterized these projections using the retrograde transport of fluoro-gold and fast blue. In 30 male Sprague-Dawley rats, a 30-40 nl injection of one of the dyes (4% in dH-O) was placed into a small area of the bed nucleus of the stria terminalis and the rat was sacrificed 10 days later. The brains were perfused, sectioned and stained for AChE, Nissl or left unstained. The results demonstrate that the projection from the presubiculum and parasubiculum to the bed nucleus of the stria terminalis originates in the AChE rich layer V. The projection neurons are all pyramidal in shape and ipsilateral to the injection in the bed nucleus. Further, the data indicate that only the medial subdivision of the bed nucleus receives this projection. These data demonstrate a topographic projection from pre- and parasubiculum to the bed nucleus of the stria terminalis in the rat.

370.15

Limbic cortex and astrocytic glioarchitectonics: retrieval of Golgi-like GFAP immunoimpregnation from formalin fixed brain. B. Quinn*. Division of Neuropathology, NYU Medical Center, New York, NY 10016.

Recent studies focus new attention on the potentially diverse roles of astrocytes in neuromodulation, including calcium wave propagation, perisynaptic ion modulation, glutamate release and metabolism, amyloid processing, neurotrophin production, and neuromodulatory microvasculature control[1]. Traditional pathologic studies recognize fibrous and protoplasmic astrocytes, while in vitro studies demarcate Type I and Type II astrocytes. However, rare classical Golgi studies recognized many astrocytic subtypes with complex, intriguing regional architectonics[2]. We have developed a nearly Golgi-like "immuno-impregnation" from human brain both *routinely* immersion fixed for weeks, or alcohol fixed, using PEG embedding, hydrated autoclave treatment of floating sections sandwiched between lens paper and slides, GFAP or CD44 immunostaining of thick floating sections, and a novel silver immunointensification of the DAB product. Our preliminary studies have defined at least seven reliable astrocytic forms in human meso-temporal lobe/hippocampus; this morphologic diversity seems to increase from rodent to man. Other limbic areas such as cingulate and orbital cortex reveal highly distinctive laminar glial architectures. The findings invite correlation with molecular differentiation markers, for example analysis of glial heterogeneity in neocortical regions with different predispositions to amyloid plaque formation, and the comparative study of limbic system glioarchitectonics in schizophrenia and controls. Sponsored by the Stanley Foundation and the NYU Alzheimer Disease Center. (1) Smith, Prog. Brain Res. 94, 1992. (2) Retzius, Biol. Untersuch., 1894.

ASSOCIATION CORTEX AND THALAMOCORTICAL RELATIONS: ANATOMY

371.1

MUSCARINIC m2 ACETYLCHOLINE RECEPTOR EXPRESSION IN SELECTED PROJECTION AND LOCAL CIRCUIT NEURONS IN THE MONKEY CEREBRAL CORTEX: AN IMMUNOHISTOCHEMICAL AND IN SITU HYBRIDIZATION ANALYSIS. L. Mrzljak*, A.I. Levey, S. Belcher and P.S. Goldman-Rakic. Section of Neurobiology and Dept. of Pharmacol., Yale Sch. of Med., New Haven, CT 06510 and Emory Univ. Sch. of Med., Atlanta, GA 30332.

Pharmacological and physiological studies have emphasized the role of the muscarinic m2 acetylcholine receptor as an autoreceptor on cholinergic afferents that modulates the release of acetylcholine in the cerebral cortex. Using an antibody against acetylcholine m2 muscarinic receptor protein, we have recently described postsynaptic as well as presynaptic localization of this receptor in the cerebral cortex (Levey et al. J.Neurosci. 11:3218-3226,1991; Mrzljak et al. Proc.Natl.Acad.Sci. 90:5194-5198,1993). We have now combined immunohistochemistry and in situ hybridization using ^{35}S labeled riboprobes to validate postsynaptic localization and evaluate the expression of m2 protein and mRNA in prefrontal and premotor neurons. In nine rhesus monkeys ranging from 4 months to 15 years of age immunohistochemistry revealed either a positive band of labeling across layer V in areas 9,10,11 and 46 or a bilaminar pattern of labeling in layers III and V of areas 6,8, SMA and 24. A subset of nonpyramidal neurons exhibiting the characteristics of local circuit neurons in layers II-VI expressed m2 protein indicating a role of this receptor in inhibitory intracortical projection. However, pyramidal neurons in layers III and V were the most predominant cell type stained. The apical dendritic trees of these pyramidal neurons were intensely labeled, whereas immunolabeling in the cell bodies was barely detectable. In situ hybridization revealed m2 mRNA expression in numerous layer III and V cells and further displayed the same areal banding pattern. The postsynaptic localization of m2 receptor protein in deep layer pyramidal neurons suggest a selective role of this receptor in the regulation of corticofugal (corticostriatal, corticotectal) projections.

371.2

DIVERSE MESENCEPHALIC DOPAMINERGIC CELL GROUPS PROJECT TO THE MACAQUE FRONTAL CORTEX. Patricia S. Goldman-Rakic* and S. Mark Williams. Section of Neurobiology, Yale University School of Medicine, 333 Cedar Street, C303 S.H.M., New Haven, CT 06510

Although the topography of the mesocortical DA system has been extensively studied in rodents, few studies have examined the origin of this projection in primates. We have employed a combination of retrograde tracing, using fluorescent dyes, and tyrosine hydroxylase (TH) immunohistochemistry to identify frontal cortex projecting DA neurons. Injections of fast blue (FB) were made in areas 46, 9d, 8B, 6M, 4, 24 and the prelimbic area (PL) of the rhesus monkey. Neurons containing both FB and TH (FB/TH+) were plotted from the level of the mammillary bodies to the locus coeruleus. Although it is commonly assumed that the ventral tegmental area (VTA) is the major source of DA afferents to the neocortex, our results demonstrate that the frontal cortex is innervated by a widespread continuum of neurons distributed in all three of the mesencephalic DA cell groups (A9, A10 and A8, corresponding to the substantia nigra pars compacta [SN], VTA, and the retrorubral area [RRA], respectively). For all frontal areas examined, approximately 1/3 of midbrain FB/TH+ were located caudally and dorsally to the SN_{pc}, in the dorsal retrorubral area. The remaining 2/3 of FB/TH+ neurons were distributed primarily in the full medial-lateral extent of the dorsal SN and in the n. parabrachialis pigmentosus of the VTA. Although, this pattern of distribution is observed for all frontal areas examined, some evidence of topography was evident. The medial prefrontal cortex (area PL) receives more DA input from the midline VTA and less from the lateral SN and RRA than do dorsolateral prefrontal areas (e.g. area 46). As has been reported in other species, a large number of of retrogradely-labeled neurons in the ventral mesencephalon are non-dopaminergic (FB/TH-). These cells are primarily distributed in the rostromedial VTA; however, FB/TH- are observed in A8 and A9 as well. The evidence of robust projections of DA neurons from the A8 and A9 cell groups to the primate frontal cortex implicates midbrain regions beyond the VTA in the modulation of neocortical processes.

371.3

OVARIECTOMY AFFECTS MONOAMINE INNERVATION IN PRIMATE PREFRONTAL CORTEX. M. Kritzer*, Dept. of Neurobiology & Behavior, SUNY at Stony Brook, Stony Brook, N.Y.

The association cortices have well known roles in memory, motivation, and emotion. Monoaminergic circuits are critical to these processes, and are implicated in their dysfunction in cognitive/ affective disorders. However, gender differences noted in some of these same functions and disorders indicate that gonadal hormones also influence association cortex. Findings are presented suggesting this influence could stem from hormone modulation of monoamines.

Immunolabeling for tyrosine hydroxylase (TH) and serotonin (5-HT) in the prefrontal cortex of an adult rhesus monkey ovariectomized (OVX) eight months before sacrifice, and two age-matched controls revealed striking differences in monoamine innervation. Thus, whereas labeling in controls corresponded to published descriptions, immunolabeling in the OVX case was qualitatively and/or quantitatively different. TH labeling in layer I, for example, which is normally divided into inner and outer strata of high and low fiber density, was uniformly dense in the OVX case. Quantitative analysis revealed that TH-innervation was decreased in OVX cortex in all but layer Ia, with fiber density reduced by more than 50% in layer II, and by 10-30% in remaining layers. 5-HT labeling on the other hand was more dense in every layer of the OVX vs. control cortex, with density increased by 15% in layer I, and by 40-60% in remaining layers.

The opposing effects of OVX on 5-HT vs. TH argues against roles for non-specific factors, e.g., tissue preservation, in producing observed differences in immunolabeling, and argues for layer- and transmitter-specific effects of the hormonal milieu on monoamines in prefrontal cortex. This influence may be important in gender differences seen in the maturation, adult function and dysfunction of the association cortices in human and non-human primates.

371.4

OVERLAP AND SEGREGATION IN PREFRONTAL TERRITORIES CONNECTED WITH PARIETAL AND TEMPORAL VISUAL ASSOCIATION AREAS IN MACAQUE MONKEYS. C. Cavada*, J. Tejedor, A. Hernández-González and F. Reinoso-Suárez. Dept. Morfología, Fac. Medicina, Univ. Autónoma de Madrid, 28029 Madrid, Spain.

The dorsolateral and ventrolateral sectors of the prefrontal cortex are usually related to the posterior parietal and temporal visual cortices, respectively, regarding both connections and function. However, following injections of HRP-WGA in either sector, neuronal and terminal labeling was present in both posterior parietal and temporal areas. This observation led us to examine whether there is segregation, overlap, or both, in the prefrontal cortical territories connected with visual association areas of the parietal and temporal lobes. Multiple labeling experiments were performed using anterograde and retrograde tracers placed in posterior parietal areas 7a and 7ip, and in the temporal areas STP (superior temporal polysensory area), TE and TEO. Following pairs of injections in 7a and intermediate or posterior STP, segregation was the rule in the prefrontal labeled territories. These were often interdigitated, and some mingling of projection neurons was only observed in restricted portions of the dorsolateral prefrontal convexity, lower bank of the principal sulcus and area 45 in the anterior bank of the lower limb of the arcuate sulcus. Conspicuous overlap of connectional territories was observed, however, following injections in the posterior parietal areas and anterior STP, TE and TEO. Overlap was most prominent in the cortex of the principal and arcuate sulci, again in the lower bank of the former and anterior bank of the latter. Pairs of injections in 7ip and TE additionally produced overlap of connectional territories in the ventrolateral prefrontal convexity. These territories where projections to and from posterior parietal and temporal cortices coincide may play a key role in global visual perception through integration of both spatial and object-related visual cues.

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371.5

SELECTIVE CONNECTIONS OF THE SUPERIOR TEMPORAL AUDITORY REGION WITH THE PREFRONTAL CORTEX IN THE MACAQUE MONKEY. L.M. Romanski*, J.F. Bates and P.S. Goldman-Rakic, *Yale Univ. Sch. Med. Section of Neurobiology, New Haven, CT 06510.*

A recent study from this laboratory has revealed multiple, parallel anatomical pathways linking specific visual areas in the temporal lobe with particular regions of the prefrontal cortex (Bates et al., 1994). To examine the possibility that similarly organized processing streams exist in the auditory domain we have examined the connections between the superior temporal gyrus (STG) and the supratemporal plane with the prefrontal cortex. WGA-HRP was placed into cytoarchitectonically defined regions within the frontal lobes of rhesus monkeys and the retrograde cells and anterograde terminals were localized within cytoarchitectonically defined areas of the superior temporal auditory region (Pandya and Sanides, 1973). Most regions of the prefrontal cortex had connections with some portion of the STG or the supratemporal plane. However, prefrontal cortical areas 12 lateral, 11/12 orbital and the frontal pole (area 10) showed the greatest connectivity with superior temporal auditory regions with retrograde cell labeling being densest in areas TS2 and TS3 of the STG. In addition, many regions of the prefrontal cortex were connected with area TPO, the polysensory area located in the dorsal bank of the STS. In all cases, retrogradely labeled cells in the superior temporal region were most prominent in layers III and V. Terminal labeling was apparent in all areas which had retrograde cells. This labeling was particularly dense in layer I but columns of terminals which spanned all layers of the cortex were also present. Our results confirm and extend previous studies detailing the connections of the superior temporal auditory regions with the prefrontal cortex in the primate. These temporo-frontal connections indicate that there may also be multiple, parallel processing streams to convey auditory information to the prefrontal cortex in a similar manner as that seen with visual processing.

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371.6

DIRECT COMPARISON OF THE ORIGINS OF THALAMOCORTICAL PROJECTIONS TO AREAS 4, 6 AND 46: MULTIPLE NEUROANATOMICAL TRACING IN SINGLE MONKEYS. E.M. Rouiller[(1)]*, V. Moret[(1)], J. Tanne[(2)], N. Zeller[(2)] and D. Boussaoud[(2)]. (1) Institute of Physiology., Univ. of Fribourg, 1700 Fribourg, Switzerland; (2), Vision et Motricité, INSERM U94, 69500 Bron, France.

To clarify to what extent the various subdivisions of the cortical areas 4, 6 and 46 receive segregated or overlapping thalamocortical inputs, multiple retrograde tracing experiments were conducted in individual monkeys. Typically, distinct retrograde tracers were deposited in the hand representations of the primary (M1) and supplementary (SMA) motor areas, in the anterior (PMda) and posterior (PMdp) zones of the dorsal premotor cortex, in the ventral premotor cortex (PMv), and in the superior (46s) and inferior (46i) zones of area 46. The corresponding clusters of labeled thalamocortical neurons formed complex mosaics in the thalamus, transgressing cytoarchitectonic boundaries. PMdp and SMA received inputs from thalamic territories presenting a large degree of overlap; their main nucleus of origin was VLo, but projections also came from VA, VLm, VPLo, VLc, CL, CM and MD. The origins of inputs to 46i and 46s often overlapped, but were clearly segregated from those reaching PMdp and SMA, occupying more medial regions of the thalamus (mainly in MD). As a general rule, thalamic territories directed to 46i were larger than those projecting to 46s. Inputs to M1 originated from territories located in the same thalamic nuclei as those directed to PMdp and SMA, but the degree of overlapping was less prominent (about half of the cross-sectional area); the main nucleus of origin of the projection to M1 was VPLo. The inputs to PMv originated mainly from the area X where they partially overlapped with those to PMda. In other thalamic nuclei these inputs to PMv only partially overlapped with those directed to PMdp, SMA and M1. The thalamocortical projections to these various cortical areas, in spite of the complex arrangement of the thalamic territories of origin, exhibit distinct patterns of segregation and/or overlapping, consistent across monkeys.

371.7

CORTICOTHALAMIC PROJECTIONS FROM DIFFERENT LAYERS OF THE CINGULATE CORTEX TO THE ROSTRAL RETICULAR NUCLEUS AND RELATED THALAMIC NUCLEI. C. Lizier, S. DeBiasi and G. Battaglia*. Dept. of Neurophysiology, Neurological Institute "C. Besta", Milano, Italy; University of Milano, Italy.

The anatomical connections between the cortex and the thalamus have been traditionally regarded as strictly ipsilateral. Recent studies, however, revealed extensive crossed corticothalamic projections from limbic cortical areas (Lizier et al., 1994), and commissural intrathalamic connections arisinf from the rostral pole of the reticular nucleus (Raos and Bentivoglio, 1993; Battaglia et al., 1994). The present work reinvestigated, by means of sensitive retrograde and anterograde axonal tracers, the corticothalamic projections from different layers of the rat cingulate cortex. Small iontophoretic injections of Fluorogold (FG) or biotinylated dextran amine (BDA) were delivered in different regions of the rostral reticular nucleus, or in different layers of the cingualte cortex, respectively. After a survival time of 7 to 10 days, the rats were perfused, and the brains coronally cut with a vibratome. The sections were then processed with an antibiotin antibody to reveal the anterogradely transported BDA, or directly observed with a fluorescence microscope for the retrogradely transported FG.

After FG injections in the medial region of the rostral pole of the reticular nucleus, retrogradely labeled neurons were found in the ipsilateral cingulate cortex (CG1 and CG3: Zilles, 1985), mainly concentrated in the inferior part of leyer VI. After injections in the lateral region of the rostral pole, retrogradely labeled neurons were found in the same ipsilateral cingulate areas, particularly concentrated inthe superficial part of leyer V. These results were confirmed by the anterograde tracing data: after BDA injections in the deep layer VI of CG1, labeled terminals were found in the medial region of the ipsilateral rostral pole of the reticular nucleus, and bilaterally and almost symmetrically distributed in the anterior, intralaminar, and midline nuclei. After injections in superficial layer V, labeled fibers were confined to the lateral rostral pole, and predominantly distributed in ipsilateral dorsal thalamic nuclei.

The present results reveal a different anatomical organization of the corticothalamic pathways arising from different limbic cortical layers, and suggest an important role for the deep layers of the cingulate cortex in the subcortical link between the cerebral hemispheres.

371.8

CORTICAL CONNECTIONS OF THE RETROSPLENIAL GRANULAR B CORTEX. J.M. Wyss* and T. van Groen. Dept of Cell Biology, University of Alabama, Birmingham, AL 35294

Although the retrosplenial cortex is situated in a critical position between the hippocampal formation and the neocortex, few studies have examined its cortical connections. The present experiments used anterograde tracing techniques to characterize the efferent and afferent cortical connections of the retrosplenial granular B cortex (Rgb). Rgb projects to the ipsilateral anterior cingulate (infraradiata, IR), retrosplenial dysgranular (Rdg), Rga, postsubicular and presubicular cortices, and to the contralateral Rgb, Rdg, Rga, and postsubicular cortices. The axons from Rgb terminate in specific cortical layers, in IRβ predominantly in layer I and V, in Rgb in I and III/V, in Rga in layers I and III, in postsubiculum in layers I and V, however, in presubiculum predominantly in layer I. Axons from contralateral Rgb form a dense terminal plexus in layers IV and V of Rgb, with a smaller number of terminals in layers I and VI. Rgb projections to the thalamus terminate primarily in the anteroventral, and the laterodorsal nuclei with less dense projections to anterodorsal nucleus. Cortical projections to Rgb originate in the ipsilateral area infraradiata, Rdg and Rga cortices, the dorsal subiculum, and in contralateral Rgb. Each cortical projection to Rgb terminates in distinct layers of the cortex. The axons arising from Rdg terminate primarily in layers I and III-V, the axons from Rga terminate predominantly in layers I and II-III, the projections arising from the subiculum mainly end in layers II-III. The axons arising from the thalamus terminate in layers I and III/IV. Together, these data provide insight into the connections that link Rgb to the limbic learning and memory circuit.

371.9

ANALYSIS OF CORTICAL ORGANIZATION AND INDIVIDUAL VARIABILITY USING COMPUTERIZED FLAT MAPS.
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We illustrate several ways in which computerized three-dimensional reconstruction and automated flattening of the cerebral cortex can facilitate the analysis of cortical organization and of variability across individual brains. Our analysis was based on reconstructions of the right hemisphere from two macaque monkeys. Both hemispheres were flattened using a multi-resolution approach reported at last year's meeting (Anderson et al., Soc. Neurosci. Abstr. 20:428, 1994). The two hemispheres were similar in total surface area of neocortex (60 cm^2 vs. 69 cm^2), which is significantly smaller than previous estimates of neocortical surface area based on manually flattened cortical maps. The pattern of sulcal and gyral folding, represented by maps of mean curvature in the three-dimensional reconstruction, were broadly similar in the two maps, but there were numerous differences in detail. The borders of different cortical areas can be readily displayed on individual sections, in the three-dimensional reconstruction, and on the flat cortical map. Alternative partitioning schemes can be directly overlaid in order to facilitate precise comparisons between different schemes.

Comparisons between different individual hemispheres can be facilitated using shape-based warping algorithms that transform one structure to match the shape of another (Miller et al., PNAS 90: 11944; 1993). Application of this method to two-dimensional maps, using sulcal and gyral boundaries to drive the warping process, represents an efficient transformation strategy that preserves topological relationships on the transformed surface. It can be applied in conjunction with a surface-based coordinate system to establish a valuable alternative to standard (three-dimensional) stereotaxic coordinate systems.

ASSOCIATION CORTEX AND THALAMOCORTICAL RELATIONS: PHYSIOLOGY

372.1

OPIOID PEPTIDES INHIBIT THALAMIC RELAY CELLS
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In rat brain slices, we used the whole-cell recording technique, in current and voltage-clamp modes, to characterize the response of thalamic relay neurons to opioid peptides. Of 89 cells located in the centrolateral nucleus, 82 were inhibited by [D-Ala^2,$NMePhe^4$,Gly-ol^5]enkephalin (DAMGO), the agonist selective for μ-opioid receptors. In current-clamp mode, the inhibition resulted in a hyperpolarization accompanied by a decrease in membrane input resistance. The effect of DAMGO was reversible, concentration-dependent (a 23 % decrease in input resistance was measured at the ED50 (about 2.5 10^{-7} M)) and was antagonized by CTOP. No cell tested (n=11) responded to either [D-Pen^2,L-Pen^5]enkephalin (2.5 μM) or to U50469 (2.5 μM), respective agonists for δ and κ-opioid receptors. The response to DAMGO could be reversed at potentials negative to -90 mV and the reversal potential shifted with the extracellular K^+ concentration according the Nernst equation. Our results demonstrate that opioid peptides inhibit centrolateral neurones principally through an increase of a voltage-independent potassium conductance. Relay cells located in the reuniens, rhomboid, ventrolateral, ventromedian and ventroposterior nuclei were similarly inhibited by DAMGO. We thus hypothesize that morphine-like agonists induce a specific brain state where the thalamo-cortical inputs are globally depressed.

372.2

DEPTH-PROFILE ANALYSIS AND SPATIAL EXTENT OF SYNCHRONY OF FAST (20-40 Hz) AND SLOW (<1 Hz) CORTICOTHALAMIC OSCILLATIONS.
F. Amzica* and M. Steriade. Lab. Neurophysiol., Sch. Med., Laval University, Quebec, Canada G1K 7P4.

Coherent fast activities were recorded within intracortical and corticothalamic networks (Steriade et al., this meeting). Here we report the temporal and spatial relations of fast (generally 20 to 40 Hz) and slow (<1 Hz) activities. Data resulted from multisite extracellular and field potential recordings in cortical and related thalamic foci, in cats implanted chronically or anesthetized with ketamine and xylazine. Fast oscillations were present throughout activated patterns (waking and REM sleep, or after stimulating midbrain activating systems), and were related to the depolarizing phase of the slow (<1 Hz) sleep oscillation. Simultaneous recordings at different depths in the same cortical column showed high, in-phase synchrony between fast activities at all levels, from most superficial to deep cortical layers. In contrast, the depolarizing and hyperpolarizing components of the slow (<1 Hz) sleep oscillation were annulled at, and reversed below, a depth of ≈300 μm. This suggests a uniform vertical distribution of fast oscillating pools of neurons, and a massive cortical sink of the slow oscillation below 300 μm. The negative waves composing the fast oscillations were crowned by action potentials throughout the cortical depths. In some instances, however, in a circumscribed deep cortical region, around 0.8 mm, negative field potentials of fast oscillations, overridden by action potentials, showed phase opposition with related surface activities. The horizontal, intracortical synchrony was conversely affected by the distance and the observation time. The coherence of fast rhythms was almost abolished at distances exceeding 5 mm, while the coherence of the slow oscillation extended over the whole cortical surface. Spontaneous fast activities may remain synchronous beyond 5 mm, if only short epochs (<1 *s*) are observed. This led us to the development of an analysis paradigm that discloses the dynamic evolution of synchronized processes.

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372.3

FAST (20-40 HZ) SPONTANEOUS OSCILLATIONS ARE SYNCHRONOUS IN CORTICOTHALAMIC NETWORKS DURING BRAIN-ACTIVATED EPOCHS.
M. Steriade*, F. Amzica and D. Contreras. Lab. Neurophysiol., Laval Univ., Sch. Med., Quebec, Canada G1K 7P4.

We present experimental evidence that, in contrast to the widely used term of "EEG desynchronization" defining arousal and REM sleep, fast activities are coherent within intracortical and corticothalamic networks. Extracellular and intracellular activities were recorded, together with local field potentials, from multiple sites in sensory, motor and association neocortical areas and related thalamic nuclei of cats under ketamine & xylazine anesthesia as well as in chronically implanted, unanesthetized cats. The electrographic patterns of quiet sleep in behaving animals were similar to those during anesthesia. Natural arousal or EEG activation elicited by stimulation of mesopontine cholinergic nuclei were associated with the blockade of sleep oscillations (<15 Hz) and the occurrence of fast rhythms (generally 20 to 40 Hz) whose coherency among various cortical foci or between simultaneously recorded cortical and thalamic sites was assessed by means of cross-correlation analyses. The strength of corticothalamic synchronization depended on the presence of direct reciprocal projections between cortical and thalamic recorded sites, as identified by conventional physiological procedures. The synchronized fast activities were also observed during sleep patterns; however, they were selectively suppressed during the prolonged, rhythmic, simultaneous hyperpolarizations of cortical, thalamic reticular and thalamocortical neurons. This aspect contrasted with the sustained fast rhythms during brain-activated states when all these neuronal types were depolarized. These results on spontaneously occurring, state- and voltage-dependent fast rhythms, challenge the notion that fast rhythms are contingent on optimal sensory stimuli and are exclusively generated intracortically. Our data also indicate that "EEG desynchronization" is an obsolete term, since fast spontaneous activities are synchronized within corticothalamic circuits.

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372.4

THALAMOCORTICAL FOS EXPRESSION AND BEHAVIOURAL ACTIVATION: MODULATION BY CATECHOLAMINE DEPLETION.
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The medial prefrontal cortex (PFC) is characterized by converging input from thalamic and dopaminergic afferents. We used the transsynaptic expression of the immediate early gene c-fos (measured as Fos-like immunoreactivity [Fos-LI]) and behavioural parameters to analyze the interaction of these afferent systems in freely moving rats. To this aim we compared the effects of activating the thalamocortical pathway in intact rats and in rats with 6-hydroxydopamine (6-OHDA) lesion of the right ventral tegmental area. A dialysis probe that was implanted into the mediodorsal thalamus was perfused with the GABA-A antagonist bicuculline (20 min, 0.03 or 0.1 mM) which induced Fos-LI in the mediodorsal and more pronounced in the midline thalamic nuclei. In the PFC the transsynaptic expression of Fos-LI was dose-dependently increased both in controls and in 6-OHDA-lesioned rats which had a unilateral reduction of dopamine (-70%) and noradrenaline (-80%) in the PFC and of dopamine in the dorsal and ventral striatum (-90%). In lesioned rats perfused with 0.1 mM bicuculline the amount of Fos-LI was increased when compared to correspondingly treated controls. Bicuculline perfusion also induced a dose-dependent behavioural activation consisting of stationary (0.03 mM) or ambulatory (0.1 mM) activity that was not modified by 6-OHDA lesion. These findings confirm that forebrain catecholamines exert an inhibitory control over thalamocortical functions and they indicate that the interaction of prefrontal afferent systems can be shown *in vivo* at the level of immediate early gene expression.

Supported by the Van den Houten Foundation.

372.5

COLUMNAR AND LAMINAR TRANSMISSION OF THE ACTIVITY IN THE MONKEY PREFRONTAL CORTEX REVEALED BY OPTICAL IMAGING IN VITRO. K Nakamura*[1] and T. Sawaguchi[1,2]. [1]Department of Behavioral and Brain Sciences, Primate Research Institute, Kyoto University, Inuyama, Aichi 484, Japan, [2]PRESTO, Research Development Corporation of Japan.

To examine local transmission of the activity in the prefrontal cortex (PFC) of primates, we examined the spread of neuronal activity followed by an electrical stimulation in slice preparations of the monkey PFC, using an optical recording technique. Slices (400 μm) were prepared from the principal sulcal region (area 46) of the PFC and were stained with an absorptive voltage-sensitive dye, RH482 (0.1 mg/ml). The electrical stimulation (single pulse, 0.3 ms in duration, usually 200 μA) was applied at various layers and neuronal excitation was optically recorded using a 128 x 128 photodiode array covering an area of 4 x 4 mm (Fuji Film Microdevice, SD1001). We found that excitation of neurons spread in a columnar fashion from the stimulated site to the surface and bottom of the cortex when the site was at middle layers (layers III and IV). The width of the column increased as the current intensity of stimulation was increased (40-300 μA) but it was saturated at ~600 μm when the current intensity exceeded a certain level (i.e., >160 μA). The stimulation at different sites usually activated different columns, and there appeared to be discrete, perpendicular boundaries between columns. When stimulated sites were close in horizontal distance (<~0.3 mm), almost the same area was activated. Further, the spread of the activity differed in direction and range by the stimulation at different layers. The stimulation at deep layers (V and VI) activated deep layers, that at middle layers (III and IV) activated all layers, and that at upper layers (II and III) activated upper and middle layers. The activation disappeared when an antagonist of excitatory amino acid, CNQX (40 μM), was applied on the slice preparation. These findings suggest that the PFC of monkeys consists of relatively distinct functional columns formed by excitatory connections and the activity transmits laminarly within the columns.

372.6

CURRENT SOURCE DENSITY ANALYSIS OF NEOCORTICAL SLEEP SPINDLES, SPIKE-AND-WAVE EPILEPSY, DELTA, AND GAMMA WAVES IN THE FREELY MOVING RAT. A Kandel* and G Buzsáki. CMBN, Rutgers University, Newark, N.J. 07102.

Thalamocortical neuronal oscillations underlay various field potential patterns that are expressed in the neocortex. Many of these patterns are thought to be initiated in the thalamus and/or in discrete regions within the neocortex. The thalamus is known to be the initiator of various neocortical patterns (e.g. sleep spindles and high voltage spike-and-wave spindles (HVS)) and is believed to be involved in other patterns as well (e.g. delta waves, slow waves, and 40 Hz oscillations (gamma)). In this study a 16-site silicone probe (100 μm site intervals) was fixed to a moveable platform and implanted medial to the barrel cortex. Animals were able to move freely and field potentials and extracellular unit recordings were obtained simultaneously during sleep spindles, HVS, delta, and gamma wave EEG activity patterns. Current source density (CSD) analysis of the spike component of HVS and sleep spindles indicated sinks in the dorsal most aspect of layer IV, which corresponds to the location of major inputs from thalamic relay cells. Another possible current sink was in layer I/II with corresponding sources in layers I and III, where non-specific thalamocortical efferents diffusely synapse. CSD for delta and gamma waves suggests that they have current sink-source pairs located in layers II/III, as well as in layers V/VI, the major neocortical pyramidal cell layers. Extracellular units fired preferentially with the spike components of sleep spindles and HVS in each electrode. The results of this study support previous hypotheses regarding thalamic relay inputs to layer IV as the site of origin of sleep spindles and HVS in the neocortex but also postulates the involvement of layers I/II. These results suggest that specific and non-specific thalamic relay cells are differentially involved in various thalamocortical oscillation patterns.

372.7

OSCILLATORY AUTO-STIMULATION OF THALAMIC NEURONS *IN VITRO*, USING FICTIVE RECURRENT INHIBITION (FRIN) REVEALS A NEW TYPE OF INTRINSIC PLASTICITY. R. Llinás and R. Rai*. Dept. Physiology & Neuroscience, New York University Medical Center, 550 First Avenue, New York, New York 10016.

The electrophysiology of thalamic relay neurons was investigated using *in vitro* guinea pig brain slices. The bridge amplifier was modified such that a thalamic neuron action potential would trigger an inhibitory feedback current injection resembling the amplitudes and time courses of GABAa and GABAb IPSPs. Single action potentials in the recorded neurons were used to trigger the fictive recurrent inhibitory current pulse (FRIC). Such transient return feedback did not trigger rebound responses sufficiently robust to ensure oscillatory firing at all membrane potentials and for all fictive IPSP durations and amplitudes. At membrane potentials negative to the resting level, the auto-stimulating frequencies could be from as low as 0.5Hz to near 2Hz. As the duration of the inhibitory current was reduced, the frequencies for any given resting potential increased until the cell was incapable of supporting an oscillatory event. In tabulating the frequencies of oscillation as a function of FRIC amplitude, time course, and cell resting potential, it became clear that some frequencies are favored by these neurons. These are the ultra-low 0.5Hz, and 2Hz, 4Hz, 10Hz and 40Hz. These oscillatory frequencies correspond quite nicely to those seen in thalamic neurons in other preparations and under other recording conditions. Surprisingly, certain frequencies, when entrained for a short time, resulted in subthreshold oscillations which persisted after the stimulation was discontinued. This occurred particularly at high frequencies (35 to 55Hz) where driven oscillations lasting on the order of 5 seconds could produce continuing oscillations for up to 4 to 6 seconds. This 40Hz oscillation may be important for thalamocortical re-entry pathway function. Indeed, the issues of a computational optimization believed to be implemented by thalamocortical resonance and its possible relation to sensory binding would be greatly facilitated by such intrinsic plasticity. Supported by NIH-NS13742

372.8

ANTICIPATORY HEAD-DIRECTION CELLS IN THE ANTERIOR THALAMUS OF THE RAT. H.T. Blair* & P.E. Sharp. Dept. of Psychology, Yale University, New Haven, CT 06520-8205

A head-direction cell fires action potentials only when a rat's head is facing in a specific, preferred direction with respect to the static surrounding environment. Head-direction cells have been observed in several regions of the rat brain, including postsubicular cortex (PSC) (Ranck, 1984, *Soc Neurosci Abstr*), and the anterodorsal nucleus (ADN) of the thalamus (Taube, 1995, *J Neurosci*). Here, we recorded head-direction cells from PSC and ADN of freely-moving rats. Neural activity was analyzed in relation to both head direction and angular head velocity. We found that 17 of 19 cells in PSC maintained the same preferred firing direction, regardless of the angular head velocity. By contrast, 15 of 21 cells in ADN shifted their directional preference systematically to the left during clockwise head turns, and to the right during counterclockwise head turns, by an amount proportional to the angular speed of head turning. This firing pattern suggests that ADN cells were anticipating that the head would face a specific direction in the near future. By contrast, the behavior of PSC cells suggests that they fired only when the head was presently facing a specific direction. Supporting this conclusion, it was found that ADN cells had their best directional tuning function in relation to the rat's future head direction (at a future time displacement of about 40msec), whereas PSC cells had their best tuning function in relation to the rat's present head direction.

PSC and ADN are reciprocally connected to form a thalamocortical circuit (van Groen & Wyss, 1990, *Brain Research*). Since PSC contains both head-direction cells and neurons that encode the angular velocity of the head (Sharp, in press), ADN cells might be able to anticipate the future head direction by combining information about the present head direction, and the angular motion of the head. The anticipatory head-direction signal from ADN might then be sent back to PSC, where it could be used in computing the rat's present head direction.

372.9

SUSTAINED ATTENTION AND HABITUATION: A COMBINED PET AND EEG STUDY. **T. Paus*, R.J. Zatorre, J. Gotman, Z. Caramanos, M. Petrides, A.C. Evans. Montreal Neurological Institute, McGill University, Montreal H3A 2B4, Canada.**

This study investigated changes in rCBF and EEG during the performance of an auditory sustained-attention test. Eight healthy male volunteers listened for 60 min to 1-sec tones randomly varying in intensity (ISI:2 sec; total number:1200). The subjects' task was to detect a 5-dB decrement occurring during the last 100 ms of the tone (target probability: 0.05). With the ^{15}O-H_2O bolus technique, six 60-sec CBF scans were obtained at 1, 10, 20, 30, 40, and 50 min from the start of the test. EEG was recorded concurrently. Time-trend analysis of the normalized CBF data revealed highly significant time-dependent (across scans) CBF decreases in the thalamus. In addition, CBF decreases were observed in the prefrontal, secondary-auditory, and parietal cortex of the right hemisphere. On the other hand, time-dependent CBF increases were found in the fusiform gyrus bilaterally. Time analysis of the EEG spectrum showed subtle but significant increase in the theta and decrease in the gamma relative amplitude. There was also highly significant reduction of blood flow in the temporalis muscle bilaterally. No significant decline in the number of the detected targets was observed. We conclude that the above pattern of CBF and EEG changes is due to (1) habituation of the neural response to the repeated auditory stimulation, (2) an associated shift from an alert to a relaxed state, and (3) changes in a cognitive network related to auditory attention.

372.10

SIGNAL TRANSDUCTION AND NEURAL ASSOCIATION IN NEURONAL DISORDERS : FUNCTIONAL IMAGING BY POSITORON EMISSION TOMOGRAPHY. F.Kusuki, Y.Imahori,* Y.Ohmori, and S.Ueda. Dept of Neurosurgery, Kyoto Prefectural University of Med.

To determine the associating function of neuronal domains in human brain, we obtained signal transduction imaging by positoron emission tomography and evaluated these images with neural disorders. In normal neuronal action, C-11 labeled diacylglycerol (DAG) was incorporated into phosphoinositide selectively by DAG kinase and sequential phosphorylation enzymes, and the membrane bound radioactivity reflected its activity.

In the normal resting cerebrum (n=7), lateralization of heteromodal association areas was noted in each domain, and this tendency was more notable in the right brain. In patients with thalamic pain (n=2), high activity was observed in putamen and thalamus in the affected side, rather than frontal association area. And the treatment by extradural electric stimulation to the affected side of the motor area removed the pain and rised bilateral pre-frontal activity of phosphatidylinositol (PI) turnover. After analgesic stimulation, suppression effect was observed in the respective area for the long time. In contrast, dysinhibition was observed in the contralateral side. In patients with Parkinson's disease (n=4), potentiation of thalamus in the lesioned side was observed, and exercise loading of flexion caused suppression of the thalamic activity through GABA from putamen. In patients with cerebral ischemic disease (subcortical infarction, n=6), the signalling activity was kept in normal range in the domain of the respective cortex. After the therapy using mAChR-agonist for the long period, signalling activity of the corresponded cortex was activated, in other words, the associating domain was increased.

According to these results, it was showed that associating function is always rebuilded in cerebral association area and each domains. And it was also showed that long-term potentiation (LTP) and long-term depression (LTD), with PI turnover and protein kinase C, were highly concerned in the rebuilding of the association .

372.11

CEREBRAL CORTICAL AREAS INVOLVED IN A GO/NO-GO VISUAL DISCRIMINATION TASK REVEALED BY PET IMAGING IN MONKEYS.

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To examine which cerebral cortical areas are involved in the conditional selection between execution or inhibition of forelimb movement, regional cerebral blood flow (rCBF) was measured with ^{15}O-labeled H_2O positron emission tomography (PET) in two rhesus monkeys that performed a GO/NO-GO visual discrimination task with wrist movement. The task was initiated by the monkeys pressing a hold lever by their right hands and consisted of a warning period of 1-3 s (a white square on a CRT), a cue period of 0.5s (a yellow or blue square), a response period (a red square), and a final reward period. The monkeys were to release the lever within 0.8 s after the onset of the response period when the preceding cue was blue (GO) or continue to hold the lever for 1.8 s when the preceding cue was yellow (NO-GO). rCBF was measured for 2 min using a PET scan associated with each of the following conditions: the GO/NO-GO, GO, and NO-GO conditions (SHR-2400, Hamamatsu Photonics, 2.7 mm in-plane resolution). The rCBF measurements were performed repeatedly during the three conditions, and the rCBF findings in each condition were compared using a subtraction method to detect brain regions with rCBF increases specific to the GO/NO-GO condition. The PET images were superimposed onto the MR images that were fitted to Nissl-stained sections to obtain anatomical information. Significant increases in rCBF associated with the GO/NO-GO condition were found bilaterally in the prefrontal cortex (areas 46 and 8), dorsal and ventral premotor cortices (area 6), parietal cortex (area 7), temporal cortex (TE), cingulate cortex (areas 23 and 24), and peri-hippocampal regions. Among them, the prefrontal and premotor cortices appeared to be the most activated during the GO/NO-GO condition. Thus, the prefrontal-premotor system may recruit mnemonic and sensory information from other cortical areas to make the conditional selection between execution or inhibition of forelimb movement.

372.12

MAGNETIC FIELD RESPONSES IN PARIETAL ASSOCIATION CORTEX TO VISUAL STIMULATION IN SPACE.

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It was much reported that the parietal association cortex(PAC) was related to the function of visuo-spatial cognition. In human, the neural mechanism of the PAC was not revealed because it was difficult to record the neural responses noninvasively. However, we reported the magnetic field responses to visual stimulation in PAC (Neurosci. Res. 16 (1993);225). In this study, we investigated the magnetic field responses to visual stimuli in PAC for the purpose to reveal the neural function of visuo-spatial cognition in space. The subjects were 5 healthy adults and in a magnetically shielded room. The magnetic field responses to visual stimuli were recorded by a 37-channel SQUID magnetometer system (BTi). The LEDs lined at 3 degree interval at horizon, vertical and depth were used for the gazing (for eye position) and for the stimulating (for retinal stimuli), which located at 50 cm away from the eye. The combination of the gazing and the stimulating LEDs was #1 stimulated retinal position change in horizon at the same eye position, #2 stimulated retinal position change in vertical and depth at the same eye position and #3 eye position change in orbit under stimulating the same retinal position. Magnetic field responses were observed at 90-120 (M120), 120-170 (M170) and 180-220 (M220) msec latency after stimulating LED onset. The magnetic field responses of M170, which were observed in all subjects, were almost stable under stimulating the same retinal position at various eye position of #3 experiment but were change under stimulating the various retinal position at the same eye position of #1 and 2. The estimated current dipole sources of these magnetic field responses of M170 of #1,2 and 3 located at the deep area of PAC, along the intraparietal sulcus. These results suggest that the retinotopic topography was main in PAC.

372.13

ISLAND BIOGEOGRAPHY IN CEREBRAL CORTEX: Shaping Up New Cerebral Codes in Functional Archipelagos.

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While a darwinian process has six essential features (a *pattern* that *copies* with *variants* which *compete for a limited workspace* biased by a *multifaceted environment*, with this *selective survival to reproductive maturity* skewing the next generation's variants), another half-dozen features affect the speed (or stuck-in-a-rut stability) of evolutionary processes. We are all familiar with what the French call *l'esprit d'escalier*, thinking of the right response only after leaving the party. If plans of quality are to shaped up within the situational "windows of opportunity," some accelerating and equilibrium-escaping features may prove essential for mental versions of a darwinian process.

A well-known feature of island biogeography is relative speed, as in the differentiation of Darwin's finches unbuffered by large continental gene pools. When rising sea level converted part of the European coastline into an island (Jersey), the red deer trapped there underwent a rapid dwarfing in only 10,000 years. A second accelerating feature of islands is that a species can go extinct locally. This creates an "empty niche" which, when rediscovered, provides enough resources for even the rarer variants of the species for awhile. Should disease or climate change then shrink this boomtime population, some of those rarer variants may prove to have the right combination of features to survive the bottleneck and repopulate the island. Archipelagos allow for many parallel experiments. Episodes that recombine the islands (as when sea level temporarily lowers) create winner-take-most tournaments.

Triangular mosaics of synchronized cortical neurons not only provide the six essentials but form temporary "islands" with finchlike variant patterns; changes in cortical excitability may play a parcellation-then-tournament role similar to sea level in speeding the evolution of novel cerebral codes. (See W. H. Calvin, *Soc. Neurosci. Abstr.* 1992-94, and *Scientific American* 10/94).

COGNITION V

373.1

DISRUPTION OF DECREMENTAL ATTENTIONAL PROCESSING BY SELECTIVE REMOVAL OF HIPPOCAMPAL CHOLINERGIC INPUT.

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Recent studies with the selective cholinergic immunotoxin 192 IgG-saporin have indicated that lesions of the medial septal area (MSA) which selectively remove cholinergic innervation of the hippocampus do not disrupt spatial learning, unlike less selective lesions of the MSA or lesions of the hippocampus itself. The hippocampal formation is also involved in decremental attentional processing; studies from our laboratories have demonstrated that hippocampal lesions disrupt decremental attentional processing of conditioned stimuli in Pavlovian conditioning paradigms (Han, Gallagher, and Holland, submitted). We sought to determine if 192 IgG-saporin lesions of the MSA would mimic the effects of hippocampal lesions on decremental attentional processing. Male Long-Evans rats received microinjections of either 192 IgG-saporin (LES) or vehicle (CON) into the MSA. Each rat was tested in a latent inhibition paradigm in which one of two conditioned stimuli (CSs) was preexposed in the absence of reward, followed by a conditioning phase in which both CSs were (independently) paired with a food reward. The percent of conditioned responses during CS presentation in the conditioning phase was determined for both the preexposed and novel CSs. CON rats showed less conditioned responding to the preexposed CS than to the novel CS (latent inhibition), indicating that decremental attentional processing of the preexposed CS had occurred. MSA-LES rats showed equal degrees of conditioned responding to the preexposed and novel CSs, indicating that decremental processing of the preexposed CS did not occur. Studies are currently in progress to determine the effects of these lesions in a conditioning paradigm that produces both incremental and decremental processing of conditioned stimuli. (Supported by NIA Grant AG09973 to MG, a grant from the Human Frontiers Science Research Program to MG and PCH and an NSF Predoctoral Fellowship to MGB.)

373.2

C-*FOS* INDUCTION DURING AN INCREMENTAL CHANGE IN ATTENTION.

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Induction of the immediate-early gene, c-*fos*, was monitored during an incremental change in attention within an associative learning paradigm. Male Long-Evans rats were trained in a procedure developed by Wilson et al. (1992), in which attention is manipulated when a consistent predictive relation between two cues is shifted to a less predictive relationship. In the first conditioning phase, all rats were exposed to serial conditioning trials in which a light-then-tone sequence was followed by food half of the time (light→tone→food and light→tone trials). Rats in the "consistent" condition continued to receive these trials during the second phase of conditioning. In a second set of rats, those in the "shift" condition, the tone was omitted on non-reinforced trials, altering the predictive accuracy of the light and thereby increasing attention to that stimulus. Attention to the light was assessed during the third phase of conditioning, in which the light was paired directly with food. Increased attention to the light was evidenced by more rapid light-food conditioning in the shift-group relative to the consistent-group. The induction of c-*fos* was determined immunocytochemically in six animals from each training condition after one session of Phase 2 training, when the predictability of the light was initially altered in the shift condition but not the consistent condition. More Fos-like-immunostaining was evident in several cortical regions of rats in the shift-group as compared to those in the consistent-group. These regions were associated with the basal forebrain corticopetal system, and included both frontal and parietal cortex, and the substantia innominata. Comparable levels of Fos-like-immunostaining were observed for both groups in other areas, including the hippocampus and ventral tegmental area. (Supported by a Human Frontier Science Award, NRSA (F32-MH1099;AAC) and a Research Scientist Award (K05-MH01149;MG).

373.3

ALTERED SELECTIVE ATTENTION IN RATS WITH CHOLINERGIC LESIONS OF THE SUBSTANTIA INNOMINATA. A.A. Chiba*[1], P.J. Bushnell[2], W.M. Oshiro[2], and M. Gallagher[1]. [1]Department of Psychology, University of North Carolina, Chapel Hill NC 27599. [2]Neurotoxicology Division, US EPA, Research Triangle Park, NC 27711.

Current research has implicated a role for the basal forebrain cholinergic system in the modulation of attention on a variety of behavioral tasks in rats, monkeys, and humans. A spatial orienting task, modeled after Posner's covert orienting paradigm, was used to investigate the allocation of attention to a visual target in rats with selective cholinergic lesions of the substantia innominata (SI) or intact control rats. A given trial of the spatial orienting task required each rat to press a lever in response to the illumination of one of two visual targets in order to obtain a food reward. The targets were located on opposite sides of a test chamber and 70% of targets were preceded by a visual cue (a flash of light contiguous with a target location). On a given trial, the cue either accurately predicted the location of the target (valid cue) or appeared in the location opposite to that of the target (invalid cue). Target detection was facilitated by valid cues and degraded by invalid cues. Following pretraining on this task, eight of the rats received selective lesions of the cholinergic neurons in the SI by local infusion of 192 IgG-saporin and nine of the rats served as surgical controls. On trials in which a target was preceded either by a valid cue or by no cue, performance of SI-lesioned rats was equivalent to that of intact control rats. However, on trials in which a target was preceded by an invalid cue, SI-lesioned rats displayed reduced accuracy and prolonged latency to respond to a target, relative to intact control rats. These data support the hypothesis that the basal forebrain cholinergic system plays a modulatory role in mediating selective attention. (Supported by a Human Frontiers in Science Award, an NRSA (F32-MH1099) to AAC, and a Research Scientist Award (KO5-MH01149) to MG.)

373.4

OVERT ORIENTING IN THE RAT: EFFECTS OF UNILATERAL LESIONS OF BASAL FOREBRAIN CHOLINERGIC NEURONS ON SELECTIVE ATTENTION. P.J. Bushnell*[1], A.A. Chiba[2,4], W.M. Oshiro[1], and M. Gallagher[2,3]. [1]Neurotoxicology Division, US EPA, Research Triangle Park, NC 27711. [2]Department of Psychology, University of North Carolina, Chapel Hill NC 27599.

Detection of a peripheral visual target by rats can be modulated by a visual or auditory cue presented prior to the target stimulus. Detection is facilitated by (valid) cues spatially contiguous with the target and degraded by (invalid) cues in the contralateral visual field. Rats (n=6) were trained to press either of two targets (backlit pigeon keys: PKs) for food reward. Rats initiated each trial with a press on a central food cup door; 500 msec later, a cue (a 400 msec flash of light) was presented 10, 150, 450 or 900 msec before the target. Cues occurred on 70% of the trials; 70% of the cues were valid and 30% invalid. Effects of cues were quantified in relation to responses on no-cue trials. After training, each rat received a unilateral infusion of the immunotoxin 192 IgG-saporin into the substantia innominata (SI). Two kinds of changes in target detection were observed after the lesion. First, responses to targets in the visual field contralateral to the lesion were faster and less accurate than those to targets in the ipsilateral field, particularly with short (<1 sec) targets. Second, there was a tendency for invalid cues to reduce accuracy more for contralateral targets than for ipsilateral targets. Thus unilateral lesion of the cholinergic neurons in the SI degraded detection of visual targets in the contralateral visual hemifield, particularly when cues were presented in the ipsilateral hemifield (i.e., when targets in the contralateral hemifield were invalidly cued). These results support research indicating that the cholinergic projections from the basal forebrain to the cortex play a role in mediating selective attention to visual targets. [3]Supported in part by a Human Frontiers in Science Award and a Research Scientist Award (KO5-MH01149). [4]Supported in part by an NRSA (F32-MH1099).

373.5

DOUBLY DISSOCIABLE EFFECTS OF SELECTIVE MEDIAN RAPHE AND DORSAL RAPHE LESIONS ON VISUAL ATTENTION
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We have previously reported that global forebrain 5-HT depletion resulted in enhanced probability of responding and reduced latencies to respond correctly without affecting discriminative accuracy as measured by a 5-choice serial reaction time task. The current set of experiments assessed the behavioural effects of selective median raphe and dorsal raphé lesions on the same behavioural task.

Rats were trained to detect brief flashes of light presented randomly in a spatial array of five apertures with food reinforcement. When performance stabilised (>80% correct, <20% omissions) animals received infusions of either 5,7 dihydroxytryptamine or vehicle solution to either the median or dorsal raphé. The lesions induced different patterns of 5-HT depletion primarily in the hippocampus and striatal areas respectively.

Median raphé lesions produced a transient increase in premature responding and a more robust reduction of the latency to collect the reward. These results are suggestive of enhanced motivation in these animals.

Lesions of the dorsal raphé, however, induced a similar but not identical pattern of behaviour to that observed following global forebrain 5-HT depletion. Enhanced probability of responding as indexed by both increased premature responding and reduced numbers of omitted trials resulted from 5-HT depletion in striatal areas. Dorsal raphé lesioned animals also transiently exhibited superior accuracy of detection of the visual stimuli on baseline task parameters, compared to control.

The results indicate that the two ascending 5-HT systems mediate different behaviour on this task and that impulsive responding observed following global forebrain 5-HT depletion is primarily mediated by dorsal raphe 5-HT transmission.

373.6

UNILATERAL EXCITOTOXIC LESION OF THE NUCLEUS BASALIS OF MEYNERT INDUCES CONTRALATERAL NEGLECT IN THE RAT. B. Lannes, T. Humby and T.W. Robbins (SPON: European Neuroscience Association). Department of Experimental Psychology, University of Cambridge, U.K. CB2 3EB.

Previous work in this laboratory has provided evidence for a role of the cholinergic nucleus basalis of Meynert (NBM) in attentional processes. As the NBM sends projections in particular towards cortical regions implicated in neglect, such as the posterior parietal cortex and the prefrontal cortex, we investigated whether unilateral lesions of the NBM could induce a neglect syndrome in the rat.

Rats were trained in a task in which they have to sustain a nose poke in a centre hole for one of 4 variable foreperiods. At the end of each foreperiod, a stimulus appeared in either of 2 locations on one side or the other. After the stimulus appeared, the rats were required to report its presence by responding at a panel at the rear of the chamber for food reward. When rats had reached a criterion of 70% correct over at least 3 consecutive sessions, unilateral NBM lesions were performed using the excitotoxin AMPA (0.01M). The lesions were performed against the preferred eye as determined, where possible, from the baseline performance. When tested after 2 weeks of recovery, the NBM lesioned rats displayed neglect contralateral to the lesion, exhibited as a slowing of reaction or response time to contralesional stimuli. The magnitude of the deficit depended upon the baseline degree of side dominance. Results will be discussed in the context of theories of neglect and the role of ascending cholinergic projections in attentional function.

COGNITION VI

374.1

A FUNCTIONAL MAGNETIC RESONANCE STUDY OF SELECTIVE AND DIVIDED ATTENTION DURING VISUAL DISCRIMINATIONS OF SHAPE, SPEED AND COLOR.
G. Bush, B. Rosen, J. Belliveau, J. Reppas, S.L. Rauch, D. Kennedy, J. Sutton*, R. Tootell. NMR Center, Massachusetts General Hospital-East, CNY-9, Charlestown, MA 02129.

Attention is the cognitive mechanism by which humans select which of a myriad of sensory stimuli to process at any given moment. We used functional magnetic resonance imaging (fMRI) to examine the modulatory effects of selective and divided attention on regional cerebral activation during a visual discrimination task.

Four normal human subjects were scanned while engaged in the visual task developed for a similar PET study by Corbetta et al. (J. of Neuroscience, 1991, 11, 2383-2402). The subject's task was to indicate by button-press if two frames of computer-generated small bars were the same or different with respect to the cued attribute. At the start of a block, subjects were cued to attend either only to subtle (threshold) changes in shape, speed, or color (selective attention), or to changes in any parameter (divided attention). A passive-viewing/random button press block was used as a control. All subjects were scanned on two separate occasions to evaluate test-retest reliability. Three of the subjects also were scanned in a third session to directly identify extrastriate regions activated by visual, smooth pursuit, saccade, motion detection and object-recognition tasks.

Activation patterns were qualitatively similar to those of the aforementioned PET study, i.e. we found analogous discrete regions in extrastriate visual cortex which were activated by each of the selective attention conditions, and confirmed activation of right dorsolateral prefrontal and anterior cingulate cortex in the divided attention condition. While intrasubject test-retest reliability was strong, there was wide intersubject variability in activation patterns.

Selective and divided attention modulated neuronal activity in extrastriate and extravisual regions. The data support a top-down enhancing modulation theory of attention. Advantages of using fMRI included the ability to examine issues of test-retest reliability and intersubject variability, and to directly compare regions characterized by tests of visual function.

374.2

ARE THE SAME ATTENTIONAL MECHANISMS USED FOR TARGETS DEFINED BY COLOR, ORIENTATION, AND MOTION? M. Girelli and S.J. Luck*. Department of Psychology, University of Iowa, Iowa City, IA, 52242-1407.

Previous studies have shown that the N2pc component of the event-related potential (ERP) waveform reflects an attentional filtering process that operates during color and form discrimination, and the present study sought to determine if this same attentional process is used for targets defined by motion. We recorded ERPs from young adults in a visual search paradigm using stimulus arrays composed of either 8 vertical green bars moving in a downward direction (homogeneous arrays) or 7 of these bars and 1 bar that differed in its color, orientation, or direction of motion (pop-out arrays). One of the three pop-out types was the target for a given trial block, and the homogeneous arrays and other pop-out types served as nontargets. A robust N2pc component was observed for all three target dimensions, indicating that the same neural attentional systems are used across dimensions. In addition, we found that motion pop-outs elicited an N2pc component even when another feature was the target, whereas color and orientation pop-outs elicited an N2pc only when they were targets. This result suggests that motion pop-outs might automatically attract attention, even when they are not task-relevant. Together, these results indicate that: a) information from the parvo and magno streams converges before the stage of attentional filtering indexed by the N2pc component; and b) stimuli within the magno stream may attract attention more automatically than stimuli within the parvo stream.

374.3

THE EFFECTS OF DORSOLATERAL PREFRONTAL LESIONS ON VISUAL SEARCH. D. L. Woods* and K. H. Ogawa, Dept. of Neurology and Neurosciences Center, UC Davis, Northern California System of Clinics, Martinez, CA 94553.

Dorsolateral prefrontal frontal (DLPF) cortex is thought to play a key role in the control of visuospatial attention. In the current experiments, we compared performance of patients with DLPF lesions and age-matched control subjects in visual search tasks. Rectangular bars were presented in equiluminant displays of 4, 9 or 16 elements varying randomly in color (green, red, or yellow) and orientation (right-diagonal, horizontal, and vertical). In different conditions, subjects detected targets defined by Color, Orientation, or by the Conjunction of color and orientation features. Search rates were similar for DLPF patients and controls in single feature conditions. However, in Conjunction conditions DLPF lesions impaired (1) the detection of targets at peripheral display positions; and (2) the discrimination of targets from distractors of target color. These results suggest that the DLPF lesions narrow the focus of attention in both spatial and feature domains.

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374.4

COMMON AREAS OF PARIETAL ACTIVATION FOR SHIFTS OF SPATIAL ATTENTION AND TASKS INVOLVING THE CONJUNCTION OF VISUAL FEATURES. D.L. Hunton, M. Corbetta, G.L. Shulman, F.M. Miezin, and S.E. Petersen*, Washington Univ. Sch. of Med., Box 8111, St. Louis, MO 63110.

Theories of visual search postulate that tasks showing an increase in search functions involve either a serial analysis of objects in the field or a spatially parallel system that has reduced efficiency with an increase in the number of items analyzed. We have previously reported that areas in the superior parietal lobule (SPL) are active when attention is shifted across different visual locations. We used PET to determine whether SPL is differentially active during feature vs. conjunction tasks in two experiments.

In expt 1, subjects saw a display consisting of four square windows containing moving colored dots. In the color task, subjects searched for the presence of red-orange dots, in the motion task for the presence of fast moving dots, and in the conjunction task, for the combination of red-orange fast moving dots. Behaviorally only the conjunction task produced search times increasing with display size. In expt 2, subjects saw a similar display containing four differently colored rectangles. They searched for targets defined either by color, or the combination of a color and orientation. In both experiments, more activity was apparent in SPL for conjunction than for feature tasks. Coordinates obtained from the previous study on spatial attention were tested on the present data sets for replication. SPL was consistently more active in the conjunction than feature tasks, with one locus on the right (x,y,z=21,-61,50) showing significant differences between conditions in both experiments. This correspondence provides functional anatomical evidence that increasing search functions can involve shifts of spatial attention, and supports serial models for visual search.

374.5

A POSITRON EMISSION TOMOGRAPHY (PET) STUDY OF ATTENTION TO AUDITORY STIMULI. M.L. Kesler*, D.S. O'Leary, R.R. Hurtig, and N.C. Andreasen, Mental Health Clinical Research Center, Dept. of Psychiatry, University of Iowa College of Medicine, Iowa City, Iowa 52242.

A correlational analysis approach was used to relate attention-associated changes in superior temporal gyrus (STG) blood flow to changes in blood flow in other brain regions. Regional Cerebral Blood Flow (rCBF) was measured with PET in 16 right-handed control subjects using eight bolus injections of ^{15}O-labelled water. Cognitive activation tasks involved the detection of a target during the binaural or dichotic presentation of different types of auditory stimuli, and allowed examination of directing attention to the left or right ear. The baseline task involved monitoring for an infrequent tone. Dorsolateral frontal, inferior parietal, and STG regions of interest (ROIs) were drawn on magnetic resonance (MR) images, followed by individual registration of the MR scans with the PET scans. Using BRAINS software, average rCBF in each ROI was calculated for each of the eight PET conditions per subject. Spearman partial correlations (controlling for whole brain flow) were computed for pairs of regions across subjects. For these 16 subjects, there was a significant correlation between left STG flow and right inferior parietal flow during the attend-left condition ($r=0.75$, $p<.01$), as well as during the attend- right condition ($r=0.93$, $p<.01$). No such correlation was significant for the baseline condition. This suggests that the left STG - right inferior parietal coupling might be involved in selective attention or complex target detection. Alternately, this association may be the result of complex auditory stimulus processing, which was not required in the baseline task.

374.6

A FRONTO-PARIETAL NETWORK FOR RAPID VISUAL INFORMATION PROCESSING : A PET STUDY OF SUSTAINED ATTENTION AND WORKING MEMORY. JT Coull*, CD Frith and P Grasby, Wellcome Dept. of Cognitive Neurology, MRC Cyclotron Unit, Hammersmith Hospital, DuCane Road, London W12 ONN, UK.

The Rapid Visual Information Procesing (RVIP) task is a test of sustained attention, which also requires working memory (WM) for its successful execution. It is known to be sensitive to manipulations of the cholinergic and noradrenergic neurotransmitter systems, both of which have been widely implicated in the modulation of attention. Previous neuroimaging studies have implicated the frontal and parietal cortices in performance of simple sustained attention tasks. However, the neuroanatomical substrates of RVIP performance are not yet known. Therefore, this study investigated the functional anatomy of the RVIP task using Positron Emission Tomography (PET) derived measures of rCBF in 6 healthy volunteers. Subjects were required to perform variants of the RVIP task which manipulated the level of WM load and the speed of stimulus presentation. Changes of rCBF induced by these cognitive challenges were compared to that produced by a control and a rest condition. Comparison of all RVIP tests to the control condition produced increases in rCBF in the right inferior frontal cortex/precentral gyrus, the right inferior parietal cortex, and the lateral occipital cortex bilaterally. Conversely, significant decreases in rCBF were observed in the cingulate gyrus, Sylvian sulcus bilaterally, and the left middle temporal gyrus. A specific comparison of fast versus slow rates of stimulus presentation produced additional increases in rCBF in the lateral occipital cortex. These results support a fronto-parietal network for sustained attention, and provide the basis for investigating the neuroanatomical correlates of the noradrenergic modulation of attention.

374.7

MULTIMODAL AND UNIMODAL SENSORY INTEGRATION IN HUMANS: BEHAVIORAL AND ERP EVIDENCE. D. Costin*, H.J. Neville, A.M. Meredith%, B.E. Stein#. UCSD Cog Sci, La Jolla, Ca, %Med Col of VA, #Bowman Gray Sch Med, Wake Forest Univ, Winston Salem, NC.

In our previous studies ERPs elicited by multimodal stimuli (auditory and visual) displayed components not observed during the presentation of each unimodal stimulus alone: coincident multimodal stimuli elicited new patterns of activation when compared to ERPs elicited by stimulation with the separate unimodal components, or when compared to the addition of the separate unimodal responses. Moreover, the effects of spatial disparity on these additional ERP components were similar to those observed in single-cell responses of multimodal cells in the cat superior colliculus and polysensory cortex (anterior ectosylvian sulcus). Behavioral measures in both cats and humans have also shown similar effects of coincidence and disparity, providing further evidence that similar organizing principles may govern multimodal stimulus processing across species and levels of analysis.

In this study we compared ERP components elicited by multimodal stimuli with those elicited by multiple different stimuli within the same modality in order to distinguish activation patterns linked to multimodal processing from those that may be indices of general integration processes. Multiple unimodal stimuli consisted of concurrent presentations of red and green lights or noise and tone sounds at the same location. Multimodal stimuli consisted of one light and one sound at the same location.

Overlap in the ERP activity elicited by multiple unimodal stimuli and multimodal stimuli may index activity linked to modality-independent integration processes. Patterns of activation elicited by multimodal stimuli that are distinct from those elicited by multiple unimodal stimuli may provide evidence for distinct multimodal integration processes. These ERP indices will allow further characterization of multisensory integration processes.(McDonnell-Pew Grad, DC 00128, NS 22543)

374.8

DIFFERENTIAL EFFECTS OF SPATIAL AND EXPECTANCY CUES ON COVERT SHIFTS OF ATTENTION D.R. Gitelman*, S. Weintraub, Z.M. Grujic, J.R. Meyer, A.M. Farina, E.J. Russell, M.M. Mesulam Center for Behavioral & Cognitive Neurology, Northwestern University, Chicago, IL 60611

The focus of visual attention can be shifted covertly, i.e. without shifting visual fixation, permitting study of spatial attention without eye movements. Mechanisms that control covert attentional shifts include peripheral cues to prime spatial locations for targets, and central cues to generate directional expectancy for target appearance. We investigated the effects of cue type on reaction times (RTs), in two patients with right hemisphere strokes and their matched controls.

Eye movements were monitored via an infrared reflection system. We found that: 1) Valid expectancy cues (central arrow pointing towards subsequent target site) generated faster RTs than valid spatial cues (change in luminance at site of subsequent target) in all subjects. 2) In one patient with a right fronto-parietal-cingulate lesion, RTs were 25% slower towards the left vs. right for valid expectancy cues, but only 8% slower towards the left for valid spatial cues. 3) In a second patient with a right frontal-parietal but not cingulate lesion, the opposite pattern was obtained. RTs were 32% slower towards the left for valid spatial cues vs. 11% slower towards the left for valid expectancy cues. 4) Invalid expectancy cues (central arrow pointing opposite to subsequent target site) produced 72% slower leftward RTs in the first patient, but only 20% slower RTs to the left in the second patient.

These results indicate that peripheral space-priming cues and central expectancy-generating cues influence covert shifts of spatial attention through potentially different mechanisms. Attentional shifts in response to expectancy cues may be particularly vulnerable to lesions in cingulate cortex as part of a proposed distributed network for spatial attention.

374.9

MULTIMODAL HEMISPATIAL EXPLORATORY-MOTOR NEGLECT FOLLOWING DAMAGE TO THE BASAL GANGLIA. Z.M. Grujic*, D.R. Gitelman, S. Weintraub, J.R. Meyer, A.M. Farina, E.J. Russell, M.-M. Mesulam. Center For Behavioral & Cognitive Neurology,Northwestern Univ. Sch. of Med.,Chicago, IL 60611.

The substrate of spatial attention takes the form of a distributed network with interconnected cortical (frontal, parietal,cingulate) and subcortical (striatal, and thalamic) components. We had predicted that damage affecting the frontal component of this network (or the striatum with which it is interconnected) would cause a predominantly exploratory-motor neglect whereas injury to the parietal component would cause a predominantly sensory-representational neglect.

A stroke patient with a right subcortical lesion was studied with computerized paradigms designed to selectively probe the exploratory and representational components of neglect. The lesion involved the caudate nucleus, the internal capsule, and the ventrolateral nucleus of the thalamus.

There was no auditory, visual, or tactile extinction. No disengagement asymmetry was seen during covert shifts of attention, but reaction times were 15% slower to left valid cues, suggesting the existence of a mild left hemispatial deficit in the sensory-representational component of spatial attention. Exploratory-motor deficits in the left hemispace were much more prominent. On a blindfolded manual exploration task (using the unaffected right hand) the patient omitted 30% of the targets on the left (compared to 10% of the targets on the right), and touched 18 targets in the left field compared to 33 targets in the right. On a target detection task requiring visual search she was 34% slower in the left space than in the right, although simple lateral eye movements were symmetrical and normal. Our results show that there can be a dissociation between behavioral components of neglect, and that exploratory-motor neglect can have multimodal manifestations that involve manual as well as oculomotor exploration.

374.10

DISSOCIATION OF OVERT FROM COVERT SHIFTS OF SPATIAL ATTENTION IN PATIENTS WITH FOCAL LESIONS OF THE RIGHT HEMISPHERE. S. Weintraub*, D.R. Gitelman, Z.M. Grujic, A.M. Farina, M.-M. Mesulam. Center for Behavioral and Cognitive Neurology, Northwestern University Medical School, Chicago IL 60611

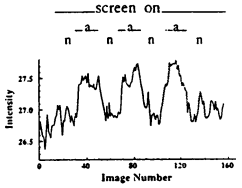
The focus of visuospatial attention can shift through overt changes in the line of gaze or through covert shifts that do not involve eye movements. Studies in two patients with supranuclear ophthalmoplegia (Rafal et al, 1988) suggested that the two mechanisms may be linked in patients with brainstem disease but the generality of this phenomenon has not been investigated systematically in patients with other types of lesions.

Reaction times to peripheral targets were measured in 3 right hemisphere stroke patients and in 9 control subjects with paradigms that elicited overt or covert shifts of attention. In the covert shift paradigms, subjects maintained central fixation and gave a rapid response to left- or right-sided targets that were preceded by a preparatory cue which was either a peripheral change of luminance or a central directional arrow. Control subjects did not show significant left-right asymmetries of reaction time in either type of task. In two patients with right hemisphere lesions and clinical left hemispatial neglect, reaction times were significantly prolonged to validly cued targets in the left side in covert attentional shifting paradigms whereas no asymmetry was detected in the overt attentional paradigm. In one pa-tient without hemispatial neglect, a right hemisphere lesion caused a slight prolongation of leftward overt attentional shifts but no asymmetry in covert attention shifts. These initial observations in patients with hemispheric lesions suggest that the mechanisms for shifting spatial attention covertly can be dissociated from mechanisms for shifting the line of gaze and focus of attention through overt eye movements.

374.11

VISUAL ATTENTION INDUCED BOTTOM UP AND TOP DOWN SIGNAL MODULATION IN FMRI. W. Chen[1], S. Ogawa*[2], P. Mitra[2], X. Hu[1] and K. Ugurbil[1] [1] Univ. of Minnesota Med. School, Minneapolis, MN 55455 and [2] AT&T Bell Laboratories, Murray Hill, NJ 07974

Using fMRI (echo planar multi slice imaging at 4T) of the human brain , we studied feed-forward and feed-backward effects on the signal activation associated with visual attention. Colored box patterns on a screen were presented to a subject during the measurement. He was instructed to pay attention at a cue to a box pattern flickering at the center of the screen (1x2 degrees in the visual field) and count the event where a pair of patterns (200 msec on and 200 msec apart and the pair appeared at every sec.) had the same color. The periods (45 sec each) of attention and non-attention were alternately repeated 3 times in an experiment. During the period of not paying attention to the visual pattern, the subject kept fixating at a cross at the center of the screen but listened to the sound generated by the field gradient during the imaging. With paradigm I where the screen presentation was always on, there was not appreciable modulation due to the attention-non attention task performance at the fovia area of V1. Although the task did not involve spatial information in any special way, activation patterns associated with the visual attention was observed at areas 7, 39,40 in the parietal area in 6 subjects among 8 tested. A typical activation time course of a site at area 39 is shown in Fig. 1. With paradigm II, where attention and no attention periods were separated by a dark (no screen display) period, sustained activation in the V1 area during each task period was larger with attention than without. With the same display as in paradigm II but without attentiion, such signal modulation due to attention was not observed in V1 area nor parietal area. This work done at U. of MN was supported by NIH grant RB08079.



374.12

ERP MEASURES OF AUTOMATIC ATTENTIONAL ORIENTING. J.B. Hopfinger, G.R. Mangun*. University of California at Davis, Department of Psychology & Center for Neuroscience, Davis, CA 95616.

Attentional cueing studies have shown that at short cue-to-target intervals, a non-predictive peripheral cue appearing at the location of a subsequent target (valid trial) facilitates response times to the target. At longer cue-to-target intervals, however, responses are actually slower for valid as compared to invalid targets (i.e., Inhibition of Return - IOR). The mechanisms which underlie these effects remain unresolved. In this experiment, we used ERPs elicited by valid and invalid targets to investigate the mechanisms underlying the facilitation and inhibition seen in peripheral cueing tasks.

A left or right field peripheral cue (offset and onset of four marker dots) preceded each target (a "tall" or "short" bar), but did not predict target location. The cue preceded the target at either a short (68-268 msec) or long (600-800 msec) cue-to-target interval (i.e., SOA). ERPs recorded over occipital scalp sites showed differential modulations of early, sensory-evoked components, as a function of cue validity and SOA condition. In the short SOA condition, validly cued targets showed an enhanced amplitude P1 component (110-130 msec) relative to invalidly cued targets. However, in the long SOA condition, the P1 to validly cued targets was reduced in comparison to the invalidly cued targets. These data suggest a possible facilitation of early visual processing at the cued location immediately following a peripheral cue, which is replaced later in time by a relative inhibition at this same location. Such a pattern implies that reaction time patterns obtained in similar peripheral cueing studies may be the result of spatial attention effects on early visual processing.

374.13

EVENT-RELATED POTENTIAL (ERP) CORRELATES OF UNEXPECTED JUMPS OF A CONTINUOUSLY MOVING AUDITORY STIMULUS

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Multichannel ERPs were recorded from human subjects participating in an auditory free-field experiment. A total of nine equally spaced loudspeakers were arranged to form a semi-circle of an arc of about 120°, facing the subject at a distance of about 1,2 m. The typical stimulus sequence consisted of brief broadband noise bursts shifted through consecutive spatial locations from left to right and vice versa, thereby producing the illusion of a train passing. Occasionally, the perceived moving stimulus would violate the pattern and skip two spatial locations instead of moving continuously onwards as expected. Control sequences presented the same stimuli in other patterns that did not create expectancies for continuous movement.

The ERP elicited by the stimuli marking the violation of the sequential ordering showed a distinct waveform, as compared to the ERP elicited by the same spatial location in one of the control conditions. ERPs to violations were more negatively displaced at a latency of about 130 ms and showed a broad frontocentral scalp distribution. The results are discussed in terms of a mismatch-type brain process that is elicited in a broad range of situations where expectancies are violated.

374.14

SEQUENTIAL CHANGES IN AUDITORY EVOKED POTENTIALS DURING TARGET DETECTION IN HUMANS. A. Starr*, T. Aguinaldo and H. J. Michalewski. Department of Neurology, University of California, Irvine, California 92717-4290.

The amplitude of potentials evoked by non-target signals change in an orderly manner as a function of their position relative to the target. The negative components, N100 and a pre-stimulus negativity (Readiness Potential, RP) are markedly attenuated to non-targets immediately following the target, and then regain their amplitudes to subsequent non-targets in an exponential manner for N100 and a linear manner for the RP. In contrast, the amplitude of the positive potentials (P50 and P200) increase immediately following the target but are of normal amplitude thereafter. The effects of instruction ("press to targets" or "count targets") influenced these sequential amplitude changes on component amplitudes for the negative but not the positive potential changes. Thus, there are dynamic changes in both traditional sensory and in Readiness Potentials to stimuli that follow a detected target.

374.15

EYE BLINK RATE AS A PRACTICAL PREDICTOR FOR VIGILANCE S. R. Quartz*, M. Stensmo, S. Makeig[†] and T. J. Sejnowski Howard Hughes Medical Institute, The Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92137. U.S.A. [†]Naval Health Research Center, P.O. Box 85122, San Diego, CA 92186, U.S.A.

Performance on signal detection tasks depends on a subject's state of alertness. We are developing methods to automatically assess the state of alertness of human subjects using electrooculogram (EOG) signals. Eye movements were monitored on human subjects in 28-minute sessions during which they responded to randomly-occurring targets (rate 10/min) consisting of barely audible noise-bursts. The responses were used to compute a local error rate (percent of recognized targets during the past minute). Ten subjects performed two or more experiments on different days. The vertical EOG (VEOG) signal was analyzed to extract eye closure and opening events, which were also translated into per minute rates. In an initial study only eye closure events were used. This simple measure of alertness nonetheless showed good correlation over the entire sessions to local error rate (max. -0.85). Further analysis of the VEOG showed that error rate was also correlated with amplitude and blink rate (where a blink is defined as an eye closure followed by an opening within 1 second). We trained a nonlinear neural network predictor on the first experiment session, and tested it by prediction of the local error rate from blink events on the second session, for six subjects with meaningful blink rate/performance correlations in both sessions. We were able to predict the local error rate of the subjects with an average accuracy of 0.176 ± 0.03 s.d. root-mean-square error. This was not significantly different from the accuracy of estimates based on spectral data from two channels of the electroencephalogram (EEG), 0.156 ± 0.05 s.d. Psychophysical experiments from other laboratories support our findings. However, our study is, so far as we know, the first in which eye blink data were used to predict as well as analyze error rates. A measure based on eye blinks, and also perhaps eye movements, could be used in a practical device to monitor the alertness of, e.g., sonar operators, truck drivers or air-traffic controllers.

374.16

CNV IS AFFECTED BY UNILATERAL FRONTAL EXCISION IN HUMANS. C. Verkindt, M. Robert and F. Richer*. Labo. Neuroscience Cognition, Université du Québec, Montréal, QC, H3C-3P8.

We examined the hypothesis of a frontal lobe involvement in the generation of the contingent negative variation by recording slow potentials in frontal lobectomy patients performing delayed conditional response tasks. nine patients who had undergone a unilateral frontal excision for the relief of epilepsy were compared to 9 temporal lobectomy patients and 9 age-matched controls. Subjects were tested on two tasks involving speeded responses to a visual stimulus (S2) which followed another visual stimulus after a 4-sec delay. One task required a two-alternative color discrimination at S1 and delaying response until S2, whereas the second task involved the color discrimination at S2 and a neutral stimulus as S1. Response times (but not error or omission rates) were increased in both patient groups compared to controls. Compared to the other two groups, 6 frontals showed a large attenuation of both the early and the late portion of the slow potential in the S1-S2 delay. The three other frontal patients presented a normal early CNV. One of them exhibited a moderate attenuation of the late portion of this potential while the two others did not. The young age of these subjects at operation (12-16 years old), suggests that some plasticity effect may contribute to the reduced lesion effects in these frontal patients. Although all the frontal lesions were unilateral, no lateralization of the potential attenuation was observed. This absence of asymmetry in frontal lesion effect was previously found on attentional waves (Knight et al. 1981). The present results may indicate that the integrity of both frontal lobes is necessary to preserve some neural mechanisms leading to the generation of the CNV.

374.17

COGNITIVE INHIBITION OF OBJECT VERSUS LOCATION IN YOUNG AND OLD ADULTS. P.M.Simone*. Department of Psychology, Santa Clara University, Santa Clara, CA 95053

Selective attention involves activation of a target and inhibition of any distractors. Some evidence suggests that older adults are not as able as young adults to inhibit distractors. Connelly & Hasher (1993) found that this cognitive inhibition can be associated with the distractor object OR its location. Additionally, older adults are impaired only when required to inhibit the object, and not the location of the distractor.

This study explored young (18-22) and older adults' (55-74) ability to inhibit the object or location of distracting information. Negative priming trials were presented in prime and probe pairs. Stimuli were 9 colored squares. A target and distractor were presented for 150 msec. After a 500 msec delay subjects were presented with 4 colored squares and were to press the target as quickly as possible. A similar probe trial began 500 msec later. The probe target was either the same color as the prime distractor but in a new location, a new color located in the same position as the prime distractor, or the two were unrelated. Sustained inhibition was measured in the probe trial.

As predicted, older adults did not inhibit the distractor object but did show effects of sustained inhibition when required to inhibit the location of the distractor. Young adults also showed a differential pattern of responding when the distractor object or location was inhibited. These results suggest that separate mechanisms exist for the inhibition of an object and the inhibition of a location.

374.18

THE EFFECT OF DISTRACTORS ON VISUALLY GUIDED REACHING BY PATIENTS WITH HEMISPATIAL VISUAL NEGLECT L. L. Baylis, Dept. Psychology, Presbyterian College, Clinton, SC 29325, G. C. Baylis*, Dept. Psychology, University of South Carolina, Columbia, SC 29208, and R. D. Rafal, Dept. Neurobiology, U. C. Davis, Davis, CA 95616.

Patients suffering from left hemispatial visual neglect following damage to the right parietal lobe were tested on a visually guided reaching task. In this task, nine locations (in a 3 x 3 array) could be illuminated red (target items) or green (distractor items).

Consistent with the observed neglect, reaction times to reach to targets located to the patients left were elevated compared to central or right targets.

In addition, it was found that the location of irrelevant distractor items could affect reaction times to reach to targets. So, for target items located directly in front of the patient, reaction times were elevated when distractors were located to the right of them, but were reduced when distractors were located to their left.

These results suggest that both relevant (target) and irrelevant (distractor) information are used preattentively to demarcate visual space. Neglect represents a failure to allocate attention within this parsed visual space.

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374.19

ATTENTIONAL ORIENTING ALTERED BY NICOTINE FROM TOBACCO SMOKE. E.A. Witte*, J.T. McCracken, and J. Wilkins, Psychiatry Department, University of California, Los Angeles, CA 90024.

Attentional orienting, as measured by performance in a cued covert target detection task has been shown to be affected by alterations in nicotinic cholinergic transmission[1,2]; the difference in reaction times between validly and invalidly cued trials (validity effect) was reduced by nicotine administration. While these studies have established a preliminary link between facilitation of nicotinic cholinergic neurotransmission and altered attentional orienting, they did not examine the dose-response relationship between these variables.

To this end, we examined the relationship between salivary nicotine levels and measures of attentional orienting, alerting, and overall response times in five smokers and five nonsmoker controls. Smokers were tested after an overnight abstinence from smoking, and then one, two, three, five, and seven hours after beginning *ad libitum* smoking on that day. Nonsmoker controls were tested at the same intervals. At each session, a saliva sample was collected for later analysis for nicotine levels (ng/ml).

The results of this study show a clear relationship between nicotine levels and the validity effect. No apparent relationship was observed between nicotine levels and the alerting effect (difference in reaction times between non-cued trials and double-cue trials), as would be predicted by previous research[1,2].

This study provides important support for a direct role of the nicotinic cholinergic system in mediating attentional orienting. However, increases in nicotine levels did not affect the alerting measures.

1. Witte, E.A., and Marrocco, R.T. (1993). *SFN Abstract* 19: 233.3.
2. Marrocco, R.T., and Witte, E.A. (1993). *SFN Abstract* 19: 233.2.

375.1

DENTATE GYRUS GLYCOGEN METABOLISM IS INVERSELY CORRELATED WITH EEG THETA ACTIVITY. A. Uecker*, G. Rao, C.A. Barnes, B.L. McNaughton and E. Reiman. ARL Division of Neural Systems, Memory and Aging, Univ. of Arizona, Tucson, AZ 85724 and Samaritan Pet Center, Good Samaritan Regional Medical Center, Phoenix, AZ 85006.

Glycogen phosphorylase a (GPa), an enzyme marker of neuronal activity, has been used to study behaviorally-related metabolism in the hippocampus (Wallace, 1982; Harley & O'Keefe, 1986) and other brain regions. GPa activity is least in the molecular layer of the dentate gyrus in exploring animals and greatest in anesthetized animals (Uecker et al., 1993), and is also systematically reduced by different levels of restraint (exploration < manual restraint < towel restraint < anesthetization; Uecker et al., 1994). Thus, GPa activity appears to be inversely correlated with behaviors associated with the theta rhythm. We addressed this question directly in nine 6 month old Long-Evans female rats with electrodes implanted in the region of the hippocampal fissure. During 10 behavioral sessions, 5 minute recordings were made of hippocampal EEG during exploration, manual restraint, towel restraint, and rest. Power in the theta frequency (6-10 Hz) exhibited a significant decline in amplitude across these conditions. Molecular layer glycogen metabolism increased across behavioral conditions (as observed previously) and was inversely correlated with the theta rhythm (r=-.50). Although it is known that hippocampal CA1 pyramidal cells are more active during rest than during exploration (Thompson & Best, 1989; Ranck, 1973), Foster et al. (1989) found that CA1 CS cells became almost completely silent during towel restraint. If this effect occurs also in the granule cells it would suggest that it is the theta rhythm or factors associated with it, rather than cell discharge *per se* that regulates the metabolic rate in the dentate gyrus granule cells. Supported by the Flinn Foundation and MH01227.

375.2

BEHAVIORAL CORRELATES OF RAT PREFRONTAL CORTICAL UNITS DURING SPATIAL WORKING MEMORY TASKS. M.W. Jung*, Y-L. Qin, B.L. McNaughton and C.A. Barnes. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Single units were recorded with "tetrodes" in regions of the prefrontal cortex, including those which are targets of hippocampal afferents, while rats were performing an 8-arm radial maze, spatial working memory task or a figure-8 maze, delayed spatial alternation task. Virtually no place-specific firing was observed on the 8-arm maze among approximately 200 recorded units, except to the extent that different spatial locations were related to distinct phases of the task. A small, but significant number of cells exhibited spatially biased firing on the figure-8 maze. Also, few units showed continuous neural activities that might mediate working memory of spatial locations. The units had diverse behavioral correlates that were generally associated with different stages of the task, such as intertrial intervals, onset or end of trials, selection of arms on the 8-arm maze, delay periods, approach to or departure from goals, and selection of paths on the figure-8 maze. Some units were essentially silent during the tasks while others were active but had no discernible task-related behavioral correlate. Among the units that were recorded in both tasks, the majority showed no obvious relationship between behavioral correlates in the two tasks. These results, while preliminary, are consistent with previous behavioral studies that suggest a prefrontal cortical role in encoding "rules" (i.e., structural features) of a task but not in encoding allocentric spatial information or memory. Given that the hippocampal projection to this region is capable of undergoing LTP (Laroche et al., 1990), our data lead to the hypothesis that the role of this projection is not to impose spatial representations upon prefrontal activity, but to provide a mechanism for learning the spatial context in which particular behaviors are appropriate. Supported by the McDonnell-Pew Foundation and MH01227.

375.3

IDENTIFICATION OF IEGs DIFFERENTIALLY REGULATED BY LTP-INDUCING STIMULI IN YOUNG AND OLD RATS. B. Pierchala, A. Lanahan, G. Lyford, P.F. Worley, G.S. Stevenson* and C.A. Barnes. Depts Neurosci & Neurol Johns Hopkins Univ, Baltimore, MD 21205 and Depts Psych & Neurol and ARL Division NSMA, Univ Arizona, Tucson, AZ 85724.

Several lines of evidence suggest that activity-dependent mechanisms are altered with aging and that these mechanisms may contribute to age-dependent memory decline. For example, the durability of LTP in the hippocampus is reduced in aged rats and this deficit is associated with impaired performance on hippocampal-dependent spatial learning tasks. Because the initial induction of LTP appears normal in aged rats, attention is focused on the cellular mechanisms underlying long-term plasticity. In previous studies, we have used differential cloning techniques to identify genes that are rapidly induced by physiological synaptic activity and by stimuli that induce LTP in the hippocampus. We now use these same techniques to identify rapid response genes that are differentially induced following LTP in young versus aged rats. Dentate gyri were microdissected and used to prepare radiolabelled cDNA. Panels of candidate genes were then probed using the reverse Northern strategy. Most of the known IEGs are similarly regulated in young and old animals, however a small set of genes have been identified that appear to be differentially regulated. We are presently working to determine whether this pattern of results holds in larger populations of young and old rats. It is anticipated that an understanding of this gene regulation will lead to identification of signaling pathways that are functionally altered with aging. Additionally, our previous work indicates that many of these rapid response genes play a direct role in modification of signaling capacity of neurons. Thus, identification of these genes should provide insights into the cellular basis of age-dependent changes in synaptic plasticity. Supported by AG09219 and MH01227.

375.4

PRESERVED LTP INDUCTION IN CA1 OF AGED F-344 RATS. G. Rao*, J. Shen, C.A. Barnes and B.L. McNaughton. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

With supramaximal, high-frequency stimulation, no differences in magnitude of LTP induction have been observed between young and old animals in either CA1 or the fascia dentata; however, using weak stimulation both Deupree et al. (91) and Moore et al. (93) did observe a reduction in LTP induction in CA1, suggesting a deficit when more "physiological" stimulation is employed. We have previously shown a reduction in evoked, population $AMPA_R$ and $NMDA_R$ EPSPs in aged F-344 rats but no change in unitary EPSPs or apparent quantal sizes. This suggests that CA1 may undergo a loss of synapses in aging with no change in the receptor content of individual synapses, and possibly no differences in the intrinsic capability of individual synapses to express LTP. Under this interpretation, the apparent age effect would be a consequence of activation of fewer synapses, and consequently less effective postsynaptic depolarization. To test this, we paired weak orthodromic stimulation with postsynaptic depolarization via direct, intracellular current injection, in order to bypass the possible variable level of presynaptic activation with electrical stimulation. The evoked EPSP (stimulating electrode in s. radiatum) was paired with an ascending series of intracellular depolarizing current pulses (100 msec, 0 to 4 nA, .2 nA steps). LTP was not different across age groups (% increase in EPSP slope: young = 35.6 ± 3.6; adult = 37.9 ± 4.21; old = 36.6 ± 4.25). A replication of Moore et al. (n=11 rats, 9 mos; n=11, 23-28 mos) confirmed reduced LTP in old rats when using primed burst stimulation (% increase in EPSP slope: adult = 24.7 ± 2.9; old = 13.4 ± 2.1). This deficit in LTP induction may arise from insufficient depolarization by orthodromic stimulation, perhaps as a result of synapse sparsity, and appears not to reflect an intrinsic age difference in LTP induction mechanisms. Supported by AG03376 and MH01227.

375.5

HIPPOCAMPAL THETA AND THE SPATIAL FIRING OF DENTATE GYRUS GRANULE CELLS. W.E. Skaggs*, J.J. Knierim and B.L. McNaughton. ARL Division NSMA, Univ. of Arizona, Tucson, AZ 85724.

Two recent findings warrant a re-examination of the relations between the theta rhythm and firing of dentate gyrus granule cells. First, granule cells are not, as once thought, high-rate "theta cells", but rather low-rate cells with highly specific place fields (Jung and McNaughton, 1993); second, a robust interaction exists between place-related firing of complex spike cells in CA1 and theta phase (*phase precession*, O'Keefe and Recce, 1993; Skaggs *et al.*, submitted). Phase precession implies that different cells are active at the beginning and end of the theta cycle. If this occurs in one area, it is likely to induce differences in downstream areas. Therefore, a simple strategy for locating the source of the precession is to backtrack upstream from CA1 until an area is found that does not show precession. We have used parallel recording techniques to record simultaneously from granule cells and CA1 CS cells in rats running for food reward on a narrow track. CA1 CS cells fire their first spikes, as the rat enters a place field, near the end of the theta cycle, and bursts of spikes gradually precess to the beginning of the theta cycle as the rat passes through the place field. Granule cell spatial firing also shows clear phase precession, but their first spikes, as the rat enters a place field, appear approximately 90 degrees earlier in the theta cycle than would be the case for a CA1 cell; also, unlike CA1 cells, granule cells are virtually silent during approximately the last quarter of the theta cycle. Thus, the phase of peak population activity for granule cells is advanced about 90 degrees from that of CA1 cells. Furthermore, in two rats in which the fascia dentata was lesioned by multiple injections of colchicine, phase precession could still be seen in some CA1 complex spike cells. Pending verification of lesions, this suggests that phase precession may originate prior to the fascia dentata, possibly in the entorhinal cortex. Supported by MH46823 and NS09052.

375.6

DIFFERENTIAL EFFECTS OF DENTATE GYRUS LESIONS ON PYRAMIDAL CELL FIRING IN 1- AND 2-DIMENSIONAL SPATIAL TASKS. J. J. Knierim* and B. L. McNaughton. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Destruction of the dentate gyrus with colchicine leaves place-specific firing on an 8-arm maze relatively intact (McNaughton et al., 1989). In the present experiments, similarly lesioned rats were trained to forage for food in a gray cylinder (76 cm diam., 51 cm high) with a single white cue card (Muller et al., 1987). After training, CA1 and/or CA3 pyramidal cells were recorded over 4-6 days. Few cells displayed normal spatial selectivity. Some fired strongly in a ring at the edge of the cylinder, whereas others fired weakly in fuzzy "doughnut" or "crescent" shaped regions of the cylinder. In contrast, when the rats were subsequently trained to run back and forth on a linear track, with a moveable goal box at one end and a stationary goal location at the other, many cells displayed normal behavioral/spatial correlates (see Gothard, Skaggs, and McNaughton, this session). The same cells showed little selectivity when the rats foraged in the cylinder or on an open circular platform. It thus appears that in a task in which directional behavior was highly constrained, CA1/CA3 cells did not require an intact dentate gyrus in order to express normal selective activity. In a two-dimensional apparatus, in which directional behavior was less stereotyped and unconstrained, pyramidal cells apparently had access to information about distance to the edge of the cylinder/platform, but were unable to use the available heading information. These results remain preliminary pending histological verification of the lesion, but they nonetheless provide clear evidence of a differential effect of damage to the hippocampal system on place cell firing in tasks that provide different sources of heading information. Supported by NS09052 and HFSP.

375.7

INTERACTIONS BETWEEN MULTIPLE SPATIAL REFERENCE FRAMES IN THE RAT HIPPOCAMPUS. K.M. Gothard*, W.E. Skaggs and B.L. McNaughton. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Hippocampal CA1 cells were recorded from five rats, trained to run on a 1.5 m rail between a fixed food cup at one end (goal) and a sliding box containing a food cup at the other. On each trial, the rats ran from the box to the goal, and then back to the box, which, in the meantime, was moved to a different position. Each trial started at one of five equally spaced box locations and ended at another. Two main classes of cells were observed: (1) cells that fired in relation to the moveable box (box-inward and box-outward cells) and (2) cells that fired in relation to the goal and/or the global spatial reference frame (place cells), indicating that both the goal and the box defined discrete reference frames. Most cells were unidirectional in their firing, regardless of which reference frame they were bound to. The firing of cells bound to one reference frame appeared to occlude the firing of cells bound to the other. In all rats, box-outward cells, which fired in the immediate vicinity of the box, were unaffected by the location of the box, whereas those that fired at greater distances from the box showed a systematic shift and shrinkage of firing field as a function of the proximity of the box to the goal. These cells may either represent position as a fixed fraction of the distance between start and the goal, or else encode the distance from the most recently visited reference point, modulated by the proximity of the goal. These results suggest that in this behavioral context, (a) hippocampal cells represent position in multiple reference frames (the fixed frame of the goal, the moving frame of the box and a fractional frame between box and goal), (b) only one reference frame is active at a time, and (c) a given reference frame is associated reliably across rats with the same part of the task. Supported by ONR.

375.8

CONTEXT-DEPENDENT BINDING OF HIPPOCAMPAL "PLACE CELLS" TO DIFFERENT FEATURE-CENTERED REFERENCE FRAMES. B.L. McNaughton*, K.M. Gothard and W.E. Skaggs. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Hippocampal CA1 cells were recorded from three rats, trained for two different tasks in two different rooms but with an overlapping set of behaviorally relevant objects. In the first task, rats ran back and forth on a 1.5 m rail, with a fixed food cup (goal) at one end and a sliding box (containing a food cup) at the other. While the rat was at the fixed goal, the box was moved to a new position, so that each trial started at one, and ended at another of 5 equally spaced box locations on the rail. In the second, analogous task, which was performed on a 1.2 x 1.2 m open platform, rats ran from the same box to a goal location adjacent to a cylindrical landmark. There were three possible goal locations along one edge of the platform, and 3 box locations along the opposite edge. The box and landmark locations were independently randomized. Of 43 cells with correlates on at least one task, only 29 showed any behavioral correlate in both tasks. The correlates were: (1) location-specific firing (place cells), box-related firing, (inside-box, box-outward, and box-inward cells; see Gothard et al., this session) and goal-related firing. From the 29 cells, only 4 showed the same behavioral correlate for both tasks; the other 25 were allocated in an apparently nonsystematic way to different reference frames. Although the same box and food cups were used in both environments, only one cell was bound to the box in both environments. These results suggest that binding to a feature frame is not maintained across environments and tasks, and further supports the notion that the hippocampus generates orthogonal representations for similar experiences. Supported by ONR.

375.9

RECENT EXPERIENCE STRENGTHENS PRE-EXISTING CORRELATIONS BETWEEN HIPPOCAMPAL NEURONS DURING SLEEP. H.S.Kudrimoti*, B.L. McNaughton, C.A. Barnes and W.E. Skaggs. ARL Division NSMA, University of Arizona, Tucson, AZ 85724.

During slow-wave sleep, patterns of correlated neuronal firing that occurred during the preceding behavior are reactivated in rat hippocampus (Wilson and McNaughton, 1994). We confirmed and extended this result in 22 of 24 recording sessions from 3 rats in which 60-90 CA1 CS cells were recorded per session. Correlations between all cell-pairs were computed during awake behavior, and during 20-60 min sleep episodes recorded before and after each behavior session, as $Cov(x,y)/\sigma_x\sigma_y$, x and y being the firing rate vectors (100 ms bins) of any two cells in the sample. Correlations between cells that were strongly correlated during behavior (reflected in overlap of their fields) were significantly higher in the sleep period following maze running than a) correlations between the same cell-pairs in the preceding sleep session, or b) correlations between cells that were active but uncorrelated during behavior. The correlation increases returned to baseline in about 1 hr. Also, when two separate environments were visited sequentially, the effects of the earlier of the two experiences were still evident in the subsequent sleep, suggesting that not much decay occurs during waking. Firing rates did not change significantly during sleep following the experience; however cells that had place fields on the apparatus had substantially higher rates during sleep both before and after the behavior. There was a significant linear relationship between the correlations between cell-pairs during behavior and the corresponding correlations during prior sleep, even in cases in which the subsequent behavior was in a novel apparatus. This suggests that the probability that two cells will have overlapping place fields in any given environment is partly a function of a preexisting synaptic weight distribution. Support: MH46823 and MH01227.

375.10

REACTIVATION DURING SLEEP OF CORTICO-CORTICAL AND HIPPOCAMPO-CORTICAL CORRELATION STATES FROM PRECEDING BEHAVIOR. Y-L.Qin*, B.L. McNaughton, W.E. Skaggs and C.A. Barnes. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Hippocampal cells that fire together during behavior exhibit enhanced correlations during subsequent slow-wave sleep (Wilson and McNaughton, 1994; Kudrimoti et al., this meeting), indicating that information acquired during behavior is reexpressed in hippocampal circuits during sleep, as postulated by some theories of memory consolidation (Marr, 1971; McClelland, McNaughton and O'Reilly, 1995). If hippocampus reinstates patterns in neocortex, as postulated by this theory, then correlations between neocortical cells during behavior should also reemerge during sleep. This should also be true for correlations between hippocampal and neocortical cells. Parallel recordings were made either within posterior neocortex (HL), within CA1, or simultaneously in both areas. Each session involved an initial episode of sleep (S1), a period of behavior on a simple, triangular maze (M1) and a subsequent sleep episode (S2). All pairwise correlations ($Cov(x,y)/\sigma_x\sigma_y$) between rates (100 ms bins) were computed for each of the 3 states. For both cortico-cortical and hippocampo-cortical interactions, the correlations during S_2 were significantly more strongly predicted by the correlations during M_1 than were the correlations during S_1, indicating that information about the experiences during M_1 was reexpressed during S_2. This suggests that traces of recent experience are reexpressed in both hippocampal and neocortical circuits during sleep, and moreover, that at any given moment during slow-wave sleep, the representations in the two areas tend to correspond to the same experience. It remains to be determined whether the effect in one area depends on the other. Supported by MH46823.

375.11

MATCHING PURSUIT ANALYSIS ENABLES TEMPORAL-LOCALIZATION OF FREQUENCY/PHASE PATTERNS OF HIPPOCAMPAL EEG AND THEIR RELATIONS TO THE NEURONAL STATE VECTOR DURING SLEEP. B. Shen, B. L. McNaughton and C.A. Barnes*. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

EEG signals embed a wide range of patterns over the time-frequency domain. Linear expansions in a single complete basis, (e.g., Fourier, wavelet), can provide accurate characterization in either the time or frequency domain, but not in both simultaneously. Matching pursuit analysis (Mallat and Zhang, 1993) provides a decomposition of signals into components that are simultaneously localized in both time and frequency. The output of this analysis, which can be appreciated visually in 3-D Wigner plots of relative energy in both frequency and time, can provide a powerful basis for automated EEG analysis, for example, the characterization of transitions between hippocampal theta and LIA.

During REM sleep, theta oscillations in the hippocampal EEG were decomposed into relatively discrete segments of different dominant frequencies and phases. We examined the dynamics of a population of 54 simultaneously recorded CA1 pyramidal cells during these frequency/phase transitions. Firing rates were binned in 100 ms intervals and the state vector correlation matrix was constructed. As shown previously (Moore et al., Soc. Neurosci Abs, 1994), this matrix is characterized by periods in which the firing rate vector is relatively stationary over time, punctuated by rather abrupt transitions to different regions of neuronal state space. We observe that these state transitions tend to be correlated with the transitions of the frequency/phase of the theta rhythm during REM revealed in the matching pursuit analysis.Supported by MH46823 and the McDonnell-Pew Foundation.

375.12

ON THE PHASE RELATION BETWEEN EXCITATORY AND INHIBITORY POPULATIONS DURING HIPPOCAMPAL THETA RHYTHM. M. Tsodyks*, W.E. Skaggs, T.J. Sejnowski and B.L McNaughton. Computational Neurobiology Lab, Howard Hughes Medical Institute, The Salk Institute, La Jolla CA 92037 and ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson AZ 85724.

We examine the dynamical behavior of a neural network consisting of two groups of cells, one whose members are excitatory to all other cells and the other inhibitory, with external oscillatory inhibitory input to the inhibitory population such as is known to occur in the septal GABAergic input to hippocampal interneurons (Freund and Antal, 1988). Using simulations and phase plane analysis, we find that over a substantial range of parameters, the behavior of the network is apparently paradoxical, in that increasing the direct external *inhibitory* input to the inhibitory cells leads them (as well as the excitatory cells) to synchronously *increase* their activity, and thus to oscillate out of phase with the external drive. The range of parameters over which this occurs corresponds closely to the range in which the excitatory network is unstable when all inhibition is removed. In the opposite regime, the inhibitory population oscillates in phase with the external drive, and out of phase with the excitatory population.

For a network operating in the vicinity of the transition between these two regimes, the inhibitory population exhibits intermediate phase shifts relative to the excitatory one. This phenomenon may be important for understanding the behavior of inhibitory interneurons ("theta cells") during the hippocampal theta rhythm, where unit recordings have revealed all three possible situations. The model may also be relevant to understanding the effects of neuromodulation in several brain regions, including the neocortex. Supported by MH46823 and the McDonnell-Pew Foundation.

375.13

COOPERATIVE ROLE OF PERIODIC DRIVING AND RANDOM BACKGROUND INPUT IN GOVERNING THETA CELL OUTPUT IN RAT HIPPOCAMPUS. D.R. Chialvo, C.A. Barnes B.L. McNaughton, K.M. Gothard and M. Mehta*. ARL NSMA, Univ. of Arizona, Tucson, AZ 85724.

The interspike interval distributions of hippocampal interneurons vary continuously between Poisson-like (random) and multimodal (periodic) as a function of behavioral state. This suggests the simultaneous presence of both oscillitory and random inputs. The theory of stochastic resonance predicts that a cell's response can be exponentially sensitive to the uncoherent (noise) input, but only quadratically sensitive to the oscillatory source (Chialvo, J. Stat. Phys. 70:1993). We analyze the spike time-series of theta cells from rats during either sleep or running for food on a rectangular track. We define the spectral density of the point process at the theta frequency as Signal(S) and the background spectral density as Noise (N). The quantity 10*Log10(S/N) estimates the signal to noise ratio (SNR), reflecting how many spikes are coherent with the periodic input compared with how many are not. From this we derive the input magnitude due to N and S. We find, paradoxically, that during both activity and sleep, as the noise increases, there is a substantially larger *increase* in the SNR, even for rather small changes in mean rate. This paradoxical improvement of the coherent spiking in the presence of augmented noise is the signature of stochastic resonance (SR), a phenomenon in which noise and periodic modulation play a cooperative role. Although SR has been demonstrated artificially in a variety of systems, our results are the first evidence of this phenomenon occurring spontaneously in a living system. These results suggest that SNR as defined here is a more sensitive indicator of changes in the state of theta cells than mean firing rate, and may be useful in characterizing physiological changes that occur in hippocampal networks during abnormal states such as drug-states, disease or aging. Supported by MH50064, MH46823, AG12609 and MH01227.

375.14

ACCURACY OF HIPPOCAMPAL PLACE FIELDS IN PREDICTING LOCATION IS ENHANCED IN THE PRESENCE OF THETA RHYTHM. J.L. Gerrard*, I. Ginzburg, C.A. Barnes, T.J. Sejnowski and B. L. McNaughton. Howard Hughes Medical Institute, Salk Institute, La Jolla, CA 92037 and ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Theta rhythm recorded from the hippocampus is observed mainly when a rat is running or exploring its environment. It has been observed that the place-specific activity of single hippocampal pyramidal cells is more robust in the presence of theta rhythm in the EEG (Kubie et. al., Soc. Neurosci. Abst., 1984), but no quantitative analysis has been performed on how this change affects the accuracy of a population of place cells in predicting the rat's location in space. We have estimated the effectiveness of the hippocampal place cells in coding for the location of the rat in space in two conditions: (1) the place fields are defined using only spikes that appear when theta rhythm is present in the EEG, and (2) the place fields are defined using all the spikes that occurred when the rat is running on the maze. We analyzed data from a large ensemble of simultaneously recorded hippocampal cells and reconstructed the path made by the rat when running in the maze, using the population activity vector, as described by Wilson and McNaughton, 1993. The reconstruction error is reduced by 40% when using only the spikes that appear when theta rhythm is present in the EEG, in comparison to the case when all spikes are taken into account. Clearly, the presence of theta rhythm is well correlated with relatively high accuracy of the place fields in predicting location. Supported by MH46823, AG12609 and MH01227.

375.15

MILLISECOND TEMPORAL STRUCTURE OF MEMORY REPRESENTATIONS AND HIPPOCAMPAL-DEPENDENT COGNITIVE MECHANISMS. A. Samsonovich* and B.L. McNaughton. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Experimental studies of hippocampus and neocortex show temporal correlations of spikes at the scale of milliseconds. The ability of a network of spiking units to store and to retrieve such patterns has been demonstrated numerically (W. Gerstner et al., Biol. Cyb. 69, 503, 1993). The present study investigates possible neural mechanisms of information processing that depend on the millisecond temporal structure of memory representations. A model network of leaky integrate-and-fire neurons with dynamic delayed Hebbian synapses has been studied numerically in discrete time. Two basic kinds of dynamics are observed: (quasi) periodic and chaotic spatio-temporal patterns of spikes. The period is estimated analytically. Different self-developed periodic spatio-temporal patterns were stored in the network and selectively retrieved later. Retrieval was possible by initiation of the temporal sequence as well as by a steady stimulation with the appropriate spatial distribution. The period of the pattern can be set by global sinusoidal modulation of neuronal thresholds and tends to be commensurate with the period of modulation (i.e., the period ratio is a rational number). Endowing spatially correlated patterns with fixed uncorrelated temporal structures may substantially reduce their interference thus substantially increasing the memory capacity of the network. In addition, the obtained results imply that formation of cyclic attractors in the phase space of the CA3 network may account for such phenomena as phase precession of place cell firing during the theta rhythm (O'Keefe and Recce, 1993) and formation of archetypal spatial maps. Supported by the McDonnell-Pew Foundation.

375.16

TOWARDS A COMPUTATIONAL MODEL OF THE HIPPOCAMPAL FORMATION INCORPORATING REALISTIC ANATOMICAL CONNECTIVITY: AMMON'S HORN AREA CA1 AND THE SUBICULUM. P. Patton* and B. L. McNaughton. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

A computational model of the rat hippocampal region incorporating realistic anatomical connectivity is being developed. This report focuses on Ammon's horn area CA1 and the subiculum. Each of these structures is represented as a rectangular sheet, through which points, representing neurons, are distributed. CA1 is assumed to contain 380,000 pyramidal cells and the subiculum 280,000 (West et al., Anat. Rec. 231: 482-497). Intrinsic circuitry of area CA1 and inputs from area CA3 are based on Ishizuka et al. (JCN 295: 580-623) and Bernard and Wheal (Hippocampus 4(5): 497-529). Major connections of CA1 and the subiculum with each other and the entorhinal cortex (EC) are also included in the current model. These connections are topographically organized with respect to a septotemporal axis running parallel to the boundaries between the cytoarchitectonic subdivisions, and a perpendicular proximodistal axis, with proximal being the direction towards area CA3 along the hippocampal surface. Fibers originating in the medial EC (MEC) terminate in the proximal half of area CA1 and distal half of the subiculum, and lateral EC (LEC) fibers terminate in an opposite fashion (Witter, Hippocampus 3:33-44). CA1 projects to the subiculum, with more proximal portions projecting more distally (Tamamaki and Nojyo, JCN 291:509-519). CA1 and the subiculum project to the EC, with the proximal portion of CA1 and distal portion of the subiculum projecting to MEC and opposite portions to LEC (Witter ibid.). The connectivity matricies resulting from these anatomical models will serve as a basis for models of operations such as associative memory. Supported by MH42683.

375.17

REVISITING THE PAPEZ CIRCUIT: THE ROLE OF HIPPOCAMPUS AND ITS AFFERENT AND EFFERENT STRUCTURES IN RODENT NAVIGATION. A.D. Redish* and D.S. Touretzky. Computer Science Dept. and Center for the Neural Basis of Cognition, Carnegie Mellon Univ., Pittsburgh PA. 15213.

We present a hypothesis compatible with the neurophysiological, anatomical, and lesion literature, which details the role of hippocampus and its afferent and efferent structures in rodent navigation. Place cells in hippocampus are sensitive to sensory information, which can enter from cortical areas via deep entorhinal cortex into the superior layers, and thence into hippocampus proper. But place cells continue to show place fields in the dark. We have suggested elsewhere that this is driven by path integration. We suggest here that the path integrator is located in post-subiculum and cingulate cortex, and that path integrator information enters hippocampus via connections from post-sub to sup-EC.

If place cells are involved in navigation, rodents must have a means of combining place information with information about current goals to generate motor actions. We suggest nucleus accumbens as a good candidate, receiving place information via the fornix and goal information from hypothalamus.

Although head direction cells have been found in a number of places, we suggest that these different anatomical structures are performing different functions: lateral dorsal thalamus integrates orientation cues from multiple external stimuli (represented individually in parietal cortex), while HD cells in anterior thalamic nuclei and post-subiculum are updated by instrinsic cues.

When an animal enters a familiar environment, head direction or path integrator coordinates may be reset by external cues. In this hypothesis, the place code is recombined with local view information in subiculum, and then sent to the head direction and path integration systems.

Novelty is assumed to be encoded by acetylcholine levels reaching hippocampus from the septal nuclei. New environments require dentate gyrus, while familiar ones can be processed by the perforant path.

375.18

INTRA-CORTICAL LONG-TERM POTENTIATION IN SUPERFICIAL LAYERS OF LATERAL ENTORHINAL CORTEX INDUCED BY THETA-PATTERN STIMULATION. M. Protomastro* and T.Otto. Program in Biopsychology and Behavioral Neuroscience, Dept. of Psychology, Rutgers University, New Brunswick, NJ, 08855.

It has previously been demonstrated that the entorhinal cortex (EC), a primary cortical target of the hippocampal formation, is capable of robust long-term potentiation (LTP) induced by theta-bursting stimulation (TBS) of ipsilateral CA1. The present study sought to determine the capacity within the EC itself to induce and maintain LTP. Five urethane-anesthetized rats were implanted with a bipolar stimulating electrode and a recording electrode in the superficial layer of lateral EC (LEC). Responses were characterized by a latency to peak of 6 ms. The mean maximum response was 0.9 mV (mean stim. int. = 590 uA), and all responses showed paired-pulse facilitation and followed brief trains of high frequency (4-5 pulses at 70-100 Hz) stimulation. During baseline recording, EPSPs (mean amplitude of 0.2 mV, mean stim. int. = 200uA) were evoked every 15 sec for 15-20 min; initial slope and peak amplitude were recorded on-line by computer. TBS of LEC (2 bouts of 14 bursts [4 pulses at 100Hz], 140ms between bursts [7Hz], 10 sec between bouts) was then delivered at twice the baseline stimulation intensity, and its effect on the EPSP was assessed by continued low-frequency stimulation (every 15 sec) for 60 min. TBS produced stable LTP in LEC; the average potentiation at 60 min was 140.4% of the baseline response. Histology is presently being conducted to verify placement of electrodes in the superficial layers of the LEC.

375.19

HIPPOCAMPAL NEURONS ENCODE BOTH LOCAL AND DISTAL STIMULI IN RATS EXPLORING A CUE-CONTROLLED ENVIRONMENT. M.L. Shapiro*[1], H. Tanila[2], & H. Eichenbaum[2], [1] Dept. of Psychology, McGill Univ., Montreal, Canada; [2] Cntr. for Behavioral Neuroscience, SUNY Stony Brook, NY.

Sensory and behavioral correlates of hippocampal complex-spike cell (CS) activity in rats are often assessed in environments with many distal extra-maze, but few local intra-maze stimuli. In such cases, CS cells usually respond to the constellation of extra-maze stimuli. Conversely, CS activity recorded while rats explore environments rich with intra-maze stimuli and reduced extra-maze stimuli is often correlated with the intra-maze stimuli. In the present experiment, male Long Evans rats explored a 4-arm radial maze surrounded by curtains holding large, easily discriminated visual stimuli, and in addition, each arm of the maze was covered with a unique set of visual, tactual, and olfactory cues. CA1 and CA3 CS cells were recorded using tetrodes, and the arm entries were rewarded with lateral hypothalamic stimulation. After recording CSs in a standard configuration (baseline trials), the arms and the distal stimuli were rotated 90° in opposite directions around the maze center. After determining whether local or distal cues controlled CS activity, each element of the relevant stimulus set was removed one at a time in recording trials interposed between baseline trials.

As many CS cells fired in relation to the local as to the distal stimuli, with some influenced strongly by a single local or distal cue. Groups of CS cells recorded simultaneously were influenced by the same stimulus set. Furthermore, some cells changed their firing pattern during the course of these manipulations, becoming most influenced by fixed, uncontrolled room stimuli. Thus, hippocampal neurons encode all salient features of the environment, including local intra-maze stimuli as well as distal extra-maze stimulus configurations, and many change their coding properties rapidly, ultimately learning to encode the most stable aspects of the environment. Supported by the NIMH and NIA.

375.20

HIPPOCAMPAL PLACE FIELDS IN AGED RATS WITH SPATIAL MEMORY DEFICIT. H. Tanila*, M.Shapiro, H.Eichenbaum. Cntr. Behav. Neuroscience, SUNY at Stony Brook, NY 11794-2575.

As a part of an ongoing study to characterize hippocampal neural coding, place cells in aged rats (26 months) identified as spatially-impaired or intact by water maze test were compared with those of young rats (4-5 months). Previous work has shown that hippocampal cells of aged rats have place fields, suggesting intact spatial coding, but it is not clear whether this firing is determined by relationships between distal spatial cues, or rather is controlled by single distal or local cues as occurs after explicit damage to hippocampal connections. In the present study rats performed a radial-arm maze task for lateral hypothalamic stimulation. Local cues were provided by removable arm inserts distinguished by visual, tactile, and olfactory stimuli. Distal cues were provided by different removable visual patterns on a blank curtain surrounding the maze. Place cells were characterized by response to independent rotation, deletion, and transposition of local and distal cues as described in the accompanying abstract.

In both spatially-impaired and -intact aged rats, most place fields rotated with the distal cues when local and distal cues were rotated independently, and the spatial firing patterns were not disrupted by deletion of single distal cues. Furthermore, as found in young rats, hippocampal cells of aged rats became more dependent on stable cues after repeated manipulations of other cues. Thus, although the present study did not determine whether the rate of such "learning" is slower in hippocampal place cells, it is clear that encoding of spatial relationships is accomplished by the hippocampus even in aged rats that are severely impaired in spatial learning. Supported by NIA.

375.21

NEURONAL ACTIVITY IN THE PARAHIPPOCAMPAL REGION OF RATS PERFORMING A DELAYED NON-MATCHING TO SAMPLE TASK. B.J. Young*, T. Otto, G.D. Fox and H. Eichenbaum. Center for Behavioral Neuroscience, SUNY at Stony Brook, NY 11794.

Neuropsychological and electrophysiological evidence indicates that the parahippocampal region (perirhinal and entorhinal cortex) plays a critical role in recognition memory. The present study sought to further examine the role of the parahippocampal region in recognition memory by characterizing perirhinal and entorhinal neural activity in rats performing an odor-guided continuous delayed nonmatching to sample task that requires parahippocampal function. Analyses focused on the extent to which cells demonstrated odor selective responses during memory cue sampling and at the end of the memory delay just prior to the recognition test.

Our initial analyses of 147 perirhinal and 71 lateral entorhinal cells revealed that more than 90% of the cells recorded from each area fired selectively during specific behavioral events. Greater than 40% of the cells from each area fired maximally around the time the rat sampled odor memory cues, with the activity of 11.6% of perirhinal and 28.1% of entorhinal cells displaying a statistically significant degree of odor-selective activity. Of particular importance, the activity of 8.2% of perirhinal cells was significantly influenced immediately prior to the recognition test by the odor that had to be remembered across the preceding delay. The results of this latter analysis are not yet available for entorhinal cells. These data show that neurons of the parahippocampal region encode to-be-remembered odors and that specific memory codings are maintained or reestablished at the time of a subsequent recognition test. Supported by NIA & NIMH.

LEARNING AND MEMORY: PHYSIOLOGY II

376.1

A SIMULATION OF EPISODIC MEMORY FUNCTION IN THE HIPPOCAMPAL FORMATION.

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Models of hippocampal subregions are combined in a network simulation which can sequentially store highly overlapping patterns representing component features of behavioral episodes, and can recall these patterns given partial cues. In the simulation, Hebbian modification of perforant path synapses and feedforward inhibition in the dentate gyrus and region CA1 establishes sparse self-organized representations of patterns presented sequentially to entorhinal cortex. Dentate gyrus activity passes via the mossy fibers to region CA3, where excitatory recurrent synapses mediate auto-associative memory function with attractor dynamics due to a balance of feedback excitation and inhibition (Hasselmo et al., *J. Neurosci.* in press). Activity then spreads to region CA1 via the Schaffer collaterals, which mediate storage and recall of associations between activity in region CA3 and region CA1 (Hasselmo & Schnell, *J. Neurosci.* 14: 3898-3914). In region CA1, this allows sequential comparison of recall from region CA3 with afferent input from the entorhinal cortex, as measured by the sum of activity in region CA1. Region CA1 output regulates cholinergic modulation from the medial septum to set appropriate dynamics for learning of novel patterns or recall of previously stored memories (Hasselmo, *Behav. Brain Res.* 67: 1-27).

Brain slice experiments tested the cholinergic suppression of synaptic transmission at the backprojections from region CA1 to subiculum and entorhinal cortex. In the model, combining self-organization of perforant path input with associative memory function of backprojections to entorhinal cortex required cholinergic suppression at these synapses. Extracellular synaptic potentials were recorded in the pyramidal cell layer of the subiculum during stimulation of stratum oriens in CA1. Perfusion of the cholinergic agonist carbachol (100μM) suppressed these synaptic potentials by 66.4% ± 3.54 (n=5). This supports the theory that cholinergic modulation allows combined self-organization and associative memory function in the hippocampus.

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376.2

REVERBERATING CIRCUIT IN THE ENTORHINAL-HIPPOCAMPAL SYSTEM REVEALED BY REAL-TIME IMAGING.

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Among the mechanisms proposed to underlie memory formation in the central nervous system, long lasting changes in synaptic efficacy (LTP and LTD) as well as reverberation of activity along a closed loop of excitatory neurons are considered promising candidates. Although the hippocampal formation is believed to be a crucial structure for memory processes, results of recent behavioral and electrophysiological experiments have indicated that the closely associated entorhinal cortex may execute a specific role as well.

The dynamics of neuronal circuits in the entorhinal-hippocampal system were studied in slices with the use of optical imaging with high spatio-temporal resolution. Following a focal electrical stimulation in the superficial layers of the entorhinal cortex, we detected reverberation of neural activities within the entorhinal cortex, which apparently enables this area to hold information for a certain period of time. Reverberation was more prominent and lasted longer, up to 500 msec, when the inhibition due to gamma-aminobutyric acid was slightly suppressed by applying 1 - 5 μM bicuculline. In this situation, we also occasionally observed transfer of entorhinal activity along the various routes of the perforantpathway into the various components of the hippocampal formation. The entorhinal neuronal circuit may thus contribute specifically to memory processes taking place in the entorhinal-hippocampal system by holding information and selectively gating the entry of information into the hippocampus. The latter finding may be in line with previous observations that the transmission of neural activity to the hippocampal formation is frequency dependent (Jones R.S., TINS, 16, 58-64, 1993).

376.3

A COMPUTER CONTROLLED APPARATUS FOR GENERATION OF SLOPE GUIDED PLACE NAVIGATION IN RATS. M. Moghaddam, Yu. Kaminsky, A. Zahalka and J. Bures (SPON: ENA). Institute of Physiology, Academy of Sciences, Prague, Czech Republic.

Cue directed locomotion is usually defined as approach to visible targets, but can also be studied in darkness when the goal emits non-visual signals (odor, sound). A special situation arises when the slope of terrain is used as the orienting gradient the steepness and direction of which is assessed by vestibular system and proprioception. Rats can be trained in darkness to find a feeder placed on the top of a low cone (1m diameter, 4 cm high). A device allowing continuous generation of slope guided locomotion consists of a 1m arena on the floor of which rests a styrofoam disk (1m dia) equipped with 3 equidistant feeders 17 cm from its center. A 1-5 cm long vertical rod raised under one of the feeders opens its cover and tilts the disk (balanced by solenoid operated counterweights) in a plane passing through the feeder, rat's center of gravity and a point at the disk periphery contacting the floor of the arena. The multitude of such planes generated by the rat's locomotion forms the surface of a virtual cone the top of which is represented by the feeder. A computerized infrared tracking system monitors the rat's position in darkness, closes the feeder after the rat has spent 5 s in its vicinity and returns the disk to horizontal position. After a 10 s interval the computer raises the feeder most remote from the position of the rat and the sequence is repeated. Relative path length (ratio of distance travelled to rat-target distance at the time of feeder opening) is inversely related to inclination (0° to 8°) and is a sensitive measure of performance in this type of vestibular navigation. Supporting grants IGA AVCR 711401 and BMFT 01VJ 9300215/26.

376.4

AN INFORMATION SYSTEM FOR NEURONAL PATTERN ANALYSIS
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There has been an explosion of data in the neuroscience community in recent years owing to rapid developments in multi-neuron recording techniques, computer-based data acquisition systems and large scale neuronal modeling studies. This has led to a disproportionate amount of time being spent archiving, cataloging, and searching through datasets —time that could be more productively spent on data analysis and interpretation. In addition, existing software tools are usually tailored to the specific formats of individual laboratories, forcing research groups to reinvent tools and making it difficult to share data between groups. The Information System for Neuronal Pattern Analysis (ISNPA) is being developed by neuroscientists at the Beckman Institute in collaboration with NCSA in order to address these problems.

ISNPA consists of three primary interrelated components: **1)** The *database component*, a SYBASE database engine running on a Sun Sparc 10 server with 300 Gb of online storage capacity. Users interact with the database engine by means of an extensive and individually customizable graphical user interface that allows the user to search hierarchically-organized descriptive information (metadata) about the experimental datasets. **2)** The *Time Series Data Protocol (TSDP)*, which allows neuronal time series data to be stored, manipulated and transported via the internet from one computer platform to another, independent of the format of the source data. **3)** A suite of *analysis and visualization tools* based on TSDP that provide a flexible, intuitive and efficient means of processing and interacting with large amounts of data. Further information available at http://soma.npa.uiuc.edu/isnpa.html
(Supported by NSF BIR 91-16763)

376.5

MODELS OF DENDRITIC SPINES CONTAINING ACTIVE CONDUCTANCES DISPLAY COMPLEX TEMPORAL PROCESSING CAPABILITIES. BE Peterson, EA Gale, RV Jensen and GM Shepherd* Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510

Models of dendrites with spines containing active conductances were previously used to demonstrate how these conductances can enable an arbor to perform logical functions on coincident inputs (Shepherd and Brayton, 1987). We have used similar models to investigate how such systems behave over a range of spatiotemporal input patterns. Recent work verifying the existence of active conductances in dendrites has provided evidence for the possibility of such mechanisms (Stuart and Sakmann, 1994; Magee and Johnston, 1995; Spruston et al., 1995). Theoretical work along these lines has postulated that these active conductances can have important functional roles in the way information is processed in the dendritic arbors of neurons (Softky, 1994). In particular, these properties can limit integration time and can therefore force an arbor to act as a local coincidence detector of its synaptic inputs. Our results indicate that within the model there are complex interactions between arbor and spine morphology, channel kinetics, synaptic strengths, and effective output of the system. Changing the synaptic conductance by 27% in a typical model resulted in a change of integration time from 0.4ms to 2ms. We have also found that these properties can effectively insert delays between inputs from spatially distributed spines without increasing the integration window. This mechanism could be employed by neurons to process temporally complex signals in a more sophisticated manner than simple coincidence detection. Supported by NIDCD, NIMH & NASA (Human Brain Project).

376.6

GEOMETRIC DETERMINANTS OF HIPPOCAMPAL PLACE FIELDS.
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Why do place cells fire where they fire? We examined the firing fields of 16 well-isolated place cells from 3 rats while they searched for food in quadrangular boxes formed by identical walls arranged in 4 configurations: a large square (122x 122cm), a small square (61x61cm), and 2 rectangles (61x122cm) whose long side was either parallel to the long axis of the (rectangular) testing room, or perpendicular to it. 14 cells had a single field in 2 or more of the configurations, and 2 cells had two fields in 2 or more of the configurations. No cells had fields exclusively in the large and small squares, or exclusively in the two rectangles. Fields were quantified by calculating the centroid and standard deviation of the spatial firing rates in the 2 orthogonal directions defined by the axes of the boxes. The component of the centroid in each direction was measured in 3 ways: as a distance relative to a testing room wall (M1), as a distance relative to a box wall (M2), and as a proportion of the distance between two opposite box walls (M3). The measurement with the minimum variance across configurations was taken as the major determinant of the component of the field location in that direction. The most common combinations of measurements determining field location were M2 in both orthogonal directions (4 fields), and M2 in one direction with M3 in the other (7 fields). 5 fields had M1 as the determinant in one direction combined with M2 or M3 in the other. We assume that these cells were influenced by elongated features outside of the box, e.g. the testing room walls. The fields of neighbouring neurons may have differing determinants. The results show that a place field's location is the result of at least two vector computations involving the distance in an allocentric direction to an extended cue.

376.7

SOLUTIONS OF THE SHORTEST PATH PROBLEM FROM NETWORKS OF DIRECTIONALLY SELECTIVE PLACE CELLS. M. Stead and R.U.Muller*. Department of Physiology, SUNY-Brooklyn, Brooklyn, NY 11203.

In previous work, we showed that recurrent (CA3-like) networks of place cells connected by Hebbian synapses can store enough information to solve spatial problems. The key idea is that synaptic resistance (inverse synaptic strength) is proposed to monotonically increase with of the distance between the firing fields of a pair of place cells. A cell pair with overlapping fields should fire together in time, so synaptic resistance should be low. In contrast, a cell pair with separated fields will not fire together, and synaptic resistance should stay high. When synaptic resistances are set in this way, shortest paths can be extracted from the network with standard graph theoretic algorithms.

The stated synaptic modification scheme assumes that place cells are omnidirectional, as is true in open areas surrounded by walls (Muller et al, J.Neurosci. 14; 7235-7251, 1994). Muller et al also showed that place cells show directional selectivity on an 8-arm maze. It is therefore important to test if optimal paths can be found when discharge depends on head direction as well as position.

In a first order scheme, synaptic resistance was set according to the distance between the fields of the cell pair multiplied by the similarity in the directional tuning of the two cells. When resistance is set in this way, straight-line paths between arbitrary pairs of points can still be calculated; the representational method is robust despite a fundamental change in the spatial firing properties of the underlying cells. The "behavior" along the path is, however, peculiar: the fake rat need not point in the direction in which it moves. In a more sophisticated method, resistance is lowered only if the two cells are tuned to similar directions AND the direction from the field of the presynaptic cell to the field of the postsynaptic cell is similar to the tuning direction. This method, which better mimics the behavior of real rats, yields more realistic paths.

376.8

THE FIRING OF HIPPOCAMPAL PLACE CELLS IS EITHER NOISY OR SIGNALS MORE THAN JUST POSITION. A.A. Fenton*, J.L. Kubie, R.U. Muller. SUNY Brooklyn, NY, 11203.

Current ideas of place cell (PC) properties are based mainly on time-averaged positional firing distributions, which are very smooth for long recordings. We find, however, that PCs may fail to fire as the rat crosses the firing field through its center, suggesting that firing in time is more variable than expected from averages. This impression was tested by comparing the observed spike time series to one generated by a Poisson model. The probability of a spike in each 1/60 sec interval was calculated from the average firing rate in the pixel. The match of observed to predicted spike times was good on a scale of tens of seconds but poor on a scale of 1-3 seconds, about what it takes a rat to cross the field; spikes were more clustered than predicted from the average rate.

To formally demonstrate excess firing variance, we did a Monte Carlo analysis (100000 repeats) of the number of spikes expected along a path, given the time-averaged rate in each pixel. (A path is a time series of positions.) Only paths through the field center were studied. A t-value was got for each path by seeing where the observed number of spikes fit into the Monte Carlo distribution. Such t-values were much larger (positive or negative) than t-values got from seeing where a single additional Monte Carlo run fit in the Monte Carlo distribution. An F-test of the 2 t-value distributions revealed that the variance of the observed was much greater than of the expected. The excess variance could not be explained by the tendency of PCs to fire complex spikes, by drift in average firing rate or by details of the paths. Interestingly, when firing on single field paths was compared for simultaneously recorded PC pairs with overlapping firing fields, the t-values from the two cells were uncorrelated. Thus, either the positional signal of PCs is very noisy and not modulated by any aspect of the general state of the rat, or individual PCs carry a signal in addition to position.

376.9

PERFORMANCE DIFFERENCES IN THE P AND NP SELECTED LINES OF RATS IN PLACE LEARNING IN THE MORRIS MAZE. C. R. Goodlett[1] and S. L. Morzorati*[2], [1]IUPUI and [2]Indiana Univ. School of Medicine, Indianapolis, IN 46202.

The P (alcohol-preferring) and NP (alcohol-nonpreferring) rats, selectively bred for differences in voluntary alcohol consumption, also differ in the generation of hippocampal theta rhythm. NP rats generate a consistently higher peak theta frequency than P rats during REM sleep, still-alert waking, and ambulation (Morzorati *et al., Alcohol*, 1994; Breen *et al., Alcoholism: Clin. Exp. Res.*, 1993). Frequency-specific theta activity is correlated with exploration and learning in rats, and theta sets the optimal conditions for induction of long-term potentiation in the hippocampus. Because the theta frequency generated by NP rats is higher than is typical found in outbred rat stocks, the present study compared P and NP lines on acquisition of spatial learning in the Morris water maze, a task known to depend on hippocampal function.

Adult, male P (n=10) and NP (n=9) rats were trained on the following sequence in the water maze: cue-guided learning using a visible platform (2 days); simple place learning using a submerged platform in a designated location (7 days, plus probe trials); and, place learning set with the submerged platform in a different location each day (5 days). For cue learning (conducted in a separate room), latency to escape was the dependent measure. Data for the place learning trials were acquired using a computerized video tracking system which collected escape latency, path length, swimming speed, perimeter time, and time in each quadrant (on probe trials).

No differences were found on the visible platform task, and both lines had escape latencies under 10 sec by the second day. Throughout the *place* training, NP rats swam slower than P rats, leading to a dissociation of escape latencies and path lengths. The NP rats had significantly longer escape latencies (but not path lengths) during acquisition of the simple place learning and, more prominently, during the first 2 days of learning set training. The markedly longer escape latencies when novel spatial contingencies were instituted, and the slower swimming during place but not cue-guided learning, suggests the NP rats adopt slower search behavior in the Morris maze to compensate for altered spatial processing. (Supported by AA09596 and AA07611).

376.10

ALTERED INHIBITION OF DENTATE GRANULE CELLS DURING SPATIAL LEARNING IN AN EXPLORATION TASK. E. I. Moser*, Centre for Neuroscience, University of Edinburgh, Edinburgh EH8 9LE, U.K., and Dept. of Neurophysiology, University of Oslo, Norway.

Spatial learning may require precise control of the impulse flow through the hippocampal formation. The inhibitory interneurons in the dentate gyrus may be important in this respect. To study whether inhibition on the dendrites and somata of dentate granule cells is altered during spatial learning in an exploration task, field potentials were recorded in the dentate gyrus in response to paired stimulation of the perforant path while rats explored or rested (20 ms interstimulus interval, wide range of intensities). A population spike in the first potential was consistently associated with a suppression of the field EPSP as well as the population spike of the second waveform. The suppression is likely to reflect recurrent inhibition on the dendrites and the somata of the granule cells, respectively.

During exploration, the suppression of the EPSP was enhanced relative to reference potentials sampled at the same brain temperature while the rat was resting in its home cage. The difference did not disappear when the potentials were matched with respect to the EPSP slope or the spike amplitude of the first potential. Below the spike threshold, no change in the paired-pulse ratio was observed. The suppression was largest at the start of the exploration, after which it decayed gradually. It was still present at 15 min. On the other hand, the suppression of the population spike seemed to be relaxed during the exploration. The population spike of the second waveform was significantly enhanced relative to that of temperature-matched reference potentials. This enhancement showed no decay during the session. The results suggest that exploration of an unfamiliar environment may be accompanied by a dual change in recurrent inhibition on the granule cells: Interneurons projecting to the molecular layer may be more excitable, whereas interneurons contacting the somatic region may exert less inhibition.

376.11

THE RESPONSE OF HEAD DIRECTION CELLS TO NON-VISUAL CUES. J.P. Goodridge*, K.A. Worboys, P. Dudchenko and J.S. Taube. Department of Psychology, Dartmouth College, Hanover, NH 03755.

Previous research has shown that head direction (HD) cells in both the anterior thalamic nuclei and the postsubiculum discharge in relation to prominent visual landmarks in the environment. In particular, when a familiar visual cue in the environment is rotated, the HD cell's preferred firing direction shifts by a corresponding amount. This study assesses whether HD cells are similarly responsive to non-visual cues in the environment.

Five female, Long-Evans rats were trained to retrieve food pellets thrown randomly into a gray, cylindrical chamber in which an auditory click emanating from one side of the cylinder served as the only prominent orientation cue. Following training, a recording electrode array was implanted into either the anterior thalamic nuclei or the postsubiculum. Eight HD cells were identified and recorded in a control session with the room lights off. Then, with the animals removed from the cylinder, the auditory click was rotated 90° and the animals were returned to the cylinder. All HD cells failed to shift in accordance with the click rotation. In addition, four HD cells, each in a different animal, were tested for their response to rotation of a large white card which was taped to the inside cylinder wall and which the animal had never seen before. The preferred firing direction of these cells all shifted in accordance with the card rotation. Thus, despite the fact that the animal was given extensive experience with the auditory click, it was not able to exert stimulus control over the preferred firing direction of these cells in the same manner as the cue card.

The response of three HD cells to non-visual cues was also assessed in three different blindfolded rats. Even after the animal was blindfolded, the preferred firing direction of two of the three cells remained stable while the third cell stabilized after 20 minutes. In addition, when the floor paper (on which urine spots could be identified) and the cylinder (from which the cue card had been removed) were rotated together, the preferred firing direction of two of the three cells shifted by a corresponding amount. These findings suggest that HD neurons are responsive to olfactory information as well as visual information.

376.12

THE EFFECTS OF LESIONS OF THE POSTSUBICULUM ON HIPPOCAMPAL PLACE CELL ACTIVITY. P. Dudchenko*, J.P. Goodridge, and J.S. Taube. Department of Psychology, Dartmouth College, Hanover, NH 03755.

The presence of cells in the postsubiculum (PoS) and other regions of the brain which code for an animal's allocentric head direction in the horizontal plane has been well established. The PoS projects directly to layers of entorhinal cortex which in turn project to the hippocampus. Recent work in our lab, described in a separate abstract (Golob and Taube), has shown that head direction cell activity in the PoS is generally not disrupted by lesions of the hippocampus. The current study examined the converse relationship: Are place cells in the hippocampus affected by lesions of the PoS?

To assess this question, bilateral ibotenic acid lesions were made in the PoS of Long-Evans female rats and moveable recording electrodes were implanted to assess neuronal activity in the CA1 hippocampal region. Upon recovery, animals were screened daily for place cells in a cylindrical apparatus containing a white cue card. Our preliminary results suggest that place cells can be identified in the hippocampus following destruction of the PoS, although these cells appear to possess higher rates of background firing. In addition, the firing rate maps of these place cells are less robust in terms of spatial coherence and information content when compared to values reported from place cells in the dorsal hippocampus of normal animals (Poucet et al., 1994). If these preliminary findings are borne out in subsequent animals, it may be hypothesized that head direction cells in the PoS provide an input which is necessary for the optimal firing of hippocampal place cells.

376.13

HEAD DIRECTION CELLS RECORDED FROM RATS WITH HIPPOCAMPAL LESIONS. E.J. Golob* and J.S. Taube. Department of Psychology, Dartmouth College, Hanover, NH 03755.

Neurons have been identified in the postsubiculum (PoS) and anterior thalamic nuclei (ATN) which discharge as a function of the animal's allocentric head direction in the horizontal plane (HD cells). Previous studies have also shown the presence of location-specific discharge in hippocampal cells. This study was designed to determine how HD cell activity would be affected by lesions of the hippocampus.

Six female, Long-Evans rats were trained to forage for food pellets dropped randomly into a gray cylindrical chamber containing a prominent white cue card. After training, bilateral ibotenic acid lesions were made in the hippocampus and an electrode recording array was implanted in the PoS (n=4) or the ATN (n=2). Following recovery animals were screened for cells daily within the cylinder. Eleven HD cells were identified in the lesioned animals, with at least one cell per animal. Previous experiments have shown that when an animal is introduced to a novel enclosure having a different shape, such as a rectangle, the HD cell's preferred firing direction (PFD) will often shift relative to the PFD within the cylinder. We tested whether lesioned animals, when presented with a novel enclosure, were able to maintain this new PFD across repeated sessions over several days. The variation in PFD for 7 HD cells did not appear to differ from control animals. The absolute difference between the PFD on the first and second days were 14.6 ± 3.34° for 7 cells in the cylinder and 11.6 ± 4.93° for 5 cells recorded in a novel enclosure. However, preliminary results show that the PFD of lesioned animals may be less stable as the animal walks from a familiar to an unfamiliar environment compared with control animals. Taken together, these findings indicate that the hippocampus may not be necessary for establishing and maintaining stable HD cell firing when landmarks are present, but may be important for processing path integration information.

376.14

INFLUENCE OF VESTIBULAR SYSTEM LESIONS UPON ANTERIOR THALAMIC HEAD-DIRECTION CELL ACTIVITY. R.W. Stackman* and J. S. Taube. Department of Psychology, Dartmouth College, Hanover, NH 03755.

Single-unit analyses have identified a subset of neurons, in the rat anterior thalamic nucleus (ATN) (Taube, 1995), that discharge as a function of the rat's head direction (HD) in the horizontal plane. HD cells may be an integral component of a neural network that supports spatial navigation. It has been hypothesized that vestibular information may be critical for acquiring an internal representation of space and for maintaining that representation in the absence of a familiar landmark. Further, vestibular lesions disrupt spatial navigation under conditions in which landmarks are not present. Given the potential contribution of the vestibular system to HD cells, we investigated the influence of vestibular system lesions upon the activity of HD cells.

Female Long-Evans rats were trained to retrieve food pellets thrown randomly into a cylindrical apparatus that included a white cue card that served as an orientation cue. Following acquisition of this task, rats were implanted with chronic recording electrodes directed at the ATN. Rats also received bilateral intratympanic injections of either the vestibular system toxin, sodium arsanilate (30 mg/0.1 ml/side) or 0.9% saline. Following recovery, rats were tested each day for vestibular function using a contact righting test, and then cellular activity was monitored in the cylinder to determine directional, and other spatial or behavioral correlates. Vestibular-lesioned rats exhibited persistent deficits of contact righting. Although HD cells were identified in control-treated rats, no HD cells were isolated in vestibular-lesioned rats. Cells from vestibular-lesioned rats exhibited higher background firing rates and appeared to fire in bursts, although these bursts did not correlate with the animal's HD. These data appear to suggest an important contribution of the vestibular system in the maintenance of the directional firing properties of ATN neurons.

376.15

HEAD DIRECTION CELL ACTIVITY IN THE ANTERIOR THALAMIC NUCLEI, BUT NOT THE POSTSUBICULUM, PREDICTS THE ANIMAL'S FUTURE DIRECTIONAL HEADING. J.S. Taube*[1] and R.U. Muller[2]. [1]Dept. of Psychology, Dartmouth College, Hanover, NH 03755 and [2]Dept. of Physiology, SUNY Health Science Center, Brooklyn, NY 11203.

Previous studies have identified two distinct populations of neurons which discharge as a function of the animal's head direction (HD) in the horizontal plane. One population is in the postsubiculum (PoS); the second is in the anterior thalamic nuclei (ATN). These two brain areas are reciprocally connected with one another, making it hard to determine which area is primarily responsible for organizing HD cell discharge. To help understand the origin of the HD signal, we performed a time shift analysis where the spike series was shifted forwards and backwards in time with respect to the animal's head direction in 1/60th sec intervals. A sample of 20 PoS and 17 ATN cells were selected from 8 min recording sessions during which Long-evans rats performed a food foraging task in a cylindrical apparatus containing a single visual polarizing cue. For each cell, firing rate vs. head direction plots were computed for each shift of the spike series and two parameters were measured from each plot: peak firing rate and half-width of the directional firing range (see Taube et al., 1990). It is our argument that determining the time shift which produces the optimal value for each parameter lets us determine which brain structure "leads" HD cell firing. We found that the optimal time shifts for peak firing rate and half-width were 40.00 ± 4.23 and 34.45 ± 5.50 msec for ATN cells, respectively. In contrast, PoS HD cells had optimum values of -0.83 ± 9.72 and -1.67 ± 9.43 msec, respectively. These results are consistent with preliminary experiments showing that lesions of the ATN disrupt HD cell discharge in the PoS, while lesions of the PoS do not interfere with HD cell discharge in the ATN. Taken together, these findings suggest that the HD cell signal, which was first identified in the PoS, actually originates in the ATN, and predicts the animal's future directional heading by about 37 msec.

376.16

THE POSITIONAL FIRING OF PLACE CELLS IS CONTROLLED MORE STRONGLY BY CARDS ON THE WALL THAN BY 3-DIMENSIONAL OBJECTS INSIDE THE ARENA. B. Poucet*, A. Cressant & R.U. Muller. Lab. Cognitive Neuroscience, CNRS, Marseille, 13402 France, and Dept. Physiology, SUNY, Brooklyn, NY.

Place cells recorded from the dorsal hippocampus of freely-moving rats fire the most intensely in a region of space called the firing field. Previous work has shown that a single white cue card attached to the wall of a recording cylinder controls the angular position of firing fields; when the card is rotated, fields rotate equally. In the present study, we asked whether similar control could be exerted by 3-dimensional objects placed directly in the recording arena. Single cell recordings were made while hungry rats chased pellets in a gray cylinder with 2 or 3 different, heavy objects on the floor; the locations of the objects relative to each other and their distances from the cylinder wall were fixed. Recordings were made under 2 conditions, first with the objects in a "standard" position relative to the laboratory frame and second with the objects rotated as a rigid set around the center of the cylinder. In contrast to the stability of firing field positions across sessions when the white card was in a standard position, firing fields often rotated and changed in other ways across sessions when the objects were in the standard position. Moreover, when the objects were rotated between sessions, the new angular position of the firing field was unpredictable, in contrast to the reliable control over angular position exerted by the card. Finally, when both the card and the objects were simultaneously present and rotated equally, the ensemble of stimuli exerted nearly ideal control over angular firing position. Nevertheless, when the card was withdrawn, the objects were still ineffective in controlling field position. These results suggest that the rat hippocampus is not sufficiently powerful to do the spatial processing needed to use as landmarks objects that can be viewed from all angles.

376.17

VISUAL AND VESTIBULAR INFLUENCES ON HEAD-DIRECTION CELLS IN THE ANTERIOR THALAMUS OF THE RAT. P.E. Sharp* & H.T. Blair. Dept. of Psychology, Yale University, New Haven, CT 06520-8205

Head-direction cells were recorded from the anterodorsal thalamic nucleus as rats navigated freely in a cylindrical recording chamber. The wall and floor of the cylinder could be rotated independently of one another, to deliver different combinations of visual and vestibular movement information. The wall was painted with an alternating pattern of eight vertical black and white stripes, so that any 90° rotation of the wall and/or floor left the environment visually unchanged.

We measured the responses of head-direction cells to several different rotations of the apparatus, including: 1. Wall Alone 90°. As the stripes on the wall moved around the animal, a change of head direction was signalled by visual motion cues, but not by vestibular cues. 2. Wall+Floor 90° (fast). The entire apparatus was rotated, and the animal along with it. Vestibular signals indicated a change of head direction, but visual motion cues did not, since the wall rotated with the animal. 3. Floor Alone 90°. The animal moved along with the floor, but the wall was stationary as the animal moved. Both visual and vestibular cues indicated a change of head direction. 4. Wall+Floor 90° (slow). As in #2, the entire apparatus was rotated, but the rotation was below vestibular threshold. Thus, a change of head direction actually occurred, but was signalled by neither visual nor vestibular cues.

In manipulations #1 and #2, visual and vestibular cues provided contradictory information, and cells variably rotated their preferred direction in agreement with visual cues, vestibular cues, or a partial combination of both. In manipulations #3 and #4, visual and vestibular cues provided complementary information, and cells always responded in the same way. In manipulation #3, cells never rotated their preferred firing direction, and in manipulation #4, cells always rotated their preferred firing direction along with the apparatus. These results indicate that visual and vestibular signals cooperatively influence the rat's head-direction system.

376.18

NONLINEAR DYNAMICAL MODEL OF HIPPOCAMPAL FIELD CA3 WITH REVERBERATING SHORT-TERM MEMORY. S. P. Wiebe*[1], M. Taketani[2], J. Ambros-Ingerson[3], G. Lynch[3], and R. Granger[3]. [1]Center for Neural Science, New York University, New York, NY 10003. [2] RCE2, Central Research Laboratories, Matsushita Electric Industrial Co., Ltd., 3-4 Hikari-dai, Seika-cho, Soraku-gun, Kyoto 619-02 Japan. [3]ICS Department and Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717.

The hippocampal formation can be viewed as a roughly serial chain of structures, each performing a distinct information processing operation on the input which is passed through this "assembly line." Centrally located in this pathway, the unusually dense recurrent associational system of CA3 suggests that its operation may be one of time dilation (i.e. sustained input-specific dynamical activity). Incorporation of hippocampal field CA3's dense local connectivity along with its mossy fiber, septal, and perforant path afferents and the differential time courses of excitatory and inhibitory postsynaptic potentials into a mathematical model gave rise to a system of neuronal ensembles or "patches" which exhibit oscillatory activity in response to brief excitatory input. Simulations reveal the persistence of a patch's oscillatory response to transient (~milliseconds) afferent stimulation to be dependent on (i) the strength of the converging information-bearing signal and (ii) the time of its arrival relative to the phase of ongoing periodic septal and mossy fiber input, the optimal frequency for maintaining the oscillatory activity being that of theta rhythm. Thus in the present model, the pattern of actively oscillating CA3 patches, which also cycle at theta rhythm, reflects the specific spatial and temporal characteristics of the afferent signal and extends information about the input across the duration of the recurrent activity (~seconds) The hypothesis is forwarded that this unusual form of transient memory may render CA3 capable of holding fleeting neuronal traces encoding sensory cues over the seconds-long time courses of behavioral actions via input-specific standing periodic responses.

376.19

DIFFERENTIAL EFFECTS OF SPATIAL REFERENCE AND WORKING MEMORY TESTING ON SYNAPTIC EFFICACY IN THE LATERAL SEPTUM OF MICE. R. Garcia, R.M. Vouimba and R. Jaffard*. Lab. Neurosciences Comportementales & Cognitives, CNRS URA 339, Univ. Bordeaux I, Ave. des Facultés, 33405 Talence, France.

The efficacy of synaptic transmission from the fimbria to the lateral septum of freely moving C57 BL6 mice was monitored electrophysiologically over 9 days of training in either a spatial discrimination task (Reference memory: RM) or a delayed nonmatching-to-place task (Working memory: WM) in an 8-arm radial maze. The learning specificity of the observed changes was assessed using a control group exposed to muscular effort (treadmill). Results showed that RM testing induced a progressive and persistent increase in the amplitude of the N3 averaged evoked potentials in the LS, the magnitude of which was significantly greater in fast learners than in slow learners. Conversely, WM testing induced an inverse pattern of effect. Specifically, each daily training session induced a slight but significant decrease in the amplitude of N3 with respect to the control group. Moreover, a significant correlation was observed between the magnitude of this decrease and response accuracy. The observed bidirectional effect of RM and WM testing on synaptic transmission in the LS were also observed using a task in which, on each trial, animals were forced to visit, one at a time, each arm of the radial maze. In this task animals were divided into two groups. In the first group, the probability of finding food in a given arm was either 1 (the four constantly baited arms) or 0 (the four never baited arms); in the second group, the probability of finding food in each of the 8 arm was 0.5. Preliminary results indicates that each type of testing induced opposite changes in the amplitude of N3: the first (i.e.,"invariant") group displayed a persitent increase in N3, whereas the second (i.e., "variant") group displayed an opposite pattern of change (i.e., a depression). These changes in testing-induced hippocampo-septal septal glutamatergic synaptic transmission might play a role in the previously observed task-specific (WM vs RM) testing-induced alterations of hippocampal cholinergic activity (Marighetto et al., Behav. Brain Res., 1993, 56: 133-144; Pharmacol. Biochem. Behav., 1994, 49: 689-699).

376.20

SPATIAL REPRESENTATION IN MONKEY SEPTAL NUCLEI. H.Nishijo*, T.Kita, T.Ono, K.Nakamura and K.Terasawa. Dept. Physiol., Fac. Med., Toyama Medical & Pharmaceutical University, Sugitani, Toyama 930-01, Japan.

It has been reported that septal lesion resulted in deficits of various types in place navigation tasks. In the present study, neuronal activity in the monkey septal nuclei was recorded during performance of a place-dependent Go-NoGo (PGN) task in which reward contingencies were variable with reference to the spatial location of a monkey's cab in one of four places in an experimental room. The task was initiated by a sight presentation of a place (place phase) followed by presentation of an object (object phase). A bar-press was reinforced only if each object was seen in one corresponding place (Go-trial), and the same object was never reinforced in other places (NoGo-trial). Of 349 septal neurons recorded, 42 responded differentially to location during the place phase (place-differential neurons), and 72 responded differentially to Go/NoGo responses during the object phase. The multidimensional analysis of place-phase responses of these 42 place-differential neurons indicated that the four places distributed in a two-dimensional space at relative positions were similar to those in the real experimental room. Furthermore, the distance between possible two places in the two-dimensional space significantly correlated to behavioral performance defined as a % of correct trials during discrimination of the given pair of places. These results suggest that functional subpopulation of monkey septal neurons could encode some behavioral aspects of a place.

376.21

* ELECTRICAL STIMULATION OF THE SEPTAL REGION OF AGED RATS IMPROVES PERFORMANCE IN AN OPEN FIELD MAZE. F. Jiang, J. Turnbull* and R. Racine. Dept.of Medicine and Psychology, McMaster Univ. Hamilton, Ontario, Canada L8N 3Z5.

* Memory deficits of age and disease may result from dysfunction of the temporal lobes and functionally associated structures. We have proposed that iatrogenic rhythmic electrical stimulation of the deep septal nuclei, which project to the hippocampal formations of the temporal lobes, might moderate these memory deficits. We show here that this is the case. Septal stimulation of very old rats leads to a marked improvement in performance in an open field maze task. Unilateral stimulation of the projection from the entorhinal cortex to the hippocampus was less effective. The frequency of stimulation was important: stimulation at 5 Hz and 50 Hz was effective, while 0.5 Hz was less effective (though still significantly beter than control). Hippocampal (dentate) EEG did not change significantly with septal stimulation frequency. These results may bear on the memory deficit of old age in humans. The results may also bear on the memory deficits seen in human disease states such as Alzheimer's disease, though this cannot be readily verified in the absence of a suitable animal model.

LEARNING AND MEMORY: PHARMACOLOGY IV

377.1

PHARMACOLOGICAL MANIPULATION OF HIPPOCAMPAL PLACE CELL FIRING WITH MICRODIALYSIS IN THE RECORDING SITE. N. Ludvig*, B. T. Altura, B.M. Altura and S.E. Fox. Dept. of Physiology, State University of New York, Brooklyn, NY 11203.

This study was devoted to exploring whether the microdialysis-coupled unit recording method (Ludvig et al., *J. Neurosci. Meth.* [1994] 55:31-40) can be used for studying hippocampal place cells, and whether the firing pattern of these cells can be changed by perfusing drug solutions in the recording site.

Complex-spike cells (pyramidal cells) displaying the characteristic location-specific firing of place cells could be readily detected in behaving rats during microdialysis with artificial cerebrospinal fluid (ACSF) in the microenvironment of the neurons. This provided evidence that the microdialysis procedure, although it causes unavoidable damage in the hippocampal circuitry, leaves a substantial number of neurons in the dialysis area functionally intact.

The effects of exposing identified place cells to 10 min perfusions of ACSF with high concentration (50 mM) of K^+ were also examined. These recording/dialysis sessions were preceded by *in vitro* tests to determine the time when the high concentration of K^+-solution reaches the tip of the microdialysis probe, and to measure the amount of K^+ that diffused out of the probe. For these tests, ion selective electrodes were utilized. The generated data enabled us to know exactly the time-frame and magnitude of the K^+-exposures. We have found that such exposures mostly increase the firing rate of the recorded neurons, and often activate "silent" pyramidal cells within the recording/dialysis site. Place cells appeared to fire more intensely out of their firing field than before and after the K^+-challenge. Importantly, the overall behavior of the rats were not affected by these procedures, and in each recording/dialysis session place cells could be studied for as long as 2 days.

The described method may provide a way to examine some aspects of the synaptic and molecular mechanisms that regulate place cell firing.

377.2

EFFECTS OF T-588, A NOVEL COGNITIVE ENHANCER, ON ACETYLCHOLINE AND MONOAMINE RELEASES AND SECOND-MESSENGER SYSTEMS IN RATS. S. Ono*, M. Maekawa, T. Kimura, A. Takagi, A. Nakamura, K. Kondo, H. Miyazaki, K. Mozumi, N. Matsui and H. Narita. Research Laboratories, Toyama Chemical Co. Ltd., Toyama 930, Japan.

Previous studies demonstrated that T-588 (3 mg/kg, po) ameliorated the memory and learning impairment in embolized and ischemic rats to the performance level of sham-operated rats by daily administration at the chronic phase. We have also shown that T-588 (0.1 mg/kg, iv) improved the scopolamine-induced EEG changes in rabbits. To examine the mechanism for T-588, we investigated the effects of T-588 on acetylcholine (ACh) and monoamines releases and on phosphoinositide (PI) hydrolysis and cyclic AMP formation in rat brains. Oral administration of T-588 (3 and 10 mg/kg) caused a significant increase of microdialysate ACh and noradrenaline (NA) releases from the frontal cortex and hippocampus. T-588 produced a concentration-dependent increase in the spontaneous efflux of [^{3}H] dopamine, [^{3}H]NA and [^{3}H]serotonin in the cortical, hippocampal and striatal slices. These effects were still fully detected when slices were incubated with a calcium-free medium or tetrodotoxin. T-588 (1-10 mM) stimulated PI hydrolysis and cAMP formation in a concentration-dependent manner in the cortical and hippocampal slices. 2-Amino-3-phosphonopropanoate or N-ethylmaleimide blocked T-588's actions, while atropine, prazosin, propranolol, ketanserin or SCH23390 did not. T-588 (3 and 10 μM) enhanced isoproterenol-stimulated cAMP formation without altering the cAMP level by itself. These results suggest that T-588 enhances the neuronal transmitter system not only by increasing ACh and NA releases, but also by facilitating PI hydrolysis and cAMP formation.

377.3

THE EFFECTS OF NIMODIPINE ON THE ACQUISITION AND RETENTION OF A REFERENCE MEMORY TASK. K.A. Kane[1] and G.B. Robinson*[2]. [1]Dept. of Psychology, University of New Brunswick, Fredericton, NB, E3B 5A3 and [2]Dept. of Surgery, Louisiana State University Medical Centre, Shreveport, LA, 71130-3932.

Manipulation of neuronal calcium has been found to affect learning and memory systems. The calcium channel antagonist, nimodipine, for example, improves working memory in both young and aged animals (LeVere & Walker, Neurobiol. Aging, 13:63, 1991; Levy et al., Pharm. Biochem. & Behav., 39:781, 1991). There is, however, some question as to whether or not nimodipine administration also improves reference memory (e.g., McMonagle-Strucko & Fanelli, Pharm., Biochem. & Behav., 44:827, 1993). In the present study, we investigated the effects of nimodipine administration on the performance of young rats on a circular platform reference memory task. The effects of nimodipine on both task acquisition and task retention were examined.

Male Long-Evans hooded rats (3-4 months old) were subcutaneously implanted with either nimodipine (n=14) or placebo (n=14) sustained-release pellets. Nimodipine pellets contained 30 mg of drug, slowly released over approximately 30 days. Animals received 2 trials/day on the maze until acquisition criterion (3 or fewer errors/trial over 5 consecutive trials) was met. Fifteen days after reaching the acquisition criterion, retention was tested in a subset of the rats administered nimodipine (n=6) and placebo (n=7). Retention criterion was 3 or fewer trials over 3 consecutive trials. Rats administered nimodipine required an average of 9.5 ±0.2 acquisition trials to reach criterion compared to 13.5±0.1 trials for controls. This difference was significant ($p \leq .01$). Nimodipine administration also decreased the number of trials required to meet the retention criterion from 5.0 ±0.2 for controls to 3.0±0.0. This difference was also significant ($p \leq .05$). These results suggest that nimodipine administration facilitates reference memory acquisition and retention in young rats. Supported by NSERC.

377.4

A COMPARISON OF THREE CLASSES OF CALCIUM CHANNEL ANTAGONISTS ON MEMORY IN MICE Victoria Garcia deSoria, Kevin Phelan* & David Quartermain Lab.Behavioral Neurology, NYU Medical Center and Pfizer Inc.New York, N.Y.

Calcium channel antagonists (CCA's) of the dihydropyridine class such as Nimodipine Amlodipine and Nifedipine can facilitate learning and memory but little information is available on the other types of CCA's.The purpose of the present study was to compare the effects on memory of drugs representing the three main classes of CCA's.The following drugs were used;Amlodipine,Nimodipine Nifedipine (Dihydropyridines) Verapamil (phenlalkalkyamines and Diltiazem (Benzothiazepines) 1.Different doses of the drugs were injected immediately after training in a passive avoidance task. Results showed that all agents improved retention in a dose dependent manner with Verapamil producing the weakest effects.2.The most effective doses of each drug were injected immediately after training (5 trials) in an a spatial discrimination task.Results indicated that all drugs enhanced retention with the dihydropyridines showing the strongest effects.

377.5

A COMPARISON OF THE EFFECT OF CALCIUM CHANNEL ANTAGONISTS ON SHORT AND LONG TERM MEMORY. David Quartermain* & Victoria Garcia deSoria. Lab. of Behavioral Neurology,NYU Med. Cntr.New York, N.Y.

Short and long term memory was studied using a spontaneous alternation task in a T maze. Mice were given either 1 or 4 forced choices to one arm followed by a single free choice trial at intervals ranging from 10 min to 5 d.after training.Mice given 1 trial exhibited STM (ie chose the opposite arm) at all iontervals out to 20 min. but not beyond.Animals given 4 trials showed good memory (80% to 90% correct choices) at all intervals to 24 h.By 3 d. performance had declined to 70% and became random 5 d. post training.Various Ca channel antagonists were administered before the training trial.Results showed that amlodipine, nimodipine,diltiazem verapamil & flunarazine improved STM with amlodipine showing the strongest enhancement effects.Only amlodipine and nimodipine significantly improved LTM.

377.6

EFFECT OF CHRONIC ADMINISTRATION OF THE CALCIUM CHANNEL BLOCKER AMLODIPINE ON LEARNING AND RETENTION IN MICE. Michael Reilly, Victoria Garcia deSoria,Anne Shemer* & David Quartermain Pfizer Inc. & Lab. of Behavioral Neurology NYU School of Medicine.

The calcium channel antagonist Amlodipine (AM) can facilitate memory when administered acutely immediately after training.The effect of chronic administration is not known.This study reports three experiments which show that AM does not lose its effectiveness as a memory enhancer with chronic administration. 1. Mice were injected daily with 5 mg/kg AM for 14 days prior to training on a spatial discrimination. Immediate post training AM was as effective in enhancing retention as it was in a group treated chronically with saline.2. Mice trained to 4/5 avoidances in a one way active avoidance (AA) task showed substantial forgetting 14 days later.This forgetting was alleviated in mice treated daily with AM for 14 days.3.Mice given AM daily prior to training showed an increased rate of learning of AA. These results show that chronically administered AM can facilitate retention and prevent spontaneous forgetting.

377.7

CALCIUM ENTRY BLOCKER AMELIORATE NEURONAL DAMAGE AND PLACE LEARNING IMPAIRMENT OF RATS WITH TRANSIENT FOREBRAIN ISCHEMIA. R. Tamura*, Y. Nakada, Y. Takamura and T. Ono. Dept. Physiol., Fac. Med., Toyama Medical and Pharmaceutical University, Toyama 930-01, Japan.

Effects of (±)-1-(3,4-dimethoxyphenyl)-2-(4-diphenylmethyl-piperazinyl) ethanol dihydrochloride (tamolarizine), a calcium entry blocker, on ischemic neuronal damage (selective neuronal death in the hippocampal CA1 subfield) and place learning impairment were studied in rats with transient forebrain ischemia. Rats were subjected to forebrain ischemia (occlusion of the bilateral common carotid and vertebral arteries) for 15 min, and received an intraperitoneal injection of tamolarizine (40 mg/kg) or saline just after restoration of blood flow. Place learning was tested in a spatial navigation task in which rats were required to alternate between 2 circular areas located diametrically in a circular open field to obtain intracranial self-stimulation (ICSS) rewards. In this task, rats in the ischemia+saline group showed severe learning impairment and, during the test period (30 days), they did not reach the same performance level as normal rats, while rats in the ischemia+tamolarizine group showed mild learning impairment, but reached the normal performance level. There were no significant differences of spontaneous locomotor activity and sensitivity to ICSS rewards between the ischemia+saline and ischemia+tamolarizine groups. The extent of selective neuronal death in the hippocampal CA1 subfield of rats in the ischemia+tamolarizine group was remarkably weaker than that of rats in the ischemia+saline group. These results indicate that blockage of calcium entry by tamolarizine treatment suppresses selective neuronal death in the hippocampal CA1 subfield, which consequentially prevents the impairment of place learning.

377.8

INHIBITION OF ENDOGENOUS NITRIC OXIDE SYNTHESIS FACILITATES ASSOCIATIVE LEARNING. W. Du*, A.G. Romano, V.A. Aloyo and J.A. Harvey. Div. of Behavioral Neurobiology, Dept. of Pharmacology, The Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA 19129.

Nitric oxide (NO) has been suggested to play an important role in synaptic plasticity and learning. We examined the effects of L-NAME, a specific and competitive inhibitor of NO synthetase, on classical conditioning of the rabbit's nictitating membrane response. Rabbits received injections of saline (n=11) or L-NAME (25 mg/kg, n=11), 60 min prior to each of 4 daily conditioning sessions. Standard conditioning procedures employed a tone CS and an air puff US. L-NAME-treated animals demonstrated an enhanced acquisition of CRs across conditioning days as compared with controls. L-NAME treated animals required 54 fewer trials to achieve the criterion of 5 ($p< 0.001$) and 47 fewer trials to achieve the criterion of 10 ($p< 0.05$) consecutive CRs. The enhanced acquisition of CRs produced by L-NAME was not due to an effect on nonassociative processes or changes in blood pressure. Thus, inhibition of endogenous NO synthesis results in facilitation of associative learning. These results suggest that NO normally functions as a tonic inhibitory modulator of associative learning and that procedures aimed at decreasing its production may provide a novel approach for improving learning. This work was supported by NIMH grant MH16841.

377.9

EFFECTS OF L-ARGININE ON SPATIAL MEMORY IN RATS: ROLE OF NITRIC OXIDE. K. Sato and T.J. Maher*. Div. Pharm. Sci., Massachusetts College of Pharmacy/AHS, Boston, MA 02115.

While much information is available regarding the role of nitric oxide (NO) in synaptic events measured in *in vitro* systems that are thought to play a role in learning and memory, little information exists documenting the effects of NO in whole animal behavioral paradigms. Thus, the present study attempted to manipulate NO neurotransmission via the administration of the NO precursor L-arginine (L-ARG) alone, or in combination with, the NO synthase inhibitor N-nitro-L-ARG methylester (L-NAME), while determining performance in a spatial memory task utilizing rats. Parameters of performance in the Morris water maze (latency to locate platform, swim path length, and time spent in the platform quadrant during the probe trial) were significantly improved by pretreatment with L-ARG (100-400 mg/kg, i.p.) when compared to controls ($p<0.05$). L-NAME alone, 500 mg/kg, but not 300 mg/kg, significantly ($p<0.05$) impaired performance. None of the above treatments produced their observed effects via alterations in swimming ability as determined by measuring swim speed. Co-administration of either dose of L-NAME with L-ARG (200 mg/kg) significantly ($p<0.05$) attenuated the previously observed performance enhancement. These studies document the spatial memory enhancing abilities of the amino acid L-ARG in rats, and provide further evidence for a role of NO in these behaviors.

Supported in part by a grant from the Center for Brain Sciences and Metabolism Charitable Trust.

377.10

THE ROLE OF NITRIC OXIDE IN DIZOCILPINE-INDUCED IMPAIRMENT OF SPONTANEOUS ALTERNATION BEHAVIOR IN MICE. K. Yamada*, Y. Noda and T. Nabeshima. Department of Neuropsychopharmacology and Hospital Pharmacy, Nagoya University School of Medicine, Nagoya 466, Japan.

We investigated the role played by nitric oxide in the dizocilpine-induced impairment of both spontaneous alternation behavior in a Y-maze and of passive avoidance performance in a multiple passive avoidance task in mice. Dizocilpine impaired the sponaneous alternation behavior, and the retention of passive avoidance, without affecting acquisition, in the multiple passive avoidance task. N^G-nitro-L-arginine methylester (L-NAME), an inhibitor of nitric oxide (NO) synthase, dose-dependently impaired the spontaneous alternation behavior, but had no effect on either the acquisition or retention of passive avoidance. The inhibitory effect of L-NAME on the spontaneous alternation behavior was completely reversed by the co-administration of L-arginine. Pretreatment with L-arginine ameliorated the dizocilpine-induced impairment of spontaneous alternation behavior, but not the impairment of the retention of passive avoidance. Finally, the impairment of spontaneous alternation behavior caused by dizocilpine was significantly diminished by pretreatment with dibutyryl cyclic GMP. These results suggest that, although N-methyl-D-aspartate receptors play a critical role in both spatial working memory and long-term memory processes, assessed by spontaneous alternation behavior and the passive avoidance, respectively, different neuronal mechanisms may be involved in these two processess. Further, it is suggested that the NO/cyclic GMP system may play a role in spatial working memory.

377.11

EFFECTS OF PROPENTOFYLLINE, A NERVE GROWTH FACTOR (NGF) SYNTHESIS STIMULATOR, ON CHOLINERGIC DYSFUNCTION INDUCED BY THE INFUSION OF ANTI-NGF ANTIBODY INTO RAT'S SEPTUM. T. Nabeshima*, Y. Ogiwara, A. Nitta, J. Onishi and Y. Noda. Department of Neuropsychopharmacology and Hospital Pharmacy, Nagoya University School of Medicine, Nagoya 466, Japan.

Alzheimer's disease (AD) is characterized by the loss of cholinergic neurons. Since nerve growth factor (NGF) plays an important role in the survival and maintenance of cholinergic neurons in CNS, NGF may have some beneficial effects in the patients with AD. We investigated the effects of oral administration of propentofylline, having potent stimulatory effects on NGF synthesis and secretion in mouse astrocytes, on the cholinergic dysfunction induced by the continuous infusion of anti-NGF antibody into the septum. The deficits of performance in water maze, habituation and passive avoidance tasks were produced by the infusion of anti-NGF antibody (10 μg/rat for 14 days). Further, when choline acetyltransferase (ChAT) and cholinesterase (ChE) activities, regarding as the cholinergic marker enzymes, were measured after the behavioral studies, both activities were reduced. The impairment of learning and memory of water maze task, and reduction of ChAT and ChE activities in the anti-NGF antibody-infused rats were recovered when administration of propentofylline (10 and 25 mg/kg p.o., once a day for 19 days) was commenced 3 days before the implantation of mini-osmotic pump, and continued through the period of learning and memory tasks. These results suggest that propentofylline, a NGF synthesis stimulator, may provide a new therapeutic approach to the patients with AD.

377.12

INHIBITION OF NITRIC OXIDE SYNTHASE DISRUPTS THE EXTINCTION OF CONDITIONED FEAR IN THE RAT. P.L. Malen* & P.F. Chapman. Graduate Program in Neuroscience and Department of Psychology, University of Minnesota, Minneapolis, Minnesota, 55455.

Inhibition of nitric oxide synthase (NOS) affects the induction of some forms of long-term potentiation and disrupts the acquisition of different forms of learning. We have reported that systemic administration of an NOS inhibitor disrupts the extinction of conditioned fear in rats. To more fully characterize the effects of NOS inhibition on conditioned fear, we have used a conditioning paradigm designed to isolate different components of conditioned fear. Our results indicate that NOS inhibitors administered directly to the brain are as effective as those given systemically at retarding the extinction of cued conditioned fear.

Male rats were maintained on a 22-hour water restriction schedule throughout the experiment. Rats received conditioning in a distinctive chamber designated context A. A conditioning session consisted of ten pairings of a 60 second tone coterminating with a footshock. Following testing of cued conditioned fear levels in a different chamber (context B), animals received extinction training in context A that consisted of five 60 second tones and no footshocks. The level of cued conditioned fear was then measured in context B. The conditioned fear response to the tone was measured as a relative decrease in drinking during the tone. We considered fear conditioning extinguished when animals licked at baseline levels following the repeated tone-alone presentations.

Systemic administration of 75 mgs/kg L-NAME (a NOS inhibitor) significantly disrupted the extinction of cued conditioned fear ($p<0.05$). Direct infusion of 500 nmols of L-NAME into the lateral ventricle also significantly disrupted the extinction of cued fear ($p<0.05$). Our results, although preliminary with respect to the contextual component of conditioned fear, indicate that the effects of NOS inhibition appear to be specific for the extinction of cued conditioned fear.

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377.13

AIT-082 DELAYS THE ONSET OF AGE-INDUCED WORKING MEMORY LOSS. R.F. Ritzmann*[1,2], A.J. Glasky[1,2], I. Prisecari[2], and M.P. Rathbone[3] [1] Adv. ImmunoTherapeutics, Tustin, CA 92680, [2] Olive View/UCLA MC, Sylmar,CA 91342 and [3] Depts. Biomed. Sci. and Medicine, McMaster U., Hamilton, Ontario L8N 3Z5

AIT-082, a purine derivative, has been shown to improve working memory in the win-shift test (Pharmacol. Biochem. Behav. 47:325-329, 1994). This test employs a T-maze and by increasing the time of the interval between trials, the duration of memory trace can be determined. In normal adult C57Bl/6 mice, the longest delay that these mice can remember is 120 seconds; AIT-082 (30mg/kg) increased the duration to over 180 seconds. NG-Nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO production, at 30mg/kg inhibited memory in the win-shift test but the inhibition was blocked by AIT-082. Thus, AIT-082 acts downstream from nitric oxide. Normal mice show a progressive deficit after 16 months of age and by 20 months can no longer perform at a 10 second interval. AIT-082 (30 mg/kg/day) administered in the drinking water beginning at 14 months (prior to the appearance of any age-induced deficit), maintained the average duration of the memory trace of 60 minutes at 21 months. This demonstrated a 3 month delay in the onset of age-induced deficits. Analysis of the data showed that the age-induced deficit although delayed occurred in only half the subjects. The remaining mice continued to perform at normal level at 21 months of age. The neurochemical basis for this differences in responsiveness is under investigation. In vitro, studies have suggested a carbon monoxide/guanylate cyclase site for the action of AIT-082. (Supported by NIA AG09911 and the Hospital for Sick Children Foundation)

377.14

ICV INSULIN INCREASES PERFORMANCE ON A PASSIVE-AVOIDANCE TASK. C.R. Park*, R.J. Seeley, and S.C. Woods, Depts. of Behavioral Neuroscience and Psychology, University of Washington, Seattle, WA 98195-1525

Woods and Strubbe have proposed that associative factors are major regulators of ingestive behavior. Animals may become conditioned to ingesting food when exposed to certain external or internal cues which have become associated with food presentation. Since food intake is followed by increased insulin levels, an effect of insulin on hippocampal processes involved in memory could enhance the ability to learn such an association. This experiment determined whether insulin has a modulatory role on memory formation

Naive, Long-Evans rats (n=25) were implanted with third ventricular cannula. The animals were trained on a step-through, passive avoidance procedure, in which latency to enter a darkened compartment was the dependent measure. On the training trial, after entering the darkened compartment, the animals received a mild foot shock. The animals were then removed and received an injection of either 4 mU insulin, heat-deactivated insulin or saline vehicle (4 ul). After 24 hours, the animals were tested in the apparatus again

Compared to saline or heat-deactivated insulin, animals receiving standard insulin had a statistically significant increased latency to enter the dark compartment when retested ($p=0.037$ and $p=0.001$ respectively). This difference is consistent with an increased ability to remember the negative consequences of entering.

377.15

FACILITATIVE EFFECT OF GLUCOSE ON OBJECT RECOGNITION MEMORY IN MICE. C. Messier* and V. Pelletier. School of Psychology, U. of Ottawa, (Ont) Canada K1N 6N5.

The memory-improving effect of glucose has been demonstrated in rodents and humans. In rodents, this effect has been observed using a variety of memory tests that have no direct equivalent in humans. Similarly, glucose improvement of memory in humans was observed in tests that cannot be applied to rodents. In the present experiment, we have examined in mice the effect of post-training glucose on the memory for objects. Animals were first placed in an empty open field for two 3-min habituation periods. The next day, the animals are placed for 3 min in the open field that now contained two identical objects. The time taken to observe and explore the objects was recorded. Immediately after, the animals were given either a saline or glucose (500 mg/kg) injection. Two additional groups received a glucose injection one or five hours post-training and one group of mice received no treatment. The next day, animals were placed again for 3 min in the open field with one of the previously observed object and one new object. The objects exploration time was again recorded. Results showed that the animals that received the immediate glucose injection spent more time exploring the new object while no such effect was observed for the various control groups. These results suggest that glucose retroactively improved the memory for previously observed objects in the same way as it has been shown to improve memory in other behavioral tasks.

This research was supported by a Natural Sciences and Engineering Research Council grant to C.M.

377.16

INFLUENCE OF GLUCOSE AND GLUCOREGULATION ON HUMAN MEMORY IN OLDER SUBJECTS. R. Curwin*, M. Gagnon, V. Knott and C. Messier. School of Psychology, U. of Ottawa, (Ont) Canada K1N 6N5.

We examined changes in cognitive performance following drinking a glucose (50 g) or saccharin solution (50 mg) in fasted older male and female subjects. Blood glucose levels were measured and a recovery index was computed: it was used to categorized poor and good recovery subjects within each sex. For the Wechsler paragraph immediate recall, a significant interaction was observed between gender, recovery, and glycemic (glu/sacc) condition. Post hoc analysis revealed that poor recovery males showed significantly poorer performance on immediate recall of the first paragraph under the glucose condition. Males with good recovery and females with both poor and good recovery displayed no significant differences of immediate recall between glycemic conditions. For the ADAS word list learning trials, a similar interaction was also observed for the second and third learning trials. Post hoc analysis revealed that good recovery males tended to benefit from glucose consumption in learning word lists whereas poor recovery males and both good and poor recovery females did not. There were no significant differences on the first learning trial between gender, recovery or glycemic condition. With delayed recall of paragraphs, no significant differences were observed. Nevertheless, poor recovery males tended to do poorer than good recovery males and both poor and good recovery females on delayed recall. No significant differences were found for other memory measures such as verbal paired associate learning and recognition memory.

377.17

CONTEXT-DEPENDENT EFFECTS OF GLUCOSE AND TIME-DEPENDENT EFFECTS OF FRUCTOSE ON THE MODULATION OF A REACTIVATED MEMORY. W. A. RODRIGUEZ*, C. A. HORNE, and J. PADILLA. Psychobiology Lab, New Mexico Highlands Univ., Las Vegas, NM 87110.

A passive avoidance-to-active avoidance negative transfer paradigm was used to investigate the context-dependent effects of glucose and the time-dependent effects of fructose on reactivate memories in rats. In both experiments, memory reactivation consisted of re-exposing the rats 24 after passive avoidance conditioning to environmental cues present during training; and post-reactivation injections of glucose (100 mg/kg, sc) and fructose (100 mg/kg, sc) or saline were followed 24 h later by active avoidance (reversal) conditioning. In Experiment 1 glucose or saline was administered immediately after one of three reactivation-context treatments: experimental context + US (footshock), experimental context only, or novel context + US. Glucose administered in the experimental context, but not in the novel context, impaired active avoidance conditioning (i.e., enhanced passive avoidance memory) compared with saline treatment. In Experiment 2, fructose or saline was administered in the experimental context 0, 2, 5, or 30, min, or in the colony room 60 min, after reactivation. The results showed a time-dependent decrease in the ability of fructose to modulate a reactivated memory. Together, these results indicate that modulation by sugars of old (reactivated) memories depends on where and when the sugar is administered.

377.18

INTRA-SEPTAL INFUSIONS OF A MU OPIOID RECEPTOR AGONIST IMPAIR SPONTANEOUS ALTERNATION PERFORMANCE: CONCURRENT GLUCOSE INFUSIONS ATTENUATE THE DEFICIT. M.B. Parent*, F.J. Lexcen, M.R. Stefani, V.A. Louis, P.T. Laurey, & P.E. Gold. Dept. of Psychology, Univ. of Virginia, Charlottesville, VA 22903.

Infusions of the opioid agonist morphine into the rat medial septum impair spatial working memory, including perfomance on a spontaneous alternation task. The morphine-induced performance impairments are blocked by concurrent administration of glucose, injected either systemically or intra-septally. The present study sought to identify the opioid receptor subtype(s) which may participate in these effects. With evidence that the medial septum contains a high density of μ opioid receptors, the effect of intra-septal infusions of the selective μ opioid agonist [D-ala2, N-Me-phe4, gly-ol5]-enkephalin (DAMGO) on spontaneous alternation performance was examined. Intra-septal infusions of DAMGO (0.3 nmol), administered 30 min prior to testing, significantly impaired spontaneous alternation performance. Concurrent intra-septal infusions of glucose (16.7 nmol) attenuated the DAMGO-induced deficit. These results indicate that activation of μ opioid receptors in the medial septum impairs spatial working memory. These findings suggest that intra-septal morphine infusions may impair spontaneous alternation performance via activation of μ opioid receptors. Combined with other evidence from our laboratory indicating that glucose does not reverse memory impairments induced by central injections of noradrenergic antagonists or GABAergic agonists, these findings further suggest that glucose may interact primarily with μ receptor-mediated processes to influence learning and memory. [Supported by NIA (AG 07648), NINDS (NS32914), and NIH (HDO7323)].

377.19

DIETARY SATURATED FATTY ACIDS IMPAIR LEARNING AND MEMORY IN RATS. C.E. Greenwood[1,3]* and G. Winocur[2], Dept. Nutr. Sci.[1], Univ. Toronto, Toronto, Ont., Canada M5S 1A8; Rotman Inst.[2] and Dept. Food & Nutr. Services[3], Baycrest Centre for Geriatric Care, North York, Ont., Canada M6A 2E1.

At one month of age, Long Evans rats (n= 8/group) were placed on one of four high fat diets (20 % wt/wt) varying in fatty acid (FA) composition or chow (4.5% fat). The saturated fatty acid (SFA) levels of experimental diets ranged from 9.5% to 3% of total diet, with fat blends allowing for the dissociation of the effects of the SFAs from those of either monounsaturated (MUFA) or polyunsaturated (PUFA) fatty acids. After three months of feeding, rats were tested on a variable interval delayed alternation task. Impairment in both the ability to learn the basic alternation rule as well as remembering trial-specific information over time was observed in all animals fed the experimental diets relative to those consuming chow. The degree of impairment was highly associated with the level of SFAs fed and independent of the levels of either the MUFAs or PUFAs provided. Brain membrane phosphatidylcholine FA profile was altered by the different dietary fats fed. However changes in FA profile across diets showed no direct association with the cognitive impairment. The results provide evidence that cognitive impairment in weanling rats is directly associated with intake of SFAs and suggest that the mechanism whereby SFAs mediate their effect is independent of brain membrane compositional changes. (NSERC)

377.20

THE INFLUENCE OF NIMODIPINE AND MK-801 ON THE BRAIN FREE ARACHIDONIC ACID LEVEL AND THE LEARNING ABILITY IN HYPOXIA-EXPOSED RATS. J.Mršić*,G.Župan,V.Eraković, A.Simonić, J.Varljen,Department of Pharmacology,School of Medicine, B.Branchetta 20/I,Rijeka,Croatia.

A number of reports indicated that a disturbance in calcium homeostasis induced by hypoxia is accompanied with increase in free arachidonic acid (FAA) level and behavioral deficits.The purpose of this study was to investigate the effects of calcium channel blockers, nimodipine and MK-801 on the brain FAA level and learning ability in hypoxia-exposed rats.The study was carried out on Hannover-Wistar rats weighing 250 g.They were injected with 0,03;0,1;0,3 or 1 mg/kg of tested drugs.Thirty minutes later the learning ability was tested in a passive avoidance task according to the step-through procedure. Immediately after the training trial,the animals were subjected to a period of oxygen deprivation hypoxia until the lost of the righting reflex.The retention trial was carried out 24 hours later.A part of animals was pretreated with calcium channel blockers tested and exposed to the hypoxic conditions.Fifteen minutes after loosing the righting reflex they were decapitated and brains were frozen in liquid nitrogen.The FAA was separated by thin-layer chromatography,methyl esters were prepared by methanolysis and quantified by gas chromatography.The level of FAA was measured.It has been found that nimodipin was effective in reversing the significant increase of the brain FAA level and memory decline in hypoxia-exposed rats.MK-801prevented the accumulation of the brain FAA,but did not improve the memory deficit in hypoxic animals.

377.21

THE INFLUENCE OF NICARDIPINE AND IFENPRODIL ON THE BRAIN FREE ARACHIDONIC ACID LEVEL AND BEHAVIOR IN HYPOXIA-EXPOSED RATS. V.Eraković,* G.Župan, J.Mršić, A.Simonić, J.Varljen*. Department of Pharmacology, Department of Chemistry and Biochemistry*, Medical School, University of Rijeka, Braće Branchetta 20/I, Rijeka, Croatia.

Cerebral hypoxia is associated with increase of intracellular calcium concentration and free arachidonic acid (FAA) level and followed by various behavioral disturbances. The aim of this study was to investigate the effect of calcium channel blockers, nicardipine and ifenprodil, on the brain FAA level and learning ability in rats exposed to hypoxia. The study was carried out on Hannover-Wistar rats weighing 250 g. They were injected with 0,03; 0,1; 0,3 or 1 mg/kg of tested drugs i.p.. Thirty minutes later the learning ability was tested in a passive avoidance task according to the step-through procedure. Immediately after the training trial, the animals were subjected to a period of oxygen deprivation hypoxia until loosing of the righting reflex. The retention trial was carried out 24 hours later. Other group of animals was pretreated with mentioned substances before hypoxia-exposure. Fifteen minutes after loosing of the righting reflex they were decapitated and brains were frozen in liquid nitrogen. The FAA level was quantified by gas chromatography. Our results show that both nicardipine and ifenprodil were effective in preventing a memory decline in hypoxia-exposed rats. Nicardipin did not prevent the accumulation of the brain FAA contrary to ifenprodil which produced statistically significantly decrease of the brain FAA level in hypoxia-exposed rats.

378.1

LEFT LATERAL ORBITOFRONTAL ACTIVATION DURING AVERSIVE OLFACTORY STIMULATION. D. H. Zald, P. J. Pardo* & J. V. Pardo. Psychiatry PET Unit, Psychiatry Service, VAMC, Dept. of Psychology and Division of Neuroscience Research, Department of Psychiatry, University of Minnesota, Minneapolis, MN

The perception of olfactory stimuli is dominated by a hedonic (pleasantness-unpleasantness) dimension. We utilized this hedonic characteristic of olfactory perception to study the neural correlates of emotional processing in humans. Ten psychiatrically healthy female subjects were exposed to aversive, moderately pleasant, or no odor conditions while cerebral blood flow (rCBF) was measured with a slow bolus $H_2{}^{15}O$ technique and PET. Exposure to aversive olfactory stimuli produced the greatest rCBF increase in the left lateral orbitofrontal cortex (Brodmann's area 47). The increase in rCBF in this region was highly statistically significant when the aversive condition was compared to either the pleasant odor or the no odor condition. Preliminary data from experiments with affectively valenced gustatory stimuli indicate that this same area is activated during aversive gustatory stimulation. These data provide evidence that the left lateral orbitofrontal cortex plays a role in the processing of aversive chemical stimuli.

This research was supported by the Department of Veterans Affairs and an Eva O. Miller Fellowship from the University of Minnesota.

378.2

SHOCK INDUCED ANTICIPATORY ANXIETY: IMPACT OF RIGHT OR LEFT HEMISPHERIC LESIONS IN HUMANS. B. Slomine, D. Bowers*, K. M. Heilman, R.M. Bauer, & M. Bradley. Depts. of Neurology & Clinical Psychology, University of Florida College of Medicine, Gainesville, FL 32610.

When shown pictures of affective scenes, patients with right parietal lesions fail to display normal autonomic aroused (e.g., SCR). Because such lesions are associated with visuoperceptual and hemispatial attentional disturbance, reduced autonomic reactivity to emotional pictures may be secondary to perceptual/attentional difficulties. To circumvent the "visuoperceptual" explanation, we evaluated 24 focal stroke patients (12 right, 12 left) and 24 controls on a *shock induced anticipatory anxiety task*. In this task, onset of one target tone signaled that the S would receive a mild electric stimulus 6 seconds later (shock condition), whereas onset of another target tone indicated that no shock would be forthcoming (control condition). Psychophysiologic data were collected over 40 trials along with verbal ratings of subjective affective state. During anticipation of shock, the LH and control Ss showed increased SCR arousal in conjunction with increased negative ratings of their emotional state. In contrast, the RH patients displayed a dissociation between SCR and verbal report: they failed to show increased SCR when awaiting shock, although their subjective ratings reflected increased negativity. This dissociation suggests that lesions of RH can disrupt generation of autonomic arousal during aversive situations, while leaving operational the appraisal of one's subjective affective state. *Supported by NIMH48861.*

378.3

RECRUITMENT AND RETENTION OF HISPANIC ELDERS IN A MUSCULOSKELETAL STUDY. R. Ramirez-Delay*, M.L. Villa and R. Marcus. Stanford University and PAVAMC, Palo Alto, CA 94304.

Scant information exists concerning recruitment and retention of minority elders into clinical studies, although survey non-participation is high among general elderly groups. We are conducting a study that enjoys a retention rate of 95% after 3 years, and explored reasons why subjects decided to participate and continue participation. 152 healthy Mexican-American women aged 60-85 years enrolled in a 5 year study of osteoporosis. Volunteers were recruited through community agencies, churches and word of mouth, from 4/92 to 3/93 from a group of 257 women interviewed during the recruitment campaign. Following enrollment, all subjects were contacted monthly. At the screening interview, all were asked how they heard about the study. Responses were compared with the answer to the same question 2 years later when 135 of the original cohort were interviewed. Other questions included: reasons for participation; perception of personal benefit; and opinions about quality of attention received. Subjects averaged 66±6 yr. of age and 9±5 yr. of education; 22% spoke Spanish only; 19% mostly Spanish; 30% both languages equally; 29% mostly English; and less than 1% English only. Average acculturation score (Espino and Maldonado) was 2.1 ± 0.9. Social network (friends and community centers) was reported as the primary source of study information by 71% of subjects at time of initial contact, compared to 67% recalled at the 3rd visit. The primary reason for participation and the most important benefit received from participation was information concerning health status (62%); 95% reported benefits from participation; 80% rated quality of attention received as very good and 19% as good. Conclusion: These Hispanic elders enrolled primarily due to a local community recruitment approach. Retention was enhanced by perception of personal benefit and continuity in follow-up. Funded by the NIA and the Research Service of the Dept. of Veterans Affairs.

378.4

CORTICAL AROUSAL PATTERNS IN RESPONSE TO EMOTIONAL AND NEUTRAL AUDITORY STIMULI. M. Vaningan* and J. Panksepp Dept. of Psychology, Bowling Green State University, Bowling Green, OH 43403.

Previous work has demonstrated that event related synchronization (ERS) is a sensitive index of dynamic cortical arousal patterns. In the present research ERS snalysis was used as a method for discriminating neurophysiological affective states. Cortical activity was measured in 10 adult females with a standard EEG array of 19 electrodes. Responses to happy and sad music, female voice and white noise were analyzed.

It was found that the ERS analysis in the alpha frequency band (8-12 Hz) differentiated cortical arousal in response to happy versus sad music. Overall, a trend was found for smaller synchronization increases during the sad versus happy music. The most robust statistical differences appeared during the third and fourth seconds following music-onset. More specifically, during the third second there was greater synchronization during sad versus happy music at electrode site F4. During the fourth second there was less synchronization during sad versus happy music at electrode sites F3 and C3.

The ERS analysis also unambiguously differentiated cortical responses to a female voice versus white noise. There was less synchronization at all electrode sites, at all times, in response to the voice versus noise. The most robust statistical differences appeared during the second and third seconds following stimulus-onset. During the second second, the synchronization increase was smaller during voice versus the noise conditions at electrode sites F3, F7 and T4. One second later there was less increased synchronization during the voice versus noise at electrode sites Fp2, F3, F4, C3, P3, P4, T5, Fz, Cz, Pz, O1 and O2.

The results suggest that the ERS analysis is one method for determining different cortical arousal patterns in response to affective and neutral stimuli.

378.5

FUNCTIONAL ANATOMY OF GO/NO-GO DISCRIMINATION IN MAN -A PET STUDY-. R.Kawashima*, H.Itoh, S.Ono, K.Satoh, S.Furumoto, R.Gotoh, M.Koyama, S.Yoshioka, K.Takahashi#, T.Takahashi#, T.Yanagisawa#, H.Fukuda. Dep. Nucl. Med. & Radiol., IDAC, Tohoku Univ. Sendai, 980-77 JAPAN, #Dep. Radiol, Iwate Medical Univ. Morioka, 020 Japan.

The purpose of this study was to identify the functional fields activated in relation to the NO-GO decision. Nine healthy subjects participated in the study which consisted of two test positron emission tomography scans, GO/NO-GO (GNG) task and response selection (RS) task, and one control scan. In the RS task, subjects were asked to flex their thumb of the right hand when a light emitting diode (LED) placed 60 cm from thier eyes turned on red and to flex their index finger of the right hand when LED turned on green. In the GNG task, subjects were asked to flex their thumb when the LED turned on red, however, they were asked not to move their fingers when LED turned on green. In the control state, they were asked simply to look at the LED without any movement of finger during the course of the scan. The mean regional cerebral blood flow (rCBF) change images for each task minus control and task minus task were calculated and fields of significant rCBF changes were identified. Several fields in the prefrontal cortex of the right hemisphere were specifically activated in relation to the GNG task. The results indicate that the prefrontal cortex of the right hemisphere may be a key structure to make a decision not to move.

378.6

TRYPTOPHAN DEPLETION AND AGGRESSIVE RESPONDING IN HEALTHY MALES. F.G. Moeller*, D.M. Dougherty, A.C. Swann, D. Spence, C.M. Davis, and D.R. Cherek. Human Psychopharmacology Laboratory, Dept. of Psychiatry and Behavioral Science, The University of Texas - Houston, Houston, TX 77030.

In order to study the effect of decreasing plasma tryptophan levels on aggressive responding in a controlled laboratory setting, we administered two doses (25 g and 100 g) of a tryptophan-free amino acid mixture to 10 healthy male subjects after 24 hours of a low tryptophan diet. Subjects were screened for current or past psychiatric, or non-psychiatric medical illness, Aggressive responding on a free-operant laboratory measure of aggression, (the Point Subtraction Aggression Paradigm), and plasma tryptophan levels were measured before and after drinking the amino acid mixture. There was a significant increase in aggressive responding after the 100 g mixture and a non significant increase in aggressive responding after the 25 g mixture. There was also a significant decrease in plasma tryptophan at 5 hours after ingestion compared to baseline for both doses of the amino acid mixture. This study supports the hypothesis that tryptophan depletion increases aggressive responding in healthy males in a laboratory setting, probably by decreasing brain serotonin.

378.7

CORTICAL EVOKED POTENTIAL AND fMRI MEASURES OF EMOTIONAL PERCEPTION.
P. J. Lang*, B.N. Cuthbert, M. M. Bradley, and J. R. Fitzsimmons, Center for the Study of Emotion and Attention, Department of Clinical and Health Psychology, University of Florida, Gainesville, Florida 32610.

Two related experiments were performed to study brain and autonomic responses to pictures varying along affective dimensions of valence and arousal. In one study, 81 subjects viewed 18 pleasant, 18 neutral, and 18 aversive pictures selected from the International Affective Picture System, each presented for 6 s. Cortical potentials were measured with a 10-s time constant from 9 sites along frontal, vertex, parietal, and occipital rows. Vertical and horizontal eye movements were also recorded for removal of eyeblink and saccadic eye movement effects. Slow potentials were positive throughout most of the picture viewing period at frontal, vertex, and parietal sites. These potentials were further modulated by the arousal dimension of picture content, with pleasant and unpleasant pictures (both highly arousing) showing significantly more positivity at all leads compared to neutral content. In contrast, occipital leads showed only a late positive complex peaking at about 400 msec, with minimal subsequent positivity; further, differences among affective contents were minimal. These results suggest that slow waves provide a topographically sensitive measure of affective picture processing. A second fMRI study, assessing reactions to these pictures in a 1.5T GE Signa scanner, was conducted to compare responses in these two measurement domains.

378.8

PROCESSING OF REWARD-RELATED INFORMATION IN PRIMATE ORBITOFRONTAL NEURONS.
L. Tremblay and W. Schultz*, Institut de Physiologie, University of Fribourg, CH-1700 Fribourg, Switzerland.

The involvement of the frontal cortex in the motivational control of behavior was investigated by recording the activity of orbitofrontal neurons (areas 11 and anterior 13) in monkeys performing in a conditional motor task. Animals performed, or refrained from performing, an arm movement in response to a trigger signal depending on a preceding instruction. In order to differentiate reward-related from movement-related activity, movement trials were either rewarded with fruit juice or unrewarded (correct performance indicated by a sound), depending on the preceding instruction. Correct performance in non-movement trials was also rewarded with fruit juice. All trials included delays between 1) the instruction and trigger and 2) the trigger and the reinforcer (reward or sound). Over 400 neurons were recorded from two monkeys, with about half of them exhibiting task-related activity. This study had 3 principal results. 1) Very few neurons showed delay-related activity preceding the behavioral reaction. 2) Reward-related activity was common in orbitofrontal cortex, with half of recorded neurons having activity directly associated with the expectation or reception of primary reward. This activity was absent in unrewarded trials, indicating a true relationship to juice delivery and not to a "correct" or end-of-trial signal. 3) About half of the neurons responded to the instruction. Two thirds of these responses were preferential or selective for one or two trial types, mostly for both rewarded trials (movement and non-movement) or for unrewarded movement trials. These neurons discriminated stimuli based on their reward or non-reward associations. In conclusion these results indicate that, while other prefrontal regions have been implicated in the "what" and "where" of behavioral reactions, the orbitofrontal cortex may be involved in the question of "why" a behavioral reaction is performed.

BIOLOGICAL RHYTHMS AND SLEEP: PHYSIOLOGY I

379.1

CYCLIC SPONTANEOUS LIMB MOVEMENTS IN NEONATAL RATS AND THEIR RELATION TO BEHAVIORAL ACTIVITY STATES.
M. Dyer* and C. R. Almli, Developmental Neuropsychobiol. Lab., Washington Univ. Med. Sch., St. Louis, MO, 63108.

Cyclic spontaneous motility is ubiquitous prenatally and postnatally in most species. A 1-3 min cyclicity/periodicity has been found during prenatal and early postnatal development. It has been proposed that the 1-3 min periodicity is an expression of REM sleep states. The present study examined cyclic motility in 1 day old rats as a function of behavioral states.

At PN1, individual rats were videotaped for 20-30 min. Videotapes were scored for numbers of movements of individual limbs, and for duration of active and inactive behavioral states. Active behavioral states were scored when pups displayed ambulation, grooming, or righting movements, i.e., behaviors that appeared homologous to behaviors seen in adult rats. Inactive behavioral states were scored using REM sleep criteria, e.g., limb twitches. Limb movements that occurred during active or inactive behavioral states were analyzed for periodicity with spectral analysis. Results showed that movements were more likely to be cyclic when rats were behaviorally active. When rats were behaviorally inactive, movement periodicities tended to be attenuated or abolished.

These results do not support the proposition that cyclicity of movements in the neonatal rat is restricted to REM sleep states.

379.2

HOW DOES THE STATE OF VIGILANCE MODIFY FREQUENCY RECEPTIVE FIELDS (FRF) IN THE GUINEA-PIG AUDITORY THALAMUS ?
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It is well known that thalamocortical neurons display a burst discharge in slow-wave sleep (SWS) and a tonic discharge in both wakefulness (W) and Paradoxical Sleep (PS). However, it is unknown how sensory processing is modified by these changes in functional states of thalamic neurons. In the present experiment, auditory thalamus neurons were studied during W, SWS and occasionally PS phases. Single units were recorded from restrained guinea-pigs via chronically implanted tungsten electrodes. The animals were undrugged and never sleep deprived. EEG (cortical and hippocampal) and EMG activity were continuously monitored during FRF determination. FRF was tested by presenting 11 ascending frequencies to the contralateral ear via a calibrated earphone. Up to 6 intensities were used to test each cell.

During SWS, spontaneous activity was significantly decreased in 14/27 cells (increased in 4/27). Evoked activity was decreased in 15/27 cells (increased in 2/27). The Signal/Noise ratio (S/N) was unchanged. During PS, spontaneous activity was increased in 7/16 cells (decreased in 2/16), while evoked response was decreased in 7/16 cells (increased in 2/16).The S/N ratio was significantly decreased.

Quantification of the frequency selectivity at different intensities should allow a better characterisation of sensory processing during the sleep states.

379.3

FREQUENCY AND VOLTAGE OF THETA WAVES IN THE HIPPOCAMPUS AND CORTEX AS RELATED TO DREAMING PATTERNS IN THE RAT.
C.Timo-Iaria*, A.C.Valle, K.Sameshima, G.Ballester, L.V.Venturini and V.L.L.Missen. Univ. São Paulo Medical School and Fed. Univ. São Paulo Medical School (EPM), 01246-903 São Paulo SP, Brazil.

Instantaneous voltage and frequency of hippocampal and cortical theta waves were analyzed as related to dreaming behaviors (DB) of the rat. Head (H), rostrum (R), eyes (O), ears (E) and fore- and hindlimbs (L_f, L_h) movements were recorded simultaneously with the EEG of several cortical areas and hippocampal fields. It was found that: 1) a DB may be expressed as H, R, O, E, L_f and L_h movements alone or as the various possible combinations among them (HR, HO, HRO etc.); 2) during a DB sustained desynchronization occurs only in the frontal cortex and theta waves occur over most cortical areas and in the hippocampal fields; 3) theta frequency and voltage usually increase (typical values: 140 μV and 7.5 Hz) during a DB but often both variables augment before and decrease to 5.5 or 6 Hz after DB until a new episode occurs; 4) while DB is in course both variables keep high but voltage changes continuously in distinct areas; 5) when DB is intense frequency increases while voltage decreases for either a few seconds or a few hundred milliseconds, paralleling DB duration. COMMENTS. The increase of both variables before a DB is displayed probably reflects configuration and identification of a dream. The brief dissociation between theta voltage and frequency is in fact a short desynchronization. Desynchronization of the frontal electro-oscillograms in rats and of almost the entire neocortex in primates, including man, is an electrophysiological manifestation of attentive wakefulness and of dreaming activity. Since the frontal cortex and desynchronization are phylogenetically recent acquisitions, the drop in theta voltage while frequency goes up in the rat desynchronized sleep may be related to a high degree of attention to a dream, just as desynchronization in attentive wakefulness.
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379.4

UNIQUE DYNAMICS OF RAT HIPPOCAMPAL ELECTROGRAM IN RELATION TO REM SLEEP.
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The aim of this study was to characterize the dynamics of hippocampal and cortical EEGs with respect to rapid-eye-movement sleep (REM) in the rat. Albino male rats were implanted with skull screws for recording the cortical EEG and with electrodes for recording the hippocampal EEG and the temporalis muscle EMG. Data were sampled continuously during 24-48 h and stored. Power spectral density (PSD) in the delta (1-4 Hz) and theta (5.5-8.5 Hz) bands was averaged in periods ranging from 2 to 48 sec. The vigilance states [wakefulness, non-REM sleep, and REM] were scored in two-sec epochs.

Although there was considerable waxing and waning of EEG power in the sec to sec range, there was a rising trend in the hippocampal EEG PSD in the theta band in the sleep epochs preceding REM, state in which it reached a maximum. When REM episodes lasted ca. 1.5-7 min, the rising trend continued during REM well above the mean theta PSD in the 8 min preceding REM onset. In the cortical EEG, theta PSD decreased sharply after REM onset, to continue during REM below the corresponding 8 min pre-REM average. Conversely, both structures showed similar trends in delta power in the epochs before and during REM. Therefore, the hippocampus shows unique dynamics in EEG theta PSD during sustained REM, although it shares with the cortical EEG the dynamics in delta power.

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379.5

EFFECTS OF SLEEP DEPRIVATION ON PLASMA FREE AMINO ACIDS IN THE RAT. R.K. Zoltoski* and P. Ramm. Dept. of Psychology, Brock U., St. Catherines, ON L2S 3A1, Canada.

We investigate the metabolic consequences of sleep and sleep deprivation. In this study, we examined plasma free amino acid profiles associated with different conditions of sleep deprivation. A 24 hour island platform deprivation condition (n=16) used medium-sized islands over water. Island platform deprivation replicates the classic REM deprivation paradigm. A computer-scored and driven rocker condition (n=14) produced total sleep deprivation (>99%). A third condition (n=41) was created by manually arousing rats for 4-6 hours. This established a fairly uniform group of minimally-deprived animals with a high level of sleep need. Prior to deprivation, rats received EEG/EMG electrodes and cannulae in the femoral vein and artery. Three days of post-surgical recovery were allowed.

Following the deprivation period and a 3 hour fast, the EEG was recorded for 45 mins and blood samples were taken at 0, 5, and 45 mins. Glucose, haematocrit, the animal's state of vigilance, and fourteen free amino acids were analyzed. The three deprivation conditions are associated with different profiles in five of these amino acids (Table).

Amino acid	Mild dep. (1)	Island (2)	Rocker (3)	($p < 0.05$)
glutamine	574.8±13.8	315.5±19.8	290.7±40.6	1*2, 1*3
glycine	374.0±14.0	308.8±13.5	289.5±24	1*2, 1*3
methionine	18.2±1.4	34.0±1.2	16.9±3.9	1*2, 2*3
tyrosine	52.1±1.3	58.1±1.4	62.4±3.4	1*3
histidine	70.0±2.7	71.4±1.1	54.3±4.06	1*3, 2*3

Island and rocker deprivation differ in their effects upon levels of circulating amino acids, and may be targeting different physiological processes.

379.6

THE DEVELOPMENT OF SLEEP HOMEOSTASIS IN THE RAT. M. G. Frank, H. C. Heller, W. C. Dement*, Dept. of Biological Sci., Stanford Univ., Stanford, CA. 94305

Increases in non-REM sleep (NS) slow wave activity (NSWA) after sleep deprivation (SD) provide an index of sleep homeostasis in adult and juvenile rats. We investigated the effects of SD on sleep homeostasis in neonatal rats during the period of initial NS maturation (P12-P24).

Rat pups were implanted for EEG/EMG recording and sleep deprived for 3h at light onset using forced locomotion. SD in P12-P20 rats increased NS and suppressed REM sleep in the first 2h of recovery. Spectral analyses, however, revealed that NSWA did not significantly increase until P24. These findings suggest that P12-P20 neonatal rats respond to SD by increasing NS time and not NS intensity. Therefore sleep homeostasis may be present in neonatal rats but its manifestation as increased NSWA is absent until the fourth postnatal week.

379.7

SLEEP ARCHITECTURE IN KITTENS AFTER BILATERAL MESENCEPHALIC LESIONS THAT ELIMINATE PONTO-GENICULO-OCCIPITAL (PGO) WAVES IN THE LATERAL GENICULATE NUCLEUS (LGN). James P. Shaffery*, Thomas Fitch, Samuel G. Speciale, Howard P. Roffwarg, Roseanne Armitage and Gerald A. Marks. Dept. of Psychiatry, University of Texas Southwestern Medical Center at Dallas, 75235.

We previously reported that reducing the time kittens spend in rapid eye-movement (REM) sleep during the critical period of visual system development leads to morphological changes in LGN cells. Bilateral mesencephalic lesions severing the ascending pontine cholinergic fibers that are thought to produce PGO-waves, a cardinal feature of REM sleep, while eliminating PGO in LGN, are reported to have no effect upon subsequent sleep amounts. It is possible, however, that such lesions may affect more subtle aspects of sleep architecture in these young animals.

After confirming PGO-waves in baseline records, kittens were anesthetized (Ketamine and Xylazene) and bilateral electrolytic lesions (5mA for 20 s) were preformed (P2, L2 5,3.5, H-1). Baseline sleep micro-architecture data are compared with that from post-lesion days three and seven. We report on the structure of sleep bouts and REM sleep cycles. We also quantify ECoG activity within sleep states utilizing a digital period analysis (DPA) algorithm that includes full zero-cross (sensitive to slow frequency events), first derivatives (to detect fast frequency events occurring upon a background of slow waves) and power-in-frequency measures in the following frequency bands: delta 0.5<4Hz, theta 4<8Hz, alpha 8<12Hz, sigma 12<16 Hz, and beta 16<30Hz.

These bilateral brainstem lesions effectively eliminated PGO-waves for the seven days of the experiment. Post-lesion REM sleep measures of bout and cycle, number and length remained at baseline levels. Slow wave sleep (SWS) measures were only slightly increased over baseline, by 10%. Total power was increased over baseline about 20% in both sleep states, resulting largely from increased beta power in SWS (~31%) and REM (~10%) over baseline relative to other power measures. The DPA data suggest that removal of the modulatory influence of pontine cholinergic-generated PGO-waves results in an increase in fast frequency cortical ECoG activity.

379.8

A DEVELOPMENTAL AND COMPONENTIAL ANALYSIS OF ACTIVE SLEEP. M. S. Blumberg* and D. E. Lucas. Department of Psychology and Neuroscience Graduate Program, University of Iowa, Iowa City IA 52242.

A wide variety of hypotheses have been put forth that address the functional significance of active sleep. Despite, however, the well-accepted fact that active sleep expresses itself predominantly in the perinatal period, the vast majority of these functional hypotheses are applicable largely, if not exclusively, to the adult. We build on the developmental approaches of previous researchers and propose that the individual components of active sleep (e.g., myoclonic twitches, rapid eye movements) exhibit unique developmental and phylogenetic histories and may serve independent functions in the developing organism. This dynamic perspective leads to specific experimental approaches aimed at the developmental roles of these components in the neonate, their maintenance roles in the adult, and the means by which these various components coalesce temporally in what is commonly referred to as a behavioral state.

379.9

EFFECTS OF REM SLEEP ON EXPERIENCE-DEPENDENT MORPHOLOGICAL DIFFERENCES IN THE VISUAL CORTEX. **K.E. Armstrong*, P. Sinha, C. Solomon, S. Han, W.T. Greenough. Departments of Psychology and Cell and Structural Biology, Neuroscience Program, Beckman Institute, University of Illinois Urbana-Champaign, IL 61801.**

Rapid eye movement (REM) sleep has been thought to play a critical role in information processing and memory consolidation. In animals the initial REM sleep episode following any learning task is the most important for the learning and consolidation of that information. A novel method for manipulating REM sleep, ambient temperature variation, was used (Szmusiak and Satinoff, 1985). This technique is ideal for measuring morphological changes associated with sleep manipulations because it appears to minimize stress effects relative to other nonpharmacological sleep deprivation procedures. Environmental Complexity (EC) and Individual Cage (IC) rearing conditions were used to vary the opportunity for learning. Previous studies using this paradigm found an increase in occipital cortical thickness in the EC rats (Bennett et al, 1964; Greenough et al, 1985). Our preliminary findings suggest that following as few as 10 days of EC\IC exposure, coupled with ambient temperature manipulations, the increased occipital cortical thickness seen in non-sleep deprived EC rats was reduced in rats deprived of sleep immediately following a 2hr daily exposure to the EC environment. Animals receiving delayed temperature manipulations (4 h after EC exposure) had a thicker visual cortex than did their immediate deprivation counterparts. Supported by MH35321.

379.10

DIFFERENTIAL PERIODIC EFFECTS OF BRIGHT LIGHT ON TEMPERATURE AND PERFORMANCE RHYTHMS IN RHESUS MONKEYS DEPEND ON THE TIMING OF THE LIGHT PULSE. L.A. Schultz and W.N. Tapp*, Neurobehavioral Unit, V.A. Medical Center, East Orange, NJ 07018.

Five rhesus monkeys housed in constant light were given single 2 h pulses of 1000 lux bright light (BL) at several points in their day. Each monkey earned all of its daily food aliquot by correct responding on a task that combined a measure of vigilance ability (VIG) with a measure of short-term memory (delayed same/different or DSD). Monkeys were implanted with transmitters that allowed us to continuously monitor body temperature (Tb). BL applied at the start of alpha phase-advanced the Tb rhythm while bright light at the end of alpha phase-delayed the Tb rhythm. In contrast, BL given during the middle of subjective day or during the middle of subjective night did not reliably alter the phase of Tb. The phases of the performance measures were insensitive to light given at any point.

BL also altered the period of Tb and performance rhythms. BL pulses given at the onset of alpha had similar effects on the period of Tb, VIG, and DSD though the direction of the effect varied between monkeys. However, while the direction of the period change in Tb following BL exposure at the end of the subjective day was identical to that following exposure at the start of alpha, BL had a differential effect on the direction of period changes in the performance rhythms. That is, lengthened Tb periods as a result of BL were associated with lengthened DSD periods, but with shortened VIG periods compared to baseline. Likewise, shortened Tb periods following BL exposure at the end of alpha were associated with shortened DSD periods, but lengthened VIG periods.

379.11

DAILY SCHEDULED EXERCISE CAN ADVANCE, DELAY OR INVERT ENTRAINMENT TO SKELETON PHOTOPERIODS IN HAMSTERS. Sinclair, S.V., Mistlberger R.E.* and Marchant, E.G. Psychology, Simon Fraser University, Burnaby, B.C., V5A 1S6.

Daily schedules of exercise can shift the phase of photic entrainment in hamsters. This study utilized a skeleton photoperiod (SPP, two 30 minute light pulses) to better simulate natural dawn and dusk light exposure patterns, and to facilitate observation of transients indicative of possible oscillator decoupling. Sixty-eight adult male Syrian hamsters were housed in cages with activity wheels in a 14:10 LD SPP. Exercise (3h locked in a novel running wheel) was scheduled at one of 5 phases of the SPP. Some of the animals exercised in the middle of their inactive period (ZT4-7) showed 180° inversion of their activity rhythms. Inversion in one animal appeared to be achieved by partition. Animals that did not invert showed small phase advances ($\bar{x}$ = .65h ± .72h). Exercise near the end of the active period (ZT21-24) caused large phase delays of entrained rhythms ($\bar{x}$ = 3.29h ± 5.24h). Exercise at other phases late in the activity period (ZT19-21 and ZT20-23) had minimal effects. Exercise near the beginning of the activity period (ZT9.5-12.5) on average caused phase delays ($\bar{x}$ = .64h ± 1.35h); but, some advances were also observed. The period of free-running rhythms recorded in subsequent constant dark was longer in hamsters that phase-delayed in the ZT9.5 condition, but was not otherwise related to the phase of the prior exercise schedule. There was no evidence that exercise could stably uncouple an activity component from the entrained activity bout (i.e. free-running rhythms were not split in DD). These experiments demonstrate that exercise can have powerful effects on phasing of activity rhythms entrained to SPPs that may better simulate patterns of light exposure in nocturnal and diurnal animals, such as humans, that may spend most of the day shielded from natural light. Supported by NSERC, Canada.

379.12

PHASE RELATIONSHIP OF EVENING AND MORNING OSCILLATORS REGULATES PHASE RESPONSIVITY :COMPARATIVE STUDY BETWEEN WILD-TYPE AND *TAU* MUTANT HAMSTERS. S. Yamazaki*, K. Shimomura and M. Menaker. Dept. of Biology, Gilmer Hall, Univ. of Virginia, Charlottesville VA 22903.

The length of the activity phase (α) of the hamster's circadian wheel-running rhythm is regulated by two coupled oscillators, the evening oscillator (E) which controls activity onset and the morning oscillator (M) which controls the end of activity. Kawato (personal communication) predicted that the phase relationship between E and M should regulate circadian period and phase responsivity. The hamster's α gradually lengthens in constant darkness, by inference the result of decoupling of E and M. We applied 1 h light pulses and measured the relationship between α and the magnitude of the phase shift that they produced. In *tau* mutants (period ≈ 20 h), the magnitude of the phase delay produced by a light pulse at circadian time (CT) 14 was strongly correlated with α. The critical α (CA) at which the phase delay increased dramatically was about 9.5 circadian hour (CH). However, the CA for phase advance (light pulse at CT 18) was about 12 CH. In wild-type (period ≈ 24 h), we could not find a CA for phase delay, but the CA for phase advance was about 10 CH. CAs of heterozygous mutants (period ≈ 22 h) were 12 CH for phase delay and 12.5 CH for phase advance.

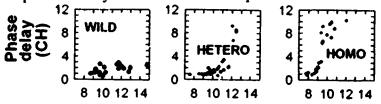

Fig. 1 Phase delays produced by light pulses given at CT 14

379.13

COLD EXPOSURE PHASE-SHIFTS CIRCADIAN RHYTHMS IN HAMSTERS: ROLE OF ACTIVITY AND SEROTONIN. E.G. Marchant*, S. Sinclair and R.E.Mistlberger. Psychology, Simon Fraser University, Burnaby, BC V5A 1S6.

Circadian rhythms in rodents can be phase-shifted by appropriately timed activity. This may be dependent on the motivational stimulus for activity; running induced by a novel cage or social interaction is effective, whereas running induced by cold has been reported to be less or ineffective. We report here, however, that cold exposure in the home cage (no novelty) induces large phase-shifts that are dependent on activity induction, despite modest activity induction. Adult, male Syrian hamsters (N=24) were housed in plastic cages with activity wheels (17cm) in a ventilated refrigerator. Every 10-14 days, LD (14:10) was replaced by 3-4 days of dim red light (DD). On the first day of DD, 6 h before usual dark onset, the refrigerator was cooled to 4°C for 3 h. Phase shifts of circadian rhythms were quantified by comparing daily activity onsets before and after the cold pulse. By contrast with control tests, cold exposure induced significant phase advances of the wheel-running rhythm (106±53 min vs. 15±21 min, p<.001). Hamsters ate during the cold pulse, but food deprivation did not prevent shifts (N=7). Most hamsters also ran during and after cold exposure (3h total 486±652, 6h total 1367±1335, range 0-4003 revolutions). These levels are low by comparison with thresholds for activity-induced phase shifts defined in previous studies, and some hamsters exhibited phase-shifts despite little running. Nonetheless, locking the wheel for 6 h prevented phase-shifting (24±53 min, p>.1, N=7). These data indicate that cold-induced running is an effective nonphotic zeitgeber, and contradict the best evidence that motivational contexts determine the phase-shifting value of physical activity. Although 5HT1A agonists induce phase shifts similar to cold-induced running, we have not been able to attenuate cold or novelty induced shifts by preinjections of the 5HT1A antagonist WAY100478 sc. at doses up to 15 mg/kg. Supported by NSERC, Canada.

379.14

IMPAIRED CIRCADIAN RHYTHM RESPONSE FOR BODY TEMPERATURE AND ACTIVITY OF OBESE ZUCKER RATS TO REVERSAL OF THE LIGHT-DARK CYCLE. T.A. Lennie, P.J. Rowsey, B. Cimprich*, and B.L. Metzger. The University of Michigan, Ann Arbor MI, 48109-0482

As part of a larger study, we compared the ability of female Sprague–Dawley and obese Zucker rats to reentrian circadian rhythms for body temperature and activity following complete reversal of the light–dark cycle (12h/12h). Body temperature (Tb) and spontaneous cage activity were measured by surgically implanted biotelemetry devices (Mini–Mitter, Sunriver, OR). Following a baseline period, the light–dark cycle was reversed at the onset of the dark cycle resulting in 24 hours of light. The rats were left undisturbed except for a brief daily inspection for an additional 5 days during which body temperature and activity were continuously monitored and recorded every 10 minutes (Dataquest III, Data Sciences). During baseline, body temperatures of the Zucker rats averaged about 0.3° C lower than Sprague-Dawley rats. However, there were no differences in the period, amplitude, or phase of the circadian rhythm for Tb. Baseline circadian activity patterns were also similar between the groups. Following reversal of the light cycle, the Sprague-Dawley rats demonstrated a more rapid response to the light cycle change. By Day 5, the Tb rhythm was entrained to the new light cycle. In contrast, reentrainment was delayed in the Zucker rats with the nadir for Tb still occurring during the light cycle on Day 5. Reentrainment of activity patterns in the Sprague Dawley rats was noted by Day 4. The pattern of activity in Zucker rats was flat following light change. Moreover, the level of activity progressively decreased such that total activity on Day 4 was 27% lower than baseline. By Day 5, only a 4 hour phase advance in the nadir for activity was noted. These results indicate that obese Zucker rats have an impaired ability to respond, both behaviorally and metabolically, to changes in environmental zietgebers.

379.15

MEALS INCREMENTALLY AND INSTANTANEOUSLY RESET CIRCADIAN ACTIVITY PATTERNS IN SUPRACHIASMATIC NUCLEUS LESIONED RATS J.M. White*, W. White, and W. Timberlake, Dept. of Psychology and Prog. in Animal Behavior (RTG), Indiana University, Bloomington, IN 47405

The nature of meal-engendered circadian processes of rats with complete lesions of the suprachiasmatic nucleus (SCN) was studied. After receiving electrolytic lesions, female Sprague-Dawley rats were housed , in constant dim light, in stations containing a running wheel, a nest, and pellet and water dispensers. In Experiment 1 rats were arrhythmic when fed ad lib, wheel turned prior to both mealtimes when fed from 1000 to 1100 and from 1600 to 1700, but wheel turned primarily around the early mealtime (the meal time preceded by the long intermeal interval) during two consecutive days of food deprivation. In Experiment 2 the rats failed to wheel turn prior to two meals scheduled at 19 or 31 hr periods (6 and 13 hr and 6 and 25 hr intermeal intervals). While these noncircadian schedules were in effect, a component of activity with a 24 hr period and a "peak" between 1000 and 1200 was observed. This component appeared to be an oscillating after-effect of the 24 hr period feeding schedule. The component was not observed when an animal was maintained on a 12/12 hr light-dark cycle and fed ad lib for 6 weeks before being placed on noncircadian schedules. When the 31 hr schedule was in effect, rats were especially active 24 hr after every early meal. Rats with complete lesions of the SCN can simultaneously manifest two meal-engendered circadian processes, each associated with an independent circadian activity pattern. Meals appear to incrementally adjust the time course of one process (Stephan, J.Bio.Rhythms, 1989) and instantaneously rest the time course of the other.

379.16

SHALLOW AND DEEP TORPOR ARE DIFFERENTIALLY ASSOCIATED WITH FOOD INTAKE AND BODY WEIGHT IN SIBERIAN HAMSTERS. C.C. Purvis* and M.J.Duncan, Dept. of Anatomy and Neurobiology, Univ. of Kentucky Med. Center, Lexington, KY 40536.

Torpor is a thermoregulatory adaptation exhibited by many small rodents in response to one or more environmental stimuli, e.g., short photoperiod, cold exposure and reduced food availability. In Siberian hamsters, short photoperiod (SD)-induction of daily torpor depends upon the pineal gland. This study examined the effect of lesions of melatonin-receptor containing thalamic nuclei, the paraventricular nucleus (PVT) and reuniens nucleus (RE), on food intake and the display of torpor. Adult male hamsters were subjected to sham surgery (controls) or received stereotaxic injections of ibotenic acid into the RE or PVT, sparing the rostral pole. One week later, animals were transferred to SD(10L:14D); after 12 weeks, they were transferred to a cold room (7°C) and daily torpor was monitored for 60 days. Then the hamsters were perfused transcardially with fixative, brain sections were prepared and the lesion sites were verified histologically. This study detected two distinct behavioral states of torpor characterized by different core body temperatures: shallow (27°C) and deep (17°C) torpor. Lesions of either the PVT or RE had no significant effect, as compared to controls, on the expression of either shallow or deep torpor. However, statistical analysis revealed that the incidence of deep but not shallow torpor was inversely correlated with food intake ($p<0.0001$) and final body weight ($p<0.001$). These results suggest that these two states of torpor are regulated by different underlying mechanisms, in particular, differential coupling to metabolic state.

379.17

COUPLING BETWEEN LIGHT- AND FOOD-ENTRAINABLE CIRCADIAN OSCILLATORS IN PIGEONS. M.E. Rashotte* and F.K. Stephan. Program in Neuroscience, Department of Psychology, Florida State University, Tallahassee, FL 32306-1051.

Two experiments were conducted to determine whether pigeons have a separate food-entrainable oscillator (FEO) and to assess the coupling strength between the FEO and the light-entrainable oscillator (LEO). Two pigeons were housed in a LD12:12 cycle with food available 9h after light onset. Body temperature (Tb), O_2 consumption and CO_2 production were monitored. Tb and O_2 increased prior to light onset and food presentation. When the LD cycle was phase delayed or advanced by 4h while feeding time remained unchanged, Tb and O_2 rises showed the expected delaying or advancing transients with respect to light-onset. The Tb and O_2 rises prior to feeding also delayed or advanced for several days but then returned to their proper phase position. In a second experiment, food was presented at 23.5h intervals for 10 days while the LD cycle continued with a 24h period. Entrainment to the LD cycle was unaffected. Both Tb and O_2 consumption continued to rise prior to feeding but with a reduced lead time. Feeding time was then delayed by 5h. Tb and O_2 consumption with regard to the LD cycle was unaffected, but both measures showed delaying transients until they re-entrained to the new feeding time. This experiment also provided some evidence for a circadian modulation of the respiratory quotient (CO_2/O_2). The results provide strong support for the existence of a separate FEO in pigeons and indicate asymmetrical coupling between the LEO and FEO, with the former having a stronger effect on the latter. *Supported by NSF-9222369.*

379.18

TIME SERIES ANALYSIS OF PERIODICITIES IN INTERSCAPULAR BROWN FAT TEMPERATURE PATTERNS. L. Kelly* and C. Bielajew. School of Psychology, Neuroscience group, University of Ottawa, Ottawa, Ont. K1N 6N5 Canada.

There is evidence that brown adipose tissue (BAT) thermogenesis contributes to the ultradian warming patterns of core temperature (Closa et al, 1991). Indeed, ultradian cycles in BAT activity have been suggested as a pivitol factor underlying the feeding bouts of ad-lib schedules (Himms-Hagen, 1994). Diurnal patterns in BAT activity have been described by examining changes in sympathetic firing rate (Sakaguchi et al, 1988) and GDP-binding levels (eg. Redlin et al, 1992); however, results are equivocal. We chose to investigate the chronic BAT temperature pattern. A radio-frequency disc transmitter (Mini-Mitter, Inc.) was implanted in each 400g male rat, either by bonding it directly to the muscle under the interscapular deposit or to a piece of surgical teflon which was then sewn in place. After one week of recovery, BAT temperatures were recorded throughout the 24 h cycle (12h light:dark) for up to six days. Spectral analysis was performed on the raw data (half-hour intervals) and again after removing the circadian component from the series ("deseasoning"); this procedure reduces interference from harmonic frequencies. As expected, all data show a strong circadian cycle, but the ultradian rhythms are less clear. The 8h and 4h cycles are most consistent, appearing in 3, and 2, respectively, of the 8 spectral graphs. The 4h cycle may be stronger as it persists in 3 graphs when deseasoned data are used. As ultradian cycles appear less stable than circadian ones, simultaneous recording of BAT temperature and food intake/hormonal profiles, etc. would be useful in assessing the potential significance of the 4h cycle reported here.

BIOLOGICAL RHYTHMS AND SLEEP: PHYSIOLOGY II

380.1

PREOPTIC/ANTERIOR HYPOTHALAMIC (POAH) COOLING SUPPRESSES SLEEP IN RATS. D. McGinty[1]*, Md. Noor Alam[1], S. Morairty[2] and R. Szymusiak[3], Dept. Veterans Affairs Medical Center, Sepulveda, CA 94343, Depts. Psychology[1], Neuroscience[2] and Medicine[3], Univ. Calif., Los Angeles, CA.

Excitation of POAH warm-sensitive neurons (WSNs) and/or suppression of cold-sensitive neurons (CSNs) can facilitate NREM sleep, but some evidence suggests that POAH hypnogenic effects can be independent of thermoregulatory output. Noting that the majority of POAH neurons are insensitive to local temperature changes, we used bilateral POAH cooling (1.5°C) with water-perfused thermodes to selectively inactivate WSNs and activate CSNs in the rat. During three hour cooling periods in the middle of the usual daily sleep period (CT 3.5-6.5) total sleep time was reduced by 85%, including both NREM and REM sleep. EEG delta activity was also sharply reduced during cooling, but was enhanced during the first 2 hours following cooling. Body temperature was elevated during and one hour after POAH cooling. Since POAH cooling produces selective effects on thermosensitive neurons, our finding suggests that these neurons are essential for the hypnogenic output of this site leading to spontaneous sleep.
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380.2

EFFECTS OF PARADOXICAL SLEEP DEPRIVATION ON THERMOREGULATION IN THE RAT. P.Shaw, B. Bergmann, C. Landis* and A. Rechtschaffen, Dept. of Psychiatry, U. of Chicago, Chicago, IL 60637

Rats subjected to total sleep deprivation (TSD) by the disk-over-water method have shown three thermoregulatory changes: an elevated thermoregulatory setpoint (T_{set}); excessive heat loss; a reduction of wake-sleep temperature differences (dT). The present study evaluated which of these changes may be achieved by the loss of paradoxical sleep (PS) alone, and which required loss of the NREM component of total sleep as well. Hypothalamic temperature (T_{hy}) was continuously monitored in 5 rats selectively deprived of PS (PSD) and their yoked controls (PSC). All were provided with a continuously available operant to control ambient temperature (T_a). Unlike TSD rats, PSD rats did not show significant increases in T_{hy} or operant responses for heat, thus supporting previous inferences that elevated T_{set} in TSD rats resulted from the loss of the NREM sleep component. That T_{hy} remained near baseline in spite of large increases in metabolic rate is consistent with previous inferences that PSD alone can cause excessive heat loss. Large progressive decreases of dT from 0.6°C during baseline to 0.2°C near the end of deprivation in PSD rats, but not in PSC rats, confirms earlier indications that PSD alone is sufficient to reduce wake-sleep temperature differences.

380.3

DISCHARGE DURING WAKEFULNESS AND SLEEP OF RAT PREOPTIC/ANTERIOR HYPOTHALAMIC (POAH) NEURONS Md. Noor Alam[1], R. Szymusiak[2], T. Steininger[1]* and D. McGinty[1]. Dept. Veterans Affairs Medical Center, Sepulveda, CA 94343, [1] Dept. Psychology and [2] Dept. Medicine , UCLA.

Neurons in the POAH participate in the regulation of both body temperature and sleep-waking states. A significant proportion of POAH neurons recorded in cats have elevated spontaneous discharge rates during sleep with EEG synchrony (nonREM sleep) compared to waking. We have recently reported that activity of POAH thermosensitive neurons in cats is modulated by sleep. Discharge rates and thermosensitivity of most warm-sensing neurons are elevated in nonREM sleep. Activity of cold-sensing neurons is reduced during sleep.

We have extended our analysis of POAH neurons to the rat. Extracellular POAH unit activity was recorded using chronic microwire technique. In a sample of 46 cells, 30% were nonREM sleep-related (i.e., spontaneous discharge rate in nonREM sleep >120% of waking discharge rate), 15% were waking-related, and the discharge rate of 54% did not vary across the sleep-waking cycle. Of a small sample of thermosensitive neurons studied to date, the majority of warm-sensing neurons also displayed elevated spontaneous rates in nonREM sleep.

These preliminary results indicate that sleep-wake discharge of POAH neurons in rats is similar to that previously described in cats. They also support a hypothesized role for hypothalamic thermosensing cell types in sleep-wake regulation in this species.
Supported by the Dept. Veterans Affairs and HHS #RO1 MH47480

380.4

DEVELOPMENT OF CIRCADIAN RHYTHMS OF BODY TEMPERATURE AND ACTIVITY IN SPRAGUE-DAWLEY RAT PUPS. T. M. Hoban-Higgins, D. M. Murakami, C. Fermin* and C. A. Fuller. Section of Neurobiology, Physiology and Behavior, Univ. of California, Davis, CA 95616-8519.

Twenty-four albino rat pups were implanted with intraperitoneal telemetry transmitters on postnatal day 17. Immediately after surgery, they were individually housed with *ad lib* food and water. Body temperature was recorded at 10 minute intervals until postnatal day 90. Twelve of the animals (7 male, 5 female) were maintained in a light-dark cycle consisting of 12 hours of light followed by 12 hours of darkness (LD 12:12). The remaining 12 pups (6 male, 6 female) were housed in constant light of 0.5-1 lux (LL). Data were analyzed for amplitude, mean and phase of the circadian rhythm of body temperature. Small amplitude rhythms were visible on postnatal day 17. Amplitude continued to increase until days 40-50 when it reached a mature steady-state. The absence of an external light-dark cycle did not delay the appearance of rhythmicity in the pups maintained in LL. Interestingly, the pups in LL attained a mature homeostatic level of temperature regulation sooner than did the pups in LD. All pups evidenced robust rhythms in both lighting environments for the duration of the study. There was no difference between males and females in free-running period. (Supported in part by NASA Grant NCC2-886.)

380.5

THE DEVELOPMENT OF THE PHOTIC INDUCTION OF C-FOS IN THE RETINA AND SCN. D. M. Murakami*, I.-H. Tang, T. M. Hoban-Higgins and C. A. Fuller. Section Neurobiology, Physiology, and Behavior, Univ. of California, Davis, California 95616-8519.

The retinohypothalamic tract (RHT) is an important neural pathway for light to entrain and phase-shift circadian rhythms. The development of the RHT was studied by examining the ability of light to induce c-Fos reactivity within the retina and suprachiasmatic nucleus (SCN) at different ages. Rats were housed in a standard vivarium environment of LD 12:12 (weaned at P21). At CT 13 rats (ages from P1 to P35) were moved to an isolation chamber. The experimental group was exposed to a 1 hour light pulse (300 lux), while controls did not receive a light pulse. All rats were immediately sacrificed, brains and eyes removed, sectioned through the retina (5mm) and SCN (50mm), immunohistochemically stained for c-Fos, and counterstained. Rats at P1 & P3 did not exhibit the photic induction of c-Fos within the retina or SCN. However, by P7 the SCN exhibited an adultlike pattern of photically induced c-Fos reactivity. Retinal c-Fos reactivity exhibited significant photic induction within the outer and inner nuclear layers and ganglion cells. These results suggest that highly significant maturation of the RHT occurs between P3 and P7. (Supported in part by NASA Grant NAGW-2195.)

380.6

EFFECTS OF UNILATERAL OPTIC NERVE TRANSECTION ON MAMMALIAN CIRCADIAN TIMING SYSTEM. I-H Tang, D. M. Murakami, C. A. Fuller*. Section Of Neurobiology, Physiology and Behavior, University of California, Davis, California 95616-8519

This study investigated the effects of unilateral optic nerve transection (UONx) on the golden hamster's circadian rhythms and light induced c-Fos in the SCN. One group of six animals was housed under a square-wave LD (SQ/LD) cycle(14:10). Another group of six animals was exposed to a simulated natural LD(SN/LD) cycle composed of a 4 hr linearly ramped dawn-mimicking period, a 6 hr full light intensity day period, a 4 hr linearly ramped dusk-mimicking period. Unilateral optic nerve transection was performed on half of the animals from each group after stable entrainment had been established. Then all animals were kept under the SN/LD cycles. The body temperature and ambulatory activity rhythms of animals previously exposed to the SN/LD became arrhythmic for up to 4 weeks after the surgery and gradually restored circadian rhythmicity to a new circadian pattern as previously described. However, animals housed under SQ/LD before UONx retained their circadian rhythmicity and gradually established a new circadian rhythms under the SN/LD. All animals, intact and UONx, received one hour light pulse at 1500 hour and were then sacrificed at 1600 hour. c-Fos expression in the SCN was examined. Animals with UONx showed weaker c-Fos expression and different spatial distribution of the c-Fos immunoreactive cells in the SCN. (Supported in part by NASA Grant NAG2-795.)

380.7

MODULATION OF c-Fos EXPRESSION IN ORGANOTYPIC CULTURES FROM THE RAT BRAINSTEM. S.H. Winston, D.G. Rainnie, P. Shiromani, B. O'Donnell*, R.W. Greene, and R.W. McCarley. Dept of Psychiatry, Harvard Medical School and Brockton VAMC, Brockton, MA 02401.

We have previously shown that microinjection of carbachol into the pontine reticular formation *in vivo*, causes c-Fos expression in a subset of neurones in the brainstem. The purpose of this study was to develope an *in vitro* brainstem preparation that could be used to study this phenomenon.

Organotypic cultures were prepared using a modification of the method of Stoppini *et al.*, (1991). In sterile conditions, 8-10 day old Long-Evans rat pups were anaesthetised by hypothermia and decapitated. Brains were rapidly removed and placed in ice-cold MEM containing 25 mM HEPES and 10 mM TRIS, pH 7.35. A block of tissue containing the entire rosto-caudal aspect of the dorsal Raphé nucleus was prepared, 250 μm coronal slices cut on a Sorval TC-2 tissue slicer, and transfered to 35mm culture dishes containing dissection medium at 27°C. Individual slices were transfered to (30 mm) Millicell-CM inserts in 100 mm petri dishes containing 5 ml of culture medium and maintained at 36°C in an incubator with a 5% CO_2 atmosphere. Culture medium contained 50% MEM with 25mM HEPES, 25% horse serum, and 25% Hanks solution buffered with 5 mM TRIS and 4 mM $NaHCO_3$ to pH 7.2. After 3-5 days cultures were washed with sterile, serum-free, ACSF (containing in mM: NaCl 124; Kcl 3.75; KH_2PO_4 1.25; $MgCl_2$ 1.3; $CaCl_2$ 2.5; $NaHCO_3$ 26; and glucose 10) for 4 hrs, and then cultured overnight in fresh ACSF. After drug treatment, slices were fixed with 4% paraformaldehyde and stained for Fos-Li activity with a rabbit anti-Fos antibody (Santa Cruz) and visualised with DAB staining. ACSF alone caused no c-Fos expression, addition of carbachol (200 μM) evoked regionalised expression of c-Fos, which could be modulated by atropine (200 μM). Experiments are in progress to determine the mechanisms underlying carbachol-induced activation of c-Fos in the brainstem.

380.8

EFFECTS OF THE OPIATE RECEPTOR ANTAGONIST NALOXONE ON IPSPS EVOKED IN LUMBAR MOTONEURONS FOLLOWING STIMULATION OF THE NUCLEUS RETICULARIS GIGANTOCELLULARIS DURING CARBACHOL-INDUCED ATONIA. M.-C. Xi*, R.-H. Liu, J. Yamuy, F.R. Morales and M.H. Chase. Department of Physiology and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Anatomical data demonstrate that spinal cord motoneurons are richly innervated with axon terminals containing the peptide enkephalin (ENK) *(Arvidsson et al., Synapse, 1992, 11:85-104)*. Functionally, ENK and other opioid peptides suppress the spontaneous and evoked activity of cat spinal motoneurons *(Zieglgänsberger et al., Brain Res., 1976, 115:111-128)*. These data have led us to hypothesize that opioid peptides may be involved in modulating the activity of motoneurons during active sleep. We were therefore interested in examining the large amplitude, long-latency, glycinergic IPSPs which are induced in lumbar motoneurons by electrical stimulation of the medullary nucleus reticularis gigantocellularis (NRGc). Specifically, we wanted to determine whether these IPSPs, which are evoked exclusively during active sleep and carbachol-induced atonia, would be affected by ENK.

Intracellular recordings were obtained from lumbar motoneurons in the decerebrate cat during atonia induced by the microinjection of carbachol into the rostral pontine reticular formation. The non-selective opioid peptide receptor antagonist naloxone was applied, juxtacellularly, to the recorded cells. Electrical stimulation of the NRGc elicited IPSPs in lumbar motoneurons with a mean amplitude of 1.8 ± 0.3 mV (S.E.M.), a latency to onset of 28.8 ± 1.8 ms, a latency to peak of 53.2 ± 1.8 ms, a half-width of 27.6 ± 1.5 ms and a duration of 57.1 ± 1.5 ms (n=8). Naloxone, when applied microiontophoretically (50-400 nA, 4-11 min), reversibly reduced by 33% the amplitude of these NRGc-induced IPSPs (to 1.2 ± 0.3 mV; n=8; $p < 0.01$), but had no effects on the resting membrane potential, or the latency or time course of the IPSPs. The mean latency of the naloxone effect on the NRGc-induced IPSPs was 2.3 ± 0.4 min (n=8).

The present results suggest that opioid peptides modulate the NRGc-induced IPSPs during carbachol-induced atonia, and that they may participate in the regulation of lumbar motoneuron inhibition during active sleep. Supported by USPHS Grants NS 23426, NS 09999 and MH 43362.

380.9

ELICITED DIAPHRAGMATIC FRACTIONATIONS AND PGO WAVES ACROSS STATES. W. K. Hunt, L. D. Sanford, A. R. Morrison, R. J. Ross*, and A. I. Pack. Lab. for Study of the Brain in Sleep., Dept. of Psych., and Ctr. for Sleep and Resp. Neurobio., Univ. of Penna., Philadelphia, PA 19104

Fractionations (FR) are 20-100 ms pauses in diaphragm activity that occur spontaneously during rapid eye movement sleep (REM), often in association with ponto-geniculo-occipital (PGO) waves. Tones elicit FR and PGO waves during REM, non-REM, and waking with similarities in timing suggesting generation by the same neural mechanisms. We examined elicited PGO waves and FR across states. Cats (n=4) were implanted with electrodes to record EEG, EOG, neck EMG, diaphragm EMG, and PGO waves from the lateral geniculate nuclei. Auditory tones (n=50-150, 90dB, 20ms duration) or blanks were presented in mid-inspiration across a 4-8 hr recording session. Tones elicited PGO waves and FR at similarly high rates across all states (62-79% of trials). The majority of stimuli elicited both a PGO wave and a FR (60-68% of trials), but each could be elicited alone. The latencies and durations of elicited FR were similar across states ($\bar{x}$ latency: 23-25ms, $\bar{x}$ duration: 21-25ms), but the mean latency of elicited PGO waves in REM ($\bar{x}$ = 63 ± 7ms) was longer ($p<.01$) than in waking or non-REM ($\bar{x}$ = 37 ± 7ms and 41 ± 7ms, respectively). In addition, a second, long-latency FR (LFR) ($\bar{x}$ latency: 82 ± 6ms and $\bar{x}$ duration: 22 ± 2ms) was elicited at a higher ($p<.05$) rate in REM (35% of trials) than in other states (5% of trials in waking, and 3% in non-REM). We found that elicited FR consistently occur with shorter latencies than elicited PGO waves, in contrast to spontaneous FR which have a variable relationship to PGO waves, but usually occur 10-40 ms after the onset of the PGO wave. The LFR elicited during REM resembles a spontaneous FR in its relationship to the PGO wave and its prevalence in this state may reflect the bias toward motoneuronal inhibition characterizing REM. In conclusion, this study suggests that PGO waves and FR may be generated by different neuronal populations that are similarly influenced by auditory stimuli. Supported by MH42903, HL07713, and HL42236.

380.10

AFFERENT STIMULUS-INDUCED IPSPs ARE TIME-LOCKED TO PGO WAVES FOLLOWING THE INJECTION OF CARBACHOL INTO THE NUCLEUS PONTIS ORALIS. K.A. Kohlmeier, F. López-Rodríguez, F.R. Morales and M.H. Chase* *Neuroscience Interdepartmental Program, Dept. of Physiology, Dept. of Anatomy & Cell Biology, and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90095.*

We have recently found that auditory clicks and sciatic nerve stimulation result in the generation of IPSPs in masseter motoneurons in the α-chloralose-anesthetized cat (*Sleep Research* 1995 24:9; *Soc. Neuro. Abs.* 1994 20:1218). The production of these IPSPs by afferent stimuli is state-dependent insofar as they occur only in conjunction with the carbachol-induced state and its accompanying pattern of muscle atonia. Consequently, we were interested in determining (1) whether afferent stimuli elicit PGO waves in the α-chloralose preparation following carbachol-induced muscle atonia in order to demonstrate whether such stimuli activate the startle network (*Acta. Neurobio. Exp.* 1975 35:821) and, if so, (2) whether there is a temporal relationship between the afferent stimuli-induced increase in inhibitory drive on motoneurons and afferent stimuli-elicited PGO waves. Following the microiontophoresis of carbachol (200 mM) into the nucleus pontis oralis (NPO) in seven cats anesthetized with α-chloralose (40 mg/kg I.V.), either an auditory click (1.5 ms duration, 95 dB) was presented or the sciatic nerve was stimulated via an implanted nerve cuff at an intensity sufficient to induce an H reflex. Both types of afferent stimuli were found to produce PGO waves. The average latencies to onset of the stimuli-induced PGO waves were not significantly different (sciatic nerve stimulation-induced PGO wave, 21.6 ± 1.2 ms; n=27; click-elicited PGO wave, 21.4 ± 0.6 ms, n=35; $p>.05$). The sciatic nerve stimulation- and click-induced IPSPs also had similar latencies to onset (30.4 ± 1.7 ms and 32.1 ± 0.8 ms, respectively). The delay between the onset of the sciatic nerve stimulation-elicited IPSP and the click-elicited IPSP from the foot of the elicited PGO wave was 8.8 ± 0.7 ms and 10.9 ± 0.8 ms, respectively. This delay was not significantly different from the latency to onset of 1) the spontaneous IPSPs which are time-locked to PGO waves in the α-chloralose-anesthetized cat following the injection of carbachol into the NPO ($p>.05$) and 2) the IPSPs that are time-locked to the PGO waves which are present during spontaneously-occurring active sleep in the chronic cat (Kohlmeier et al., submitted for publication; *Brain Research* 1994, 653:31). The data in this report suggest that the spontaneous production of PGO waves during active sleep represents the neuronal signature of internal activation of the startle network.

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380.11

CORTICAL EEG-RELATED ACTIVITY OF BASAL FOREBRAIN NEURONS AND THEIR RESPONSES TO BRAINSTEM AND SENSORY STIMULATION IN URETHANE-ANESTHETIZED RAT. K. Semba*, L. Détári and D.D. Rasmusson. Depts. of Anat. & Neurobiol.(K.S.) and Physiol. & Biophys.(D.D.R.), Dalhousie Univ., Halifax, N.S. B3H 4H7 Canada; Dept. of Comp. Physiol., Eötvös Loránd Univ., Budapest, Hungary (L.D.).

The cholinergic projection from the basal forebrain to the cerebral cortex has been suggested to have a crucial role in cortical activation. The activity of basal forebrain neurons appears to be regulated by ascending inputs from the brainstem. In this study we examined the effects of electrical stimulation of the pedunculopontine tegmental (PPT) and the dorsal raphe (DRN) nuclei as well as sensory stimuli (tail pinch) on the activity of desychronization (D)- and synchronization (S)-active basal forebrain neurons under urethane anesthesia (initial dose: 1.25-1.35 g/kg, i.p.). Fifty-two of 169 basal forebrain neurons tested were identified as D-cells and 14 as S-cells on the basis of correlation between firing rate and EEG power. Single pulses to PPT and DRN elicited short-latency (2-13 msec), short-duration (9-15 msec) excitatory responses in most of D- (73%) and S-cells (67%). Inhibitory responses, longer in duration (24-131 msec), were seen only in 8% of D-cells but more frequently (22%) among S-cells. In contrast, short train stimulation (0.5 sec, 100 Hz) of the same structures or tail pinch (1-2 sec) excited most D-cells (80-96%) but inhibited the majority of S-cells (55-67%). These results indicate that the majority of basal forebrain neurons showing EEG-related firing patterns increase their activity during cortical EEG desynchronization. Together with previous anatomical and physiological data, the predominantly excitatory effects of PPT and DRN stimulation on D-cells suggest that, in addition to the well known cholinergic and serotonergic projections, glutamatergic or other excitatory pathways from these structures to the basal forebrain may be important for cortical activation. (Supported by Soros Foundation, MRC and Scottish Rite Charitable Foundation of Canada.)

380.12

HIGH FREQUENCY BURST FIRING IN BASAL FOREBRAIN NEURONS RECORDED *IN VIVO*: RELATIONSHIP TO SLEEP-WAKING STATE. R. Szymusiak[2*], Md. Noor Alam[1] and D. McGinty[1]. Dept. Veterans Affairs Medical Center, Sepulveda, CA 94343, [1] Dept. Psychology and [2] Dept. Medicine , Univ. Calif., Los Angeles, CA 90024.

Cholinergic neurons of the basal forebrain (BF) recorded *in vitro* display the voltage-dependent calcium current I_t, that underlies high frequency burst (HFB) firing (Khateb et al., Neurosci. 51:489, 1992). I_t is also present in thalamocortical relay cells, and HFB firing in relay cells recorded *in vivo* occurs primarily during sleep (Steriade et al., Science 262:679, 1993). Using chronic microwire technique, we compared extracellular single unit discharge within the thalamus and BF of adult cats and rats to look for evidence of sleep-related HFB firing in BF neurons.

In cats, 76% of a population of thalamic neurons displayed HFB firing during nonREM sleep and tonic, single spike firing in waking. Presence of sleep-related HFB firing was indicated by a >50% increase in the variance about the mean discharge rate (calculated in 250 msec bins) during sleep compared to waking. Analysis of BF neurons was confined to those displaying relatively tonic discharge (>5 Hz) in waking. In a sample of 64 such neurons, 16% had a state-dependent pattern of discharge variability comparable to that found in the majority of thalamic neurons. In rats, a similar proportion of BF neurons (14%) displayed evidence of HFB firing in sleep. Extracellularly recorded HFB firing during sleep may be useful for identifying BF cholinergic neurons recorded *in vivo*.
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380.13

SINGLE UNIT RECORDING FROM THE MESOPONTINE TEGMENTUM DURING DIFFERENT BEHAVIORAL STATES Chiara M. Portas, Mahesh Thakkar* and Robert W. McCarley. VA Medical Center and Harvard Medical School, 940 Belmont St, Brockton, MA 02401

Neurons of the mesopontine tegmentum play a significant role in the control of behavioral states; it has been proposed that projections from cholinergic neurons in the laterodorsal and pedunculopontine tegmental nuclei to the thalamus and medial pontine reticular formation are crucial to the regulation of REM sleep. In order to clarify the firing characteristics of neurons in these regions across different behavioral states, we have recorded single units in freely behaving cats.

Male adult cats, anesthetized with sodium phenobarbital (35 mg/kg), were implanted with chronic sleep recording electrodes and a mechanically driven microdrive targetted at the mesopontine tegmentum. Two bundles of 7 microwires (32 μm each) were used to record single units. 7-10 days were allowed for post-operative recovery before recordings began. Extracellular single unit activity was amplified and fed into a window discriminator. The microwires were advanced in steps of 25-50 μm until a signal to noise ratio of at least 3:1 was encountered. Once an acceptable single unit was found, neuronal activity was recorded along with polygraphic indicators of behavioral states *viz.* EEG, EOG, and EMG. The cat was continuously observed by video (without disturbance) throughout the recording session. Two to five wake/slow-wave sleep/REM cycles were recorded for the units reported here. Histological verification of the recording site is pending.

Polygraphic records were classified into active waking, quiet waking, slow-wave sleep, REM sleep with eye movements and REM sleep without eye movments. The firing patterns of all recorded neurons were then categorized in relation to behavioral state. Preliminary results from this study show a low firing rate for most neurons and that majority of these alter their firing rate with regard to sleep and wakefulness. Majority of these neurons showed increased firing during wakefulness and REM.

380.14

EFFECTS OF DISCRETE IBOTENIC ACID LESIONS OF MESOPONTINE TEGMENTUM ON SLEEP AND WAKING STATES IN THE RAT. M. Thakkar, W.L. Inglis, D.G. Rainnie, R.W. Greene, R.W. McCarley* and K. Semba. Dept. Psychiatry, Harvard Medical School & VAMC, Brockton MA 02401, USA, and Dept. Anatomy & Neurobiology, Dalhousie University, Halifax NS, Canada B3H 4H7.

Several lines of evidence indicate that the cholinergic neurones in the laterodorsal (LDTg) and pedunculopontine (PPTg) tegmental nuclei play a crucial role in the control of behavioral state. It is possible to make relatively circumscribed lesions in the rat mesopontine tegmentum using ibotenic acid (e.g. Inglis *et al.*, Neuroscience 58, 817-833). Using a similar lesion protocol, this study investigates the extent to which neurons in the PPTg or LDTg are involved in the regulation of sleep and wakefulness.

Rats were implanted with EEG electrodes on occipital and frontal cortices, wires into the neck muscles for EMG measurements, and bilateral guide cannulae aimed at either LDTg or PPTg. Following recovery from surgery and habituation to the test environment, 2 or 3 baseline recordings of 4h were taken during the same period of each day, in order to establish normal activity patterns for each rat. Lesions were made by lowering a microinjection needle through a guide cannula into the mesopontine tegmentum under pentobarbitone anaesthesia, and pressure-injecting 0.1 M ibotenic acid into the region. Polygraphic recordings were made, during the same 4 h period as baseline recordings, on days 1 and 4 after excitotoxic infusion and thereafter at weekly intervals for up to two months. Polygraphic records showed that lesioned rats spent more time awake or in a drowsy state and their sleep pattern was more fragmented. EEG power spectral analysis revealed striking changes in activity within identifiable frequency bands (<25 Hz) in different. These data indicate that destroying *either* the PPTg or LDTg is sufficient to disrupt the pattern of sleep and wakefulness and alter cortical EEG patterns in characteristic ways. *Supported by The Wellcome Trust (038117/Z/93), MRC of Canada (MT-11312) and the Veterans Administration.*

380.15

INTERACTION BETWEEN TWO PHOTORECEPTORS SYSTEM IN CRAYFISH PROCAMBARUS. V. Inclán-Rubio* and A. Borgonio-Aguilar. Departamento de Fisiología. Facultad de Medicina, UNAM. AP. 70-250, CP 04510 México, D.F. MEXICO.

It is known that white-light stimuli on the caudal photoreceptors of the sixth abdominal ganglion (6th AG) in the crayfish increase the electric activity along the abdominal chain, evoking an inhibitory effect on the amplitude of the electric response to the light of the visual photoreceptors (electroretinogram, ERG). The objective of this research was to study the capability of monochromatic light applied to the 6th AG to synchronize the ERG circadian rhythm. We worked with adult crayfish Procambarus clarkii. The ERG was obtained with the conventional registration technique under a L:D (12:12) photoperiod. In the 4th day of registration, a monochromatic-light stimulus (590nm) of one-hour duration and 400 lux intensity on the 6th AG, at four different circadian times (6, 12, 18 and 24 CT), six more days after were analyzed the changes. Results show changes in the ERG circadian rhythm, which are made evident by (a) a decrease in the amplitude of the ERG after the stimulation, (b) the appearance of a transitory state lasting from 36 to 40 h, and (c) a change of phase that depends on the moment of the application of the stimuli, (advance: 12, 18 and delay at 6, 24 CT). These results show a functional communication between the extraretinal photoreceptors of the 6th AG and the retinal photoreceptors in the compound eye. (PAPIIT-DGAPA:IN204593).

381.1

MODELLING AND MEASURING THE ACOUSTICAL FEATURES OF THE MIDDLE-EAR OF THE ZEBRA FINCH. H.-P. Rangol & W. Plassmann*
ZOOLOGISCHES INSTITUT, J. W. GOETHE-UNIVERSITÄT, FRANKFURT AM MAIN, FRG

The middle-ear cavities of the Zebra Finch *Taeniopygia guttata castanotis* have an interconnection by pneumatizations of the bony skull which can be considered as an interaural pathway: Sound which is incident to one tympanic membrane travels to the inner side of the other tympanic membrane. This causes an action of the soundwave on both sides of the tympanic membrane. This mechanism of sound receiving is described in acoustical terms as a pressure- difference-receiver.

The directionality and frequency-sensitivity of this mechanism can be used to distinguish it from pressure-receiver-mechanisms. The features are physically well understood and can be used to construct physical and mathematical models to evaluate the values of pressure-difference-receivers with different geometrical dimensions and in different sound-fields.

We have designed a mathematical and physical model for pressure-difference-receivers, checked it with hardware-models and have done physiological measurements with the Zebra Finch. We recorded with three probe-microphones, two of them inserted through holes into the middle-ear cavities of the bird resp. into the interconnecting tube of the hardware-model. We measured the directionality on a turntable at every 10° rotation angle up to 360° azimuth. Afterwards the transferfunctions between the microphones were evaluated and compared with the calculated values.

The results show that the middle-ear of the Zebra Finch acts as a pressure-difference-receiver with good agreement to the theoretical expectations about directionality and frequency-sensitivity. The comparison of the measurements with published behavioural studies about hearing-range and directionality from other labs shows good agreement too.

381.2

FUNCTIONAL HIERARCHY DEFINED BY SINGLE UNITS IN SINGING BIRDS: HVc REPRESENTS SYLLABLES, AND RA REPRESENTS NOTES. A.C. Yu, D. Margoliash*, Com. Neurobiol., Univ. of Chicago, Chicago, IL 60637

We have characterized the motor properties of single neurons in forebrain nuclei of the song system in male zebra finches. Birds chronically implanted with electrodes mounted on miniature microdrives were recorded during singing.

Single unit study of HVc activity confirmed prior results (Yu and Margoliash, 1993). In all cases, neuronal activity in HVc preceded each syllable of song, and was dominated by tonic excitation. Histograms of neuronal activity were unique for each syllable type and independent of the position of the syllable in the song.

In contrast, activity of RA neurons was characterized by strong inhibition and highly phasic excitation, producing very precise sequences of 1-7 distinct bursts/syllable. The number of bursts was related to syllable complexity. Bursts appeared to be associated with notes (subunits of syllables) or individual acoustic features of a syllable, although activity was also dependent on the position of the syllable within the song. Syllables that shared notes also shared the corresponding bursts. The time-registry of sequences of bursts from pairs of vocalizations was improved by aligning the spike traces using the "warp-path" derived from dynamic time warping of the syllable acoustics. Within bursts, timing was more variable for introductory syllables, whereas the number of spikes and the inter-spike interval (ISI) in bursts associated with major syllables were highly consistent across vocalizations (e.g., between pairs of spikes ISI = 1.4 ms • 106 •s SD). For such bursts, typically the first ISI was longer than the rest, and the second and following spikes exhibited increasingly diminished amplitude.

In zebra finch, HVc is organized at a syllable-type level, and is independent of the context within a song. The motor code in RA is organized around notes or smaller acoustic features of a syllable, and depends on song context. Context-dependent RA activity suggests a coarticulation mechanism for song production.

381.3

CHARACTERIZATION OF SLOW INHIBITORY POTENTIALS IN NUCLEUS HVc OF ADULT MALE ZEBRA FINCHES. Marc F. Schmidt* and D. J. Perkel, Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

Nucleus HVc of songbirds is anatomically situated at the intersection of motor and auditory pathways. It is thought to be a primary candidate for integrating song motor activity and auditory feedback. Chronic neural recordings from adult zebra finches (McCasland and Konishi, 1981) have shown that auditory responses in HVc become inhibited during and following singing. Because auditory feedback is critical for song learning, it has been hypothesized that this vocalization-induced inhibition may not occur until the onset of song crystallization since such inhibition would prevent auditory feedback to HVc during learning. To investigate possible cellular mechanisms by which this inhibition may occur, we used a slice preparation to record intracellularly from adult HVc neurons.

Electrical stimulation of fiber tracts outside of HVc, or directly within the nucleus, elicited relatively rapid $GABA_A$ receptor-mediated IPSPs as well as two types of slow IPSPs. Both of these slow IPSPs were best elicited by brief stimulus trains rather than single pulses. The first IPSP had a duration of 200-400 msec (consistent with $GABA_B$ receptor-mediated events). The second type of slow IPSP was much longer in duration with a time course of several seconds. Its duration was similar to the time course of vocalization-induced inhibition of auditory responses.

To investigate candidate neurotransmitters which might mediate these slow IPSPs, we have tested various agonists for their ability to hyperpolarize HVc neurons. The $GABA_B$ receptor agonist baclofen (30 μM; n=5/5), the metabotropic glutamate receptor agonist 1S-3R-trans-ACPD (100 μM; n=4/4) and serotonin (30 μM; n=6/7) all caused hyperpolarizations of 5-10 mV. These changes were associated with a decrease in input impedance. Pharmacological characterization of the observed slow IPSPs will be crucial for testing their role in vocalization-induced inhibition of auditory responses.

381.4

SENSITIVITY TO AUDITORY TEMPORAL CONTEXT INCREASES SIGNIFICANTLY FROM FIELD L TO HVc. M.S. Lewicki* and B.J. Arthur, Computation and Neural Systems Program, Division of Biology, Caltech, Pasadena, CA 91125

Neurons in the songbird forebrain nucleus HVc have responses that are highly dependent on the auditory temporal context and can integrate information over periods of hundreds of milliseconds. An example of this integration is temporal combination sensitivity (TCS) where the response of the neuron is sensitive to both the combination and order of pairs of song syllables. Whether these properties arise in HVc or already exist in the afferent neurons is not known.

To address this issue, we compared the degree of context and temporal combination sensitivity of HVc neurons with their auditory afferents in field L (L1, L2a, L3, L2b, and L). Extracellular physiological recordings were made from adult male zebra finches. Single units were isolated with conventional methods and with spike discrimination software. The anatomical location of the recording sites was determined from electrolytic lesions.

Context sensitivity was measured by comparing the response to forward song with the response to the same syllables presented in reverse order. Neurons that showed a significant response to the bird's own song were selected for analysis. In HVc, half of these neurons showed a significant difference between the response to forward song and the response to the syllables in reverse order (15/31). This effect was seen less frequently in neurons in field L (5/25).

TCS was determined by whether a neuron showed 1) a response dependedent on the temporal order of a syllable pair and 2) a difference between the response to the syllable pair and the sum of the responses to the individual syllables. Syllable pairs were selected for presentation by comparing the response to syllable pairs in the forward song to the same syllable pair in the reverse syllable order song. Neurons that showed a significant response to the syllable pair were selected for analysis. TCS was not seen in any of the field L neurons (0/13), whereas TCS was present in one third of the selected population of HVc neurons (5/15).

381.5

INNATE CONTRIBUTIONS TO SELECTION OF ACQUIRED SONG DURING VOCAL LEARNING IN WHITE-CROWNED SPARROWS. C.S. Whaling*[1], M. Solis[2], G. Carrillo[2], J.A. Soha[1], P. Marler[1] and A.J. Doupe[2]. [1]Section of Neurobiology, Physiology, and Behavior, UC Davis, Davis, CA 95616 and [2]Keck Center and Depts of Psychiatry and Physiology, UCSF, San Francisco, CA 94143.

Young, naive sparrows tutored with tape recordings of both conspecific and heterospecific song preferentially learn conspecific song. We investigated the acoustic and physiological basis for this learning preference using both behavioral and neurophysiological techniques. White-crowned sparrows (wcsp; *Zonotrichia leucophrys nuttalli*), were collected as nestlings and raised by hand in acoustic isolation. In behavioral experiments, we measured the vocal response of fledglings to a variety of song stimuli (Nelson and Marler, 1993). These songs included normal wcsp song, "isolate" songs from wcsp raised in acoustic isolation, and heterospecific songs. We found that normal white-crown song and "isolate" songs are equally potent in eliciting a vocal response in fledglings and are more potent than heterospecific songs. The strong response to isolate songs, which are composed of a series of whistles, suggests that whistles may be critical for song recognition in this species. To investigate the neurophysiological basis of this innate preference, we recorded single and multi-unit auditory responses of neurons in HVc and auditory neostriatum of fledgling wcsp. We found many neurons that responded strongly to whistles of both normal and isolate song. Some neurons also responded well to pure tone bursts of the same duration as wcsp whistles but did not respond to the same tone bursts when their duration was shorter than normal whistle length. These results suggest that the innate ability of naive fledglings to select conspecific song from an array of stimuli may depend on the presence of whistles, which are universal in normal wcsp songs. Consistent with this hypothesis, whistles are effective stimuli for evoking neuronal responses in the auditory neostriatum of naive wcsp.
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381.6

NEUROPHYSIOLOGICAL EVIDENCE FOR SONG DISCRIMINATION IN SONGBIRD NUCLEUS HVC. S.F. Volman*. Department of Zoology, The Ohio State University, Columbus OH 43210.

Almost all neurons in the song-system nucleus HVc respond preferentially to a bird's own song (Margoliash, 1986). Most hypotheses about the function of this selectivity assume that a bird's song could serve as a useful reference, or "matched filter," for discriminating among the similar songs of other birds. Songbirds do solve this difficult auditory task, because they recognize other individuals by song, and there is often a large amount of song sharing among birds within a local area. I have tested this idea by comparing HVc auditory responses among groups of sibling zebra finches, using the songs of all siblings, and the father, as stimuli. Because song is learned, these songs are highly similar. In the HVc of each bird, the relative responses to the songs were consistent across multiple recording sites. The response profile -- i.e the relative response strength across stimuli -- was unique in each bird, although, e.g., in a group of 4 brothers, two birds could share a similar profile, which was different from that of the other two birds. Songs that produced the most response in one bird could be the worst stimulus in another. Thus, the response profiles cannot be explained by the general nature of the stimuli, but only by reference to their similarities and differences from a bird's own song. These data support the hypotheses that the unique tuning properties of HVc are useful for audition. Furthermore, because HVc neurons acquire their selectivity during song learning (Volman, 1993), this nucleus appears to be a kind of build-it-yourself perceptual filter, similar to the aquisition of phonemic categorical perception during speech learning in humans. Supported by NIMH grant R29-MH47330

381.7

LEARNED SONG DISCRIMINATION AT THE SINGLE CELL LEVEL IN ZEBRA FINCH CAUDOMEDIAL NEOSTRIATUM (NCM). R. Stripling*[1], S.F. Volman[2], and D.F. Clayton[1]. [1]Beckman Institute, Urbana, IL, 61801 & [2]Dept. of Zoology, Ohio State Univ., Columbus OH 43210.

The caudomedial neostriatum (NCM) of songbird forebrain responds to playbacks of recorded birdsong with a large and rapid induction of the immediate early genes zenk and c-jun, both of which have been implicated in learning and memory processes. We set out to investigate the biological significance of these events by recording the electrophysiological responses of single units in the NCM of awake, unanesthetized zebra finches during song playback (Stripling, et. al., Soc Neurosci Abstr, 1994). Here we show that single units in NCM can reduce their response to a song if that song is repeated, without altering their responses to other songs. This learned discrimination develops rapidly within the first few presentations, and approaches an asymptote within ~10 minutes, when one song is presented repeatedly (~2 s of song every 10s). Of particular interest, the acquired response selectivity remains in an apparently labile state for up to 30 minutes, during which time it can be disrupted or abolished by presentations of other songs.The timecourse for consolidation of this learned discrimination parallels the timecourse for consolidation observed in other systems, and may be related to the time required to produce and transport the proteins that are synthesized as a result of the genomic response to stimulation.

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381.8

THE DEVELOPMENT OF SONG- AND ORDER-SELECTIVITY IN THE ANTERIOR FOREBRAIN OF JUVENILE ZEBRA FINCHES. M.M.Solis* and A.J.Doupe, Neuroscience Graduate Program, Keck Center, Depts of Psychiatry and Physiology, UCSF, San Francisco, CA 94143

Auditory experience of both the tutor song and the bird's own plastic song is crucial to song learning. The anterior forebrain pathway (AFP) could be a site of auditory feedback, since it is also required for normal song development, and in adult birds it contains auditory neurons that are highly selective for the bird's own song. This selectivity clearly emerges during development, because these neurons respond equally well to all song stimuli in 30d. zebra finches. To understand the origin of song-selectivity in neurons of the AFP, juvenile zebra finches were studied at an intermediate stage of development, when the sensory phase was closing, and the plastic song of the sensorimotor phase was well underway.

Single unit recordings were made in LMAN and X of 60d. male zebra finches. The acoustic stimuli presented included the bird's own plastic song (BOS), its tutor song, conspecific and heterospecific songs. BOS and tutor song stimuli were the most effective stimuli for neurons, consistently eliciting stronger responses than conspecific and heterospecific songs. Responses to BOS were usually greater than those to tutor song; however, a minority of neurons preferred tutor to BOS stimuli. A small population of neurons remained untuned, responding equally well to many song stimuli. Neurons also exhibited order-selectivity for BOS and tutor song, showing weak responses when these songs were presented in reverse. Neurons in X were often less selective than those in LMAN.

These results show that neurons in the AFP change dramatically between 30 and 60 days of age. Despite the immature quality of the plastic song, BOS selectivity was already displayed by these neurons. In addition,tutor song selectivity was found, a feature not present in younger juveniles. Finally, order-selectivity had developed. Such selective properties at an intermediate stage of song learning support an auditory role for the AFP during song acquisition. Studies of birds in which motor learning has been delayed or altered, thus interfering with the similarity between juvenile and tutor song, will clarify the origin and role of selectivity in the AFP.

381.9

SENSORY ACQUISITION IN ZEBRA FINCHES REQUIRES ACTIVATION OF NMDA RECEPTORS IN THE ANTERIOR FOREBRAIN. M.E. Basham*, E.J. Nordeen and K.W. Nordeen, Dept. of Psych. and Neurosci. Program, Univ. of Rochester, Rochester, NY 14627

Activation of NMDA receptors has been implicated in experience-dependent behavioral and neural plasticity. In zebra finches systemic injections of NMDA receptor antagonists during exposure to a song tutor impair song development. In the present study NMDA receptors in one song region, the lMAN, were selectively inactivated during restricted access to a song tutor. The results suggest that song memorization requires activation of NMDA receptors located in or near the lMAN.

Zebra finch chicks were isolated from song until 30 days of age, when they received bilateral cannulae directed at either the lMAN or the cerebellum, or were given sham surgeries. They were then visually isolated from other birds and acoustically isolated from adult male song except during tutoring sessions. Between 32 and 52 days of age all birds were exposed to an adult male tutor and his mate for 90 minutes every other day. Half of the lMAN group and all of the cerebellum group received AP5 infusions (2.5 μg in .1 μl) through the cannulae 5-15 minutes prior to tutoring. The remaining lMAN birds received AP5 infusions only on non-tutoring days. At 90 days of age songs were recorded and the birds were then sacrificed and cannula placement verified. Birds that received AP-5 infusions directed at the lMAN immediately prior to tutoring were impaired in two measures of vocal learning (% of the bird's song learned from the tutor, and % of the tutor's song learned). These birds learned significantly less than shams, cerebellum infused birds or lMAN birds infused on non-tutoring days. In other birds, AP5 infusions into the lMAN did not impair the ability to discriminate zebra finch from canary song. Taken together, these results suggest that song memorization requires activation of NMDA receptors in or near the lMAN. (supported by NIMH #MH45096)

381.10

IMMUNOCYTOCHEMICAL ANALYSIS OF ZENK GENE INDUCTION BY SONG STIMULATION IN THE ZEBRA FINCH BRAIN. **Claudio V. Mello*. Laboratory of Animal Behavior, The Rockefeller University, NY, 10021**

The ZENK gene (also known as zif-268, egr-1, NGFI-A, or Krox-24) encodes a transcriptional regulator and is induced by song playbacks in specific portions of the forebrain of songbirds. ZENK induction is highest for conspecific songs, lower for heterospecific songs, and absent for pure tones (Mello et al., 1992). ZENK expression peaks at 30 min and returns to control levels about 60 min after stimulus onset. Mapping of ZENK expression in the brain after song playbacks by in-situ hybridization reveals induction in areas closely related to auditory pathways, especially in the caudomedial neostriatum (NCM), but, surprisingly, not in the song control circuit (Mello and Clayton, 1994). The study of regulation of the ZENK gene has now been extended to the protein level. An antiserum that recognizes the carboxy-terminal of the protein predicted by the ZENK sequence has been used in an immunocytochemical assay for the ZENK protein. This antibody detects a nuclear protein in telencephalic neurons of zebra finches after song or metrazole-induced depolarization. This method is being used to determine the time course of expression and anatomical distribution of ZENK protein in the brain of zebra finches hearing playbacks of conspecific song. Defining when and where ZENK protein is present is an essential step in the search for genes that may be targets of ZENK action.

381.11

PHYSIOLOGICAL EVIDENCE FOR A SELECTIVE NEURONAL LEARNING PROCESS IN SONGBIRD AUDITORY FOREBRAIN. **D.S. Vicario*, F. Nottebohm, and S.J. Chew. Lab. of Animal Behavior, The Rockefeller University, New York, NY 10021.**

We are interested in the mechanisms that allow a bird to remember and recognize a familiar vocal signal. Neurons in the caudomedial neostriatum (NCM) of adult songbirds give vigorous auditory responses to playbacks of conspecific song, but these responses decrease (habituate) when the same novel song is repeated many times (Chew et al. 1995).

The establishment and duration of this stimulus-specific habituation depended both on the nature of the auditory stimulus and on the exposure paradigm. Repeated presentation of novel complex auditory stimuli (conspecific and heterospecific songs, conspecific male and female long calls) produced habituation, but pure tone sequences did not. When each stimulus was initially presented 200 times with an 11s inter-stimulus interval (ISI), habituation was maintained for >20h for conspecific song and female calls, 17h for male calls, and <3h for heterospecific stimuli. When short ISI's (<3.5s) were used, no habituation occurred. When the bird heard only 50 presentations of a conspecific song, habituation lasted only 6h. However, if the 50 stimuli were presented in groups of 10, spaced 10 mins apart, the habituation lasted >20h.

Related work has shown that this long-lasting neuronal memory for conspecific vocalizations depends on gene expression within NCM at critical times after exposure to a novel stimulus (Chew et al. 1995). It is possible that forgetting of specific classes of stimuli after characteristic delays may be due to the lack of induction of new genes at those times. We now predict that the manner of presentation of a stimulus also influences gene expression, and through it, memory duration. (MH40900, MH18343)

381.12

LONG-TERM NEURONAL MEMORIES FOR NOVEL CONSPECIFIC SONGS REQUIRE NEW GENE EXPRESSION IN AVIAN AUDITORY FOREBRAIN. **S.J. Chew*, E.D. Jarvis, C.V. Mello, D.S. Vicario and F. Nottebohm. Lab. of Animal Behavior, The Rockefeller University, New York, NY 10021.**

Song playbacks induce expression of an immediate early gene, ZENK, in the caudo-medial neostriatum (NCM) of songbirds; this induction is higher for conspecific than for heterospecific songs. Multi-unit recordings in NCM of zebra finches have demonstrated vigorous auditory responses that habituate to repeated presentation of a novel song stimulus; this physiological memory is long-lasting for conspecific, but not heterospecific, songs.

The present study assessed the role of new gene expression in long-term habituation to song by injecting inhibitors of RNA (actinomycin-D, or ACT) and protein (cycloheximide, or CYC) synthesis, and antisense RNA molecules into NCM at the site of electrophysiological recordings. ACT, CYC and ZENK anti-sense had no effect on short-term habituation of NCM neurons. However, ACT and CYC disrupted long-term habituation (tested 3-24h after training) when injected at 1-3h, 6-7h, 15h, and 17h after training. ZENK antisense disrupted long-term habituation when injected up to 1h after training. Control injections of ZENK sense or of anti-sense to another gene not induced by song playback, but known to be expressed in the tissue, had no effect. Immunocytochemistry showed that ACT, CYC and ZENK anti-sense injections block production of the ZENK protein in a limited subregion of NCM, but ZENK sense does not; we are now determining the specificity of this anti-sense effect. Our results show that the long-term auditory memory for conspecific songs depends on new RNA and protein synthesis in NCM at defined intervals after training, and suggest that the induction of the ZENK gene is a necessary step in the establishment of this memory. (MH40900, MH18343, GM12562)

381.13

EXPRESSION OF THE ZENK GENE IN CANARY FOREBRAIN IS IMPLICATED IN THE MEMORIZATION OF VOCAL SIGNALS THAT REGULATE GOAL-DIRECTED BEHAVIOR. E.D. Jarvis*, S.J. Chew, C.V. Mello, M.V. Rivas and F. Nottebohm. Laboratory of Animal Behavior, The Rockefeller University, NY 10021.

Birds exposed to song paired with a mild shock give fear responses to the song and eventually learn to avoid the shock by hovering in the air when they hear the song. This training induces expression of the immediate early gene ZENK in the auditory NCM-HVCM region above levels seen in yoked-unpaired controls or in birds hearing song alone. Expression in other hyperstriatal subdivisions (HD, HA, rostral HV), LPO, and Hippocampus occurred from the shock alone. We examined whether the enhancement of ZENK expression in NCM-HVCM of trained birds is related to attention or to associative learning. Birds overtrained to associate song with shock gave 80-100% avoidance responses to the song stimulus and by then presumably had little new to learn about the paired stimuli. These birds jumped precisely before the shock was given, indicating that they were paying close attention to the song. In-situ analysis of brain sections hybridized with ZENK riboprobes revealed an inverse relation between the amount of overtraining and the level of ZENK expression. Lesions in NCM-HVCM impaired learning of the avoidance response but not of the fear response. Likewise, injections of ZENK antisense RNA into NCM-HVCM impaired long-term retention of the avoidance response but not of the fear response. Taken together, our results and other published data suggest that ZENK expression in NCM-HVCM is related to higher order processing and memorization of naturally occurring communication signals that regulate goal directed behaviors.

381.14

SEASONAL REGULATION OF IMMEDIATE EARLY GENE EXPRESSION IN THE SONG CONTROL NUCLEI OF THE MALE CANARY FOREBRAIN. Fernando Nottebohm* and Erich D. Jarvis. Laboratory of Animal Behavior, The Rockefeller University Field Research Center, Millbrook, NY 12545.

Adult male canaries undergo seasonal periods of song plasticity. During the late summer/early fall new song syllables are acquired, others are modified and still others disappear. We investigated whether these changes in vocal behavior were accompanied by changes in expression of the immediate early gene ZENK, which has been implicated in the formation of song auditory memories. 1 to 2 year old male canaries were sacrificed at different months of the year. Brains were removed and in-situ hybridizations were performed with ^{35}S-ZENK riboprobes. In the fall, over 40% of the birds showed ZENK expression in the song nuclei HVC and RA at levels 2-7 times higher than the barely detectable levels seen in the spring. The highest expression occurred during the month of October which coincides with 1) the peak in the recruitment of new neurons into HVC, 2) a rise in blood testosterone levels and 3) consolidation of newly learned syllables. Because expression was seasonally higher in RA, where there is no adult neurogenesis, and also in the large X projecting cells of HVC, which are not formed in adulthood, ZENK regulation does not appear to be directly related to neurogenesis. Since HVC and RA are necessary for the acquisition and production of learned song, our observations suggest that ZENK expression in the song system is related to circuit changes that underly the long-term acquisition of new song memories.

381.15

PCR-BASED mRNA DIFFERENTIAL DISPLAY REVEALS ENRICHMENT OF ALDEHYDE DEHYDROGENASE IN THE HIGH VOCAL CENTER AND IN TWO OTHER NUCLEI OF THE SONG SYSTEM OF SONGBIRDS. N. Denisenko*, F. Nottebohm and C. Mello. Laboratory of Animal Behavior, The Rockefeller University, NY, 10021.

HVC (High Vocal Center) is necessary for the acquisition and production of learned vocalizations in song-birds. PCR-based mRNA differential display was used to screen for mRNAs enriched in the HVC of adult male zebra finches. HVC and its underlying "shelf" region were dissected out for comparison in three birds and RNAs were isolated. Approximately one third of the possible primer combinations described by Liang and Pardee (Science, 257:967-970, 1992) were used to PCR-amplify mRNAs from HVC and "shelf" samples; 23 primary candidates for differentially expressed mRNAs were detected. Using in situ hybridization to brain sections containing HVC and the "shelf" region in a secondary screening procedure, we identified six clones that were expressed at higher levels in HVC than in the rest of the forebrain. Expression of one of these clones was particularly high in HVC, high in anterior neostriatum (including lMAN), lower in RA and very low or absent elsewhere in the forebrain. lMAN and RA are part of the song system and, as is the case for HVC, are necessary for the acquisition or production of learned song. Sequence analysis revealed significant homology to aldehyde dehydrogenase (ALDH) of several species. ALDH function has been linked to catecholamine metabolism and retinoic acid synthesis. We are currently investigating the possible role of ALDH in the acquisition and production of learned song.

381.16

Synelfin mRNA Expression is Differentially Regulated in Nucleus lMAN of Zebra Finches Raised Under Acoustic Isolation

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Male zebra finches form a model of a tutor's song during a "critical period" in juvenile development. Under normal conditions, the critical period for song model acquisition ends by ~35d after hatching. The closure of the critical period may be delayed, however, if birds are raised in auditory or visual isolation from potential tutors (R.G.Morrison & F.Nottebohm, J. Neurobio., 24: 1045-1064, 1993). We recently described the identification of a gene -- synelfin -- whose expression is regulated in lMAN, a telencephalic nucleus implicated in song model acquisition (Soc. Neurosci. Abstr., 19:807, 1993). Synelfin mRNA is highest early in the critical period, but then drops sharply at 30-35d. In order to examine the linkage between the differential regulation of synelfin and the progression of the critical period, we compared the levels of synelfin mRNA in lMAN in males raised either in a normal aviary or in acoustic isolation, by means of RNA *in situ* hybridization. Pilot data indicate that birds reared in isolation had significantly higher synelfin mRNA levels in lMAN at 30d than did the normal birds (p=.01, n=2 normal birds, n=4 isolated birds). Additional replications of this experiment are currently being analyzed, and will be described. This preliminary result suggests that increased synelfin mRNA levels may be closely linked to increased functional plasticity in lMAN, and that a decrease in synelfin may be correlated with closure of the critical period for song model acquisition. The potential ability of environmental manipulations to alter synelfin expression is of particular interest, since synelfin's human homolog has recently been implicated as a molecular participant in the pathology of Alzheimer's Disease. (Supported by NIH grant NS25742)

381.17

EFFECTS OF NEUROMODULATORS ON EXCITATORY SYNAPTIC TRANSMISSION IN NUCLEUS RA OF THE ZEBRA FINCH D. J. Perkel* Div. Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

Nucleus RA is a prominent brain region crucial for vocal production and learning in songbirds. Modulation of synaptic strength in RA is a leading candidate mechanism underlying behavioral plasticity. As part of a long-term goal of understanding the cellular substrates of learning, I am characterizing synaptic transmission and putative modulators of synaptic strength in RA.

Coronal brain slices were prepared from male zebra finches just beginning to sing (30 - 45 days old), and whole-cell voltage-clamp recordings were obtained from RA neurons in those slices. Antidromic stimulation of RA output fibers permitted selective activation of recurrent collaterals, which give rise to the major class of excitatory synapse (> 75%) in RA. These synapses were compared with previously described inputs from nuclei HVc and L-MAN.

The collateral synapses in RA were found to resemble those arising from HVc fibers and to differ from L-MAN connections: application of the glutamate receptor (gluR) antagonists CNQX and APV showed that transmission is dually mediated by NMDA and non-NMDA postsynaptic gluRs; transmission was blocked by activation of $GABA_B$ receptors; and transmission was relatively insensitive to norepinephrine (NE). In addition, activation of metabotropic gluRs by bath application of 1S-3R-trans-ACPD (100 µM) reversibly blocked synaptic transmission in all three (HVc, L-MAN and recurrent) synaptic populations.

The pharmacological similarity of synapses made by collaterals in RA to those made by HVc fibers is consistent with a major role for intrinsic RA circuitry in the primary motor pathway for song production. These findings extend previous observations of differential effects of neuromodulators on inputs to RA; in particular, they suggest additional complexity of modulation by selective or global adjustment of synaptic strength.

381.18

DEVELOPMENTAL STUDIES OF GLUTAMATE RECEPTOR AND PEPTIDE IMMUNOREACTIVITY IN THE ZEBRA FINCH SONG SYSTEM. G.Carrillo and A.J.Doupe*, Psychiatry/Physiology Depts., UCSF, San Francisco, CA 94143

Sensory exposure during a critical developmental period is essential for normal song learning in songbirds. During this period, lesions of the anterior forebrain pathway (AFP) disrupt song learning, while similar lesions in adults have no effect. To understand further the changing role of this pathway in song learning, we studied the development of neurotransmitters in the AFP, using immunohistochemical staining for the R1 subunit of the NMDA receptor and for two peptide neurotransmitters, leu-enkephalin and Substance P.

In 20d old male zebra finches, LMAN cell bodies and their dendrites showed intense NMDAR1 labelling, and were indistinguishable in immunoreactivity from the surrounding neostriatum. In contrast, in adult finches LMAN neurons showed a striking reduction in R1 immunolabelling, and stained much less intensely than the surrounding cells. In 40d birds, R1 immunoreactivity in LMAN was intermediate between that of 20d and adult birds. This result is in agreement with previous studies of LMAN using MK-801 autoradiography (Aamodt et al, 1993). Furthermore, in 20d old finches, the entire parolfactory lobe (LPO), including Area X, was also immunoreactive for R1. By adulthood, however, Area X showed a marked decrease in immunolabeling relative to the surrounding LPO. This developmental decline in receptor labelling in both LMAN and X may be linked to the decreased role of the anterior forebrain sensory circuit as birds mature and learn. Peptide immunoreactivity showed similar developmental changes in some song nuclei. Area X and RA were more strongly enkephalin-immunoreactive in 20 and 35d old birds than in adults, while HVc, LMAN, and NIf were well-labelled at all ages. Substance P immunoreactivity was prominent in Area X at all ages, but appeared to decrease in intensity with age. Increased knowledge of the transmitters involved in the song system and of how they change during ontogeny should lead to a more mechanistic understanding of song acquisition and suggest manipulations to assess the role of these molecules in development and learning.

Supported by Searle Scholars and the McKnight Foundation

381.19

DEVELOPMENTAL INCREASES IN TYROSINE HYDROXYLASE IMMUNOREACTIVITY AND CATECHOLAMINE LEVELS IN MALE ZEBRA FINCH SONG NUCLEI. J.A.Soha*[1], T.Shimizu[2], and A.J.Doupe[3]. [1]Dept. of Neurobiology, Physiology and Behavior, UC Davis, CA 95616, [2]Depts. of Psychology and Surgery, Univ. of So. Florida, Tampa, FL, 33620, and [3]Keck Center and Depts. of Psychiatry and Physiology, UCSF, San Francisco, CA 94143

Several of the interconnected brain nuclei which mediate song learning and production in the male zebra finch (*Taeniopygia guttata*) have been shown to contain catecholamines (CA), both by direct chemical assay and by immunolocalization of tyrosine hydroxylase (TH), the synthetic enzyme for CA. Neuromodulatory roles have been proposed for CA both in the adult song system and in a number of other neural systems, particularly during experience-dependent plasticity. We therefore investigated the appearance of CA in song nuclei of male zebra finches during posthatch development, i.e. the period during which song learning occurs. Using immunohistochemical staining, we examined the intensity of TH-immunoreactivity in the song nuclei HVc, LMAN, RA, and Area X in young males aged 20, 35, and 60 days as well as in adults (>90 days). We also visualized catecholamines directly in Area X using CA histofluorescence. Both TH immunoreactivity and CA histofluorescence were initially low in Area X relative to the surrounding parolfactory lobe (LPO), and then increased during development to become more intense than LPO by day 60-90. Similarly, TH immunoreactivity in HVc was initially low relative to the surrounding neostriatum, then increased during development to become more intense than the surround by day 60. RA and LMAN also showed large increases in TH immunostaining over the same period. These results show that CA levels increase in song nuclei during development and thus raise the possibility that these transmitters play a role in the development of the song system and/or in song learning. *Supported by the Markey Charitable Trust, NSF IBN9209538, and a Caltech Summer Undergraduate Fellowship.*

381.20

CHANGES IN CATECHOLAMINE LEVELS AND TURNOVER IN THE BRAINS OF MALE ZEBRA FINCHES OVER DEVELOPMENT. C.F. Harding*, S.R Barclay, and S. Waterman. Biopsychology Program, Hunter College, CUNY, New York, NY 10021

Catecholamine (CA) levels and turnover were quantified in ten behaviorally-relevant brain nuclei (6 vocal control [VCN], 2 auditory [AN], 2 hypothalamic [HN]) at four critical points during development of the VCN and the period of song learning. Aviary-housed finches were sacrificed at 25, 35, 55, and 90 days of age. Some birds were pretreated with α-methyl-para-tyrosine (αMPT) to allow estimation of CA turnover, and CA levels in microdissected nuclei were quantified using high performance liquid chromatography with electrochemical detection.

Patterns of CA function in VCN and AN were quite different from those in HN in which CA function showed gradual changes and no striking peaks. Norepinephrine (NE) levels and/or turnover changed significantly over development in 5 VCN, 1 AN, and 1 HN. In all VCN and AN in which significant changes were found, NE levels and/or turnover showed a striking peak at day 25, suggesting a possible role in song memorization. DA levels and/or turnover also varied significantly over the 4 time points in 5 VCN, 2 AN, and 1 HN. However, DA peaked later than NE--between days 35 and 55, suggesting a possible role in vocal practice.

In contrast to hypothalamic areas, peak NE and DA levels and turnover rates in most VCN and AN during development were dramatically higher than those seen in these areas in adulthood, reaching levels never seen in adults. In four cases, when CA synthesis was blocked by use of αMPT, tissue levels of NE and/or DA in these areas were exhausted within an hour.

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NEUROETHOLOGY: SONGBIRDS II

382.1

TESTOSTERONE MODULATION OF $GABA_A$ RECEPTOR DENSITY IN THE ZEBRA FINCH VOCAL CONTROL SYSTEM W.R. Perlman*, J.M. Casto, D.J. Bernard and G.F. Ball Dept. of Psychology, Behavioral Neuroendocrinology Group, Johns Hopkins University, Baltimore, MD 21218

Zebra finches (*Taeniopygia guttata*) possess a neural circuit consisting of discrete nuclei that mediate the learning and production of song. The production of song in males is influenced by testosterone (T): castration greatly reduces song rate. In order to identify neurotransmitter systems involved in the production of song and their possible modulation by T, we mapped the distribution of $GABA_A$ receptors with the use of an *in vitro* quantitative receptor autoradiography procedure employing the $GABA_A$ agonist [^{3}H] muscimol as the ligand. Receptors were labeled in adult (> 90 days) male and female intact birds and in castrated, and T-treated castrated, male finches. The highest densities of $GABA_A$ receptors were detected in the paleostriatum primitivum, the lateral archistriatum, and the granule layer of the cerebellum. Within the vocal control system the High Vocal Center (HVC) and area X could be defined by their high receptor density in comparison to the surrounding structures. The robust nucleus of the archistriatum and the magnocellular nucleus of the neostriatum were characterized by receptor densities that are among the lowest detected anywhere in the telencephalon. Significant effects of castration were detected in HVC, an area that contains androgen receptors, but not in other song control areas. T-treated castrates exhibited a higher receptor density than untreated controls. Previous studies in birds and mammals have demonstrated that steroid hormones influence $GABA_A$ receptor density in steroid-sensitive areas of the hypothalamus. The localization of $GABA_A$ receptors in vocal control nuclei and their modulation by T suggests that GABA may be involved in song production.

382.2

LATERALIZATION OF SYRINGEAL FUNCTION IN TESTOSTERONE-TREATED FEMALE CANARIES. R. S. Hartley*, M. S. Chinn and N. F. E. Ullrich. Biology Department, Seattle University, Seattle, WA 98122.

Male canaries (Serinus canaria) produce most of their song syllable types on the left side of the syrinx, the two-sided vocal organ. Female canaries, which seldom sing, produced small repertoires (7-14 syllable types per bird) when treated with testosterone propionate (T). To learn whether left syringeal dominance occurs in T-treated female canaries, we cut the tracheosyringeal nerve on either the right or left side, to disrupt syllable production on that side of the syrinx. Pre- and post-operative syllable types were recorded and identified visually on spectrograms by their frequency structures and repetition rates. Right nerve cuts had minor effects on the syllable repertoires of three birds (88%, 89% and 92% of syllable types remained), whereas left nerve cuts had severe effects on the syllable repertoires of three birds (22%, 27% and 29% of syllable types remained). Two additional birds, one with a right nerve cut and one with a left nerve cut, had partially altered syllable repertoires (64% and 50% of syllable types remained, respectively). We conclude that most, but not all, female canaries generate the majority of their syllable types with the left syrinx.

382.3

SEX DIFFERENCES IN VOCAL BEHAVIOR IN BUDGERIGARS ARE NOT ASSOCIATED WITH A SEX DIFFERENCE IN THE VOLUME OF THE TELENCEPHALIC VOCAL CONTROL NUCLEUS, LPOm A. A. Nespor*, G. F. Ball & R. J. Dooling. Dept. Psych., Univ.of Maryland at College Park, College Park, MD 20742 & Dept. Psych., Johns Hopkins Univ., Baltimore, MD 21218.

Budgerigars (*Melopsittacus undulatus*) produce a complex learned vocal repertoire that includes a long rambling song called warble. Both males and females warble, but there are marked sex differences in the amount of song produced. Males warble daily at a high rate, while females rarely sing. Previous investigations on songbirds have shown that sex differences in vocal behavior are associated with sex differences in the volume of song control nuclei with the volume usually being larger in males. Such relationships between sex differences in brain and behavior have not been investigated in any psittacine species such as the budgerigar. The goal of the present study is to determine if budgerigars possess a sex difference in the volume the magnocellular nucleus of the parolfactory lobe (LPOm) a telencephalic vocal control nucleus. This nucleus was chosen because it appears to be analogous to area X, a subdivision of the parolfactory lobe in the song bird vocal control circuit, that exhibits a large sex difference in volume in many species. We employed two techniques to delineate the boundaries of LPOm and re-construct its volume. Sections (25μm) were collected from male and female budgerigars and either Nissl stained or were labeled for muscarinic cholinergic (MC) receptors with the use of an in vitro autoradiography procedure. The MC antagonist, N-methyl scopolamine served as the ligand. As previously reported by Striedter (1994 JCN 343:35-56) the boundaries of LPOm were apparent in the Nissl stained sections by the more darkly staining large cells evenly interspersed among the more numerous small cells characteristic of the rest of the LPO. In the autoradiogram, the boundaries of LPOm were defined by the higher density of MC receptors in comparison to the surrounding LPO (Ball,1994 Brain, Behav, Evol 44:234-246). When the volume of LPOm was re-constructed with either histochemical marker, no significant sex difference in volume was detected. Thus, sex differences in vocal behavior in budgerigars are not associated with differneces in LPOm volume.

382.4

SEASONAL CHANGES IN THE VOLUME OF SONG CONTROL NUCLEI IN FREE-LIVING MALE AND FEMALE EUROPEAN STARLINGS

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Temperate zone songbirds sing most during the breeding season and less often at other times of year. Brain nuclei in the neural circuit controlling song learning and production undergo changes in morphology that parallel these behavioral changes. In the laboratory, birds housed on long, spring-like photoperiods have larger song control nuclei than birds on short, winter-like day lengths. Long days also induce increases in circulating testosterone (T) levels, and the observed volume changes may be mediated by T-dependent mechanisms. Because no studies to date have systematically investigated plasticity in the song control system under natural conditions, it is unclear to what extent the volume changes observed in the lab reflect changes as they occur in the wild. In the present study, we measured the volume of song nuclei in adult male and female European starlings (*Sturnus vulgaris*) collected from the field every month from March 1994 through February 1995. Based on laboratory results, we predicted that song nuclei would be largest during the breeding season when T levels were elevated. The volume of three song nuclei, the high vocal center (HVC), nucleus robustus archistriatalis (RA), and area X were significantly larger in males than in females in every month of the study. In both sexes, area X volume decreased during the breeding season (May-July), was large during the late summer and fall when T levels were low, and decreased again in volume in January and February when T levels were increasing in males. The volumes of HVC and RA did not change significantly over the study period, although the largest volumes for both nuclei were observed in April when T levels peaked. In sum, the form and magnitude of changes in volume of song nuclei differ in laboratory and field contexts in European starlings.

382.5

HORMONAL AND BEHAVIORAL CORRELATES OF SEASONAL PLASTICITY IN THE SONG NUCLEI OF A WILD SONGBIRD. G.T. Smith*†, E.A. Brenowitz†‡, M.D. Beecher†‡, S.E. Campbell‡, and J.C. Wingfield† Departments of †Zoology (NJ-15) and ‡Psychology (NI-25), University of Washington, Seattle, WA 98195

Song control nuclei are larger in captive male songbirds that are housed in breeding, vs. nonbreeding, conditions. Few studies have asked whether these changes occur in wild songbirds, and none have asked what the behavioral correlates of these changes are in species that lack adult song learning. We measured seasonal changes in the song nuclei and song behavior in wild song sparrows (*Melospiza melodia*) to address these questions.

We captured adult male song sparrows at four times of year: (1) March 1-31 (prebreeding), (2) April 1- May 15 (breeding), (3) September 1- October 15 (postmolt), and (4) December 1-31 (late fall). We collected blood samples to measure plasma testosterone (T) concentrations. We perfused the birds and measured the size of their song nuclei. We also recorded the song repertoires of the same five adult male song sparrows at each time of year to determine if there were changes in repertoire size, within-song type stereotypy, or syllable morphology.

We found seasonal changes in the size of song nuclei that were temporally correlated with changes in plasma T concentrations and with changes in within-song type stereotypy, but not repertoire size. These results demonstrate seasonal changes in the song nuclei of wild songbirds and suggest that seasonal plasticity in the song nuclei is associated with seasonal changes in song stereotypy. GTS is a HHMI predoctoral fellow.

382.6

PHOTOPERIOD REGULATION OF CELL DEATH IN ADULT AVIAN TELENCEPHALON. J.R. Kirn* & H. Schwabl. *Biology Dept., Wesleyan University, Middletown CT 06459, and Rockefeller University Field Research Center, Millbrook, NY 14857.

The High Vocal Center (HVC) in adult male canaries exhibits neuronal loss and replacement at a higher rate in fall than in spring (Kirn et al., PNAS, 91: 7844-48, 1994). In the present experiment, the effect of day length on HVC cell survival was explored as a step toward addressing the possibility that photoperiod regulates the seasonal death and recruitment of neurons. All birds experienced a photoperiod regime corresponding to natural, seasonal changes in photoperiod (in NY State) for at least 1 yr. prior to the experiment. In May, adult males (1-2 yrs. old) were either transferred from long days (14.5 hr / 9.5 hr L/D) to short days (8 hr / 16 hr) or were kept on long days for 2 weeks. Birds were then overdosed with anesthetic, perfused with 0.9% PBS followed by 4% paraformaldehyde in PBS and their brains processed for light microscopy. The number of pyknotic, degenerating cells/mm^2 in HVC was quantified in 10-12 Nissl-stained 6 µm sections from each bird. The density of dying cells was nearly 5 times higher in short day birds (mean ± SE =1.41 ± 0.31; n=5) compared to long day birds (0.29 ± 0.13; n=5; $t(8) = 3.33$; $p = .01$). These results indicate that changes in photoperiod can induce HVC cell death and suggest further that seasonal cell death in the HVC of adult canaries is regulated by naturally occurring variation in photoperiod.

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382.7

BRAIN SPACE FOR LEARNED SONG IN BIRDS DEVELOPS INDEPENDENTLY OF SONG LEARNING. E. Brenowitz*, K. Lent, and D. Kroodsma. Depts. of Psychol. & Zool., Univ. of WA, Seattle, WA 98195 & Dept. of Biology., Univ. of MA, Amherst, MA 01003.

In numerous species of songbirds, individuals or species that sing larger numbers of song types have larger song control nuclei in their brains. The direction of the cause and effect relationship between the complexity of song behavior and brain space is unknown, however. The hypothesis that birds that learn large song repertoires develop large song nuclei was therefore tested with a songbird, the marsh wren (*Cistothorus palustris*).

Males were hand-reared and tutored in the laboratory with either small (n = 8 males heard 5 song types) or large (n = 8 males heard 45 song types) repertoires. When the birds were adults, the number of song types each male sang was first determined. The birds were then sacrificed and the volume and cellular attributes of the forebrain song nuclei HVC and RA were measured from Nissl-stained sections.

Adult males in the small repertoire tutor group produced 5-6 song types, and males in the large repertoire group produced 36-47 song types. Despite these large behavioral differences in the size of their learned song repertoires, the two groups did not differ in either the volumes of HVC and RA or in neuronal size, number, or density within these nuclei. These results suggest that the relationship between behavioral song complexity and brain space found in this and other species develops largely independently of early song learning experience and the later production of those songs.

382.8

THE AUDITORY PATHWAY THROUGH NUCLEUS BASALIS IN OSCINE SONGBIRDS DOES NOT APPEAR TO BE CONNECTED TO THE VOCAL MOTOR SYSTEM. G.F. Striedter* and S. Young. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Oscine songbirds and parrots have independently evolved the ability to learn complex vocalizations and have evolved different pathways to convey auditory feedback to the vocal motor system. Specifically, previous anatomical studies have suggested that auditory information is conveyed to the vocal motor system via Field L in songbirds, but via nucleus basalis in parakeets. The auditory pathways through Field L and nucleus basalis coexist in parakeets, but it was not known whether songbirds also possess an auditory pathway through nucleus basalis.

We therefore injected biotinylated dextran into the physiologically identified auditory portion of nucleus basalis (n=3) in zebra finches. Cells were retrogradely labelled in the intermediate nucleus of the lateral lemniscus (LLi) bilaterally and terminal arborizations were labelled in the contralateral nucleus basalis. This projection pattern suggests that individual cells in LLi project bilaterally to the caudal portion of nucleus basalis. Terminal arborizations were also labelled in the ipsilateral frontal neostriatum adjacent to the caudal nucleus basalis. Tracer injections that included this portion of the frontal neostriatum (n=2) labelled terminal arborizations in the caudal pole of the ipsilateral neostriatum. An injection into this portion of the caudal neostriatum retrogradely labelled cells in the ipsilateral frontal neostriatum, confirming the hypothesis that the auditory pathway courses from nucleus basalis through the frontal neostriatum to the caudal neostriatum. None of these injections yielded labelling in the nuclei of the so-called oscine song system. These data suggest that an auditory pathway through nucleus basalis exists in songbirds but does not play a major role in the control of vocalization. The data also support the hypothesis that parrots and songbirds are using non-homologous auditory pathways to provide auditory feedback to the vocal motor system.

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382.9

A PARALLEL CIRCUIT LINKING IMAN AND AREA X IN THE AVIAN SONG SYSTEM A.D. Perera, F.Hunger, M. Gahr and J. M. Wild*, Dept. of Anatomy, Univ. of Auckland, New Zealand, and Max Planck Institute, Seewiesen, Germany.

The "recursive loop" that links the thalamic nucleus DLM to IMAN and Area X in the forebrain is thought to be necessary for song learning in the developing bird but not for song production in the adult. The neuronal pathways linking these nuclei have been described, but our data suggest that the previously published circuit may be incomplete. The experiments show that neurons in IMAN that send axons to RA also send axons to Area X, thereby establishing a "parallel" link between IMAN and Area X. Injections of biotinylated dextran amine (BDA) or fluorescent tracers into different parts of Area X in juvenile and adult zebra finches or canaries retrogradely labeled cells in IMAN. The BDA injections also produced terminal fields in different parts of RA. In comparison, injections into RA produced retrogradely cells within IMAN but only a very sparse terminal field within Area X. This latter observation was in sharp contrast to injections into IMAN which resulted in dense terminal fields in both RA and Area X. These data suggest that the nature of the axonal terminations of IMAN neurons in RA differs from that of the terminations in Area X. Furthermore, these results imply that only some of the IMAN neurons that project to Area X also send axons to RA. Double-labelling experiments are required to determine the proportion of IMAN neurons that send axons to both of these two nuclei. (Supported by the Whitehall Foundation, Inc.)

382.10

DIFFERENT KINDS OF PLASTICITY IN THE ZEBRA FINCH FOREBRAIN.

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The hyperstriatum accessorium (HA), the medial neo- hyperstriatum (MNH), the lateral neo- hyperstriatum (LNH) and the archi- neostriatum caudale (ANC) of the zebra finch forebrain are activated in arousing situations (Bischof and Herrmann, 1986, 1988).

Within these four areas changes in spine density due to rearing conditions and the first courtship encounter can be observed by means of the Golgi method.

Rearing birds in isolation *decreases* the density of spines (compared to social rearing) within HA and ANC, and *increases* the spine density within MNH and LNH. Short social contact (7 days) after isolated rearing *enhances* spine density in HA and ANC, and *reduces* the density of spines in MNH and LNH. The spine density returns to isolation values within HA and ANC, but not within MNH and LNH if the birds are isolated again after social contact. The acoustic field L and the visual ectostriatum are not activated by arousal situations, and do not show any alterations.

Our results show that plastic changes due to the social environment can only be observed in areas activated in arousing situations, and that both, reduction and enhancement, is possible. We assume that the complexity of the HA and ANC neuronal nets mirrows the complexity of the social environment. The reduction of spines within MNH and LNH, in contrast, may be the anatomical manifestation of an imprinting process which has been shown to occur in the same experimental situation as we used in our present study (e.g. Bischof and Clayton, 1991).

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382.11

DEVELOPMENT OF SONG SYSTEM STRUCTURES IN NORMAL AND SONG DEPRIVED ZEBRA FINCHES C. E. Collins*, T. E. Ahmad, S. Das and T. J. DeVoogd
Developmental Neuroscience Group, Uris Hall, Cornell University, Ithaca, NY 14853

Song development in zebra finches (*Taeniopygia guttata)* takes place during a juvenile sensitive phase. Songs heard during this early sensitive phase are used as models for the song the young male bird will later produce. As adults, only male zebra finches produce song.

To address song-specific neural development, zebra finches were raised deprived of the opportunity for song learning and compared with finches which had learned song normally. Opportunity for song learning is manipulated by removing adult males from the aviary when the oldest hatchling is 5d old. Volumes of two song structures, HVC (high vocal center) and RA (robust nucleus of the archistriatum) were measured to assess gross anatomical changes associated with song development. The volume of a non-song-related telencephalic area, ectostriatum, was measured as an experimental control. Zebra finches were perfused at 35d and 55d of age. Volume data were collected from 40 µm thick, Cresyl violet stained sections using NIH Image software.

Preliminary data from 55d old zebra finches (N=12) suggest no volumetric differences in song structure HVC, but do indicate significant differences in RA, with the deprived finches showing smaller RA volumes. Measures of control area, ectostriatum, indicate no significant differences in volume, suggesting no general telencephalic effects of song deprivation. Recent research on white-throated sparrows (*Zonotrichia albicollis*) demonstrate variations in RA volume with amount of song production (DeVoogd et al, 1995). We are currently investigating whether observed differences in RA volume between song-deprived and normally raised zebra finches correlate with amount of song produced.

382.12

DORSAL THALAMUS OF ZEBRA FINCHES RECEIVES INPUTS FROM HYPOTHALAMUS AND ARCHISTRIATUM E. F. Foster *, Ritvik P. Mehta and S. W. Bottjer. USC, Los Angeles, CA 90089-2520

The medial magnocellular nucleus of the anterior neostriatum (mMAN), is one of several afferent inputs to the High Vocal Center (HVC) in songbirds. We have previously shown that mMAN of zebra finches receives its major source of afferent input from the posterior dorsomedial nucleus of the thalamus (DMP). To determine the trans-synaptic afferent inputs of DMP, injections of the fluorescent dye, DiI, were made in DMP of adult male zebra finches. Our results confirm the existence of a projection from DMP to mMAN, which travels within the lateral forebrain bundle (FPL). In addition, DMP projects to the area of the medial neostriatum immediately dorsal and ventral to mMAN. Sparse fiber label was visible in the ventro-medial aspects of the parolfactory lobe (LPO), and scattered retrogradely labeled cells were seen dorsal to mMAN in accessory hyperstriatum (HA). Labeled cells were also seen in the lateral septal nucleus (SL), and within the lateral forebrain bundle (FPL) at the level of the septum. Many labeled cells were seen in the dorso-lateral region of the archistriatum immediately lateral to the robust nucleus of the archistriatum (RA) in a region of dorsal archistriatum (Ad), that receives input from the shell region of lMAN. In the diencephalon, retrogradely labeled cells were visible in a region of the hypothalamus corresponding to the external cellular stratum (SCE). Scattered labeled cells were also seen in the brainstem at the level of the pons. These results have not yet been confirmed in the anterograde direction, however, they strongly suggest that DMP is a source of convergent inputs to the song control system via the DMP-to-mMAN-to-HVC pathway. These results are of particular interest because they are the first report of hypothalamic regions in communication with song control regions.

382.13

EVIDENCE FOR A NOVEL REAFFERENT PATHWAY IN THE VOCAL MOTOR SYSTEM OF SONGBIRDS • G.E. Yates*, D.S. Vicario, & F. Nottebohm Lab. of Animal Behavior, The Rockefeller University, New York, NY 10021.

Songbirds learn their song by reference to auditory information. However, once song is learned, deafening has no immediate effect on the stereotypy of the learned repertoire. We wanted to test the possibility that the efferent pathway for song production receives other feedback about ongoing motor commands. The efferent pathway goes from the High Vocal Center (HVC) to the robust nucleus of the archistriatum (RA), which in turn innervates mesencephalic and medullary nuclei involved in vocalization. Neural tract tracers (biotinylated dextran amines, rhodamine-linked dextran amines, and fluorogold) were used to show that the RA of adult male canaries sends a strong projection to a part of the dorsal thalamic complex (previously named DMP), which includes many neurons that innervate the medial portion of the magnocellular nucleus of the anterior neostriatum (mMAN), which in turn innervates HVC. A weaker projection from RA was seen to another dorsal thalamic nucleus, DLM. Initial results indicate that this projection to the dorsal thalamus originates in the dorsal subregion of RA, which is known to project preferentially to the midbrain and to a lateral medullary region involved in respiratory control of song. A similar pattern of connections was seen in zebra finches, although the projection from RA to the dorsal thalamic complex does not appear to be as robust. Thus, HVC may receive feedback about specific aspects of the motor program it generates via the loop through RA, DMP and mMAN. This feedback pathway could be essential for song learning or production.

382.14

CONNECTIONS BETWEEN VOCAL CONTROL NUCLEI AND THE BASAL FOREBRAIN IN THE BUDGERIGAR. W. S. Hall*, K. K. Cookson, J. T. Heaton, S. E. Durand, W. Liang and S. E. Brauth. Dept. Psychology, Univ. MD, College Park, MD.

We used pathway tracing, acetylcholinesterase (AChE) histochemistry, choline acetyltransferase (ChAT) and enkephalin (ENK) immuno-histochemistry to investigate basal forebrain input to four vocal control nuclei in the budgerigar: the robust nucleus of the archistriatum (RA), higher vocal center (HVC) and oval nuclei of the hyperstriatum ventrale (HVo) and anterior neostriatum (NAo). HVo and NAo contain high levels of AChE, ENK, and many ChAT fibers and puncta. RA and HVC contain moderate levels of AChE, ENK, and some ChAT fibers.

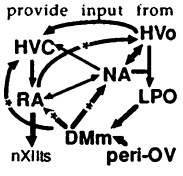
The ventral paleostriatum (VP) is a likely source of both cholinergic and enkephalinergic input to these vocal control nuclei. VP contains many ChAT and ENK immunoreactive fibers and somata. Tracer injections into RA and HVC retrogradely labeled neurons within VP, and a tracer injection into VP anterogradely labeled fibers in the rostral forebrain, including the regions of vocal control nuclei. We hypothesize that VP is an important source of both cholinergic and enkephalinergic input to vocal control nuclei.

VP lesions in nestlings disrupt the transition from unlearned food begging calls to flexible call learning (Brauth, et al., 1994). Basal forebrain projections to vocal control nuclei may provide an important input for vocal learning.

382.15

ORGANIZATIONAL FEATURES OF BUDGERIGAR VOCAL CIRCUITRY RELATIVE TO SONGBIRDS: A DISTRIBUTED VERSUS A HIERARCHICAL SYSTEM. S. E. Durand*, I.T. Heaton and S.E. Brauth. Dept. of Psychology, UMCP, College Park, MD, 20742

Vocal control circuitry of the oscine forebrain consists of a direct and an indirect pathway onto the robust nucleus of the archistriatum (RA). These two pathways are interconnected but are unidirectional and unilaterally organized. The indirect pathway onto RA incorporates nuclei within, respectively, the lobus parolfactorius (LPO), dorsal thalamus and anterior neostriatum (NA). Absence of a comparable circuit in budgerigar has been proposed as a distinguishing feature of parrot vocal pathways (Streidter, G.F., 1994, JCN 343:35-56). Using pathway tracing we have identified at least 3 such circuits by which parrot RA is connected to forebrain nuclei other than parrot "high vocal center" (HVC). These circuits incorporate nuclei within LPO, NA and dorsal thalamus, that appear comparable to oscine vocal control nuclei, as well as additional cell groups: the oval nucleus of the anterior hyperstriatum ventrale (HVo) (Streidter, 1994) and a peri-n. ovoidalis (OV) region that may provide input from accessory auditory pathways. Thus, it is not the absence of a comparable circuitry that distinguishes the parrot system but rather an organization that is distributed and more elaborate. DMm: magnicellular nucleus of the dorsomedial thalamus; n XIIts: tracheosyringeal portion of the hypoglossal nucleus; * bilateral pathway



382.16

LMAN LESIONS DECREASE INDUCED SONG PLASTICITY IN THE ADULT ZEBRA FINCH. H. Williams* and N. Mehta. Biology Department, Williams College, Williamstown, MA 01267.

Zebra finch males raised communally crystallize song at 90 days and do not change their song thereafter. However, changes to adult song do occur after denervation of the vocal organ (syrinx). These changes can take the form of silenced song syllables (syllables that disappear from the song but leave a silent interval in their place), deleted song syllables (syllables removed from the song with no remaining silent interval), and added song syllables (Williams & McKibben, 1992, Behav. Neur. Biol. **57**:67-78). We bilaterally lesioned LMAN, a nucleus that is required for song development but not for song maintenance in adults (Bottjer et al., 1984, Science **224**: 901-903), and subsequently denervated the right half of the syrinx of adult male zebra finches in order to determine whether adults can change their songs in the absence of LMAN.

Neither LMAN lesions nor sham operations were followed by changes in the songs of adult male zebra finches followed for 18 weeks postop. Five birds that received LMAN lesions followed four weeks later by right-side syringeal denervation silenced a total of four syllables over the course of 14 weeks but neither deleted nor added song syllables. Five birds that received sham surgery followed four weeks later by right-side syringeal denervation silenced 1 syllable, deleted 4 syllables, and added 6 syllables to their songs. Hence the song changes that require alterations in the timing of the respiratory pulses that drive phonation did not occur unless LMAN was intact. These results indicate that adult zebra finches that have crystallized their songs can reactivate the branch of the song system that normally affects song only during development.

Supported by NIH:DC00553 and the Essel and Hughes Foundations

382.17

LESIONS OF A VOCAL CONTROL NUCLEUS IN THE BUDGERIGAR DISRUPT PRODUCTION BUT NOT PERCEPTION OF CONTACT CALLS. J.T. Heaton*, M.L. Dent, R.J. Dooling & S.E. Brauth. Univ. of Maryland, College Park, MD 20742.

In budgerigars (*Melopsittacus undulatus*), as in songbirds, the expression of learned features of calls and warble song depend on a robust nucleus of the archistriatum (RA) which receives input from a higher vocal center (HVC). In songbirds, some HVC and RA neurons respond selectively to elements of the birds own song, and there is evidence that lesions of HVC result in perceptual deficits involving species-typical vocalizations. We used standard operant conditioning procedures to test budgerigars on several different perceptual tasks involving learned vocal signals before and after bilateral lesion of RA. First, we measured detection and discrimination thresholds for simple tones. Then we examined more difficult discriminations involving natural and synthetic species-specific contact calls. We also tested RA and Field L lesioned birds on a recognition task to determine whether lesioning affected a bird's ability to remember - not just discriminate - new calls.

Birds receiving RA lesions showed the expected disruption in contact call production; however, lesions had only small, insignificant effects on pure tone thresholds and call discrimination tests. Lesioned subjects continued to perform normally on difficult discriminations between natural contact calls and computer synthesized analogs. In addition, RA lesioned birds required the same number of trials to recognize new calls as intact birds, and subsequent Field L lesions caused only transient deficits in performance. These results demonstrate that the processing of species-specific contact calls in budgerigars does not require an intact song circuit. Budgerigars may differ from songbirds in this regard. Supported by Grants MH-10417 to JTH, NIH DC-00198 and MH-00982 to RJD, and MH-40698 to SEB.

382.18

EFFECTS OF LESIONING NUCLEUS INTERFACIALIS ON ADULT ZEBRA FINCH SONG. E. T. Vu*, Y. Kuo, and F. S. Chance. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

The nucleus interfacialis (NIf) in the songbird forebrain is thought to play a role in the control of learned vocalizations because the firing rate of neurons in NIf is elevated during singing and is time-locked to vocalizations (McCasland, 1987). We have tested the necessity of NIf in adult song control by lesioning it bilaterally with the neurotoxin ibotenic acid (6 µg/µl), which spares fibers of passage.

Animals with more than about 70% of NIf lesioned bilaterally (including two with complete bilateral lesions) produced altered songs as soon as they were recorded on the second day following surgery. Both the acoustic structure of individual song notes and the stereotyped sequencing of notes were compromised. However, in all cases (N = 16), a stereotyped sequence of notes returned within 10 days after lesioning. The acoustic structure of the notes recovered fully. In some animals, certain notes were sung less frequently 10 days after lesioning than before the lesion, resulting in a consistently shorter song phrase (or motif) than before. This relatively rapid song recovery was not mediated by auditory feedback because it was also observed in deafened animals (N = 7).

In some animals, the sparing of fibers of passage in the lesion area was confirmed by injecting a retrograde label, biotinylated dextran amine, into nucleus HVC, to which both NIf and the thalamic nucleus Uvaeformis (Uva) project. Uva fibers travel near and through NIf before terminating in HVC. A normal number of Uva neurons were retrogradely labeled in these NIf-lesioned animals.

These results suggest that neurons in area NIf do not play a necessary role in generating the song of adult zebra finches. We are investigating the nature of the transient impairment of song following NIf lesioning, and the process of relatively rapid song recovery. We are also determining whether NIf neurons are important for young birds during the process of song learning by vocal practice.

382.19

EFFECTS OF HVC LESIONS ON MOTOR CORRELATES OF COWBIRD SONG. S.L. Rich*. Program in Neural Science, Indiana University, Bloomington, IN 47405.

Adult brown-headed cowbirds (*Molothrus ater ater*) produce 2 - 6 stereotyped songs. Each song type is composed of 2-4 stereotyped expiratory bursts, corresponding to groups of notes (note clusters) and a final whistle. Song production is thought to depend on a telencephalic pathway including a "higher vocal center" (HVC).

Subsyringeal air sac pressure and vocal output were recorded in male cowbirds before and after receiving electrolytic, unilateral lesions within the rostral half of HVC. These lesions affected song production by distorting: 1) respiratory pressure patterns 2) temporal patterns of song elements and 3) the acoustic structure. The best song attempts during the first 2 days after the lesion included only one partially intact note cluster accompanied by a pressure pattern which was stereotyped but did not match pre-operative patterns. The remaining note clusters and/or the final whistle were absent. During the first 3 days after the lesion vocal intensity and sound frequency decreased and acoustic structure deteriorated until day 3 when the characteristics of preoperative song were unrecognizable. The acoustic structure and temporal patterns of the song then began to improve. By day 5 song often included 2 note clusters followed by a final whistle each accompanied by a stereotyped pressure pattern. Within the improved note clusters, notes produced by the side of the syrinx ipsilateral to the lesion were often absent or distorted while notes produced by the intact side were less affected. Throughout the post-lesion period, attempts to sing typically began with 1 or more silent expiratory bursts. Supported by NS 29467.

382.20

SYRINGEAL MUSCLE ACTIVITY DURING SONG IN BROWN-HEADED COWBIRDS. S.E. Allan* and F. Goller. Medical Sciences Program and CISAB, Indiana University, Bloomington, IN 47405.

Song in adult brown-headed cowbirds (*Molothrus ater ater*) consists of 2-3 bilaterally generated introductory note clusters, separated by inspirations, followed by a high frequency whistle (6-12 kHz) produced on the right side. We recorded electromyographic activity in up to 4 syringeal muscles together with subsyringeal air sac pressure and vocal output in 8 spontaneously singing cowbirds.

During the introductory note clusters, EMG activity in dorsal muscles (*musculus tracheobronchialis dorsalis* and *m. syringealis dorsalis*) is high during periods of full ipsilateral adduction. This activity is particularly striking at the beginning of each expiratory burst where both sides are closed. However, during the whistle EMG activity in the left dorsal muscles is weak even though that side is fully adducted, suggesting that cowbirds use different syringeal motor patterns to close the left syrinx during the whistle as compared to introductory note clusters.

EMG activity in right *m. syringealis ventralis* (vS) increases exponentially with the fundamental frequency of the ipsilaterally generated sound. Fundamental frequency of notes produced on the left side range from 300-1200 Hz and accompanying EMG activity in the left vS is variable. Much higher EMG amplitudes in the left vS were recorded during the right side generated whistle than during ipsilateral sound production. Supported by NIH #NS29467 & #NS09229 and Austrian Programme for Advanced Research and Technology.

382.21

EFFECTS OF LATERAL SEPTUM ABLATIONS ON AGGRESSION AND COURTSHIP IN MALE ZEBRA FINCHES (Taenopygia guttata). J. L. Goodson, A. C. Dukes, R. P. Eibach, M. S. Friedman, E. Adkins-Regan. Department of Psychology, Cornell University, Ithaca, New York, 14853

The role of the lateral septum in courtship and aggression has been investigated extensively only in mammals. The present study was conducted to 1) determine lateral septum behavioral function in a bird and 2) investigate functional similarities and differences in septal function between birds and mammals. Male zebra finches (Taenopygia guttata) received bilateral electrolytic ablations of the lateral septum (n=7) or sham surgery (n=10), followed the next day by 5mm silastic tubule implants of testosterone propionate (s.c.). A series of tests began one week post-surgery in which the following behaviors were measured: chases, pecks, beak fences, beak wipes, directed songs, undirected songs, allopreening, solicitation of allopreening, mounts, following females, dances, and behaviors indicative of pairing in a series of four colony tests. Ablations were histologically verified, and were largely limited to the lateral septum, with little collateral damage. Overt aggression (chasing other males in a colony) was significantly lower in the experimental group (0.111 chases/min ± .032) than in the control group (1.117 chases/min ± .365; two-tailed t-test, $p<.05$). Septal ablated birds tended to be slower to pair; only one ablated bird had paired by the second colony test, compared to seven of the control birds, a difference which approached significance (Fisher's exact probability test, $p<.08$). We conclude that the lateral septum is necessary for the normal expression of aggression, as is the case in mammals, and may be involved in the pairing process.

382.22

LEARNED ENGLISH VOCALIZATIONS AS A MODEL FOR STUDYING BUDGERIGAR (*Melopsittacus undulatus*) WARBLE SONG. P.A. Banta* and I.M. Pepperberg. Program in Neuroscience, University of Arizona, Tucson, AZ 85721.

It is commonly known that budgerigars are capable of reproducing human speech. What is not known, however, is whether such behavior can be exploited to investigate the mechanisms of budgerigar vocal production. The purpose of the present study was to determine the extent to which budgerigars can be taught English vocalizations and to assess whether these vocalizations can be used to study the vocal control system of these birds.

Seven budgerigars were taught English vocalizations using the Model/Rival technique (Pepperberg, 1981) and a variation of this technique. During training, either one or two experimenters modeled target vocalizations for the birds. Target vocalizations were single words (*e.g.* "cork" and "paper") and short phrases (*e.g.* "you be good" and "come here").

Budgerigars learned many target and non-target vocalizations and produced them singly, as elements incorporated in their warble song, or in response to presented objects. Vocalizations incorporated in the warble song were produced with high frequency (up to 13 words or phrases/min) and were also produced in a highly stereotyped manner. Learned vocalizations recognizable by ear were visually analyzed on a Kay 5500 Sonagraph with respect to fundamental frequency, first and second formants, vocalization duration and overall pattern. Three naive raters independently compared vocalization sonagrams and matched them to pre-determined vocalization classes with 90% accuracy.

We contend that the frequent and stereotypic nature with which learned English vocalizations are produced by budgerigars makes this model uniquely amenable for studying the role of specific vocal control nuclei in the production of warble song. We intend to investigate the effects of lesions in vocal control nuclei on the production of these words and phrases. Supported by the Whitehall Foundation.

383.1

INTESTINAL INFUSIONS OF FATTY ACIDS INDUCE C-FOS IN THE PARABRACHIAL N., PARAVENTRICULAR N., AND CENTRAL N. OF THE AMYGDALA. J. McCaffery, S.J. French, D. Greenberg, A.J. Strohmayer*, and T.A. Houpt. E.W.Bourne Behav. Res. Lab., Dept. Psychiatry and Neurology, Cornell Univ. Med. Coll., White Plains, NY 10605.

Induction of c-Fos-like immunoreactivity (c-FLI) in the nucleus of the solitary tract (NTS) by intraduodenal infusions of fats correlates with the satiating potency of the fats in food intake tests (McCaffery et al., 1994). Because the NTS is the first central relay for afferent information from the gut and projects to many rostral brain regions that may participate in the processing of visceral satiety signals we mapped neuronal activiation using c-FLI in the pontine parabrachial n. (PBN), the supraoptic n. (SON) and paraventricular n. (PVN) of the hypothalamus, and the central n. of the amygdala (CeN) after intraduodenal infusions of fats.

Adult male rats were surgically equipped with gastric cannulas and duodenal catheters. Rats were randomly assigned to 5 groups which received one of the following intestinal infusions (10ml; 0.44ml/min): 0.15M NaCl (Sal), 5 kcal Intralipid (IL), 0.65 kcal oleic acid, 0.65 kcal linoleic acid, 0.65 kcal linolenic acid . Infusions were performed after stomach lavage and with cannulas open allowing stomach contents to drain. One hour after intestinal infusions ended, rats were sacrificed and processed for c-FLI. The PBN, SON, PVN, and CeN were qualitatively scored for density of c-FLI-positive cells and compared to c-FLI induction in the NTS.

The induction of c-FLI in the PBN, PVN, and CeN by intraduodenal infusions of a mixture of fats (IL) or by specific long-chain fatty acids qualitatively paralleled the induction of c-FLI in the NTS. In the SON, c-FLI was infrequently observed and did not appear correlated with the type of infusion. Infusions of Sal induced little or no c-FLI in any of the brain regions. The induction of c-FLI in rostral projection sites of the NTS may represent functional activation of neuronal circuits mediating behavioral satiety caused by intestinal infusions of fat.

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383.2

INITIAL HYPOPHAGIA CAUSED BY AN IMBALANCED AMINO ACID DIET (IAAD) IS ABOLISHED IN DORSOMEDIAL HYPOTHALAMIC NUCLEUS LESIONED (DMNL) RATS. L.L. Bellinger* and D. W. Gietzen. Dept. of Biomedical Sci., Baylor College Of Dentistry, Dallas, TX. 75246 and Vet. Med. Anat. Phys. & Cell Biol. Univ. of Calif., Davis, CA 95616.

Rats show food intake (FI) suppression in as little as 3 h after giving an IAAD; this decrease in FI can be attenuated by giving Tropisetron (TROP) a $5HT_3$ receptor antagonist. Wang et al., showed c-Fos expression in the DMN 2 h after giving rats an IAAD. In the present study male S. D. rats were given DMNL or sham operations (SHAM). The rats were placed on chow for 8 d and then basal diet for 10 d; the DMNL rats were hypophagic ($p<0.01$) compared to SHAM rats. On day 11 the rats were injected i.p. with saline (SAL) 1 h prior to lights out and FI recorded 3, 6, 12 and 24 h later. On day 12 the rats were weighed and injected with SAL or TROP (9mg/kg): Grp 1, SHAM + SAL (161g BW, n=9); Grp 2, SHAM + TROP (163g BW, n= 10); Grp 3, DMNL + SAL (145g BW, n=7); and Grp 4, DMNL + TROP (146g BW, n=9) and 1 h later presented with an isoleucine IAAD; the DMNL groups weighed less ($p<0.01$) than the SHAMs. At 3 h, intakes (expressed as a % of their basal intake) were: Grp 1, 60%; Grp 2, 158%; Grp 3, 135% ($P<0.01$ greater than Grp 1); and Grp 4, 223% ($P<0.01$ greater than Grp 2). Similar cumulative intake differences were noted at 6 h, however by 12 h Grp 1 and 3 and Grp 2 and 4 were consuming similar amounts. Grp 2 and 4 intakes remained higher ($P<0.01$) than Grp 1 and 3 at 12 and 24 h. On day 2 IAAD intake of the four groups was similar and on day 3 they began to adapt, i.e., eat more of the IAAD. On days 4-7 the DMNL rats didn't adapt compared to the SHAMs. BW changes were reflective of FI. The data show that DMNL: 1. abolish the initial, but not later, FI suppression caused by the IAAD, 2. doesn't interfere with TROP's ability to enhance the intake of an IAAD and that the lesion and drug effects are additive. Supported by DK42274 to D.W.G. and BCD Research Funds.

383.3

GASTRIC LOADS OF AN AMINO ACID MIXTURE BUT NOT GLUCOSE ALTER EXTRACELLULAR AMINO ACID PATTERNS IN THE PARAVENTRICULAR NUCLEUS AS MEASURED BY *IN VIVO* MICRODIALYSIS. N. Chang, P.J. Currie and G.H. Anderson*. Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 1A8.

Brain amino acids are hypothesized to play a role in the regulation of food intake, and it is established that central amino acid concentrations are affected by the macronutrient composition of the diet. In this study, microdialysis was used to examine the profile of extracellular amino acids in the paraventricular nucleus (PVN) of awake, freely-moving rats following gavage of equicaloric loads of anhydrous glucose (0.89 g) or an amino acid mixture (0.85 g patterned after 1 g of chicken egg albumin). The microdialysis probe (perfused at a rate of 2 µl/min) was implanted into the PVN 2 hours prior to gavage and dialysates were collected every 20 min starting from 1 hr prior to and 3 h after treatment. Dialysate amino acids were derivatized with o-phthaldialdehyde and determined fluorometrically after HPLC separation. Administration of a balanced amino acid mixture evoked significant increases in dialysate levels of many essential amino acids, as well as alanine. While extracellular levels of alanine and leucine exhibited immediate (first 20 min aliquot) increases, elevations of isoleucine, methionine, phenylalanine, tyrosine, and valine were detected in the second 20 min aliquot. The concentration of most amino acids peaked within 40 min of treatment, with the exception of alanine and threonine which peaked 60 min after gavage. Most amino acids showed an increase of 125%-190% of baseline. However, dialysate levels of valine reached a maximal increase of 230%. The extracellular amino acid profile in the PVN was, in general, unaffected by glucose administration. These findings imply that dietary composition may influence the amino acid profiles of the extracellular fluid in brain (Supported by NSERC).

383.4

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

Glucoreceptors controlling food intake are known to be centrally located since administration of glucoprivic agents directly into the brain elicits feeding. Additional evidence suggests that glucoreceptors are located in the caudal hindbrain. The present study mapped the hindbrain of rats caudal to the facial nerve (n=63) for sites where feeding could be elicited by localized glucoprivation. The antimetabolic glucose analogue, 5-thioglucose (5TG, 24 ug in 200 nl) or 0.9% saline (200 nl) were injected through small diameter cannulas and food intake was measured at hourly intervals for 4 hrs. Positive sites were found in the ventrolateral medulla in the vicinity of the C1 adrenergic cell group, where feeding was increased to 1.9 ± 0.3 g above saline baseline. Positive sites were also found in the vicinity of the nucleus of the solitary tract, but the most positive sites were found at various dorsal/ventral levels within a 1 mm extent along the midline just rostral to the area postrema, an area that includes many C2 adrenergic neurons. At these sites, rats consumed 3.87 ± 0.5 g in response to 5TG and 0.9 ± 0.4 g in response to saline. Sites positive for feeding were similar to those at which 5TG stimulates adrenal medullary secretion and Fos expression. Certain hindbrain catecholamine neurons may be important effectors for glucoprivic feeding. Whether they or other neurons in close proximity to them are the putative glucoreceptor cells for feeding will require additional experimentation.

383.5

EXPRESSION OF C-FOS-LIKE IMMUNOREACTIVITY IN THE RAT BRAIN ONE WEEK AFTER BILATERAL ABDOMINAL VAGOTOMY. G.P. Smith*, R.A. Berlin, and T.A. Houpt. E.W. Bourne Behav. Res. Lab., Dept. Psychiatry, Cornell Univ. Med. Coll., White Plains NY 10605

Total subdiaphragmatic vagotomy eliminates much of the afferent information flow from the gut to the brain. Although the drinking respons to angiotensin II (sc) is decreased after vagotomy (Simansky & Smith, 1983), the drinking response to hypertonic saline (ip) does not decrease until 7-10 days after vagotomy (Jerome & Smith 1984). Gradual changes in neuronal activity may account for the delayed changes in the response to hypertonic saline. To search for changes in central neuronal activity after abdominal activity, we used c-Fos-like immunohistochemistry (c-FLI) to identify cells in the medulla, pons, and forebrain that were activated 1 week after vagotomy.

Both major vagal trunks of adult male rats (n=5) were cut between 2 silk ligatures below the diaphragm and above the hepatic, accessory celiac, and celiac branches. Control rats (n=5) were sham-vagotomized. One week later, rats were overnight food-deprived, perfused, and the brain processed for c-FLI.

Numerous c-FLI-positive cells were observed in the caudal nucleus of the solitary tract (NTS) and area postrema. The pattern of c-FLI expression in the NTS appeared to parallel the receptive fields of subdiaphragmatic vagal afferents. The lateral parabrachial nucleus, the central nucleus of the amygdala, the hypothalamic paraventricular nucleus, and the supraoptic nucleus also showed intense c-FLI staining. Little or no c-FLI cells were seen in sham-vagotomized rats in these regions.

Because c-FLI was observed not only in the receptive field of the vagus in the NTS but also in rostral projection sites of the NTS, some of the c-FLI observed must have been induced transsynaptically. Explanations for c-Fos expression 1 week after vagotomy include peristent activation of axotomized afferent vagal fibers, or a release of NTS neurons from inhibition. Because c-Fos can regulate gene expression, it may participate in intracellular mechanisms mediating neuronal plasticity after vagotomy.

Supported by theWhitehall Fdtn. and NY Obesity Ctr. (TAH) and MH00149 (GPS).

383.6

CENTRAL cFOS EXPRESSION INDUCED BY MILK DEPRIVATION AND INGESTION IN NEONATAL RATS. L. Rinaman*, J.G. Verbalis, & E.M. Stricker. Depts. of Neuroscience & Medicine, University of Pittsburgh, Pittsburgh, PA 15260.

During the first week of postnatal life in rats, dehydration is the sole stimulator of independent ingestion and gastric distension is the sole inhibitor. We analyzed central expression of cFos in 3-day-old rats following mild dehydration (induced by milk deprivation) and gastric distension (induced by feeding after deprivation) to localize neurons whose activity is heightened by these conditions. Control pups (n=4) were removed from their dam, anesthetized, and perfused with fixative; their stomachs contained varying amounts of milk (0.36±0.13g) at sacrifice. Other pups (n=6) were deprived of their dam for 5 hr and then were anesthetized and perfused; their stomachs contained no milk at sacrifice. A third group of pups (n=5) was deprived of their dam for 5 hr and then was allowed to suckle for 30 min. These deprived-refed pups were anesthetized and perfused 1 hr after completion of suckling; their stomachs were moderately distended with milk (0.71±0.12g) at sacrifice. Brain sections from control, deprived, and deprived-refed pups were processed for immunocytochemical detection of cFos. Some sections also were processed for detection of oxytocin (OT), vasopressin (VP), or tyrosine hydroxylase (TH). Little cFos was seen in the forebrain or caudal medulla of non-deprived controls. In contrast, cFos was elevated in osmosensitive forebrain regions and in hypothalamic OT and VP neurons in deprived pups, although cFos remained sparse in the caudal medulla (similar to controls). In deprived-refed pups, forebrain cFos was similar to that in deprived pups, as expected (since cFos remains detectable for several hr after induction). However, in contrast to the lack of medullary cFos in control and deprived pups, many TH-positive neurons and other neurons in the nucleus of the solitary tract, area postrema, and ventrolateral medulla exhibited cFos in refed pups. SUMMARY: Mild fluid deprivation activates specific forebrain neurons but appears not to activate caudal medullary neurons in neonates, suggesting that forebrain activation is sufficient for stimulating ingestion at this age. In contrast, refeeding does activate medullary vagal relay neurons in neonates, implicating these neurons in distension-induced inhibition of ingestion.

383.7

INTEGRATION OF ORAL AND GASTRIC SIGNALS: C-FOS ANALYSIS. Emond, M.*, & Weingarten, H.P. Dept. Psychology, McMaster University, Hamilton, Ontario, Canada, L8S 4K1.

Control of food intake requires the coordination of oral and gastric afferent signals. We used Fos immunohistochemistry, as an index of neural activity, to identify properties of neuronal populations activated during oral and gastric stimulation presented in isolation or in combination. We tested nine triads of male rats that were 24 hr food deprived before, and anaesthetized with urethane (1.2 g/kg) during, the experiment. Animals with restricted oral stimulation had 1 M sucrose perfused over the tongue at a constant rate of 0.63 ml/min for 19 min; gastric stimulation was accomplished by identical infusions directly intragastrically. The third member of every triad received the oral and gastric infusions simultaneously. 90 min later rats were killed, the brains were removed and processed for Fos-like immunoreactivity (FLI) using standard protocols. FLI was assessed by counting the number of Fos-labelled cells in the caudal, medial and rostral levels of the nucleus of the solitary tract (NST) and dorsal motor nucleus (DMN) and in the paraventricular nucleus (PVN). Overall, the isolated oral and gastric infusions produced similar degrees of Fos activation. However, in the PVN and all 3 levels of the NST the combined infusions resulted in significantly less FLI than either infusion presented alone. Overall, there were no differences in FLI across treatments in the DMN. Our results indicate first, that Fos expression can be elicited in anaesthetized rats and the profile of results replicate our previous finding in freely-behaving rats that combined oral and gastric stimulation results in significantly less FLI in NST than oral stimulation alone. Second, our results suggest the conclusion that preparations used frequently to isolate the roles of oral (sham feeding) or gastric (IG loads) afferents may not adequately capture the contribution of these signals when they are embedded in the context that approximates normal eating, ie. these signals in combination.

Supported by Natural Sciences and Research Council of Canada.

383.8

METABOTROPIC GLUTAMATE RECEPTOR INVOLVEMENT IN FEEDING STIMULATION: EVIDENCE FROM LATERAL HYPOTHALAMIC INJECTION OF ACPD. M.A. Duva*, L.C. Spears & B.G. Stanley. Departments of Neuroscience & Psychology, University of California, Riverside, CA 92521.

We have recently shown that agonists of several ionotropic glutamate receptors elicit intense eating when injected into the lateral hypothalamus (LH) of satiated rats. To investigate whether metabotropic glutamate receptors, which can modify gene expression, enzyme pathways, and cellular growth, might be involved in feeding stimulation, we employed LH injections of the metabotropic agonist 1S,3R-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD). Adult male Sprague-Dawley rats with chronically implanted LH cannulas were injected with 1S,3R-ACPD (0.1, 1, 10, 33, 100 or 300 nmol/0.3 µl) or vehicle in ascending order and food intake was measured 1, 2, and 4 hrs later. Injections of 1S,3R-ACPD produced dose-dependent feeding responses, with 33 nmol to 300 nmol doses yielding mean intakes of 3.9 to 4.6 gm 1 hr postinjection, and 6.3 to 6.8 gm at 4 hrs postinjection. To examine the consistency of the response, tests with 100 nmol of 1S,3R-ACPD were repeated every 2 to 3 days. The feeding response declined somewhat after the first test and was consistent thereafter. To examine the chemical specificity of this effect, we also tested 33 to 300 nmol doses of the less active stereoisomer 1R,3S-ACPD and found that it was significantly less effective, producing a maximum eating response of 3.7 gm 4 hr after injection of the 300 nmol dose. These findings suggest that metabotropic glutamate receptors may be involved in the stimulation of eating behavior.

383.9

BEHAVIORAL ACTIVITY AND MEAL INTAKE ELICITED BY PERIFORNICAL HYPOTHALAMIC INJECTION OF 8-BROMOADENOSINE 3',5'-CYCLIC MONOPHOSPHATE (8-br-cAMP) IN THE RAT. E.R. Gillard*, B. Mouradi and B.G. Stanley. Dept. of Neurosci. & Psychol., Univ. of CA, Riverside, CA 92521, USA.

We have shown that 8-br-cAMP elicits robust feeding when injected into the perifornical hypothalamus (PFH) and nearby lateral hypothalamus, suggesting that some neurotransmitters known to influence feeding in these regions may do so via the cyclic AMP second messenger system. To begin to address whether injection of 8-br-cAMP simulates a process that may occur in PFH neurons during normal feeding, we examined meal patterns and behavioral activity in male rats for 4 hrs following injection of 8-br-cAMP (100 nmol/0.3 µl) into the PFH. 8-br-cAMP injected animals consumed 90% of their total intake in a large (11.9 ± 4.3 g), short-latency (all but one subject ate within 6 min.) first meal lasting an average of 7 minutes; this was followed by a much smaller meal occurring in the second hour postinjection. Behavioral observations showed that these animals spent significantly more time (30 ± 7% of total time) eating during the first 10 minutes than did controls (0%). In contrast, neither drinking nor gnawing behavior were stimulated by 8-br-cAMP injection. Activity level was increased in 8-br-cAMP injected animals relative to controls during the 2 hrs following injection, with a consequent decrease in the time spent resting and sleeping. However, peaks in activity occurred later than the dramatic stimulation of eating observed in the first 10 minutes postinjection. These findings suggest that stimulation of the cAMP second messenger system in PFH neurons might contribute to intense, rapid eating responses, such as those elicited by food deprivation.

383.10

EFFECTS OF FEEDING AND INSULIN ON EXTRACELLULAR ACETYLCHOLINE IN THE AMYGDALA OF FREELY MOVING RATS. Andras Hajnal[a,b], Emmanuel N. Pothos*[a], Gregory P. Mark[a], Pedro V. Rada[a], Laszlo Lenard[b] and Bartley G. Hoebel[a]. [a]Department of Psychology, Princeton University, Princeton, NJ, 08544 and [b]Neurophysiology Research Group of the Hungarian Academy of Sciences at the Institute of Physiology, Pecs University, Medical School, H-7643 Pecs, Hungary.

Extracellular levels of acetylcholine (ACh) were measured in the central nucleus of the amygdala (AMY) by using microdialysis in 20 min intervals before, during and after 1 hr feeding in 20 hr food-deprived rats. The effects of peripheral injections of glucose or "low" (200 mU/kg) and "high" (1 U/kg) doses of insulin on ACh in the AMY were also observed in non-deprived animals. Feeding caused a 40% increase in extracellular ACh in the AMY which recovered 1 hr after feeding. Injections of both glucose and insulin resulted in an increase in ACh levels (50-60%) but the dynamics were different: glucose caused ACh to plateau within 80 min after the injection; whereas both doses of insulin caused a peak in ACh release in the first 20 min interval followed by a two step recovery. The "low" and "high" doses of insulin had similar effects on ACh release even though they had different hypoglycemic potency. These results suggest that ACh in the AMY is involved in feeding, and that peripheral insulin may influence ACh in the AMY in a way that is partially unrelated to hypoglycemic effects.

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383.11

EFFECTS OF HYPONATREMIA ON FOS-LIKE IMMUNOREACTIVITY (FLI) IN THE SUBFORNICAL ORGAN AND MAGNOCELLULAR NUCLEI IN THE RAT. L. Han*, N.E. Rowland, B.-H. Li & G.C. Smith. Dept. Psychology, Univ of Florida, Gainesville, FL 32611

The subfornical organ (SFO) and the supraoptic (SON) and paraventricular (PVN) nuclei are key brain structures in the control of fluid balance. We previously found that treatment with the natriuretic agent, furosemide, which causes salt appetite, was associated with FLI expression, a marker of neuronal activation, in the SFO but not in the SON and PVN. This study was designed to determine whether the absence of FLI in the SON and PVN after furosemide treatment is caused by hyponatremia subsequent to water ingestion. Male adult SD rats, either on regular or sodium (Na)-free diet for 2 days, received either saline or furosemide (two SC injections of 5mg, 2 hr apart). Water was then withdrawn from half of the subjects. A 1-hr, two-bottle (water vs. salt) drinking test was performed 22 hrs after furosemide treatment. Two weeks later, all the rats were treated the same way as above, except that they were perfused for Fos immunocytochemical staining 22 hours after receiving furosemide without the drinking test. It was found that 1) there was no difference between regular diet groups and Na-free diet groups in terms of plasma renin activity, urine sodium or FLI expression; 2) furosemide significantly increased salt intake, but not water intake, whether water was withdrawn or not; 3) furosemide induced a strong FLI in the SFO in all rats, whether or not allowed to drink; 4) when water was withdrawn, furosemide-treated rats showed weaker FLI in the SON and PVN than saline-treated rats. The results suggest that a 3-day Na-free diet dose not enhance FLI expression in the SFO after Furosemide treatment, and that hyponatremia caused by water intake contributes to inhibiting FLI expression in the SON and PVN.

383.12

CHRONIC INTRACEREBROVENTRICULAR (ICV) MICROINFUSION OF CYTOKINES: i) DIFFERENTIAL EFFECTS ON FEEDING; ii) PROFILE OF Gα-PROTEIN SUBUNIT SUBCLASSES IN THE VENTROMEDIAL HYPOTHALAMIC NUCLEUS (VMN). C. R. Plata-Salamán*[1], G. Sonti[1], C.D. Wilson[2] and J.M.H. ffrench-Mullen[2]. [1]Sch. Life Hlth Sci., Univ. Delaware, Newark, DE 19716, and [2]Dept. Pharmacol., Zeneca Pharmaceuticals, Wilmington, DE 19897.

Anorexigenic cytokines include IL-1, IL-6, IL-8 and TNF-α. Increased levels of cytokines in the CSF are present during various diseases. Cytokine-induced feeding inhibition is proposed to participate in the long-term anorexia observed during disease. We studied in rats the effects of the chronic ICV (into the third ventricle) infusion of various cytokines for 7 days (through osmotic minipumps) on behavioral parameters. The results during the 7-day pump period were: IL-1β decreased nighttime feeding dose-dependently (0.5 ng/24 h, n=8; 2.0 ng/24 h, n=10; 8.0 ng/24 h, n=10) relative to the vehicle infusion (n=11)($p<0.001$). The decrease of feeding and body weight persisted during the 7-day infusion (contrary to the peripheral IL-1β chronic infusion which has been associated with tolerance); feeding and body weight increased toward baseline following the end of IL-1β infusion. Total daily food intake decrease was less prominent relative to the nighttime decrease because daytime food intake did not change or increased. Water intake was not decreased in any group suggesting specificity of action. Chronic ICV administration of TNF-α (20 ng/24 h, n=9; 100 ng/24 h, n=8; 300 ng/24 h, n=7), IL-6 (100 ng/24 h, n=9), or IL-8 (20 ng/24 h, n=9) was significantly less effective than IL-1β to induce behavioral modifications. Computerized analysis also showed specific effects on the behavioral patterns. Immunoblots of VMN from normal rats (n=8) showed that the levels of GαO protein subunit are >30 fold higher relative to those of Gαi1-i3. The chronic ICV microinfusion of IL-1β (2.0 ng/24 h/72 h) increased the levels of Gαi1 and Gαi2 protein by 3.8-fold detected in immunoblots of VMN (n=6). No effect was observed on GαO and Gαi3 protein levels suggesting specificity. The results suggest that IL-1β, at estimated pathophysiological concentrations in the CSF, may induce long-term anorexia and may modify specific signalling systems.

383.13

BRAIN AREAS ACTIVATED BY INGESTION USING FUNCTIONAL MRI OF AWAKE RATS. E. Tabuchi[1,2], T. Yokawa[1], T. Ono[2], A. Niijima[3]*, and K. Torii[1]. [1]ERATO, R&D Corp. of Japan, Yokohama 221, [2]Dep. of Physiology, Toyama Med. & Pharmaceu. Univ., Toyama 930-01, [3]Dep. of Physiology, Niigata Univ. Sch. of Med., Niigata 950, Japan.

To understand spatial and temporal dynamics of activated areas in the brain, functional magnetic resonance imaging (fMRI) was undertaken using awake rats. Under anesthesia, each rat had a receptacle put on its head to immobilize the animal's skull to be later fixed painlessly in the correct stereotaxic position. After recovery, they were trained to accept the restraint in a stereotaxic apparatus without struggling, and were allowed to lick a solution from a spout when manually extended close to its mouth, following water deprivation for one day. T2* weighted MRIs, in which intensity increases in area with high consumption of oxygen (oxy-hemoglobin), were then taken before (control), during, and after ingestion of various solutions; water, saline, 5% glucose, 0.2 M lysine, 0.15 M MSG, 0.05 M arginine, 0.5 M glycine, and 0.05 M histidine. During ingestion of most solutions, the signal intensity in T2* weighted MRI raised in most brain areas and increased significantly in the lateral hypothalamic area, the ventromedial nucleus of the hypothalamus, the central nucleus of the amygdala, the piriform cortex, and the CA1 subfield of the hippocampus, and it gradually returned to the control level after ingestion. These findings directly indicate that these brain regions cooperate and work in parallel during drinking behavior.

383.14

THE EFFECT OF AD LIB FOOD PLACEMENT ON BODY WEIGHT FOLLOWING MEDIAL SEPTAL LESIONS IN RATS. **A. Poplawsky.* Dept. of Psychology, Bloomsburg Univ. of Penna., Bloomsburg, PA 17815.**

To assess changes in body weight, rats with control operations or medial septal lesions were weighed for 120 days following surgery. At the end of this period, rats with medial septal lesions weighed less than rats with control operations. For the next 25 days, half the rats from each group received their ad lib food in a feeder attached to the outside of the cage, while the remaining rats continued to have their food placed inside the cage. Placing the food outside the cage resulted in a decrease in body weights for control rats that became equivalent to medial septal rats, while the rats with medial septal lesions were not affected by this change. This suggests that ad lib food placement can interact with the effects of medial septal lesions on body weight regulation.

383.15

EFFECTS OF ARCUATE NUCLEUS LESIONS ON BODY WEIGHT, FOOD INTAKE, & REPRODUCTIVE BEHAVIOR IN OBESE ZUCKER FEMALE RATS. C.L.M. Bivens* & D.H. Olster. Dept. of Psychology & Neuroscience Research Institute, Univ. of Calif., Santa Barbara, CA. 93106.

Genetically obese Zucker female rats are hyperphagic, overweight, and infertile. It has been speculated that overproduction of neuropeptides in the cell bodies of the arcuate nucleus of the hypothalamus may contribute to obesity and infertility in obese Zuckers. To test this hypothesis ovariectomized (OVX) adults received either bilateral, electrolytic lesions (0.2 mA, 30 sec, obese Zuckers) or sham-lesions (no current, 30 sec, obese and lean Zuckers) in the arcuate nucleus and subsequently monitored for food intake and body weight for 7 weeks. Twenty-four hour food intake in lesioned obese females was lower than that observed in sham-lesioned obese females, but not different to that found in their sham-lesioned lean counterparts (e.g., week 5, lesioned obese 60±2 g; sham-lesioned obese 76±2 g; sham-lesion lean 54±4 g, $p<0.0001$). Furthermore, body weights were lower in lesioned obese, compared to sham-lesioned obese females (e.g., at 7 weeks, 522±11 g sham lesion vs. 477±10 g lesion, $p<0.02$). At 8 weeks postsurgery, OVX adults received either 15 µg/kg estradiol benzoate (EB) or EB plus 2 mg/kg progesterone (P) and tested for sexual receptivity and proceptivity. All groups showed uniformly low levels of lordosis after EB alone. However, following EB plus P treatment obese lesioned females and sham-lesioned lean females displayed increased sexual receptivity as compared to sham-lesioned obese females (LQ= 38±6%, 56±11%, 23±6%, $p<0.02$, respectively). These data suggest that the arcuate nucleus contributes to hyperphagia, weight gain, and suppressed reproductive behavior in obese Zucker females. It is proposed that neurochemicals overproduced by the arcuate nucleus, such as neuropeptide Y and β-endorphin, may contribute to the obesity and infertility in obese Zucker females (Supported by NIH HD 28636 and American Psychological Association)

383.16

INTRA-PARAVENTRICULAR INJECTIONS OF PROLACTIN INCREASE FOOD INTAKE IN FEMALE RATS D Sauvé* and B.C. Woodside. Centre for Studies in Behavioral Neurobiology, Concordia University, Mtl., Canada, H4B 1R6

Lactation in mammals is characterized by a marked hyperphagia and significantly elevated levels of Prolactin (PRL). Several recent studies in our laboratory have provided evidence for a causal relationship between PRL and the hyperphagia of lactation. For example, PRL injected intracerebroventricularly (twice daily for ten days to virgin females rats) produced a dose-dependent increase in food intake without disrupting vaginal cyclicity. Doses of 2 and 10 µg were effective in increasing food intake, whereas doses of 400 and 800 ng were not. Recently, PRL receptors have been located in the paraventricular nucleus of the hypothalamus (PVN) an area that has also been implicated in the control of food intake. In this study, therefore, the effect on food intake of prolactin administration into the PVN was determined. Doses that were ineffective when administered into the ventricles, were injected directly into the PVN of virgin female Wistar rats, and food intake monitored. Results revealed that whereas 400 ng of PRL did not cause increases in food intake, 800 ng produced a robust effect. These results confirm once more that PRL has a central effect on food intake, and that it is highly likely that the hyperphagic effect of PRL is mediated, at least in part, by the PVN.

383.17

Activational Hormones Are Not Necessary For Expression Of Prolactin's Sexually Dimorphic Effects On Food Intake In Rats. S.H. Heil*, C.P. Cramer, Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

Exogenous prolactin (PRL) administration increases food intake only in female rats (Heil and Cramer, 1992). The present experiment was designed to examine what role adult gonadal hormones play in PRL's sexually dimorphic effects on food intake. Ten male and ten female Long-Evans rats were gonadectomized at 30 days of age, before the commencement of puberty. In addition, at 85 days of age, ten intact females were implanted with subcutaneous Silastic capsules filled with testosterone proprionate, to test the possibility that male insensitivity to PRL is due simply to circulating testosterone. Gonadctomized males and females were implanted with blank capsules of the same dimensions.

At 90 days of age, animals were housed in hanging cages with ad lib access to water and a jar of ground chow. Food intake was measure daily for five days of baseline, followed by ten days of PRL injections. Injections were administered twice daily at 0800 and 1900 hours at a dose of 1 mg/kg. Results were analyzed by repeated measures ANOVA.

Both gonadectomized females and intact females + Silastic testosterone significantly increased intake in response to exogenous PRL injections. Gonadectomized males' intake was not significantly different from baseline intake. Thus, activational hormones are not neccesary for the expression of PRL's sexually dimorphic effects on food intake, nor is male insensitivity to PRL due solely to circulating testosterone. Moreover, these results suggest that the dimorphism in response to PRL is organized much earlier in development, a possibility we are currently testing.

383.18

REGULATION OF RENAL OXYTOCIN (OT) RECEPTORS BY ADRENAL STEROID HORMONES. L.M. Flanagan-Cato*, R.R. Sakai, C.N. Moga, and S.J. Fluharty. Depts. Psychology, Animal Biology and Institute for Neurological Sciences, University of Pennsylvania, Philadelphia, PA, USA. Supported by MH43787.

Aldosterone, released in response to sodium deficiency, promotes sodium retention in the kidney and augments sodium appetite in the brain. In contrast, OT released in response to hypernatremia promotes natriuresis in kidney while inhibiting sodium appetite centrally. The present study tested the hypothesis that aldosterone-induced sodium retention may in part reflect blunted natriuretic mechanisms. Previous studies had indicated that in a renal cell line, LLC-PK$_1$, OT receptor binding was reduced by treatment with aldosterone. We determined whether in vivo aldosterone reduced the levels of OT receptor binding in the kidney. Three groups of adult male rats were studied: control (C, n=6), 4 day adrenalectomized (ADX, n=6), and 4-day desoxycorticosterone acetate treated (DOCA; 2.5 mg/day, n=5). OT receptor binding studies were performed on kidney membranes. Results demonstrated that after ADX, there was a significant increase in the levels of OT receptors detected, whereas after DOCA treatment there was a significantly reduced number of OT receptors detected. More specifically, compared with control, OT receptor binding was increased by 43.2 ± 11.9 % in ADX rats ($p<0.005$), and decreased by 29.1 ± 9.3 % in DOCA-treated rats ($p<0.02$). Taken together, these results suggest that the increased level of renal OT receptor binding in the ADX group represents a disinhibition from endogenous aldosterone. Thus, it appears that aldosterone has the complementary actions of blunting OT-mediated natriuresis and directly stimulating sodium retention. This would parallel the effects of adrenal steroids in the brain to simultaneously stimulate and disinhibit sodium appetite.

383.19

BRAIN OXYTOCIN (OT) RECEPTOR ANTAGONISM DISINHIBITS SODIUM APPETITE IN PREWEANLING RATS. S.Y. Chow*, S.J. Fluharty and L.M. Flanagan-Cato. Depts. Psychology, Animal Biology, Pharmacology, and Institute for Neurological Sciences, University of Pennsylvania, Philadelphia, PA, USA. Supported by MH43787.

Adrenalectomy (ADX) stimulates sodium appetite in the adult rat, mediated in part by angiotensin II (AngII) activity in the brain. This behavioral response to ADX does not emerge until postnatal day 12 (PN12). However, as early as PN3 AngII receptors are present in brain, and these animals are physiologically responsive to ADX, as demonstrated by elevated plasma levels of AngII. The absence of sodium appetite until PN12, therefore, has not been readily explained. In the adult rat, centrally-released OT inhibits sodium appetite. We examined whether this inhibitory signal may prevent neonatal rats from displaying sodium appetite in the face of an excitatory stimulus. PN10 rat pups were infused intraorally with 4% NaCl, and intakes were determined by measuring changes in body weight. Prior to the intraoral infusions, pups were pretreated icv with either vehicle, AngII (5 ng), or AngII plus the OT receptor antagonist ornithine vasotocin (OVT; 5 µg). Central AngII increased intake of NaCl (0.7% ± 0.3 vs. 2.2 % ± 0.3; $p<0.005$, intakes expressed as % change in body weight). This intake was further enhanced by giving OVT in conjunction with AngII (3.2% ± 0.3, $p<0.05$ compared with AngII alone). When sodium appetite was induced by ADX in PN10 rats, OVT also augmented intakes (1.7% ± 0.2 vs. 2.6% ± 0.3, $p<0.05$). Together these results suggest that endogenous OT may mediate the inhibition of sodium appetite in the preweanling rat. Release from this oxytocinergic inhibition reveals a previously unsuspected ability of PN10 rats to display sodium appetite after ADX.

DRUGS OF ABUSE: AMPHETAMINES AND OTHER STIMULANTS I

384.1

BEHAVIORAL SENSITIZATION TO AMPHETAMINE AND THE CHOLINERGIC SYSTEM. J.B. Bedingfield, L.D. Calder, L.H. Thai and R. Karler*. University of Utah School of Medicine, Salt Lake City, Ut 84132

The results of our previous studies suggest that sensitization consists of at least two pharmacologically separable components, induction and expression, and that both of these components involve a dopaminergic-glutamatergic-GABAergic interaction within the striatum. The present study was designed to investigate the role of the cholinergic system in sensitization to amphetamine-induced stereotypy in CF-1 mice. The nicotinic antagonists, mecamylamine and DBE given systemically were ineffective against amphetamine-induced stereotypy in naive mice, but they blocked both the induction and expression of sensitization. The antagonists administered intrastriatally also blocked induction but were ineffective against expression. The systemic data indicate that sensitization involves the recruitment of at least one neuroeffector system not normally involved in the action of amphetamine. The intrastriatal data imply that the striatum is a locus for the nicotinic involvement in induction and that the nicotinic role in expression occurs at a different brain locus. These data add support to the concept that behavioral sensitization involves the interaction of multiple neuroeffector systems in multiple brain loci. (Supported by NIDA grant DA00346.)

384.2

SCOPOLAMINE PREVENTS BEHAVIORAL SENSITIZATION TO METHAMPHETAMINE. T.Ohmori*, T.Abekawa and T.Koyama. Dept. Psychiatry, Hokkaido Univ. Sapporo 060 Japan

Cholinergic neurotransmission has been implicated in various forms of neural plasticity such as kindling and learning. We have previously shown that blockade of muscarinic cholinergic receptors prevents the development of locomotor sensitization to methamphetamine (Life Sci 56: 1223-29,95).

The present study was conducted to examine whether scopolamine, a muscarinic cholinergic antagonist, would also block augmentation of stereotypy induced by chronic methamphetamine (MA) treatment. Rats treated with MA (2.5 mg/kg, sc) for 10 days indicated significantly enhanced stereotyped behavior when tested with MA (2.5 mg/kg) after a 7-8 day withdrawal. Pretreatment with scopolamine (3 mg/kg) prior to MA administration prevented the augmentation of stereotypy. Rats treated with scopolamine alone showed no difference in MA-induced stereotypy compared to those treated with saline. Scopolamine methylbromide, a derivative of scopolamine that does not easily cross the blood-brain barrier, had no effect on the augmentation of stereotypy.

These results suggest that stimulation of central muscarinic cholinergic receptors plays a role in the development of sensitization to the stereotypy stimulating effect of methamphetamine.

384.3

ATTENUATION OF THE LOCOMOTOR ACTIVATING EFFECTS OF D-AMPHETAMINE, COCAINE, AND SCOPOLAMINE BY POTASSIUM CHANNEL MODULATORS. S. Rosenzweig-Lipson*, S. Thomas, J.E. Barrett. Wyeth-Ayerst Research, CNS-Biology, Pearl River, NY 10965.

The locomotor activating effects of d-amphetamine, cocaine, and scopolamine were determined alone and after pretreatment with K-channel modulators in mice. When administered alone, d-amphetamine (1-30 mg/kg) and cocaine (3-56 mg/kg) produced inverted U- shaped dose-effect curves characteristic of psychomotor stimulant drugs. Low to intermediate doses produced dose-dependent increases in locomotor activity, whereas high doses either increased locomotor activity less than an intermediate dose or decreased locomotor activity. When administered alone, scopolamine (3-56 mg/kg) also produced dose-dependent increases in locomotor activity but these effects plateaued, with similar increases in locomotor activity induced by 10 - 56 mg/kg of scopolamine. Pretreatment with the K-channel blockers 4-aminopyridine (0.3-1.7 mg/kg), quinine (30-100 mg/kg) or apamin (0.3-1 mg/kg) attenuated the locomotor increases induced by d-amphetamine, cocaine, and scopolamine. Pretreatment with the K-channel openers cromakalim (1-3 mg/kg) and pinacidil (3-10 mg/kg) also attenuated the locomotor increases induced by d-amphetamine and scopolamine but did not modify the locomotor activating effects of cocaine. These results demonstrate that K-channel modulation can modify the behavioral effects of d-amphetamine, cocaine, and scopolamine and suggest that K-channels may play an important role in central nervous system pharmacology. Further, K-channel openers differentially alter the behavioral effects of d-amphetamine and cocaine, suggesting a fundamental difference in the manner in which K-channel openers interact with the neurochemical mechanisms related to these two psychomotor stimulant drugs.

384.4

EFFECTS OF THE 5-HT2A/C ANTAGONIST RITANSERIN ON AMPHETAMINE-STIMULATED DOPAMINE RELEASE IN THE RAT PREFRONTAL CORTEX. E.A. Pehek* and Y. Bi. Dept. Psychiatry, Case Western Reserve University and Cleveland VAMC, Brecksville, OH 44106.

Administration of relatively high doses of ritanserin (RIT) has been reported to increase extracellular concentrations of dopamine (DA) in the rat medial prefrontal cortex (mPFC). However, the mechanism for this effect is unknown. One possibility is that blockade of cortical 5-HT2 receptors enhances impulse-dependent DA release. It is known that the D2 antagonist haloperidol enhances impulse-dependent DA release in the striatum, and potentiates the effects of agents like amphetamine (AMPH) that act on the DA transporter to enhance DA efflux. The present study examined whether RIT acts similarly to augment AMPH-stimulated DA efflux in the mPFC. *In vivo* microdialysis coupled with HPLC/ED was used to collect and assay extracellular fluid in the mPFC for DA content before and after drug treatment. A dose of RIT was chosen (1.0 mg/kg s.c.) that does not alter basal extracellular DA levels by itself but should effectively block 5-HT2 receptors. 2-4 days following implantation of chronic, indwelling guide cannulae, microdialysis probes were lowered through these cannulae into the mPFC of awake rats. Following collection of baseline samples, RIT or vehicle was administered 30 min before d-AMPH (5.0 mg/kg i.p.). AMPH increased basal extracellular DA levels from 1.41 pg/20 µl to 4.52 pg/20 µl 60 min following AMPH injection (n = 7). In the group pretreated with RIT, basal extracellular DA levels increased from 1.34 pg/20 µl to 3.78 pg/20 µl 60 min following AMPH injection (n = 7), a non-significant difference from the AMPH alone group. Thus, at this dose, RIT did not potentiate carrier-mediated DA release in the mPFC and thus behaves differently than drugs which enhance impulse-dependent DA release in the striatum.

384.5

PHENTERMINE AUGMENTS THE LONG-TERM SEROTONIN DEPLETING EFFECTS OF (+) FENFLURAMINE IN THE RAT. R. Lew* and L.S. Seiden, Department of Pharmacological & Physiological Sciences, University of Chicago, 947 E58th St Chicago IL 60637.

Clinical studies have reported that the appetite suppressants phentermine (DA releaser) and (+) fenfluramine (5-HT releaser) are more effective together for weight reduction than either drug alone. However, the present study demonstrates co-administration of these drugs in rats causes significantly greater depletion of CNS 5-HT levels than either drug alone and cautions against their clinical use. Male Holtzmann Sprague-Dawley rats (275 - 300 g) were injected ip at 1 hr intervals for a total of 4 injections with one of the following treatments: 1) saline (0.9 % NaCl), 2) phentermine (20 mg/kg; PHEN), 3) Low (+) Fenfluramine (3.125 mg/kg; Lo-FEN), 4) High (+) Fenfluramine (12.5 mg/kg; Hi-FEN), 5) Phentermine (20 mg/kg) & Low (+) Fenfluramine (3.125 mg/kg; Phen/Lo-Fen) and 6) Phentermine (20 mg/kg) & High (+) Fenfluramine (12.5 mg/kg; Phen/Hi-Fen). After 7 days recovery, animals were sacrificed and brain regions assayed for 5-HT. PHEN/Lo-FEN and PHEN/Hi-FEN treated animals showed a significantly greater weight reduction (86 - 87% of initial body weight) than animals treated with PHEN, Lo-FEN or Hi-FEN (91-95%) ($P < 0.05$). In 5-HT studies, PHEN reduced 5-HT levels in striatum and hippocampus but not in other brain regions. Lo-FEN and Hi-FEN significantly reduced 5-HT in a dose-dependent manner in all brain regions, with H-FEN causing approximately 80 % depletion. Serotonin depletion by PHEN/ Lo-FEN in all brain regions was significantly greater than Lo-FEN alone ($P<0.05$) and was comparable with Hi-FEN. Serotonin depletion by PHEN/Hi-FEN was not different from Hi-FEN alone. In conclusion, the present study cautions against the combined use of these drugs due to their severe depleting effect on central 5-HT levels. Further studies examining the long-term and neurotoxic effects of these drugs are in progress. (This work was supported by research grant DA00085; L.S.S is supported by RSA MH-105 62)

384.6

POTENTIATION OF THE PATHOLOGICAL EFFECTS OF SUBTOXIC DOSES OF SUBSTITUTED AMPHETAMINES BY INHIBITION OF MONOAMINE OXIDASE TYPE-A. **E.T. Kokotos Leonardi*, H.K. Kramer, X.P. Hou, and E.C. Azmitia. Department of Biology, New York University, New York, New York, 10003.**

MDMA., PCA and FEN have been reported to be selective inhibitors of MAO-A, the subtype found outside serotonergic cells. These agents, therefore may increase levels of extracellular serotonin by stimulating carrier-mediated release , blocking re-uptake and inhibiting serotonin catabolism. Increased levels of extracellular serotonin are believed to stimulate protein kinase C (PKC) activity and glycogenolysis in astrocytes. We now describe the potentiation of the pathological effects of sub-toxic doses of substituted amphetamines by prior inhibition of MAO-A

An established fetal raphe microculture system was used to determine whether inhibition of MAO-A would potentiate the toxicity of the ring substituted amphetamines, MDA, MDE, MBDB. Briefly, amphetamines at 10^{-7} M to 10^{-5} M with or without 10^{-10} M clorgyline were added into 8 day old raphe cultures and left in the culture plates for four days. The toxicity of these agents to fetal raphe neurons was determined by measuring uptake capacity with 50 nM [3]-H-5-HT. MDA, MDE and MBDB toxicity was significantly potentiated in the presence of 10^{-10} M clorgyline. The role of MAO-A inhibition in the toxic mechanism of the substituted amphetamines FEN and MDMA was determined *in vivo*. Animals were pretreated with clorgyline 3 hr. prior to administration of FEN or MDMA. Four experimental groups were established, saline controls, FEN at 2 mg/kg, clorgyline at 1 mg/kg and a final group receiving both FEN and clorgyline (n=4 per group). Animals were injected (s.c.) at 10 hr. intervals and killed by decapitation 2 weeks after the last injection. The density of serotonergic terminals in cortex was quantified by [3]-H-paroxetine binding. Neither FEN nor MDMA resulted in a reduction of paroxetine binding sites. However, clorgyline pretreatment significantly potentiated the toxicity of FEN and MDMA ($p<0.05$, 0.01). These results support the involvement of MAO-A inhibition in the mechanisms by which substituted amphetamines produced serotonergic neuropathies. (NIDA# 271-90-7403)

384.7

DOI DOES NOT MODIFY, 5-HT-ACTIVATED K^+-OR Ca^{2+} CURRENTS, OR EXCITATION BY AMINO ACIDS IN ACUTELY ISOLATED RAT SEROTONERGIC NEURONS N.J.Penington* Dept. of Pharmacol. SUNY (H.S.C) Brooklyn, NY. 11203.

Previous studies of the phenethylamine hallucinogenic drugs have reported that i.v. injection of DOI produced an inhibition of the firing rate of serotonergic dorsal raphe (DR) neurons of the rat. Direct application by iontophoresis demonstrated only weak non-specific effects, but injection into the nucleus produced slowing of cell firing, possibly due to the reduction of on-going synaptic excitation. The effect of DOI (10μM) was tested on adult isolated DR neurons that responded to 5-HT with the activation of an inwardly rectifying K^+ current, but it was without effect. Under conditions that isolated Ca^{2+} current that could be inhibited by 5-HT; DOI (10μM) was similarly without effect, neither blocking nor mimicking the action of 5-HT. Brief applications of glutamate produced an inward current similar to those elicited by synaptic excitation, but DOI (10μM) failed to influence the size or duration of these responses. These results suggest that DOI does not directly influence the cell bodies of DR neurons. Further studies using DR slices, subject to synaptic influences, will be required to reveal the mechanism that may underlie the effects of DOI on DR neurons. (Supported by the Aaron Diamond Foundation).

384.8

IN VIVO EVIDENCE FOR CARRIER-MEDIATED RELEASE OF SEROTONIN BY 3,4-METHYLENEDIOXYMETHAMPHETAMINE. J.F. Nash[1]* and G.A. Gudelsky[2]. [1]The Procter & Gamble Co., 11511 Reed Hartman Hwy., Cincinnati, OH 45241 and [2]Dept. of Psychiatry and Pharmacology, Case Western Reserve University, Cleveland, OH 44106.

In vivo microdialysis was used to determine whether the 3,4-methylenedioxymethamphetamine (MDMA)-induced increase in the extracellular concentration of serotonin (5-HT) involves a carrier-mediated process and to further investigate the state-dependent interaction between 5-HT and dopamine (DA). A concentric microdialysis probe was inserted into the striatum (A: 1.2, L: 3.1, V: -6.5) on the morning of the experiment and dialysate samples were collected every 30 min after a 2 hour equilibration period. MDMA (2.5 - 20 mg/kg, ip) produced a dose-dependent increase in the extracellular concentration of 5-HT. The administration of 20 mg/kg MDMA resulted in extracellular concentrations of 5-HT that were at least 25 times those determined during the baseline period; 5-HT efflux remained significantly elevated for at least 180 min after drug administration. Treatment with the 5-HT uptake inhibitor, fluoxetine (10 mg/kg, ip), alone did not significantly alter the extracellular concentrations of 5-HT or DA. However, the increase in both 5-HT and DA produced by MDMA was attenuated significantly by treatment with fluoxetine. Infusion of the sodium channel blocker tetrodotoxin (TTX 10 μM) through the dialysis probe did not alter MDMA-induced 5-HT release. In contrast, TTX significantly diminished MDMA-induced DA release, consistent with previous reports. The co-administration of carbidopa (25 mg/kg, ip) and L-5-hydroxytryptophan (5-HTP; 50 mg/kg, ip) resulted in a marked increase in 5-HT efflux (e.g., 65 times basal concentrations) but no increase in DA release. The administration of MDMA to rats treated with carbidopa and 5-HTP resulted in a synergistic elevation of 5-HT and DA. These data are consistent with the view that MDMA increases the extracellular concentration of 5-HT by facilitating carrier-medicated 5-HT release which can be enhanced greatly under conditions in which 5-HT synthesis is stimulated. Moreover, these data are supportive of a stimulatory role of 5-HT on state-dependent DA release.

384.9

ANTAGONISM OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE-INDUCED FACILITATION OF BRAIN STIMULATION REWARD BY A D2, BUT NOT A 5HT2, ANTAGONIST. S.L. Fledderjohn, C.J. Schmidt, R.A. Frank*, Department of Psychology, University of Cincinnati, Cincinnati, OH, 45221-0376, and Marion Merrill Dow Research Institute, 2110 E. Galbraith Rd., Cincinnati, OH, 45237.

The effect of 3,4-Methylenedioxymethamphetamine (MDMA), known colloquially as "ecstacy", "X", or "XTC", on central reward mechanisms was measured using thresholds for brain stimulation reward (BSR). Since MDMA is self-administered, is structurally similar to amphetamine, and releases both dopamine and serotonin, it was predicted that it would facilitate BSR. Specific D2 and 5HT2 antagonists were used to determine how MDMA-induced release of dopamine and serotonin contribute to the hypothesized facilitation of BSR.

Bipolar stainless steel electrodes were implanted into the ventral tegmental area (VTA) of Sprague-Dawley rats that were then trained to nose poke for stimulation. The number of pulses delivered in each train of stimulation was then varied from 0 to 15 to determine self-stimulation threshold using a modified version of the method of constant stimuli. Once the rats achieved a stable BSR threshold, the first phase of the study began. In this phase, rats (n=24) were injected with three doses of MDMA (1.0, 3.0 and 6.0 mg/kg, IP) on successive days with the dose of MDMA randomized across subjects. MDMA produced significant, dose-dependent decreases in self-stimulation thresholds which was similar to those produced by amphetamine, nicotine and cocaine.

In the second phase of the study, the effects of the specific D_2 antagonist, eticlopride, and the specific $5HT_2$ antagonist, MDL 100,907, on MDMA-induced facilitation of BSR were evaluated. Eticlopride (0.066 mg/kg) blocked the effects of MDMA, while MDL 100,907 (2.0 mg/kg) had no effect. These data are consistent with the hypothesis that the rewarding effects of MDMA are mediated by dopaminergic rather than serotonergic systems.

This research was supported by NIDA grant DA04483, RA Frank, PI.

384.10

LONG-TERM ATTENUATION OF INHIBITORY EFFECTS OF SEROTONIN AND DOPAMINE ON GLUTAMATE-EVOKED FIRING OF NUCLEUS ACCUMBENS CELLS FOLLOWING REPEATED INJECTIONS OF METHYLENEDIOXYMETHAMPHETAMINE (MDMA). S. R. White*, K. M. Imel and T. Obradovic. Dept. of Vet. & Comp. Anat., Pharmacol. and Physiol., Washington State Univ., Pullman, WA 99164.

MDMA ("ecstasy") releases both dopamine and serotonin from forebrain axon terminals when applied acutely and appears to be selectively neurotoxic to fine-diameter serotonin terminals in the forebrain when administered repeatedly (Steele, et al., Addiction, 1994, 89:539-551). The purpose of this study was to investigate whether repeated exposure to MDMA might alter serotonin and dopamine neurotransmission in the nucleus accumbens (NAc), a forebrain region that is an important component of brain reward circuitry. Male Sprague-Dawley rats received twice daily injections of MDMA (20 mg/kg, s.c.) or saline for 4 successive days. Two weeks later, the rats were anesthetized with urethane and effects of microiontophoretically applied serotonin, dopamine or the D1 agonist SKF38393 on glutamate-evoked firing of cells in the nucleus accumbens core were tested. The inhibitory effects of all three drugs were markedly attenuated in the MDMA-pretreated rats compared to the saline-pretreated control rats. For example, dopamine (40 nA, 60 s) decreased glutamate-evoked firing 47.5 ± 3.4% for cells from the saline-pretreated rats compared to only 21.7 ± 3.5% for cells from the MDMA-pretreated rats. Similarly, serotonin (40 nA, 60 s) decreased glutamate-evoked firing 37.5 ± 6.8% in control rats compared to 14.2 ± 4.1% in MDMA-pretreated rats. These findings indicate that repeated exposure to MDMA produces long-term alterations in responses of NAc cells to serotonin and dopamine. Since both of these monoamines have been implicated in depression and psychosis as well as drug abuse, alterations in their effects on cells in the NAc (and perhaps other forebrain areas) following repeated exposure to MDMA might be expected to have wide-ranging consequences. (Supported by DA08116)

384.11

DIFFERENTIAL EFFECTS OF METHYLENEDIOXYMETHAMPHETAMINE (MDMA) IN THE NUCLEUS ACCUMBENS CORE AND SHELL. T. Obradovic*, K. M. Imel and S. R. White. Dept. of Vet. & Comp. Anat., Pharmacol. and Physiol., Washington State Univ., Pullman, WA 99164.

The nucleus accumbens (NAc) has been partitioned into core and shell regions based on differences in neuronal morphology, anatomical connections, neurotransmitter distribution and receptor distribution. It is well-established that the NAc is an important locus for mediating the rewarding properties of abused drugs, but little is known about whether these drugs may have differential effects in the shell and core. This study compared the effects of the euphoria-producing amphetamine derivative MDMA ("ecstasy") on glutamate-evoked firing of cells in the shell (54 cells) and core (102 cells) regions of the NAc using microiontophoresis combined with extracellular recording of single unit activity. Because MDMA increases extracellular concentrations of monoamines in the accumbens, effects of serotonin, dopamine, and the selective D1 agonist SKF 38393 were also tested in the shell and the core. MDMA (20-60 nA, 60 s) inhibited glutamate-evoked firing of cells in a dose-dependent manner in both core and shell. However, the magnitude and duration of the inhibition was significantly greater in the core than the shell. Dopamine, SKF 38393 and serotonin also inhibited firing of the NAc cells, but, in contrast to MDMA, these drugs did not have a differential effect in the core and shell regions. Therefore, the differential effect of MDMA in the two regions did not appear to be mediated by differences in serotonin or dopamine receptors. Whether MDMA may have a differential effect on monoamine release and/or reuptake from terminals in the shell compared to the core is not known. Although the mechanisms responsible for the differential effects of MDMA on core and shell cells remain to be determined, the results of this study indicate that abused drugs may have markedly different effects on neuronal excitability in the subregions of the NAc. (Research supported by grant DA08116.)

384.12

INDUCTION OF LOCOMOTOR ACTIVITY BY GLUTAMATE ANTAGONIST (DNQX) INJECTED IN THE VENTRAL TEGMENTAL AREA. A.Dalia,* N.J. Uretsky, and L.J. Wallace. Division of Pharmacology, College of Pharmacy, Ohio State University, Columbus, OH 43210.

The ventral tegmental area (VTA) dopamine neurons (A10) play a critical role in the etiology of schizophrenia as well as in the reinforcing properties of drugs of abuse. Since AMPA injected into the VTA elicits locomotor activity (LMA) similar to that of amphetamine (AMPH). Therefore, we hypothesized that AMPA/KA receptors in the VTA might control DA neuronal activity. To test this hypothesis, DNQX, an antagonist of these receptors, was injected bilaterally into the VTA immediately before the administration of AMPH (1.0 mg/kg). Contrary to our hypothesis, bilateral injection of DNQX (1.0 ug) into the VTA of both control and AMPH- treated rats increased LMA. In addition, unilateral injection of DNQX produced contraversive turning. Experiments were then designed to determine if dopamine is involved in this effect of DNQX. The locomotor stimulation produced by DNQX was not associated with a change in DOPAC/DA level in the nucleus accumbens or the striatum However, complete dopamine depletion achieved by administration of α-methyl-para-tyrosine and reserpine blocked DNQX induced LMA. These results suggest that basal activity but not activation of dopamine neurons is required for the locomotor response to DNQX. (Supported by DA07722 and DA06776).

384.13

FURTHER CHARACTERIZATION OF THE EFFECT OF AMPHETAMINE ON EXTRACELLULAR GLUTAMATE LEVELS IN THE VENTRAL TEGMENTAL AREA. J. Ng*, C.J. Xue and M.E. Wolf. Dept. of Neuroscience, Finch Univ. of Health Sciences/The Chicago Medical School, N. Chicago, IL 60064.

Considerable evidence supports the idea that excitatory amino acid (EAA)-containing projections from the prefrontal cortex to the ventral tegmental area (VTA) are important in the development of behavioral sensitization to amphetamine (AMPH). To determine whether AMPH activates this pathway, *in vivo* dialysis was used to measure extracellular levels of glutamate (GLU) and aspartate (ASP) in VTA after systemic administration of AMPH. We have reported previously that low AMPH doses (1-2.5 mg/kg, s.c.) fail to alter extracellular GLU or ASP levels in VTA. A higher dose (5 mg/kg, s.c.) similarly failed to produce immediate alterations in GLU or ASP, but resulted in a delayed increase in GLU levels which attained statistical significance 3 hrs after AMPH injection. The magnitude of this delayed increase was similar in control and AMPH sensitized rats. It is possible that repeated elevation of GLU levels in VTA leads to compensatory alterations in EAA receptors on VTA dopamine (DA) neurons, ultimately resulting in increased excitatory drive to VTA DA neurons and thereby contributing to the initiation of sensitization. Consistent with this hypothesis are: 1) the ability of intra-VTA MK-801 to prevent sensitization (Kalivas and Alesdatter, JPET 267:486, 1995), 2) the increase in sensitivity to GLU exhibited by VTA DA neurons in sensitized rats (White et al., JPET 273:445, 1995), and 3) the increased firing rate and autoreceptor subsensitivity exhibited by VTA DA cells in sensitized rats (White and Wang, Brain Res. 309:283, 1984). Studies are underway to characterize the pharmacological basis of the delayed increase in GLU levels elicited by AMPH, and to determine whether inhibitors of the GLU transporter unmask additional effects of AMPH on GLU or ASP levels in the VTA. Support: DA 07735.

384.14

INCREASE IN DOPAMINE TRANSPORTER mRNA IN THE VENTRAL TEGMENTAL AREA OF AMPHETAMINE SENSITIZED RATS. W. X. Lu* and M.E. Wolf. Dept. of Neuroscience, Finch Univ. of Health Sci /The Chicago Medical School, North Chicago, IL 60064.

Because of the importance of the dopamine transporter (DAT) in the action of psychomotor stimulants such as amphetamine (AMPH) and cocaine (COC), there has been interest in the possibility that behavioral sensitization to psychomotor stimulants involves alterations in DAT function. Studies of the effect of repeated COC administration on DAT function have yielded inconsistent results, depending on the COC treatment regimen and the length of withdrawal. However, it seems clear that repeated COC can alter the expression of the DAT. In contrast, very few studies have examined the effect of repeated AMPH on DAT levels. We have therefore examined the effect of AMPH sensitization on DAT mRNA levels in rat midbrain. Rats were treated for 5 days with water or 5 mg/kg AMPH, and perfused 3 or 14 days after the last injection. Using a new quantitative method of in situ hybridization (see abstract by Wolf, Chen and Lu), coronal brain sections were hybridized with a ^{35}S-labeled oligonucleotide probe for the DAT. Preliminary data suggest increased levels of DAT mRNA in the ventral tegmental area (VTA) of AMPH sensitized rats at both 3 and 14 days off. The region with increased DAT mRNA, which includes the paranigral nucleus, the interfascicular nucleus and a portion of parabrachial pigmented nuclei, is lateral and dorsal to the rostral interpeduncular nucleus and medial to substantia nigra. These data suggest that altered DAT levels in dopaminergic projections originating in the VTA may contribute to AMPH sensitization. Supported by USPHS Grant DA 07735.

384.15

EXPRESSION OF mRNA FOR GLUTAMATE RECEPTOR SUBUNITS IN NUCLEUS ACCUMBENS AND PREFRONTAL CORTEX OF AMPHETAMINE SENSITIZED RATS. C.J. Xue, L.M. Monteggia, H.Y. Chen, J.R. Mathiasen*, W.X. Lu and M.E. Wolf. Dept. of Neuroscience, Finch University of Health Sciences/The Chicago Medical School, N. Chicago, IL 60064.

Electrophysiological studies have shown that sensitization to amphetamine (AMPH) is accompanied by altered responsiveness of both ventral tegmental area dopamine neurons and nucleus accumbens (NAc) neurons to iontophoretically applied glutamate, suggesting sensitization-related alterations in glutamate receptor function (White et al., JPET 273:445, 1995). The function of both AMPA and NMDA receptors is known to be affected by differential incorporation of subunits. We therefore compared levels of mRNA for AMPA and NMDA receptor subunits in rats treated for 5 days with water or 5 mg/kg AMPH. Brain tissue was perfused 3 or 14 days after the last injection. A novel *in situ* hybridization protocol that allows quantification of mRNA levels was employed (see abstract by Wolf, Chen and Lu). ^{35}S-labeled oligonucleotide probes to AMPA subunits GluR1,2,3,4 and NMDA subunits NR1,2A,2B,2C were obtained from DuPont. NIH Image software was used for quantitative analysis of autoradiographs. In NAc, preliminary data suggest decreased levels of GluR2 mRNA and no change in GluR1 and GluR3 mRNAs in AMPH sensitized rats at 3 days off. At 14 days off, no differences between water and AMPH treated rats were apparent for GluR 1-3 mRNAs. In prefrontal cortex (PFC), there was no change in GiuR1,2,3 mRNAs at 3 days off, but modest increases in GluR1 and GluR3 mRNAs at 14 days off. GluR4 mRNA levels in NAc and PFC were very low. Studies with NR1,2A,2B,2C probes are in progress; preliminary data suggest alterations in NR1 mRNA levels in both NAc and PFC of AMPH sensitized rats. Thus, altered levels of AMPA and NMDA receptor subunit mRNAs may play a role in AMPH sensitization. Support: DA 07735.

384.16

EFFECTS OF DOPAMINERGIC AND GABAERGIC MANIPULATIONS OF VENTRAL PALLIDUM ON VTA SELF-STIMULATION. W. Gong*, D. B. Neill and J. B. Justice, Jr. Depts. of Psychology and Chemistry, Emory University, Atlanta, GA 30322

The ventral tegmental area (VTA) dopamine systems have well established roles in drug reward. Ventral pallidum (VP) receives a GABAergic projection from nucleus accumbens (NAS), which receives a massive dopaminergic projection from VTA. In addition, VP also receives a smaller dopaminergic projection directly from VTA. The aim of the present study was to examine the involvement of VP dopaminergic and GABAergic neurotransmissions in VTA electrical self-stimulation reward in rats. We used the autotitration procedure, in which the intensity of VTA stimulation was stepped down 3 uA every fifth depression of a stimulation lever. The rat was free to travel to another lever that, when depressed, reset the intensity to the maximum value for that rat. The major variable of interest was the stimulation intensity at which the rat reset. VP drug injections of 0.5 μl were bilateral. Amphetamine (2.5-10 μg) produced a dose-dependent decrease in reset current threshold without significantly changing total lever pressing in the 20 min test session. Picrotoxin (0.05-0.2μg), on the other hand, did not significantly change the reset current or total lever pressing. These findings suggest that dopamine at VP is involved in VTA self-stimulation reward but GABA is not, which is consistent with our previous findings that intra-VP injection of amphetamine induced conditioned place preference but intra-VP injection of picrotoxin did not.

384.17

MICRO-INJECTION OF CHOLERA TOXIN INTO THE VENTRAL TEGMENTAL AREA PRODUCES ENHANCED LOCOMOTOR RESPONSES TO SUBSEQUENT AMPHETAMINE ADMINISTRATION. J.J. Byrnes*, D.M. Weinstein, N.J. Uretsky, and L.J. Wallace, Neuroscience Program and Division of Pharmacology, The Ohio State University, Columbus, OH 43210.

The neural substrates involved in behavioral sensitization to psychostimulants include the dopamine (DA) neurons in the ventral tegmental area (VTA) and their axon terminals in the nucleus accumbens (N.Acc). In the VTA, activation of DA D1 receptors appears necessary for the development of sensitization as this phenomenon is blocked by intra-VTA administration of a D1 receptor antagonist (Stewart and Vezina, 1989). In many brain regions D1 receptors are positively coupled to adenylate cyclase (AC) activation. Therefore, we tested the hypothesis that sensitization to psychostimulants requires enhanced AC activity in the VTA. On Day 0, rats received bilateral injections of cholera toxin (CTX), a known activator of AC, or its vehicle into the VTA followed by either repeated (Days 1, 3, 5, 7, and 18) or acute (Day 18 only) administration of AMPH (0.5 mg/kg, i.p.). On Day 19, tissue slices of the VTA and N.Acc were prepared and AMPH-induced [^{3}H]DA release was measured. CTX (0.1-1.0 ug/side) treatment resulted in a dose-dependent enhancement of AMPH-stimulated locomotor activity on Day 1. The locomotor responses to subsequent AMPH treatments in high dose CTX-treated animals were markedly augmented beyond the response seen on Day 1. This long-lasting sensitivity to AMPH was associated with increased AMPH-induced [^{3}H]DA release in the VTA but not the N.Acc. Interestingly, there was no effect of CTX treatment on either AMPH-stimulated locomotor activity or [^{3}H]DA release when the first AMPH treatment was on Day 18. Moreover, repeated treatment with this low dose of AMPH in the absence of CTX pretreatment did not produce a sensitized locomotor response. These results indicate that intra-VTA CTX treatment facilitates sensitization to low doses of repeated AMPH which is associated with the increased ability of AMPH to release DA in the VTA. In addition, these effects may be mediated by alterations in cellular mechanisms linked to D1 receptors in this brain region. (Supported in part by DA07722, DA06776, and Sigma Xi)

384.18

COMPARISON OF SENSITIZED LOCOMOTOR BEHAVIOR IN RATS ELICITED BY AMPHETAMINE AND PERTUSSIS TOXIN. D.M. Weinstein, S. Narayanan, J.J. Byrnes, L.J.Wallace, and N.J.Uretsky, Division of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, OH 43210.

Amphetamine (AMPH) administered in a repeated, intermittent fashion produces a persistent, enhanced behavioral response to a subsequent challenge dose of AMPH. Administration of pertussis toxin (PTX) into the ventral tegmental area (VTA) has also been shown to produce an enhanced behavioral response to an acute challenge of AMPH. The question arises as to whether AMPH and PTX produce comparable behavioral responses and thus can be considered equivalent models of the same phenomenon. We therefore conducted experiments designed to compare sensitizations elicited by AMPH and PTX in the context of the magnitude of enhanced response to an AMPH challenge. Rats pretreated with 4 doses of AMPH (5mg/kg i.p.) showed a 70% elevation of locomotor activity relative to control in response to a 0.5mg/kg AMPH challenge dose given 10 days later. However, rats pretreated bilaterally in the VTA with PTX (0.3μg/side) and challenged with AMPH (0.5mg/kg i.p.) 15 days later, exhibited locomotor activity greater than 900% over control. To determine whether we could approach the level of activity produced by PTX pretreatment, we implemented 2 additional AMPH pretreatment paradigms: 10mg/kg i.p. or escalating 5,10,15,20mg/kg i.p. every other day for 4 doses. Rats were challenged with AMPH, 0.5mg/kg i.p., 7 days later. Although these animals also exhibited sensitized behavior, the magnitude was not different than the original amphetamine regimen of 5mg/kg, suggesting a maximal locomotor response to AMPH had been reached. These results suggest that AMPH-based locomotor sensitization plateaus well below that of the PTX-based response, and hence may imply that AMPH and PTX produce sensitized behavior through disparate mechanisms. Further studies are underway to investigate the in vitro dopamine release characteristics of these two drug models. (Supported in part by DA07722 and DA06776)

384.19

ENHANCED RESPONSES TO DIRECT ACTING DOPAMINERGIC AGONISTS AFTER PRETREATMENT WITH PERTUSSIS TOXIN IN THE VENTRAL TEGMENTAL AREA. S. Narayanan*, L.J. Wallace, and N.J. Uretsky, Division of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, OH 43210.

The development of sensitization to psychostimulant drugs may involve a transient reduction in the G/G_o proteins linked to D_2 receptors in the ventral tegmental area (VTA). Supporting this hypothesis is the observation that the administration of pertussis toxin (PTX), which inactivates the G/G_o proteins, into the ventral tegmental area (VTA) results in an enhanced locomotor response to the systemic administration of amphetamine (Steketee et al. 1991). We have demonstrated that the administration of PTX into the VTA also produces an enhanced locomotor response to apomorphine administered either systemically or directly into the nucleus accumbens, suggesting that postsynaptic as well as presynaptic changes have developed at dopaminergic synapses in the N.Ac. The purpose of the present study was to evaluate the role of postsynaptic D_1 and D_2 receptors in the N.Ac in the enhanced response to dopaminergic agonists in PTX-treated rats. Rats injected bilaterally with PTX (0.3 μg) into the VTA showed an enhanced locomotor response to amphetamine (0.5 mg/kg, i.p.) 2 weeks after PTX administration. The stimulant effects of amphetamine in these animals were antagonized by SCH23390 (0.1 mg/kg), a D_1 receptor antagonist, but not by eticlopride (0.1 mg/kg), a D_2 receptor antagonist. Both compounds antagonized the effects of amphetamine in vehicle-treated animals. In addition, the PTX-treated animals showed a markedly magnified locomotor response to SKF38393, a D_1 receptor agonist administered either systemically or directly into the nucleus accumbens at doses (10 mg/kg i.p and 0.1 μg/side respectively) which produced minimal effects on the locomotor activity of the vehicle-treated control animals. High doses of quinpirole (1 mg/kg, s.c.), a D_2 receptor agonist induced a small but significant locomotor response in PTX-treated animals. These results suggest that the administration of PTX into the VTA produces an increase in sensitivity of postsynaptic D_1 and D_2 dopamine receptors in the N.Ac.

384.20

ROLE OF VENTRAL TEGMENTAL AREA cAMP SYSTEMS IN BEHAVIORAL SENSITIZATION TO AMPHETAMINE. B.K. Tolliver*, K. Hsu, L. Ho, and S.P. Berger, University of California, San Francisco and SFVAMC, San Francisco CA 94121.

Considerable evidence has implicated ventral tegmental area (VTA) G protein signal transduction systems in the development of behavioral sensitization to psychostimulants. Sensitization has been associated with alterations in G proteins in the VTA following chronic stimulant treatment, and can be induced by persistent inactivation of G_i proteins following VTA microinjection of pertussis toxin. A recent report of sensitization following microinjection of cholera toxin into the nucleus accumbens has demonstrated that cAMP systems may be involved in this process. However, the role of cAMP systems of the VTA in sensitization have not been elucidated. In the present study, bilateral microinjection of cholera toxin (500 ng / 500 nl / side) into the VTA of male Sprague-Dawley rats resulted in a significant baseline hyperactivity and sensitized locomotor response to 1.5 mg/kg d-amphetamine relative to rats receiving bilateral VTA microinjections of saline (500 nl / side) when assessed 24 hours after microinjection. In addition, the development of sensitization evident 72 hours after a single bilateral VTA microinjection of d-amphetamine (5 μg / 500 nl / side) was prevented by coadministration of the cAMP-dependent / cGMP-dependent protein kinase inhibitor H8 (at a dose of 10 μg / 500 nl / side, but not at 5 μg / 500 nl / side). These results extend previous studies which have established the importance of the ventral tegmental area in the development of behavioral sensitization and suggest that cAMP systems in the VTA may play a crucial role in this neuroadaptive response to psychostimulant drugs. (Supported by USPHS Grant DA 07376-04)

DRUGS OF ABUSE: AMPHETAMINES AND OTHER STIMULANTS II

385.1

AMPHETAMINE, COCAINE AND MORPHINE PRODUCE LARGER INCREASES IN DOPAMINE RELEASE IN THE N. ACCUMBENS OF ROMAN HIGH-AVOIDANCE (RHA) *VS.* LOW-AVOIDANCE (RLA) RATS. **M.G. Corda*, O. Giorgi, D. Lecca, G. Carboni, V. Frau, G. Piras, V. Valentini and G. Di Chiara. Dept. Toxicol., Univ. of Cagliari, Italy.**

RHA and RLA rats are selected and bred for rapid versus poor acquisition of two-way avoidance behavior in a shuttle box. Numerous behavioral studies have found that RLA rats are more "emotional" than RHA rats. Further, anxiolytics improve the performance of RLA rats in different behavioral tests. The present study was undertaken to analyze line-related differences in the effects of psychotropic drugs with rewarding properties. To this aim, we used brain microdialysis to compare the effects of amphetamine (AMPH, 0.25 mg/kg, s.c.), cocaine (COC, 5 mg/kg, s.c.), and morphine, (MORPH, 0.5 mg/kg, s.c.) on the release of dopamine (DA) in the n. accumbens (NA) in both rat lines. No line-related differences were observed in the basal DA release (RHA: 73 ± 4, RLA: 67 ± 3). The effect of AMPH on DA release was more robust in RHA rats (maximal increase above basal value 40 min after treatment: RHA, +200 %; RLA, +125 %, $p < 0.05$). Likewise, the maximal increment caused by COC at 20 min after injection was more pronounced in RHA (+117 %) than in RLA rats (+55 %), whereas the peak effect of MORPH 80 min after treatment was +64 % and +24 % in RHA and RLA rats, repectively. These line-dependent differences persisted for 2 h with AMPH and COC and 4 h with MORPH. Interestingly, no significant line-related differences were observed with larger doses of AMPH and MORPH. Because the mesolimbic DAergic system plays a pivotal role in operant behavior by giving motivational relevance to environmental and pharmacological stimuli, our results suggest that RHA and RLA rats may have different dependence liabilities when exposed to drugs of abuse.

385.2

PARALLEL STRAIN-DEPENDENT DIFFERENCES FOR AMPHETAMINE-INDUCED LOCOMOTION AND DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS AS MEASURED BY TRANSCEREBRAL MICRODIALYSIS IN FREELY MOVING MICE. A. Zocchi*, C. Orsini, S. Cabib and S. Puglisi-Allegra, Dip. Psicologia, Universita' "La Sapienza", and Istituto di Psicobiologia e Psicofarmacologia (CNR) Roma, Italy

Mice DBA/2 and C57BL/6 are known to respond differently or even in opposite ways to DA agonists. In particular C57 show higher sensitivity to the stimulant effects of amphetamine on locomotor activity (Cabib S. Behavioral Pharmacology, 1993, 4, 367-374). The present study investigated the role of mesolimbic Dopamine (DA) release in the different sensitivity to the psychomotor stimulant effects of amphetamine in mice of the C57 and DBA inbred strains. Male mice (8-10 week old) living in groups of 6 in standard breeding cage were implanted with transversal microdialysis tubes within the nucleus accumbens and singly housed for 48 hr before testing. On the test day the home cage of each mouse was placed on an activity meter (ANIMEX) and basal levels of activity and DA outflow were collected each 15 min for at least 180 min, then mice were injected (i.p.) with either saline or D-amphetamine (2.5 mg/kg). Samples and activity scores were collected every 15 min for 120 min. Saline injection did not affect either activity or DA outflow in any strain. Amphetamine significantly enhanced DA outflow and activity beyond saline levels in mice of the C57 strain. The effect of amphetamine on activity and DA outflow was time-dependent, peaking 45 min following injection. By contrast no significant effects were found in DBA mice. These results strongly suggest that these strain-dependent differences relate to a different susceptibility of the mesolimbic DA system to the psychostimulant.

385.3

COMPARISON OF METHAMPHETAMINE-HCl AFTER INHALATION AND I.V. ROUTES OF ADMINISTRATION IN MICE. Y. Meng, A.H. Lichtman, D.T. Bridgen and B.R. Martin*
Department of Pharmacology and Toxicology, Medical College of Virginia-Virginia Commonwealth Univercity, Richmond, VA 23298.

Smoking of methamphetamine has been a very popular method of administration among drug users. The purpose of the present study was to investigate the volatilization and pyrolysis of methamphetamine-HCl, and to compare the relative pharmacological potencies and the biodisposition of this drug between inhalation and i.v. routes of administration in mice. At 200°C, 46% of the drug was volatilized, and the formation of pyrolytic products was insignificant. In the inhalation studies, 6 mice at a time were exposed to various amounts of volatilized methamphetamine-HCl for 5 min, and then assessed for locomotor activity. The maximum stimulation of activity was approximately 8 fold after either i.v. injection (ED_{50}=0.88 µg/kg) and inhalation exposure (ED_{50}=3mg) to methamphetamine-HCl. In addition, the biodisposition for the ED_{50} doses of methamphetamine-HCl by i.v. or inhalation route of administration were very similar. Intravenous injection of 1 mg/kg and inhalation exposure of the volatilization of 3 mg of methamphetamine-HCl resulted in drug equivalents of 1.2 and 1.0 µg/g in the brain, 1.0 and 0.8 µg/g in the whole body, and 0.24 and 0.28 µg/ml in the plasma, respectively. These data suggest that inhalation exposure and i.v. injection are equipotent routes of administration for methamphetamine. This research was supported by NIDA grant DA-02396.

385.4

SENSITIZATION TO LOCOMOTOR EFFECTS OF METHYLPHENIDATE IS INFLUENCED BY THE TIME OF ADMINISTRATION O. Gaytan, A.Swann, and N. Dafny* Dep. of Neurobiology, UT Medical School at Houston, P.O. Box 20708, Houston, TX 77225

Chronic administration of stimulants has been reported to produce reverse tolerance, or sensitization, to its effects on motor activity an stereotypy. This adaptation may be related to the pathophysiology of recurrent psychiatric disorders. Since disturbances in circadian rythms are also found in many of these disorders, the relationship between sensitization and chronobiological factors became of interest. This study was designed to investigate the effect of repeated treatment with methylphenidate (MPD) on the circadian pattern of motor activity, and whether the development of sensitization differs if MPD is given at different times of the light/dark cycle. Male Sprague-Dawley rats were housed in activity monitoring system test cages after 7 days of acclimation to L/D cycle (07:00/1900) and monitored continously for 16 days as follows: Baseline (Day 1- 2), Saline Injection (Day 3), Challange Doses (Day 4) - either 0.6, 2.5, or 10 mg/kg of MPD, Maintenance dose (Day 5-9) - 2.5 mg/kg once a day, Withdrawal (Days 10-14), Re-Challenge (Day 15) - same doses as day 4. This regimen was run at 4 times (0800, 1400, 2000, 0200). Two locomotor parameters are presented (Horizontal Activity and Total Distance). In general, sensitization occurred at all the time points, and the parameter of total distance was more affected. There were differences between the time points with the 2000 displaying the weakest sensitizion. Adminstration during the middle, but not at the beginning, of the light and dark phase (1400 & 0200) caused changes in the levels of diurnal activity. In conclusion, the development of sensitization and daily pattern of motor activity were dependent on the time of administrsation.

385.5

INDIVIDUAL DIFFERENCES IN NOVELTY SEEKING ON THE PLAYGROUND MAZE PREDICTS AMPHETAMINE-INDUCED LOCOMOTOR ACTIVITY. J.E. Klebaur, P.A. Abner, and M.T. Bardo. Dept. of Psychology University of Kentucky, Lexington, KY 40506-0044.

Previous research has shown that a rat's level of activity in a novel environment can predict the strength of amphetamine-induced locomotor behavior and self-administration, but not amphetamine conditioned place preference. The increase in activity observed when a rat is exposed to an inescapable novel environment is thought to reflect escape behavior due to stress. To assess approach to novelty in a free-choice test, we have examined the ability of a new test the playground maze (Nicholls, Springham, & Mellanby, 1992), to predict individual differences in response to amphetamine (0.25 or 1.0 mg/kg). Using the playground maze to categorize rats as either high or low novelty seekers, it was found that high novelty seekers were more sensitive to the locomotor stimulant effects of amphetamine, but not to amphetamine conditioned place preference. These results suggest that individual differences in a free-choice test of novelty preference can predict the psychostimulant effects of amphetamine, but not the rewarding effects.

385.6

INDIVIDUAL DIFFERENCES IN RESPONSE TO AMPHETAMINE. P. Clausing, S.A. Ferguson, and J.F. Bowyer. NCTR/FDA, Jefferson, AR 72079-9502.

In spite of efforts to minimize experimental variation, considerable individual differences in response to amphetamine (AMPH) are commonly observed. To further elucidate this phenomenon we recorded the behavior of adult male Sprague-Dawley rats after 1 x 2.5 mg/kg AMPH s.c., and simultaneously determined extracellular AMPH-levels in the caudate/putamen. The pharmacokinetic profile revealed the existence of "LOW AMPH" rats with peak AMPH concentrations less than 80 pg/µl microdialysate and "HIGH AMPH" rats with peak AMPH concentrations up to 150 pg/µl microdialysate. The "HIGH AMPH" group dissociated into *RESPONDERS* and *NONRESPONDERS* according to their behavior after AMPH administration. Circling (*i.e.*, the number of complete turns) in the microdialysis bowl was used as an accurate behavioral criterion which can be easily recorded. *RESPONDERS* exhibited a significant positive correlation (r=0.875, p=0.023, n=6) for peak number of Circlings *vs.* peak AMPH levels in the microdialysate. Currently we are conducting experiments to see if the same individuals which are behavioral *NONRESPONDERS* after 1 x 2.5 mg/kg also display an attenuated hyperthermic response after multiple doses of 5 mg/kg AMPH. In conclusion, earlier suggestions (Segal & Kuczenski, J. Pharmacol. Exper. Ther. 242:917-926, 1987) that a pharmacokinetic explanation is inconsistent for individual differences in behavioral response to AMPH are partially corroborated. It needs to be determined if NONRESPONDERS have lower extracellular dopamine levels or if they have the same levels but are less sensitive to dopamine.

385.7

INTRAPERITONEAL 3-NITROPROPIONIC ACID (3-NPA) AND AMPHETAMINE (AMPH) CAN COMBINE TO PRODUCE DAMAGE TO TERMINALS AND CELL BODIES IN THE STRIATUM AND MUSCLE RIGIDITY. J.F. Bowyer*, W. Slikker Jr., L. Schmued, Z. Binienda, A.C. Scallet and P. Clausing. Div. of Neurotoxicol., NCTR/FDA, Jefferson, AR 72079-9502.

Neither 2 doses (2 hrs apart) of 3.75 mg/kg AMPH alone nor a single dose of 30 mg/kg 3-NPA i.p. produced signs of neurotoxicity in the striatum or lowered striatal dopamine content in adult rats. Administration of a higher dose of 3-NPA alone (40 or 50 mg/kg) produced either lethality within 24 hrs or, in surviving rats, minimal neurotoxicity in the striatum. However, when 30 mg/kg 3-NPA was administered with 2 doses of 3.75 mg/kg AMPH a wide range of neurotoxicity to the striatum was produced. Over 30% of the rats showed muscle rigidity/catatonia, decreased food consumption for several days after dosing and degeneration of axons and cell bodies in the medial caudate/putamen of the striatum with little damage to the globus pallidus, cerebral cortex, hypothalamus or thalamus. The 5% that exhibited hind-limb paralysis and survived 24 hrs after combined dosing had axonal and neuronal degeneration in the caudate/putamen, globus pallidus, thalamus and cerebellar nuclei. In 30% of the rats dosed with both AMPH and 3-NPA only transient torpidity with minimal muscle rigidity was observed 1 to 3 hrs after the combined dosing. Such rats did not show prominent histological signs of neuronal degeneration in the striatum but did show dopamine depletions (50% of control) 8 to 10 days after exposure. The combined effect of mitochondrial dysfunction produced by 3-NPA along with the activation of specific neuronal pathways by AMPH may have predisposed axons and cell bodies in these pathways to degeneration.

385.8

METHAMPHETAMINE DEPLETES DOPAMINE AND DECREASES GLUTAMATE IMMUNOREACTIVITY WITHOUT INDUCING TERMINAL DEGENERATION. K. B. Burrows* and C. K. Meshul. Dept. of Medical Psychology and Pathology, Oregon Health Sciences University, and VA Medical Center, Portland OR 97201.

Methamphetamine (METH) treatment results in long-lasting depletion of dopamine (DA) in the striatum (Str). Glutamate (GLU) is believed to play a role in METH neurotoxicity. Blockade of GLU overflow or pretreatment with GLU antagonists protects against DA depletion. The goal of this study was to determine if METH effects the concentration of GLU in presynaptic nerve terminals in rats. METH treatment (4-5 mg/kg X 2 hrs X 4 inj) depleted striatal DA (40% of control) and decreased the density of GLU immunolabeling in presynaptic terminals as determined by post-embedding immuno-gold electron microscopy. Decreased immunoreactivity was present 24 hrs after METH treatment only in Str. Significant decreases were found 72 hrs after METH in Str, nucleus accumbens (NAc), and motor cortex (Ctx). The reduction in GLU immunoreactivity suggests that METH treatment may result in either prolonged release, or decreased uptake and/or synthesis of GLU. Although it has been assumed that METH results in the destruction of DA-containing terminals in Str, there is little direct electron microscopic evidence to support this hypothesis. Therefore, the effect of METH (4-5 mg/kg X 2 hrs X 4 inj) on synaptic morphology in the Str, NAc, and Ctx was examined. Despite the loss of DA observed in the current study, no evidence of cell body, terminal, or axonal degeneration was found ultrastructurally. (Supported by Dept. of Veterans Affairs and a N.L. Tartar Fellowship)

385.9

BEHAVIORAL AND BIOCHEMICAL ASPECTS OF GM_1 ATTENUATION OF MDMA-INDUCED NEURONAL TOXICITY. E. P. Finnerty*, R. Dunbar and D. Hoganson. Univ. of Osteopathic Med. & Health. Sciences and Drake University, Des Moines, IA 50312

MDMA (Ectasy), a methylamphetamine derivative, has been found to produce severe and somewhat selective damage to CNS serotonergic (5-HT) neurons. This damage, though extensive, does not involve cell death. The ganglioside GM_1 has demonstrated potential neurotrophic properties which may enhance the rate of recovery of the neurons from the MDMA-induced effects. To assess this GM_1 effect, MDMA (20 mg/kg x 4 days), GM_1 (40 mg/kg), MDMA and GM_1 or saline was injected (i.p.) into male rats. Behavioral activity was determined for 24 hours on day 5 or day 12 following the beginning of the injections. A general increase in activity on day 5 was noted for the MDMA group (consistent with a release of 5-HT and/or catecholamines) while the activity of the MDMA/GM_1 group was lower, at the level of the control. At day 12 the MDMA group's activity was decreased relative to the control (consistent with a 5-HT depletion) while the MDMA/GM_1 group's activity was at or above that of the control. Biochemical analysis of brain tissue obtained from the animals sacrificed on day 7 or 14 following the beginning of the injections demonstrated a profound depletion of 5-HT, dopamine and norepinephrine with administration of MDMA. The MDMA/GM_1 group also showed this depletion, though to a smaller degree, suggesting some attenuation of the toxic effects.

We gratefully acknowledge FIDIA Pharmaceutical Co. for their generous gift of the GM_1.

385.10

Bcl-2 PROTECTS NEURAL CELLS FROM METHAMPHETAMINE-INDUCED NEUROTOXICITY *IN VITRO*. S. Ordonez*, J. Ordonez, J. Cadet Molecular Neuropsychiatry Section, Division of Intramural Research, NIH/NIDA IRP, Baltimore, MD 21224

Methamphetamine (METH) causes neurotoxic effects on monoaminergic neurons. Previous studies from this laboratory had suggested a role on oxidative stress in METH-induced toxic effects. Specifically, transgenic mice with high superoxide dismutase (SOD) activity are protected against these deleterious effects of the drug. We have to continue to dissect the pathway through which superoxide radicals cause the changes after METH administration. In order to do so, we have used immortalized bcl-2 neural cells in which the proto-oncogene has been introduced because bcl-2 is known to work in an oxidant pathway to protect cells against oxidative stress. We predicted that these neural cells expressing bcl-2 will be protected against METH-induced toxicity. Cells expressing bcl-2 and cells expressing vector alone were treated with various doses of METH for various time points. Flow cytometry was then used to assess cellular viability. All cells exposed to METH develop cytoplasmic vacuoles. In addition, METH caused dose-dependent increase in cell death which was observed both after 24 and 48 hours of exposure. However, bcl-2 inhibits these toxic effects of the drug. These results provide further evidence of a role of an oxidant pathway in METH-induced neurotoxicity. Data will be presented to document if cell death caused by METH is via apoptosis or necrosis.

385.11

INVOLVEMENT OF SUPEROXIDE RADICALS IN METHAMPHETAMINE INDUCED AP1 DNA BINDING ACTIVITIES. P. Sheng,* X.-B. Wang, B. Ladenheim, C. Epstein, and J. L. Cadet Molecular Neuropsychiatry and Molecular Neurobiology Sections, Division of Intramural Research, NIH/NIDA IRP, Baltimore, MD 21224

Stimulants are known to cause increases in immediate early genes. The mechanisms by which they do so have not been completely characterized. In the present study, we tested the idea that this activation might involve production of free radicals by using nontransgenic (Non-Tg) and CuZn SOD transgenic (SOD-Tg) mice. Methamphetamine (METH) caused dose-dependent increases in AP-1 DNA-binding activity in both Non-Tg and SOD-Tg mice. However, the increases in SOD-Tg mice were less prominent than those observed in Non-Tg animals. The time-course of METH-induced AP-1 changes was similar in both strains of mice. The AP-1 binding activity showed an initial increase at 1 hour, peaked at 3 hours, and then gradually declined. AP-1 binding activity was back to normal by the 72-h time point. Regional analyses of METH effects revealed increases in the caudate-putamen, frontal cortex, and cerebellum, with the striatum showing relatively higher METH-induced AP-1 DNA-binding activation. These regional effects were also attenuated in the SOD-Tg mice. These data indicate that METH-induced stimulation of AP-1 DNA-binding depends on cellular redox status. These results are consistent with *in vitro* studies 3that have reported that several transcription factors are regulated through redox mechanisms.

385.12

PROTECTION OF 3,4-METHYLENEDIOXYMETH-AMPHETAMINE(MDMA)-INDUCED NEUROTOXICITY BY PHENYL-t-BUTYL NITR ONE (PBN), BUT NOT BY SALICYLATE (SAII), IN RATS. **S. Y. YEH,*Neuropsychiatry lab. Div. Intramural Research, NIDA/NIH, P.O. Box 5189, Baltimore, MD 21224**

MDMA-induced neurotoxicity is hypothesized to be due to hydroxyl free radicals and/or metabolites of MDMA. PBN was used to trap possible free radical metabolites of MDMA and other free radicals while SALI was used to trap hydroxyl free radicals. Rats received two doses of PBN (50, 100, 200 and 400 mg/kg dissolved in 25% of ethanol, 50 mg/ml, i.p.) and MDMA (20 mg/kg, s.c.) given concurrently 6 h apart. Control rats received saline or 25% ethanol as vehicle. For the SALI studies, SAL(3, 6, 12, 25, 50, 100, 200 mg/kg dissolved in saline) was injected i.p. 1 h prior to the s.c. injection of MDMA (20mg/kg for acute study; 10mg/kg x 2 doses x 4 days for chronic study). Rectal temperature was measured before drug and after the first injection for 5 h. Rats were sacrificed 5 or 7 days after the last injection for the PBN or SALI, respectively. Brain tissues were dissected and frozen until used for measurement of monoamines by HPLC. Rectal temperature of rats treated with PBN decreased whereas PBN plus MDMA, ethanol (0.5 to 2 ml/kg) and ethanol plus MDMA were not significantly altered. Temperature increased after SALI plus MDMA. Levels of monoamines in the frontal cortex, hippocampus, striatum and brain stem of rats treated with PBN and MDMA were not significantly changed as compared with saline and vehicle controls. Administration of SALI failed to attenuate the toxic effects of MDMA. These results suggest that the neurotoxicity of MDMA might be related to MDMA metabolite free radicals other than hydroxyl radicals.

385.13

BCL2 EXPRESSING DOPAMINERGIC CELLS ATTENUATE METHAMPHETAMINE- NO- AND CO- INDUCED TOXICITY

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Molecular Neuropsychiatry Section, NIDA/ARC, Baltimore, MD.

Dopaminergic systems are very sensitive to chemically very different toxic agents. Methamphetamine (METH) is one of such neurotoxin. We have shown that METH-induced toxicity in DA neurons involves the formation of free radicals such as superoxide and nitric oxide (NO). We then investigated whether METH-induced toxicity can be attenuated by BCl2 expressed in a dopaminergic cell line. Toxicity was assessed by trypan blue exclusion.
METH-induced toxicity was dose dependent in wild type and BCl2 cells. However, the percentage of dead cells was signicantly higher in wild type cells. Moreover, exposure to sodium nitroprusside, a NO-releaser, induced a dose dependent toxicity which was significantly more pronounced in wild type cells. Interestingly, the application of a medium enriched in CO also induced a more pronounced toxicity in control cells.
Thus, the neurotoxicity induced by METH or the direct exposure to NO or CO, can be attenuated by the expression of BCL2 gene in dopaminergic cells. The survival of dopaminergic neurons in a context such as transplantation, could be dependent on the expression of such genes that confer a resistance to toxic environment.

385.14

INVOLVEMENT OF THE NITRIC OXIDE IN AMPHETAMINE-EVOKED CHANGES IN THE PRODYNORPHIN SYSTEM ACTIVITY. B. Przewlocka, J. Turchan, W. Lason* and R. Przewlocki. Dept. Neuropeptide Research, Inst. Pharmacology, 31-343 Kraków, Poland.

A groving body of evidence suggests involvement of the prodynorphin system in the neurochemical mechanism of psychomotor stimulants. To better understand this issue, in a time-course study we measured the prodynorphin mRNA and α-neoendorphin tissue levels, as well as the in vitro release of the peptide in the nucleus accumbens and caudate putamen of rats treated with amphetamine (5mg/kg i.p., twice a day for 5 days). Acute and chronic amphetamine administration increased the prodynorphin mRNA level in both those structures, that effect being associated with an increase in the α-neoendorphin tissue level and in vitro release. The amphetamine-induced increase in the prodynorphin mRNA was attenuated in rats pretreated with the nitric oxide synthase inhibitor L-NAME (10 and 50 mg/kg i.p.). The obtained results indicate that amphetamine increases the prodynorphin system activity; moreover, the drug-induced prodynorphin gene expression appears to be under regulatory control of the nitric oxide.

385.15

ROLE OF NITRIC OXIDE ON BEHAVIORAL CHANGES INDUCED BY PHENCYCLIDINE IN MICE. Y. Noda[1], K. Yamada[1], Y. Komori[2], H. Sugihara[2], T. Kameyama*, H. Furukawa[3] and T. Nabeshima[1]. [1]Dept. of Neuropsychopharmacol. and Hosp. Pharm., Nagoya Univ. Sch. of Med., Nagoya 466, Japan. Dept. of [2]Miclobiol., *Chem. Pharmacol., and [3]Med. Chem., Faculty of Pharmaceut. Sci., Meijo Univ., Nagoya 468, Japan.

The role played by nitric oxide (NO) on the behavioral changes induced by phencyclidine (PCP) in mice was investigated by using a NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME). Acute PCP treatment induced hyperlocomotion, motor incoordination, sniffing, head movement and ataxia in a dose-dependent manner. Hyperlocomotion and ataxia induced by acute PCP (3 mg/kg s.c.) treatment were enhanced by L-NAME (50 mg/kg i.p.), the effects were reversed by L-arginine (1 g/kg i.p.). In mice treated with PCP repeatedly (10 mg/kg/day s.c., once a day for 14 days), the ataxia and motor incoordination induced by acute PCP (10 mg/kg) treatment were attenuated (indicating development of tolerance) whereas hyperlocomotion was enhanced (indicating development of sensitization). Tolerance to PCP-induced ataxia and motor incoordination was significantly attenuated by combined treatment with L-NAME (50 mg/kg/day i.p.), while sensitization to PCP-induced hyperlocomotion was further enhanced. NO synthase activity in the cerebral cortex and cerebellum, but not in the striatum and hippocampus, was significantly decreased by acute PCP (10 mg/kg) treatment, and such changes in activity in the cerebral cortex and cerebellum were reversed by subacute PCP treatment. These results suggest the involvement of central NO production in the mediation of behavioral changes induced by acute and subacutePCP treatment in mice.

385.16

PROTECTION AGAINST MDMA-INDUCED SEROTONIN NEUROTOXICITY IN RATS PRETREATED WITH KETANSERIN AND ALPHA-METHYL-PARA-TYROSINE: ROLE OF HYPOTHERMIA J. E. Malberg*, K.E. Sabol and L. S. Seiden. Dept. of Pharmacological and Physiological Sciences, University of Chicago, Chicago IL 60637

We looked at the role temperature plays in protection against MDMA-induced serotonin depletions. This study demonstrates that the hypothermia seen in alpha-methyl-para-tyrosine and ketanserin-pretreated rats is an important factor in protection against neurotoxicity. We administered MDMA (40 mg/kg, s.c.) to male Holtzmann rats pretreated with either alpha-methyl-para-tyrosine(AMPT; DA synthesis inhibitor, 75 mg/kg, s.c. 1 and 5 hrs prior to MDMA) or ketanserin (KET; 5-HT_2 antagonist, 6 mg/kg, 1 hr prior to MDMA). Body temperature was measured in all rats using a peritoneally implanted temperature probe and one week after injections rats were sacrificed and brain regions analyzed by HPLC. When rats pretreated with AMPT or KET were injected with MDMA, a significant hypothermia of 2.3°C was seen 20 minutes after the MDMA injection which continued for 2 1/2 hours. The KET and AMPT pretreatment significantly ($p<.05$) protected against decreases in 5-HT and 5-HIAA levels in the frontal cortex, somatosensory cortex, striatum, and hippocampus seen in rats treated with MDMA alone. The transmitter levels of pretreated rats did not differ from saline controls, and AMPT or KET alone had no effect on temperature nor transmitter levels. In a second experiment, rats were given the same drug treatments and placed under a heat lamp immediately after the MDMA injection. The temperature of these rats was maintained at 38.5-39. for 4 hours to prevent the hypothermic response. The KET and AMPT pretreated rats whose body temperature was maintained had levels of 5-HT and 5-HIAA that were significantly ($p<.05$) lower than saline controls and the drug-pretreated rats that exhibited hypothermia. This demonstrates that prevention of the hypothermia abolishes protection against neurotoxicity. One hypothesis is that the hypothermia exerts its protective effect by preventing oxidative stress or other reactions that lead to neurotoxicity. (Supported by NIDA Training Grant DA-07255-01)

385.17

EFFECTS OF VARIOUS PHARMACOLOGICAL AGENTS ON METHAMPHETAMINE-INDUCED HYPERTHERMIA AND NEUROTOXICITY IN MICE. L. Manzino*, D.S. Albers and P.K. Sonsalla. Dept. Neurology, UMDNJ-RWJ Med. Sch., Piscataway, NJ 08854.

The administration of methamphetamine (METH) to experimental animals causes a significant increase in core temperature. The damage sustained to dopaminergic nerve terminals can be reduced by attenuating the METH-induced hyperthermia by environmental cooling or pharmacological manipulation. The purpose of the present study was to determine if the protection from METH-induced decrements in striatal tyrosine hydroxylase activity and DA content by several pharmacological agents with diverse actions could be attributed to the blockade of this hyperthermia. Core temperature was significantly increased in mice during the treatment period with METH. Concurrent treatment of mice with METH and neuroprotective agents (DA receptor antagonists, dizocilpine, fenfluramine, propranolol, phenytoin, α-methyl-p-tyrosine) prevented this hyperthermic response. Moreover, agents which were unable to suppress the METH-induced temperature elevation whether from normal basal temperature (ibuprofen, salicylate, 8-OH DPAT) or from a drug-induced hypothermia (reserpine), were unable to protect against the neurotoxic actions of METH. Taken together, these data suggest that the rapid induction of core temperature by METH contributes to its neuropathology regardless of basal temperature.

385.18

THE NEUROPATHOLOGY PRODUCED BY METHAMPHETAMINE OR MPTP IN MICE IS POTENTIATED BY INTRASTRIATAL MALONATE. D.S. Albers*, G.D. Zeevalk, and P.K. Sonsalla. Dept. Neurology, UMDNJ-RWJ Med. Sch., Piscataway, NJ 08854.

The treatment of mice with neurotoxic doses of methamphetamine (METH) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) produces damage to nigrostriatal dopaminergic neurons and causes a transient loss in striatal ATP levels. These observations suggest that perturbations of energy metabolism may contribute to their neurotoxicity. The purpose of the present study was to determine if reduced energy production would potentiate the neurotoxic actions of systemically administered METH or MPTP. Mice received a unilateral infusion of saline or malonate (0.5-3.0 μmol), a reversible inhibitor of succinate dehydrogenase, into the striatum prior to treatment with METH or MPTP at doses which produced no significant DA loss. Malonate, by itself, produced minimal or no decrease in striatal DA content in animals sacrificed 5-7 days after treatment. However, in combination with METH or MPTP, malonate dose-dependently potentiated the DA depletion in the infused side (by 30-70%) compared to the contralateral side. These data suggest METH-induced perturbations of energy metabolism create a state of metabolic stress for dopaminergic neurons which may ultimately contribute to the neurodegenerative effects of METH. Furthermore, the potentiation of MPTP toxicity by malonate is consistent with the widely held view that MPTP-induced neurotoxicity is mediated by energy impairment due to the blockade of Complex I by MPP^+. This work was supported by a grant from the National Institute of Drug Abuse.

385.19

ALTERED AMPHETAMINE (AMP) METABOLISM AND ABUSE LIABILITY IN A RAT PHENOCOPY OF THE HUMAN CYTOCHROME P450 2D6 (CYP2D6) DEFICIENCY. E.M. Sellers, T. Berns, W.A. Corrigall, D.M. Tomkins, J.B. Kamien*, N. Joharchi, R.F. Tyndale and S.V. Otton. Depts. Pharmacology & Psychiatry, Univ. of Toronto; & Addiction Research Foundation, Toronto, Canada.

Humans missing CYP2D6 (about 8% of Caucasians) may have altered drug dependence characteristics because this enzyme is important in the metabolism of some drugs of abuse including AMPs. In rats d-AMP conversion to 4-OH AMP is by CYP2D1. Quinine (QN) and budipine (B) are inhibitors of male Wistar rat CYP2D1 metabolism of dextromethorphan and d-amphetamine by liver microsomes (Ki = 190 nM and 1.8 µM). *In vivo* QN (20 mg/kg i.p.) and B (10 mg/kg i.p.) increased the plasma AUC of d-AMP (5 mg/kg i.p.) by 290% and 260% respectively (and decreased 4-OH d-AMP by 68% and 88%) compared to AMP alone ($p < 0.01$). QN and B increased and prolonged rat locomotor activity after d-AMP (0.3 mg/kg i.p.) compared to controls ($p < 0.01$) in a pattern paralleling the observed changes in plasma kinetics of d-AMP. In a two-choice drug discrimination study, QN but not B increased generalization of low AMP doses to d-AMP ($p < 0.05$) and both prolonged the duration of > 90% d-AMP-appropriate responding up to 90 min post d-AMP injection. In contrast, in self administration studies, B but not QN caused a parallel shift to the left of the number of infusions vs. d-AMP injection dose response curve ($p < 0.05$). These studies indicate that d-AMP > 4-OH AMP is responsible for the behavioral effects seen in rats. In humans, CYP2D6 phenotype should be important for AMPs that undergo substantial ring hydroxylation or O-demethylation. Deficient individuals should show higher AMP toxicity at a fixed dose and use lower doses during chronic abuse. Conversely, individuals with high enzymatic activity may have lower AMP (but higher metabolite) toxicity and use higher doses than individuals with lower or absent CYP2D6 activity. Supported by NIDA Grant DA 086889.

385.20

PROTECTION AGAINST METHAMPHETAMINE-INDUCED DEPLETION OF STRIATAL DOPAMINE IN RATS BY GDNF. W. A. Cass* and D. M. Gash. Department of Anatomy and Neurobiology, University of Kentucky, Lexington, KY 40536.

Repeated methamphetamine (METH) administration to animals can result in decreased dopamine (DA) levels, tyrosine hydroxylase activity and DA uptake sites in the striatum. These effects appear to be long-lasting and in the rat may still be present six months after treatment. Glial cell line-derived neurotrophic factor (GDNF) has pronounced effects on dopaminergic systems in vivo, including partial neuroprotective effects against 6-OHDA and MPTP-induced degeneration of DA neurons. The present study examined the ability of GDNF to prevent METH-induced reductions in striatal DA. Male rats were administered METH (4 mg free base/kg, s.c.) or saline 4 times in one day at 2 hour intervals. One week later the animals were killed and striatal DA levels determined by HPLC. The METH treatment produced decreases in DA levels of 30-40% in the dorsal striatum and 50-60% in the ventral striatum. Using in vivo electrochemistry, similar decreases were found in potassium-evoked release of DA in the striatum of METH-treated animals. In another group of animals GDNF (Synergen Inc., Boulder, CO) was injected into the right striatum (10 µg in 2 µl buffer at a rate of 0.2 µl/min) 24 hours prior to the METH treatment. GDNF completely prevented the reduction in striatal DA levels on the right side of the brain, while levels on the left side were still decreased. While these results are preliminary they suggest that GDNF can prevent the toxic effects of METH on DA neurons. In vivo electrochemistry experiments are underway to determine if GDNF can also prevent METH-induced reductions in potassium-evoked DA release.

386.1

EFFECTS OF CHRONIC FLUOXETINE AND TIANEPTINE ADMINISTRATION ON MONOAMINE LEVELS AND TURNOVER IN DISCRETE BRAIN NUCLEI. C.R. McKittrick*, M. Frankfurt, C.J. Schindler, B.S. McEwen, and V.N. Luine. Laboratory of Neuroendocrinology, Rockefeller University and Department of Psychology,Hunter College, New York, NY 10021.

We investigated the effects of chronic antidepressant treatment on serotonin (5HT) and norepinephrine (NE) levels and metabolism in brain regions involved in affective states. Fluoxetine and tianeptine are both clinically active antidepressants even though they have opposing effects on 5HT reuptake: fluoxetine inhibits 5HT reuptake, while tianeptine increases it. Tianeptine (10 mg/kg), fluoxetine (10 mg/kg) or saline were administered i.p. to male rats daily for 21 days. Forty-eight hours after the last injection, half of each treatment group were injected with the monoamine oxidase inhibitor, pargyline (75 mg/kg) to assess the rates of 5HT and NE accumulation. Monoamine levels in discrete brain nuclei were measured by HPLC. Following tianeptine treatment, NE levels in frontal cortex (FCTX) were decreased by 44% compared to control, while NE turnover was increased, suggesting enhanced synaptic release of NE; tianeptine also increased 5HT turnover in FCTX by 83%. Fluoxetine, on the other hand, had no effects on 5HT or NE turnover in this region. However, in median raphe, fluoxetine increased both 5HT and NE turnover, while tianeptine also tended to increase NE turnover in this area. Finally, fluoxetine increased 5HT levels by 71% in dorsal raphe, with tianeptine having no effect. These data suggest that the therapeutic effects of the two drugs may be mediated by alterations of both 5HT and NE neurotransmission. *[Supported by GM07524-18 (CRM) and PSC-CUNY 666244 (VNL)]*

386.2

FLUOXETINE IMPAIRS ACQUISITION OF AN AVOIDANCE RESPONSE IN RATS

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Selective-serotonin reuptake inhibitors such as fluoxetine (Prozac) have become one of the most common treatments for clinical depression. While investigating the effects of fluoxetine within a learned-helplessness paradigm, control animals not made helpless but receiving fluoxetine (10mg/kg daily) were unable to learn to shuttle over and back across a low barrier to escape footshock. Of ten animals, only one successfully escaped on more than 33% of the trials during 3 days of training (mean=13%, median=1%). A majority of the vehicle animals always escaped the shock within 30s (mean=66%, median=100%).

Although a few studies have noted impairments in avoidance learning following fluoxetine administration, none have systematically investigated the extent of the debilitating effects. Subsequent tests in the present study appear to rule out changes in sensory threshold or motor dysfunction as causes for the impairment in the avoidance task. Latency to lick a hindpaw when placed on a 45°C hotplate was unaffected by daily injections of either 5 or 10mg/kg fluoxetine. Tests of jump thresholds to footshock are underway. Open field behaviors also were unaffected by an acute dose of fluoxetine (10mg/kg).

If peripheral effects do not account for fluoxetine's impairment in the avoidance task, central motivational or learning mechanisms may be affected adversely by doses of fluoxetine used in clinical applications.

386.3

CHRONIC CITALOPRAM AND FLUOXETINE TREATMENTS UP-REGULATE 5-HT_{2C} RECEPTORS IN THE RAT CHOROID PLEXUS. A. Laakso, E.-P. Pälvimäki, M. Kuoppamäki, M. Koulu*, E.K.G. Syvälahti and J. Hietala. Dept. of Pharmacology, Univ. of Turku, Turku, Finland.

The effects of chronic (for 14 days) citalopram and fluoxetine treatments with three doses (2.5, 10 and 20 mg/kg) and withdrawal times (24 hours, 68 hours and 14 days) on 5-HT_{2C} (formerly 5-HT_{1C}) receptors in the rat brain choroid plexus were studied with quantitative receptor autoradiography in two separate experiments. Chronic citalopram treatment caused a consistent and dose-related increase in the density of 5-HT_{2C} receptors (up to 90 %). This effect was slightly more pronounced when measured with an antagonist ligand ([^{3}H]mesulergine) compared to measurements with an agonist ligand ([^{125}I]DOI). The up-regulation was most evident 24 hours after the last dose and disappeared thereafter rather rapidly. Chronic fluoxetine treatment also increased the density of 5-HT_{2C} receptors 24 hours from the last dose, but the increase was accompanied by a reduced affinity and was less marked than that observed with citalopram. The changes in receptor characteristics were not observed consistently after the 68 hour withdrawal from fluoxetine. Furthermore, the up-regulation of fluoxetine appeared not to be dose-related, nor reflected by an increase in agonist binding. In conclusion, the results show that chronic citalopram and fluoxetine treatments induce an up-regulation of choroid plexus 5-HT_{2C} receptors, but the effect is more marked with citalopram. The data also suggest that 5-HT_{2C} receptors are significantly occupied during chronic fluoxetine treatment (by fluoxetine or more likely by an active metabolite, norfluoxetine), proposing that the actions of chronic fluoxetine treatment may not reside only in serotonin uptake inhibition. This direct interaction by fluoxetine may trigger additional regulatory mechanisms of the 5-HT_{2C} receptor leading to pharmacodynamic differences between chronic citalopram and fluoxetine treatments.

386.4

A-80426, A POTENT AND SELECTIVE α_2-ADRENOCEPTOR ANTAGONIST WITH SEROTONIN UPTAKE BLOCKING ACTIVITY AND PUTATIVE ANTIDEPRESSANT-LIKE EFFECTS. W.J. Giardina[1]*, S.A. Buckner[1], M. E. Brune[1], A. A. Hancock[1], C. T. Wismer[1], I. Milicic[1], A. J. Rattin[1], S. Roux[2], J. G. Wettstein[2], M. D. Meyer[1], R. D. Porsolt[2], J. E. Kerwin, Jr[1]., and M. Williams[1]. [1]Neuroscience Discovery, Abbott Laboratories, Abbott Park, IL 60064 and [2]ITEM-Labo, 93 ave de Fontainebleau, 94276 Le Kremlin-Bicetre Cedex, France.

A-80426 (N-[2-(benzofuran-6-yl)ethyl]-N-[(R)-(+)-5-methoxy-1,2,3,4-tetrahydro naphthalen-1-yl methyl]-N-methylamine) is a selective inhibitor of serotonin synaptosomal uptake and an antagonist of [^{3}H]-paroxetine binding to serotonin uptake sites and [^{3}H]-rauwolscine binding to α_2-adrenoceptors (Hancock et al. FASEB, 1995, 9:A408). A-80426 was evaluated *in vivo* for its ability to block serotonin uptake and α_2-adrenoceptors; its antidepressant potential was also assessed. In rats, A-80426 significantly reduced p-chloroamphetamine-induced hyperactivity, a measure of *in vivo* blockade of serotonin uptake, after acute (ED_{50}=13 µmoles/kg, po) and chronic (14 day) (ED_{50}= 4.1 µmoles/kg, po) dosing. At doses of 6.7 and 22 µmoles/kg, po, A-80426 was effective in this test procedure for at least 12 hours. Doses of 6.7 to 224 µmoles/kg, ip, of A-80426 failed to block hypothermia and hypoactivity produced by the α_2-adrenoceptor agonist, clonidine, and doses of 100 and 300 µmoles/kg, po, were required to blocked clonidine-induced mydriasis. A-80426 reversed the step-down passive avoidance deficit in olfactory bulbectomized rats (ED_{70} = 7.1 µmoles/kg, po), a finding suggesting that the compound has antidepressant potential. It was, however, inactive in the tail suspension and forced swim behavioral despair models of antidepressant activity in mice at doses up to 72 µmoles/kg, ip. Despite its favorable *in vitro* profile, A-80426 is not an effective α_2 blocker *in vivo* and is inactive in classical animals models of depression. It is unlikely that A-80426 would have antidepressant activity equivalent to other serotonin uptake inhibitors.

386.5

REGULATION OF BDNF AND trkB IN RAT BRAIN BY CHRONIC ANTIDEPRESSANT TREATMENTS.

M. Nibuya*, S. Morinobu, and R.S. Duman. Lab. of Molecular Psychiatry, Dept. of Psychiatry and Pharmacology, Yale Univ. Sch. Med., New Haven, CT.

Recent studies demonstrate that chronic antidepressant treatments (ADTs) activate the cAMP signal transduction system. This includes increased expression of CREB (cAMP response element binding protein), suggesting that ADTs regulate specific target genes (see Duman et al., this volume). One potential gene target is brain derived neurotrophic factor (BDNF), a possibility supported by the finding that CREB antisense oligonucleotides block the induction of BDNF in hippocampus (see Duman et al., this volume). Previously we reported that chronic electroconvulsive seizures (ECS) enhance the induction and prolong the expression of BDNF and its receptor, trkB. We now report that chronic (21 d), but not acute (1 d), administration of antidepressant drugs, including tranylcypromine, sertraline, fluoxetine, desipramine, or mianserin, also significantly increase the expression of BDNF and trkB mRNA in hippocampus by 30-75%. In contrast, chronic administration of nonantidepressant drugs, including morphine, cocaine, or haloperidol, did not increase levels of BDNF and trkB in this brain region. Chronic (21d), but not acute (1d), pretreatment of rats with antidepressants (i.e., ECS, tranylcypromine, sertraline, or desipramine) also completely blocked restraint stress-induced down-regulation of BDNF in hippocampus. The results indicate that expression of BDNF is a common gene target of different types of ADTs. Regulation of BDNF may lead to neuronal plasticity and thereby increase the survival and function of neurons, a hypothesis that is currently under investigation (see Vaidya et al., this volume).

386.6

CHRONIC ELECTROCONVULSIVE SEIZURE ALTERS CYTOSKELETAL PROTEINS (NEUROFILAMENTS, NF-200, NF-160 AND NF-68) IN THE RAT HIPPOCAMPUS.

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Previous work has shown that chronic electroconvulsive seizure (ECS) and antidepressant drug treatments lead to an increase in BDNF and trkB within the hippocampus (Nibuya et al, this volume). Since BDNF is reported to promote the growth, differentiation and function of hippocampal neurons, these findings suggest that ECS and antidepressant drugs, via regulation of BDNF, may influence neuronal growth and structure in the hippocampus. Following administration of ECS for 10 days we observed decreases in neurofilament immunoreactivity by western blotting. These decreases, ranging from 20-50%, were observed for all three forms of neurofilaments, NF-200 (NF-H), NF-160 (NF-M) and NF-68 (NF-L). There were no alterations in levels of glial fibrillary acidic protein (GFAP). In the peripheral nervous system it is known that neurofilament levels decrease and GAP-43 levels increase during nerve regeneration. In order to determine if similar changes are occurring in the CNS we are now characterizing effects on GAP-43 following chronic ECS. In addition, morphometric studies of single hippocampal neurons (i.e. CA3 pyramidal cells and dentate granule cells) are under way. Given the reports (Watanabe *et al*, Brain Res.1990;588:341-345; Uno *et al*, J.Neurosci 1989;9(5):1705-11) that chronic stress leads to the atrophy of CA3 pyramidal neurons, these results suggest that the action of ECS as well as antidepressant drugs might be related to their ability to influence neuronal morphology or to induce synaptic remodelling.

386.7

CHRONIC ANTIDEPRESSANT TREATMENTS INCREASE THE FUNCTION AND EXPRESSION OF CREB. R.S. Duman* and M. Nibuya. Lab. of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale Univ. Sch. Med., New Haven, CT.

Recent studies demonstrate that chronic antidepressant treatments (ADTs) activate the cAMP signal transduction system, including increased levels of adenylyl cyclase and nuclear translocation of cAMP-dependent protein kinase (PKA). These findings raise the possibility that ADTs regulate CREB (cAMP response element binding protein). The function of CREB is regulated by PKA, as well as Ca^{2+}-activated protein kinases, and may be influenced by regulation of total CREB expression. The present study demonstrates that chronic administration of electroconvulsive seizures (ECS) for 10 d or tranylcypromine, sertraline, fluoxetine, or desipramine for 21 d significantly increased the expression of CREB mRNA in hippocampus. Chronic ECS also increased levels of CRE binding, determined by gel shift analysis, demonstrating a corresponding increase in levels of functional CREB. One potential gene target of CREB is brain derived neurotrophic factor (BDNF), which is increased by chronic ADTs (see Nibuya et al., this volume). To directly determine the role of CREB in regulation of BDNF, we employed an antisense (AS) oligonucleotide strategy. Local infusion of CREB AS, but not sense, oligonucleotides decreased basal and ECS-induction of BDNF mRNA in hippocampus. CREB AS did not influence c-jun expression, which is independent of CREB, and blockade of BDNF induction was reversible, demonstrating that there was no permanent cellular damage by AS treatment. The results demonstrate that CREB expression is increased by ADTs, and indicate that CREB mediates the regulation of target genes, such as BDNF, in response to chronic ADTs.

386.8

ANTIDEPRESSANT-INDUCED c-*FOS* IN CENTRAL AMYGDALA AND PARAVENTRICULAR NUCLEUS. J. Demotes-Mainard, C. Henry, J. Arsaut, R.M. Bluthé*, M. Bourgeois#, E. Arnauld. INSERM U-394 and # Department of Psychiatry, Université de Bordeaux II, 33077 Bordeaux, France.

Tricyclic antidepressants are known to impair both serotonin (5-HT) and norepinephrine (NE) reuptake, but their exact neural and molecular targets remain poorly understood. Both 5-HT and NE systems are widely distributed within the brain, and the corresponding receptors exhibit a wide molecular diversity. Antidepressants act on both somatic and axonal transporters, interfering with pre- and post-synaptic actions of monoamines on their receptors. However 5-HT and NE receptors are coupled, through various transduction pathways, to transcripton factors whose induction reflects the resultant action of a given drug on both 5-HT and NE systems. Therefore we investigated in mice, through *in situ* hybridization using a [^{35}S]-labelled oligonucleotide probe, the induction of the transcription factor c-*fos* by the tricyclic antidepresssant clomipramine. Forty-five minutes after a single injection of clomipramine (30mg/kg, i.p.), c-*fos* induction was prominent in two forebrain structures, namely the central nucleus of the amygdala and the paraventricular nucleus. There was no evidence for desensitization since a similar pattern of c-*fos* induction persisted after repeated injections of clomipramine (30mg/kg.day, i.p.) for 15 days. This observation emphazises the role of central amygdala and paraventricular nucleus as targets of antidepressant actions on the limbic system and hypothalamo-pituitary-adrenal axis, respectively, and suggests that the delayed therapeutic effects of antidepressants may depend on drug-induced transcription and subsequent alterations of neuronal phenotype.

386.9

SEROTONERGIC MECHANISMS INVOLVED IN THE EFFECTS OF FLUOXETINE IN THE RAT FORCED SWIMMING TEST (FST). M.J. Detke*, L. Kirby and I. Lucki. Departments of Psychiatry, Pharmacology and Psychology, University of Pennsylvania, Philadelphia, PA 19104.

Fluoxetine has previously been shown to reduce immobility and increase swimming in the Porsolt behavioral despair test or FST using a time-sampling scoring technique (Detke, Rickels and Lucki; Psychopharmacology, in press). This behavior was used to examine serotonergic mechanisms involved in fluoxetine's antidepressant effects. Water depth was 30 cm, to prevent the rats from supporting themselves with tails or hind limbs. After a 15-min pretest, drugs were administered 24, 5, and 1 hr prior to the 5-min swim test.

Fluoxetine (20 mg/kg SC) reduced immobility and increased swimming in a dose-dependent manner. These effects were prevented by pretreatment with p-chlorophenylalanine (PCPA, 150 mg/kg, IP X2), which produced a 95% reduction of serotonin content in the frontal cortex. Fluoxetine's effects were also blocked by pindolol (2 mg/kg SC) and propranolol (5 mg/kg SC), but not WAY 100,478 (10 mg/kg SC), NAN 190 (1 mg/kg SC), ketanserin (3 mg/kg SC), or ondansetron (3 mg/kg SC). None of the antagonists had any effects on their own. Behavioral effects similar to fluoxetine's were produced by 8-OH-DPAT (0.125 - 0.5 mg/kg SC) and CGS 12066 (1 - 10 mg/kg SC), but not by bromo-dimethoxyamphetamine (DOB; 1 mg/kg SC) or phenyl bi-guanide (PBG; 30 mg/kg SC). Low doses of pindolol (0.06 - 2 mg/kg SC) did not enhance the effect of fluoxetine (20 mg/kg SC) in this animal model of depression.

These results confirm earlier findings showing that SSRIs produce antidepressant-like effects in the FST, and that these effects are behaviorally specific. The PCPA experiment demonstrates that serotonin is necessary for these effects. The other experiments suggest that the 5-HT_{1A} and 5-HT_{1B} receptors mediate the antidepressant-like effects. The preliminary findings with pindolol do not agree with the findings of Artigas et al. (1994) in depressed humans.

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386.10

LONG-TERM TREATMENT WITH MOCLOBEMIDE NORMALIZED THE BEHAVIOR OF COGNITIVELY IMPAIRED TRANSGENIC MICE WITH REDUCED GLUCOCORTICOID RECEPTOR FUNCTION. A. Montkowski, N. Barden*, J.M.H.M. Reul, R. Landgraf and F. Holsboer. Max Planck Institute of Psychiatry, Clinical Institute, Kraepelinstr. 2-10, D-80804 Munich, Germany and CHUL Research Centre, Québec, Canada.

Impaired cognitive function and enhanced activity of the hypothalamic-pituitary-adrenocortical system are among the cardinal symptoms of major depression in humans that resolve after successful antidepressant treatment. To test the hypothesis that reduced GR function could cause these clinical disturbances, we used a transgenic mouse model expressing antisense RNA complementary to glucocorticoid receptor (GR) mRNA. The transgenic mice have elevated plasma corticotropin concentrations in response to stress and show profound behavioral changes in a number of tests that are indicative of cognitive impairment. Both the behavioral deficits and the hormonal alterations disappeared after long-term treatment with moclobemide, a reversible inhibitor of monoamine oxidase type A that is used clinically as an antidepressant. Following moclobemide treatment, the behavior of transgenic mice no longer differed from that of normal mice in both the elevated plus maze, which is used as a test of anxiety in animals, and in the forced swim test. Furthermore, the deficient olfactory short-term memory of transgenic mice, as assessed in the social recognition paradigm, was significantly improved by moclobemide. The behavior of normal mice was not influenced by antidepressant treatment. These observations suggest that a transgenic mouse with GR dysfunction may be a useful model for investigation of drug effects on both cognitive and neuroendocrine aspects of depression.

386.11

BEHAVIORAL EFFECTS OF ANTIDEPRESSANT DRUGS IN RATS SELECTIVELY BRED FOR LOW ACTIVE RESPONDING IN THE SWIM TEST. C.H.K. West*, L.M. Lester and J.M. Weiss. Lab. of Behavioral Neuroscience & Neuroimmunology, Dept. Psychiatry, Emory Univ. Sch. Med., Atlanta, GA 30306.

To attempt to study aspects of reduced active responding seen in human clinical depression by constructing an animal (rodent) model, rats have been selectively bred for low activity in a swim test (SwLo); these animals now show little struggling and much floating in the swim tank. In parallel, animals showing high activity (much struggling, little floating) have also been selectively bred (SwHi). The effect of antidepressant (AD) treatment was tested on SwLo rats; drugs were also administered to SwHi rats for comparison. Groups of SwLo males were administered vehicle, 3 non-AD psychoactive compounds and 7 ADs (from several pharmacological classes) for 1,12, or 26 days via osmotic pumps. In response to 5 of the 7 ADs [desipramine (DMI), imipramine, venlafaxine, phenelzine (PHE) and bupropion (BUP)], SwLo's showed a pattern of no significant change in struggling at 1 day but significant enhancement of struggling at 12 and 26 days of AD treatment, consistent with the clinical observation that therapeutic effectiveness of ADs appears only after prolonged treatment. Fluoxetine and amitriptyline failed to increase struggling in the SwLo's. The non-ADs produced either little effect on struggling (haloperidol, caffeine) or the largest effect at 1 day (amphetamine). ADs that increased struggling also decreased floating, but usually to a lesser degree. These two effects were not correlated as DMI produced the largest increase in struggling whereas PHE and BUP produced the largest decreases in floating. Unlike swim test behaviors, activity in the open field was not consistently affected by AD treatment. These results suggest that struggling behavior of SwLo rats is increased by prolonged, but not acute, treatment with most types of ADs and that other, perhaps less vigorous, behaviors (floating, open field activity) are affected by ADs in a manner less reflective of therapeutic potential.

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386.12

A COMPARISON OF THE EFFECT OF IMIPRAMINE ON ICSS OF VTA AND LATERAL HYPOTHALAMUS. K. Sweet, S. Murray, and D. Neill*. Dept. Psychology, Emory Univ., Atlanta, GA 30322.

Anhedonia, the loss of the appreciation of pleasure, is a notable component of severe depression in humans. We examined whether administration of the classical tricyclic antidepressant imipramine would enhance the reward value of electrical self-stimulation of the ventral tegmental area and lateral hypothalamus. Sprague-Dawley rats bearing electrodes in one of these sites were trained on autotitration self-stimulation. In our version of this procedure, the intensity of the 100 pps, 0.5 msec biphasic stimulation dropped 3 μa every 5th lever press. A single response on a separate lever reset the intensity to an individually-determined maximum. We found that daily treatment with 10 mg/kg imipramine HCl, I.P., 20 hr before testing, produced resetting at lower intensities in both LH and VTA groups. For the LH group, this enhanced reward was very small (3 μa), and slowly developed over 7-10 days. For the VTA group, the enhanced reward was larger (8 - 10 μa) and developed within 2 days. We interpret these results as indicating differential effects of the drug on non-dopaminergic hypothalamic and dopaminergic mesolimbic reward systems.

386.13

CHRONIC FLUOXETINE TREATMENT ALTERS 5-HT_{1A} MEDIATED RESPONSE IN AREA CA1, BUT NOT AREA CA3, OF HIPPOCAMPAL SLICES. S.G. Beck, K. Choi, S. Birnstiel, W. A. Pouliot, J. Crayton*, Departments of Pharmacology and Psychiatry, Loyola University Medical Center, and Hines VA Biological Psychiatry, Maywood, IL 60153.

The 5-HT neurotransmitter system and the raphe-hippocampal pathway are implicated in regulating complex emotional responses such as depression. The raphe-hippocampal 5-HT system is a likely target for psychoactive drugs used to treat such disease states. To obtain information on the discriminant action of antidepressant drugs, 5-HT receptor-elicited responses were recorded by intracellular recording techniques in areas CA1 and CA3 of the hippocampus. SD rats were treated with vehicle or the anti-depressant fluoxetine in 50% DMSO by mini-pumps at an average dose of 6.8 mg/kg for three weeks. Rats were decapitated, trunk blood collected for plasma fluoxetine and norfluoxetine determinations, and brain slices prepared for electrophysiological recording. The EC_{50} for the 5-HT_{1A}-mediated hyperpolarization was shifted to the left, i.e., more potent, in cells from fluoxetine treated rats. In CA3 there were no significant differences in the Emax, EC_{50} or slope of the 5-HT_{1A} concentration-response curves between cells from sham and fluoxetine treated rats. There was no change in the 5-HT_4-mediated inhibition of the slow afterhyperpolarization elicited by a train of action potentials in area CA1 between cells from sham and fluoxetine treated rats. These results support our hypothesis that antidepressant treatment can have discriminant actions on different 5-HT receptor subtype-mediated responses (i.e., 5-HT_{1A} versus 5-HT_4) and on a particular 5-HT receptor evoked response (i.e., 5-HT_{1A} between CA1 and CA3). This differential action may be important for understanding the clinical efficacy of antidepressant drugs. Supported by PHS NS28512 and MH 00880 to SGB.

386.14

NEONATAL CLOMIPRAMINE(CLI) DECREASES ACOUSTIC STARTLE IN ADULT RATS. I.L. Stafford-Segert*, S. Dickerson, & J. Ingham, Dept. of Psychology. Univ.of Missouri, Columbia, MO 65211

Neonatal exposure to CLI has been shown to produce behavioral symptoms in adult rats which mimic those of human endogenous depression: e.g. altered activity levels, sleep patterns, and pleasure-seeking behavior(Vogel,et.al.,1990). We examined the effect of neonatal CLI on the acoustic startle response in adult rats. Male Sprague-Dawley rats were injected with CLI(15mg/kg, s.c.) twice daily for 14 days, beginning at post-natal day 8; control rats received equivalent vehicle injections. Whole body startle was tested at age 3 months. All rats were first tested in 30 min sessions consisting of 90 presentations of a 115-dB, 90 ms white noise stimulus on a 20 sec ISI. Next, rats were tested following administration of the serotonin agonist drug 5-methoxy,N,N-dimethyltryptamine (5-MeODMT; 2mg/kg,i.p), which has been previously shown to increase acoustic startle. Preliminary data indicate that the CLI-treated rats show a smaller baseline startle response compared to controls. 5-MeODMT increased startle in the control rats, but not in the CLI-treated rats. These data suggest that neonatal CLI exposure may produce alterations in adult serotonin neurotransmission, supporting the validity of this as an animal model of depression.

386.15

PHARMACOLOGICAL DIFFERENTIATION OF SELECTIVE SEROTONIN REUPTAKE INHIBITORS (SSRIs) BY RECEPTOR PROFILING. J. Lancaster, T. Hartman, S. Faraci[1], P. Sweetnam[1] and J. Ferkany*. NovaScreen, A Division of Oceanic Biosciences Corp., Hanover, MD 21076 and [1]Dept. of Explor. Lead Discovery, Pfizer Central Research, Groton CT 06340.

The antidepressants fluoxetine, paroxetine, and sertraline elicit their therapeutic action by the inhibition of serotonin reuptake, Ki's = 1 nM, within synapses. SSRI's provide a therapeutic benefit with a low incidence of side effects although a comprehensive receptor selectivity profile for each of these compounds has not been reported. Such a profile will be valuable as these drugs are further evaluated for their utility in the treatment of obsessive compulsive disorder and premenstrual syndrome. All three structurally distinct compounds were tested in over 70 different receptor binding assays to assess receptor selectivity. A comparison and contrasting of these profiles is reported here. Briefly, the data show all three to be highly selective reuptake inhibitors. However, each appears to interact with other receptors types, IC50 values ≤ 1 µM. The rank order potency for sertraline at these ancillary receptors is [3H]DTG(sigma), [3H]WIN35,428 (dopamine), and [3H]GR65630(5HT3); for paroxetine [3H]pirenzipine(M1), [3H]WIN35,428(dopamine), [3H]AF-DX384(M2), [3H]DTG(sigma), [3H]GR65630(5HT3), and [3H]batrachotoxin (sodium site 2); and for fluoxetine [3H]DTG(sigma), [3H]ketanserin(5HT2), and [3H]pirenzipine(M1). The minor differences in selectivity profiling can be used to develop an even more selective second generation SSRI's.

386.16

RANDOMIZED CONTROLLED TRIAL OF PINDOLOL IN TREATMENT NAIVE AND TREATMENT REFRACTORY DEPRESSION. R.M. Berman, A.M. Darnell, H.L. Miller, A. Anand, D.S. Charney*.Affective Disorders Program, West Haven VA Medical Center, West Haven, CT 06516

We now report our preliminary findings from two double-blind, placebo-controlled trials examining the use of pindolol, a serotonin1A antagonist, in treatment naive (Study A) and treatment refractory (Study B) depressed patients. In Study A, treatment naive patients are concurrently started on fluoxetine and either active pindolol (2.5 mg po tid) or placebo. In Study B, fluoxetine-refractory patients are maintained on fluoxetine with the addition of either active pindolol (2.5 mg po tid) or placebo; after 3 weeks, the placebo group is placed on active pindolol.

To date of this writing, 33 patients have finished Study A, all but 2 completing at least two weeks on protocol: After two weeks of medication, 3 of 15 patients on active pindolol demonstrated $\geq$50% decreases in Hamilton Depression Rating Scores (HDRS). At the same time point, 3 of 18 patients on placebo demonstrated $\geq$50% decrease in HDRS. Response (defined by $\geq$50% HDRS reduction and HDRS $\leq$15) rates in patients completing at least 4 weeks of medication were comparable in groups receiving active versus placebo pindolol (8 of 11 vs. 8 of 12, respectively). Six patients have completed study B: HDRS decreases of $\geq$50% were noted in 2 patients after 3 weeks on pindolol and another patient after 6 weeks on pindolol. A fourth patient demonstrated a robust response after 3 weeks on pindolol with a HDRS decrease from 28 to 15. None of 2 patients randomized to a placebo period responded by three weeks on placebo. Small sample sizes in these ongoing studies limit statistically valid conclusions; however, analyses with more subjects will be presented.

386.17

FLUOXETINE INCREASES PLASMA LEVELS OF ALLOPREGNANOLONE IN DEPRESSED PATIENTS. A. Pasini*, G. Spalletta, F. Di Michele, C. Furnari, P. Fucci, N. Ciani, and E. Romeo°, Dept. San. Pubblica and °Dept. Med. Sperimentale, "Tor Vergata" Univ., Roma, Italy 00133.

Acute fluoxetine treatment was reported to increase allopregnanolone (ALLO) content in rat brain (D.Uzonov et al. Neuroscience Abst. 1995). Here we examined in DSM-IV depressed patients, whether the treatment with fluoxetine (20mg/day) may alter the synthesis of neurosteroids. The plasma levels of progesterone (PROG), 3α and 3ß isomers of ALLO (3α-ALLO, 3ß-ALLO), pregnenolone (PREGN), 5α-dehydroprogesterone (DHP), were measured by gas chromatography-mass spectrometry. After a wash-out period of 7 days, blood samples were taken on days 0, 10, 20, 30, 40, 50, 60 of fluoxetine administration. On the same days patients were administered auto and hetero evaluation psychometric tests to measure depression, anxiety and aggressiveness. On day 0 the plasma levels of 3α-ALLO (250-300pg/ml) were below the control values (800-1000pg/ml) , while the 3ß-ALLO levels (300-400pg/ml) were increased compared to control values (10-50pg/ml). After fluoxetine administration, 3α-ALLO levels showed a progressive increase until day 30 after which they stabilized at control values. Conversely 3ß-ALLO levels normalized progressively between day 20 and 30. The content of PROG and PREGN was not altered by fluoxetine, while the levels of DHP, the immediate precursor of ALLO, slightly decreased until day 30. A highly significant negative (3α-ALLO) and positive (3β-ALLO) correlation existed betwen ALLO and symptoms of anxiety and depression. These data support the hypothesis that fluoxetine may selectively provide a fine control in the production of 3α and 3ß ALLO trough DHP. Since 3α-ALLO is a potent positive modulator of $GABA_A$ receptors, our working hypothesis is that the antidepressant, antidysphoric action of fluoxetine could be in part mediated by $GABA_A$ receptors active neurosteroids.

386.18

5-HT_{1A} AUTORECEPTOR ANTAGONISTS RESTORE SEROTONERGIC NEURONAL ACTIVITY AFTER ACUTE FLUOXETINE TREATMENT IN BEHAVING CATS. C. A. Fornal *, C. W. Metzler, F. J. Martín and B. L. Jacobs. Prog. in Neuroscience, Princeton University, Princeton, NJ 08544.

The lack of clinical antidepressant efficacy of selective serotonin reuptake inhibitors (SSRIs) after acute treatment may be due to a reduction in the activity of central serotonergic neurons resulting from the indirect activation of somatodendritic 5-HT_{1A} autoreceptors. It has been hypothesized that the co-administration of a 5-HT_{1A} autoreceptor antagonist with an SSRI might lead to a more rapid or more effective antidepressant response by attenuating the feedback action of serotonin at the 5-HT_{1A} autoreceptor. The present study examined the effects of two selective 5-HT_{1A} receptor antagonists (WAY-100635 and (S)-WAY-100135) on the activity of serotonergic neurons after acute treatment with the SSRI fluoxetine. Serotonergic neurons in the dorsal raphe nucleus were recorded and identified using methods previously described (Fornal et al., JPET 270: 1345-1358, 1994). Administration of fluoxetine (5 mg/kg, i.v.) decreased neuronal activity to approximately 5% of baseline values. Administration of WAY-100635 (0.1 mg/kg, i.v.) rapidly reversed this suppression, and increased neuronal activity above pre-fluoxetine baseline levels. Unit activity gradually returned to fluoxetine pretreatment levels within 2 hr after WAY-100635 administration. Subsequent injections of the same dose of WAY-100635 produced similar effects. (S)-WAY-100135 (0.5 mg/kg, i.v.) also reversed the effect of fluoxetine on neuronal activity, but was less effective and had a shorter duration of action (~ 30 min) than WAY-100635. These results indicate that (S)-WAY-100135 and in particular WAY-100635 block the action of endogenous serotonin at 5-HT_{1A} autoreceptors and increase the firing rate of serotonergic neurons during sustained reuptake inhibition. Supported by grants from NIMH, AFOSR, and Miles Laboratories.

386.19

ANTIDEPRESSANTS AND ELECTROCONVULSIVE SEIZURES AFFECT NEUROTROPHIC FACTOR EXPRESSION IN THE CNS.
M. A. Smith*[1], S. Makino[2], and R. M. Post[1]. Biological Psychiatry[1] and Clinical Neuroendocrinology Branches[2], National Institute of Mental Health, Bethesda, MD 20892

The mechanisms by which antidepressants and electroconvulsive seizures (ECS) relieve clinical depression are not well understood. However, potential candidates might include neurotrophic factors which regulate the development, plasticity and survival of neurons. To explore this hypothesis we examined the effects of antidepressants and ECS on neurotrophin expression in the rat brain. Using *in situ* hybridization we found that chronic treatment with tricyclic antidepressants decreased basal levels of neurotrophin-3 (NT-3) mRNA in the locus coeruleus (LC) and brain-derived neurotrophic factor (BDNF) in the hypothalamic paraventricular nucleus (PVN). Desipramine for 21 days partially attenuated the increases in LC NT-3 and PVN BDNF mRNA levels that occur in response to repeated immobilization stress. However, desipramine did not block the effects of immobilization stress on NGF, BDNF or NT-3 expression in the hippocampus and cortex. ECS also decreased NT-3 in the LC, but in addition, dramatically decreased NT-3 and increased NGF and BDNF mRNA expression in the hippocampus and cortex. Thus antidepressants and ECS affect neurotrophic factor expression in an opposite manner compared to stress which exacerbates depression. These results raise the possibility that some of the effects of antidepressants and ECS could be mediated through alterations in neurotrophic factor expression in areas such as the hippocampus, PVN and locus coeruleus that are important for regulating mood.

386.20

PHARMACOLOICAL PROFILE OF HT-90B, A PUTATIVE ANXIOLYTIC WITH POTENT ANTIDEPRESSIVE ACTIVITY (II).
K.Inagawa*, H.Uchida, C.Tameda, and T.Miyauchi, Fuji-Gotemba Research Labs.,Chugai Pharmaceutical Co.,Ltd., 412, Japan.

HT-90B, (-)-N-{[2-(8-methyl 1,4,-Benzodioxane-2-ymethyl) amino]ethyl}triclo[3.,3.1.13,7]Decanel-carboxamide, has been shown to be a putative anxiolytic with potent antidepressive action with minimal side effects (Miyauchi et al, (1993) Soc Neuroci Abstr,10:1867). The present study evaluated the effects of HT-90B on 1) the light-dark exploration in mice and 2) the increased motor activity in the olfactory bulbectomized rats. Receptor binding profile using autoradiography (in vitro and ex vivo) was also studied. In the light-dark exploration in mice, HT-90B (1 and 10 mg/kg, po) shortened duration time spent in the dark compartment without inhibiting the motor activity. Although buspirone (30 mg/kg, po) showed the effect on the duration time spent in the dark compartment, it inhibited the motor activity. Single oral dose of HT-90B (10 mg/kg) reduced the increased motor activity in the olfactory bulbectomized rats, as multiple i.p. injection of despramine did (10 mg/kg X 4). Both HT-90B and desipramine did not change the motor activity. In in vitro labeling autoradiography, HT-90B displaced [3H]-8-OH-DPAT (pIC50=9.6) and [3H]-ketanserin specific binding in concentration dependent manner . Results from ex vivo labeling autoradiography will also be presented.

GENETIC MODELS OF HUMAN NEUROPSYCHIATRIC DISORDERS III

387.1

NEURONAL LOSSES IN THE BRAIN OF MICE TRANSGENIC FOR THE HUMAN NF-L NEUROFILAMENT PROTEIN.
D. Ma *[1], K. Micheva [1], L. Descarries [1], J.-P. Julien [2] and G. Doucet [1]. [1] Département de pathologie et CRSN, Université de Montréal; [2] Center for Research in Neuroscience, Montreal General Hospital and McGill University, Montréal, Québec, Canada.

Mice transgenic for the human form of the neurofilament protein NF-L display abnormal perikaryal immunoreactivity for NF-L in layers II/III of the parietal cortex (Par) and in the ventrobasal complex of the thalamus (VB). This aberrant immunoreactivity is associated with an accumulation of disarrayed filaments in the perikarya as well as dendritic and axonal processes of the affected neurons. It is transient in the cortex (maximal on postnatal day 10), but persistent in the thalamus. To determine its consequences on neuronal survival, cell counts were obtained from Par and the VB of transgenic and normal mice 10 days to 18 months of age. The dissector method was used on methylene blue-stained semi-thin sections after fixation of the brain by perfusion with aldehydes and post-fixation with osmium tetroxide. A loss of neurons was apparent in the parietal cortex of transgenic mice as soon as 20 days postnatal (-10% by comparison to controls). In older transgenic mice (14 to 18 months), this loss of neurons had slightly increased in magnitude (-18%) and could be attributed mainly to layers II/III. In the VB, considerable reductions in the number of neurons were detected in the transgenic mice, but only after 3 months (21%) and later (14 to 18 months: -39%). The volume of the VB was then significantly reduced and the lateral ventricles were enlarged. These results indicate that the abnormal accumulation of NF-L leads to premature neuronal death in these transgenic mice. The time course of these neuronal losses depends on the anatomical region examined, presumably reflecting different mechanisms, which remain to be defined. [Supported by FCAR (GRSNC) and MRC grants MT-10982 and MT-3544].

387.2

ACCELERATED AND WIDESPREAD NEURONAL LOSS OCCURS IN *MOTOR NEURON DEGENERATION (mnd)* MICE EXPRESSING A NEUROFILAMENT-DISRUPTING TRANSGENE.
J.Plummer, A.Peterson and A.Messer*. Wadsworth Ctr. for Labs and Research, N.Y. State Dept. of Health and Dept. of Biomed. Sci., SUNY, P.O. Box 509, Albany, NY 12201-0509; and Dept. of Neurology and Neurosurgery, McGill Univ., Montreal, Canada H3A1A1.

To examine the effects of multiple stressors on the rate and specificity of a neurodegenerative disease, we derived *mnd/mnd* mice expressing a neurofilament-H/lac Z transgene. The *mnd* mutation produces abnormal ubiquitous accumulation of autofluorescent lipopigment, with retinal degeneration and late-onset motor neuron degeneration. The neurofilament H-ß-galactosidase fusion protein causes endogenous neurofilament subunits to precipitate in perikarya, without significant neuronal degeneration until advanced age. In *mnd/mnd*-transgenic animals, lipopigment accumulation and motor neuron loss were substantially accelerated. Also, newly vulnerable populations of neurons degenerated, including cerebellar Purkinje cells and dorsal roots. This study helps define factors underlying the specificity of neuronal degeneration in a system displaying a ubiquitous defect. It further indicates that cytoskeletal abnormalities similar to those observed in late-onset human neurodegenerative disorders can interact with other cellular defects and contribute to pathogenesis. Transgenic *mnd/mnd* mice expressing wild type and mutant forms of human Cu,Zn superoxide dismutase are currently being bred, to study the effects of SOD mutations (which themselves produce motor neuron loss in transgenic mice) on the neurodegenerative process triggered by the *mnd* mutation.
(Supported by the ALS Association, NIH NS29110, the Medical Research Council of Canada, and the Muscular Dystrophy Association)

387.3

A POSSIBLE IN VITRO MODEL FOR ALZHEIMER'S DISEASE BASED ON IMMORTALIZED CELL LINES FROM THE TRISOMY 16 MOUSE.
O.F.Olesen[1], A. Thorpe[2], K. Frederiksen*[1], L. Lärkfors[1] and S-J. Richards[2]. 1) Pharmacological Research, H.Lundbeck A/S, Ottiliavej 9, DK-2500 Copenhagen-Valby, Denmark and 2) Dept. of Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 2QQ, UK.

An additional copy of chromosome 21 in humans (trisomy 21) results invariably in Down syndrome and early development of neuropathological lesions indistinguishable from those of Alzheimer's disease (AD). Mouse chromosome 16 is partly syntenic to human chromosome 21 and the trisomy 16 (Ts16) mouse has therefore been suggested as an animal model for Down syndrome and AD. However, the utility of this model is severely hampered by the fact that Ts16 embryos die in utero. To overcome this obstacle, we have immortalized precursor cells from embryonic Ts16 mouse brain using a retroviral construct based on the temperature sensitive mutant of SV40 large T antigen. This procedure yielded a number of stable cell lines that could either be maintained at the permissive temperature of 33°C or alternatively be induced to halt proliferation and begin differentiation at the nonpermissive temperature of 39°C. Southern blotting confirmed that the cell lines were indeed clonal and the trisomic karyotype was confirmed by fluorescent in situ hybridisation with mouse chromosome 16 paints. Initial immunocytochemical analysis revealed that most cell lines expressed GFAP, MBP, nestin and APP at 33°C. After 2 weeks of cultivation at 39°C, most cell lines had lost T antigen and nestin immunoreactivity, whereas two cell lines (TH173 and TH176) demonstrated an upregulated expression of GFAP and exhibited classical astrocytic morphology. Following receptor studies and further immunochemical characterization, these cells can be useful in studying the expression of APP and other molecules involved in the pathogenesis of AD.

387.4

AGED MICE TRANSGENIC FOR THE HUMAN β-AMYLOID GENE: BEHAVIOURAL CHARACTERIZATION.
F.G. Momoli*, I. Nalbantoglu, S.A. Welner. Douglas Hospital Research Centre, Departments of Psychiatry and Neurology & Neurosurgery, McGill University, Montreal, Canada, H4H 1R3

Alzheimer's Disease (AD) is characterized by a progressive deterioration of cognitive function as well as certain alterations in affective behaviours. Since excessive deposition of β-amyloid in areas of hippocampus and neocortex is also characteristic of AD, aged mice (18-24 mo), transgenic for human β-amyloid, compared to aged and young (2-8 mo) controls, were tested in several behavioural paradigms designed to assess cognitive and affective performance. A cDNA fragment of amino acids 591-695, spanning the amyloid and C-terminus portion of the β-amyloid precursor protein, was inserted into the human neurofilament NF-L gene, under transcriptional control of the NF-L promotor. Mice with the same transgenic construct were previously found to be deficient in spatial memory performance in the Morris Water Maze (Tirado-Santiago et al, Soc. Neurosci. Abst. 18: 1437, 1992). In the present experiments, using the Forced Alternation T-Maze with a zero delay, a task which involves working and reference memory, the transgenic mice demonstrated poor acquisition, failing to attain criterion performance of 70% correct. In the Porsolt Forced Swim Test, comparing the first minute of immobility-time on the training day and the retention day, the transgenic mice were found to be less immobile than controls on the second day of testing, suggesting poor retention. Although there were no deficits in performance on Step-Down Passive Avoidance, this was likely due to the ease of the learned task and the salience of the electric shock reinforcement. In comparison to the young controls, the aged transgenics, but not aged controls, displayed longer latencies to begin eating in the Thatcher-Britton Novelty Conflict paradigm, suggesting a higher degree of anxiety. These test differences were not due to a confound of motor-performance since no apparent group differences in locomotor activity were observed. Therefore, aged transgenic mice appear to have cognitive and affective impairments, and thus may provide an advantageous model in which to assess AD-related pathological and behavioural symptoms.

387.5

ACCUMULATION OF β AMYLOID FIBRILS IN PANCREAS OF TRANSGENIC MICE. T. Kawarabayashi[1], M. Shoji*[1], M. Sato[2], A. Sasaki[3], L. Ho[4], C. B. Eckman[4], C-M. Prada[4], S. G. Younkin[4], T. Kobayashi[2], N. Tada[2], E. Matsubara[1], K. Kasai[2] and S. Hirai[1]. [1]Department of Neurology, and [3]Department of Pathology, Gunma University School of Medicine, Maebashi, Gunma 371, Japan; [2] Laboratory for Animal Center, Pharma Research and Development Division, Hoechist Japan Limited, Kawagoe, Saitama, 350-11, Japan; [4]Departments of Pathology and Neuroscience, Case Western Reserve University, Cleveland, Ohio 44106.

Overproduction of amyloid β protein (Aβ) derived from amyloid β protein precursor (βAPP) is one of the causes of Alzheimer's disease. In an effort to produce substantial Aβ *in vivo*, we generated mice expressing the transgene of signal peptide and ninety-nine residues of carboxyl-terminal fragment (CTF) of βAPP under control of the cytomegalovirus enhancer / chicken β-actin promoter. The transgenic mRNA was detected in many tissues of these mice, but the levels of transgenic mRNA, CTF, and Aβ did not correlate well. Aβ was detected biochemically in brain, kidney, pancreas, and plasma with the largest amount present in pancreas. Thus these mice produce and secrete considerable Aβ. Despite the elevated Aβ in brain, immunocytochemistry revealed no consistent cerebral Aβ deposition. In pancreas, however, intracellular Aβ deposits were detected immunocytochemically in acinar cells and interstitial macrophages, some of which showed severe degeneration. Examination of these cells by electron microscopy revealed many putative amyloid fibrils (7-12 nm) that were stained by anti-Aβ antibodies. Our findings indicate that cell-specific posttranslational processing may play a pivotal role in Aβ production and amyloid fibril formation *in vivo*. These mice could be a useful model to investigate the factors that determine the location and amount of Aβ that accumulates as amyloid.

387.6

ALZHEIMER'S DISEASE CYBRIDS MANIFEST A CYTOCHROME OXIDASE DEFECT. S. Glasco[1]*, S. W. Miller[1], and L.J. Thal[2], R. E. Davis[1]. [1]Applied Genetics, Inc., San Diego, CA 92121; [2]Department of Neurology, VA Medical Center, San Diego, CA 92161.

Defects in cytochrome oxidase (Complex IV) activity have been found in the blood and brain of patients with Alzheimer's disease. The cause of these defects remain unknown. Because Complex IV is the encoded by both nuclear and mitochondrial genes, decreases in Complex IV activity could arise from genetic lesions in one or both of these genomes. We have created mtDNA deficient SH-SY5Y neuroblastoma cell lines, $\rho^0$118/5 cells (Applied Genetics, San Diego, CA). These cells lack mtDNA encoded electron transport chain activities and normal respiration but are still able to differentiate in response to various differentiation factors. We have created disease specific cybrids by fusion of $\rho^0$118/5 cells with the platelets from AD and age-matched control platelets. These cybrids have nDNA with origins in the parental SH-SY5Y cells and mtDNA with origins in the human donor platelets.

Alzheimer's disease cybrids show a 30% reduction in cytochrome oxidase activity when compared to age-matched control cybrids. However, Complex I activity, another mitochondrial encoded ETC enzyme, was equivalent in both AD and age-matched control cybrids, demonstrating the specificity of the mtDNA defect. Upon differentiation for three weeks with retinoic acid the AD cybrids maintain the cytochrome oxidase defect.

We have modeled cytochrome oxidase defects in AD cybrids. The defect probably reflects mutations in cytochrome oxidase subunits encoded by mtDNA. These cybrids will be useful as a model for elucidating the role of the mitochondrial genome in AD and other neurodegenerative diseases.

387.7

PRODUCTION OF REACTIVE OXYGEN SPECIES CORRELATES WITH DECREASED CYTOCHROME OXIDASE ACTIVITY IN ALZHEIMER'S DISEASE CYBRIDS. J. Lakis[1]*, S. Glasco[1], S.W. Miller[1], and L.J. Thal[2], R. E. Davis[1]. [1]Applied Genetics, Inc., San Diego, CA 92121; [2]Department of Neurology, VA Medical Center, San Diego, CA 92161.

Defects in cytochrome oxidase (Complex IV) activity have been associated with Alzheimer's disease but the cause of these defects are unknown. Complex IV is the product of two genomes, with protein constituents being encoded by both mitochondrial (mtDNA) and nuclear DNA (nDNA). Decreases in Complex IV activity could arise from genetic lesions in one or both of these genomes. To evaluate the contributions of the mitochondrial genome to AD associated Complex IV defects, we created mtDNA deficient SH-SY5Y neuroblastoma cell lines, $\rho^0$118/5 cells (Applied Genetics, San Diego, CA). These cells lack mtDNA encoded electron transport chain activities and normal respiration. Respiratory function can be recovered by repopulation of $\rho^0$118/5 cells with the exogenous human platelet mitochondria (cybrid). We have created disease specific cybrids by fusion of $\rho^0$118/5 cells with the platelets from AD and age-matched control platelets. These cybrids have nDNA with origins in the parental SH-SY5Y cells and mtDNA with origins in the human donor platelets. Alzheimer's disease cybrids show decreased cytochrome oxidase activity when compared to age-matched control cybrids. However, Complex I activity, another mitochondrial encoded ETC enzyme, was equivalent in both AD and age-matched control cybrids, demonstrating the specificity of the mtDNA defect. The decrease in COX activity in AD cybrids was directly correlated with a 75% increase in reactive oxygen species (ROS) as measured by oxidation of dichloro-fluorescein-diacetate in cultured cybrids. We have modeled AD mtDNA defects in cybrids and measured biochemical defects in COX activity and ROS production. These cybrids will be useful as models for dissecting the role of the mitochondrial genome in AD.

387.8

EXTRACELLULAR APP DEPOSITS IN NEURAL TRANSPLANTS HARBORING A MUTANT HUMAN APP. T.A. Bayer [a], A. Foßgreen [b], C. Czech [b], K. Beyreuther [b] and O.D. Wiestler [a]*. [a] Department of Neuropathology, University of Bonn Medical Center, Sigmund-Freud-Strasse 25, D-53105 Bonn, FRG; [b] ZMBH, Im Neuenheimer Feld 282, D-69120 Heidelberg, FRG.

Using retrovirus-mediated gene-transfer, we have introduced a mutant cDNA of human APP 695 into rat brain transplants. This mutant APP (with point mutations at positions 670/671) has been associated with Alzheimer's disease in a Swedish kindred and was shown to induce the formation of ß-A4 peptide in human cell lines. A minor population of recombinant neural cells with expression of APP was identified immunohistochemically in the grafts. The expression was maintained for a period of six months, i.e. the longest post-transplantation interval studied so far. Neurons with strong immunoreactivity for human APP exhibited a markedly swollen cell body and showed evidence for cellular degeneration. After a six months period, extracellular APP positive deposits were observed. These precipitates had a plaque like appearance. Studies to characterize the morphology and molecular composition of affected neurons and extracellular deposits are in progress. The data indicate that a disease-related mutant form of human APP has the potential to induce morphological features of Alzheimer's disease in the rodent CNS.

Supported by the Fritz-Thyssen-Stiftung.

387.9

BDNF, BUT NOT NGF, RESCUES TRISOMY 16 CORTICAL NEURONS IN VITRO. M.T. Caserta*, J. Muller and A. Guillozet. Dept. of Psychiatry, Northwestern University Medical School, Northwestern University Institute of Neuroscience and Evanston Hospital, Chicago, IL 60611

Murine trisomy 16 (ts 16) is a well-accepted model of human trisomy 21 or Down Syndrome. Since all individuals with Down Syndrome develop the neuropathological findings of Alzheimer's Disease (AD) by their third or fourth decade, the ts 16 mouse has been used as a susceptibility model for AD. Our laboratory has previously shown that ts 16 cortical neurons in vitro are morphologically undifferentiated and over-express somatostatin and neuropeptide Y and are more susceptible to the toxic effects of the beta A4 peptide fragment 25-35 when compared to euploid cortical neurons. It is not known whether ts 16 cortical neurons are responsive to nerve growth factor (NGF) or other known neurotrophic factors, such as BDNF. In this study, we investigated the effects of varying concentrations of NGF and BDNF (0-100 ng/ul) on trisomic and euploid cortical neurons in vitro when applied to the growth media for 2-5 days. Cultures were fixed and stained with an antibody to neuron-specific enolase (NSE) and NSE positive neurons were counted and photographed. The results indicate that BDNF rescued trisomy 16 cortical neurons in a dose-dependent manner while NGF had no effect. The addition of BDNF was found to have a deleterious effect on the survival of euploid cortical neurons. These results suggest a possible role for BDNF in the treatment of AD.

387.10

INCREASED LEVELS OF KYNURENIC ACID IN BRAINS OF GENETICALLY DYSTONIC HAMSTERS. A. Richter, W. Löscher, M. Gramer and †H. Baran (SPON: European Neuroscience Association). Dept. of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine, 30559 Hannover, Germany; † Childrens Hospital, Vienna

Recent pharmacological studies have shown antidystonic effects of NMDA and non-NMDA receptor antagonists in an inbred line of Syrian hamsters (dt^{sz}) with primary generalized dystonia, i.e. a neurological syndrome of sustained muscle contractions which occurs in the absence of any pathomorphological alterations. This prompted us to examine the levels of kynurenic acid, an endogenous non-selective antagonist of the excitatory amino acid receptors. The concentrations of kynurenic acid were determined by HPLC in forebrain, cerebellum, brain stem and plasma in dystonic hamsters and age-matched non-dystonic controls. Dystonia in mutant hamsters is transient and disappears completely at the age of 70 days. In order to prove if neurochemical changes are related to dystonia, kynurenic acid was determined at the age of maximum severity (30 days) and after remission (70 days). The levels of kynurenic acid were significantly increased in forebrain, cerebellum and brain stem (37-130%) in dystonic hamsters at the age of maximum severity of dystonia (30 days of life) compared to both a genetically related non-dystonic inbred line and a non-related outbred line. The increase of kynurenic acid in brain regions was accompanied by enhanced plasma levels. However, there was no correlation between brain and plasma levels. Since the changes disappeared in parallel with dystonia (70 days), the present data provide further evidence that abnormal activity of excitatory amino acids may be pathogenetically involved in dystonia in mutant hamsters. The recent finding of antidystonic effects of glutamate receptor antagonists can not be explained by a loss of the endogenous inhibitor kynurenic acid. The increased levels of kynurenic acid could be related to an overactivity of NMDA receptors.

Supported by grants from the Deutsche Forschungsgemeinschaft (Lo 271/4-2)

387.11

KAINIC ACID-INDUCED SEIZURES IN INBRED MICE: QUANTITATIVE TRAIT LOCI (QTL) MAPPING. T.N. Ferraro*, G.T. Golden, C. Ballas, G.G. Smith, N.J. Schork, W.H. Berrettini, Thomas Jefferson University, Philadelphia, PA, DVAMC, Coatesville, PA and Case Western Reserve Univ., Cleveland, OH.

Mature DBA/2J (D2) mice are very sensitive to seizures induced by various chemical and physical stimuli whereas C57BL/6J (B6) mice are relatively seizure-resistant. We have studied the sensitivity of an F2 intercross population of D2 and B6 mice (n = 257) to the seizure-inducing effect of the glutamate agonist kainic acid (KA) and have begun to map quantitative trait loci (QTL) which influence this phenomenon. Subsequent to dose-response and pharmacokinetic studies in parental mice, F1 and F2 mice (8-10 weeks of age) were injected (sc) with a KA dose of 25 mg/kg and observed for 2 hours. Latencies to clonus, tonic/clonic seizure and *status epilepticus* were recorded and an overall seizure score calculated. Mean ($\pm$ SD) seizure scores are as follows: D2: 1.17 ± 1.00 (n=10); B6: 0 (n=10); B6D2F1/J (F1): 0.93 ± 0.58 (n=10); and F2: 1.06 ± 0.99 (n=257). Distribution of maximum seizure responses among F2 mice (*status epilepticus*: n=77; tonic/clonic: n=70; clonus: n=40: no seizure: n=70) suggests that the difference in KA seizure susceptibility between the D2 and B6 mice is a polygenic phenomenon with about 65% of the variance in seizure score between parental strains due to genetic factors and 35% due to environmental factors. First-pass screening of the murine genome (10 cM marker intervals) using F2 mice with the highest (n=77) and lowest (n=70) seizure scores has documented a QTL of moderate effect on chromosome 1 between D1Mit30 and D1Mit16 (LOD score = 5.46 with Mapmaker/QTL; 11% of variance explained) indicating the presence of a gene in this region which has a significant influence upon KA seizure susceptibility in D2 and B6 mice. Putative QTL of small effect have been detected on chromosomes 11, 15, and 18; however, these loci require further evaluation. Supported by NINDS grant RO1-NS33243-01 and VA funds.

387.12

STRAIN DIFFERENCES IN COCAINE-INDUCED STEREOTYPY IN DBA/2J AND C57BL/6J MICE: DOSE RESPONSE. G.T. Golden*, T.N. Ferraro, G.G. Smith, W.H. Berrettini. DVAMC, Coatesville, PA 19320 and Jefferson Medical College of Thomas Jefferson University, Philadelphia, PA 19107

Cocaine-induced stereotypy (CIS) and cocaine-induced locomotor activity may be mediated by separate neural pathways. The C57BL/6J (B6) inbred mouse prefers cocaine more than other inbred strains, such as the DBA/2J (D2). D2 mice develop a high degree of CIS after chronic injections of cocaine (32mg/kg, ip) whereas B6 mice show no CIS (Tolliver & Carney, 1994). As a step toward characterization of genetic elements which may be involved in differential CIS in these mice, CIS was compared in 8 wk old D2 and B6 mice (n = 6-8/strain/dose) injected ip once daily with cocaine (30 or 40mg/kg) or saline for 10 days. CIS was rated for 50 min after injection on days 3, 7 and 10 by two blinded observers using a five point stereotypy scale. StrainXDose ANOVA with CIS scores as the dependent variable showed highly significant strain and dose main effects and significant strainXdose interaction effects for day 7 and day 10 CIS scores.

CIS SCORES IN DBA/2J AND C57BL/6J MICE, MEAN (sd)

STRAIN	3/1N.I	7/1N.I	10/1N.I	DOSE
DBA/2J	4.3 (3.2)	4.3 (2.3)	8.3 (1.1)	saline
C57BL/6J	7.7 (1.1)	7.0 (1.0)	6.7 (2.1)	saline
DBA/2J	15.8 (9.8)	26.4 (5.8)	33.1 (4.0)	30mg/kg
C57BL/6J	10.4 (2.4)	10.4 (3.6)	11.0 (2.3)	30mg/kg
DBA/2J	24.2 (8.1)	33.0 (3.7)	36.0 (2.1)	40mg/kg
C57BL/6J	9.5 (1.1)	11.8 (3.4)	12.5 (5.3)	40mg/kg

Results confirm the previously reported large strain differences in CIS between D2 and B6 mice and suggest that quantitative trait loci mapping strategies would be a feasible approach for identifying the gene(s) which influence this complex trait. This may lead to new avenues of research, improved treatment and identification of individuals at high risk for cocaine addiction. Supported by the Department of Veterans Affairs.

387.13

ESTABLISHMENT AND CHARACTERIZATION OF MICE LACKING Cu/Zn SUPEROXIDE DISMUTASE. E.K. Hoffman, A.G. Reaume, H. Wilcox, D.G. Flood*, Y-G. Lin, and R.W. Scott. Cephalon Inc., 145 Brandywine Parkway, West Chester, PA 19380.

Oxidative damage has been implicated as a contributing factor in several neurodegenerative conditions including normal aging, stroke, Parkinson's disease, Alzheimer's disease and Huntington's disease. To establish an animal model predisposed to oxidative injury, the SOD-1 gene encoding Cu/Zn superoxide dismutase has been deleted in mice. A gene replacement strategy was utilized to remove all SOD-1 coding sequences in ES cells that were in turn used to generate Cu/Zn SOD-deficient mice lacking one or both copies of the SOD-1 gene. The homozygous SOD-1 null animals contain no detectable Cu/Zn SOD on immunoblots and have approximately 5% residual superoxide scavenging activity in a spectrophotometric assay. These animals survive to at least six months of age and show no apparent behavioral or gross neuroanatomical abnormalities. Initial biochemical surveys have revealed no significant differences in markers of oxidative damage (protein carbonyl content, lipid peroxidation) and oxidative stress (reduced glutathione and Mn-SOD) in brain and select peripheral tissues. The lack of any neuromuscular functional deficits to date supports the gain-of-function hypothesis for the SOD-1 mutations linked to familial ALS. The absence of an overt phenotype in the SOD-1 deficient mice is in marked contrast to results with other Cu/Zn SOD-deficient organisms including yeast and *Drosophila*. Several possibilities for this difference are that the presence of redundant or compensatory mechanisms protect the SOD-deficient mouse from oxidative injury, or Cu/Zn SOD plays a more crucial protective role in mammals under conditions of pathological stress. To address this issue, the animals are currently being aged and subjected to a variety of lesion and stress conditions to identify potentially vulnerable neuronal populations.

387.14

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF DEGENERATING NEURONS IN THE SPINAL CORD OF MUTANT SUPEROXIDE DISMUTASE TRANSGENIC MICE. B.M. Morrison*[1], J.W. Gordon[3], P.R. Hof[1], M.E. Ripps[2], and J.H. Morrison[1]. [1]Fishberg Res. Ctr. for Neurobiol., [2]Brookdale Ctr. for Mol. Biol., and [3]Dept. of Obs/Gyn and Reproductive Sci., Mount Sinai School of Medicine, New York, NY 10029.

Transgenic mice with a point mutation in the mouse superoxide dismutase(SOD) gene that results in a Gly86→Arg substitution, corresponding to a mutation observed in familial amyotrophic lateral sclerosis (ALS), display progressive loss of motor function and provide a valuable model of ALS. Parallel series of spinal cord sections from control and mutant SOD transgenic animals were stained with Nissl, Campbell-Switzer silver method, and immunohistochemical markers for phosphorylated neurofilament (NFP), non-phosphorylated NFP, and calcium-binding proteins (CaBP). Qualitatively, there were argyrophilic dystrophic neurites, ghost cells, a few cells exhibiting pyknosis and karyorrhexis, and a pronounced loss of motoneurons within the ventral horn of the spinal cord. Unlike other SOD transgenic mice and ALS patients, we have not observed vacuolization or disruption of the NFP cytoskeleton within motoneurons. To quantify the neuronal degeneration in these mice, neurons were counted, using the optical fractionator, in the ventral horn of both the cervical and lumbar enlargements of SOD transgenic and control spinal cords. By comparing the number of neurons in transgenic versus control animals, we estimated the loss of Nissl-stained neurons (used as a measure of total neuron number) in the SOD transgenic mice to be 25%, and the loss of NFP-immunoreactive neurons to be 50%. This finding suggests that NFP-containing neurons are particularly vulnerable to the cellular insult resulting from mutations in the SOD gene, consistent with several studies demonstrating that these neurons are particularly vulnerable in neurodegenerative diseases. Interestingly, preliminary results indicate that CaBP-containing neurons are lost in significant numbers, although they are not as vulnerable as NFP-enriched neurons. This contrasts with several studies that have reported these neurons to be resistant in neurodegenerative diseases. Supported by NIH grants AG10520 and AG06647.

387.15

THE MITOCHONDRIAL GENOME ENCODES THE COMPLEX I DEFECT IN PARKINSON'S DISEASE. R. Swerdlow, J. K. Parks, S. W. Miller, R.E. Davis* and W.D. Parker. Department of Neurology, University of Virginia, Charlottesville, VA 22908 and Applied Genetics, San Diego, CA 92121.

Proposed etiologies of Parkinson's Disease (PD) include toxic and inherited causes. Environmental toxins have been associated with higher disease incidence, but such findings have been questioned. Epidemiologic studies to define genetic contributions also have been confusing. This confusion may arise from previously unsuspected heterogeneity in PD. Mitochondrial inheritance may contribute to PD, accounting for its sporadic, non-Mendelian occurrence. To test this hypothesis, we created cybrid cell lines (PD-ρ^0) using mitochondria donated by PD patients. Cybrids are unique cells created by introducing exogenous mitochondrial DNA (mtDNA) into hosts cells depleted of mtDNA. In this case, human SH-SY5Y neuroblastoma cells were depleted of endogenous mtDNA by prolonged exposure to ethidium bromide ($\rho^0$118/5, Applied Genetics, San Diego, CA) and were repopulated with platelet mtDNA from PD patients or age-matched controls. This resulted in cell lines whose only genetic differences were in the origin of their mtDNA and therefore any differences between cell lines must arise from differences in the exogenous mtDNA.

In previous work we showed that the mitochondrial enzyme NADH:ubiquinone oxidoreductase (Complex 1) is deficient in PD. Since complex I is the target of the PD-inducing toxin MPP+, this complex I lesions may be pathologically important. PD-ρ^0 cells were complex I deficient compared to age-matched controls. Activities of other mitochondrial enzymes (cytochrome oxidase, citrate synthase) were similar in PD-ρ^0 and control-ρ^0 cells. This demonstrates that the focal defects in complex I activity are brought into the cells along with the transferred mtDNA from donor mitochondria..

387.16

IMMUNOLOGIC AND BEHAVIORAL ABNORMALITIES IN A MOUSE MODEL OF DOWN SYNDROME M.E. Coussons-Read* and L.S. Crnic. Depts. of Psychiatry and Pediatrics, Univ. of Colo. Sch. of Med., Denver, CO 80262

The Ts65Dn mouse is trisomic for a region of mouse chromosome 16 which has high homology to the Down syndrome (DS) region of human chromosome 21, and is thus a potential animal model of DS. Among the characteristics of DS are mental retardation, behavioral disturbances, impaired immune function, and increased disease susceptibility. The behavior of Ts65Dn and littermate control mice was assessed in the elevated plus maze, lighted and dark open field, and in a step-down passive avoidance task. In the elevated plus maze, Ts65Dn had more total arm visits than controls, entered the open arms more frequently than control mice, and showed no preference for the closed arms. Ts65Dn mice were more active in both open field situations, regardless of light condition, and entered the center of the arena more than controls. No significant differences were observed in acquisition and retention of the step-down passive avoidance task, indicating that this simple form of associative learning is intact in these mice. Overall, the data show that Ts65Dn mice are more active than control mice in two testing situations. Most striking, Ts65Dn mice did not respond to variations in environmental cues which induce wariness in normal mice. At the conclusion of behavioral testing, spleens were collected for immunological analysis. The level of Con-A induced lymphocyte proliferation was depressed in cultures of splenocytes from the Ts65Dn mice compared to their non-trisomic littermates. Lymphocytes from Ts65Dn mice were also much more sensitive than controls to the antiproliferative effects of interferon α (IFNα), results consistent with the immunologic anomalies reported in DS, and with the presence of the IFNα receptor gene in the trisomic region. These data begin to characterize the Ts65Dn phenotype, and support its use as a model of aspects DS, since similar behavioral and immunologic traits appear to exist in the Ts65Dn mouse and DS. Supported by HD04024, HD17449, MH49043, and MH15440.

387.17

THE NEUROHISTOLOGY OF THE SCID MOUSE BRAIN, F. J. Denaro*, TTUHSC, Lubbock, TX 79430 and R. Jones, Portland VAMC.

Human lymphocytes and hematopoietic stem cells can be transplanted into SCID mice without rejection. This has enabled the development of the SCID mouse as a model system for studying important human diseases, viral infections, transplantation and immune system development. In the present study, a neurohistological examination was conducted in SCID mice following transplantation of human PBLs or bone marrow. Histological examination two months following transplantation revealed a normal brain. Neurons, astrocytes, oligodendrocytes, microglia and the vascular system appeared normal. Stains, such as Bielschowsky, PAS, and LFB demonstrated an unremarkable brain at the cytologic and gross levels. EM has not revealed any abnormalities two months after transplantation. Longer term animals will be studied. Reactive gliosis, however, was found in animals which were reconstituted by intercerebral injections. In these cases reactive glial cells and possibly microglia were found around injection sites. NS31857,Veterans Affairs

EPILEPSY: BASIC MECHANISMS II

388.1

FIELD-POTENTIAL ACTIVITY INDUCED BY 4-AMINOPYRIDINE IN THE RAT HIPPOCAMPAL/ENTORHINAL SLICE PREPARATION. R. Köhling*, T. Nagao, A. Lücke and M. Avoli, Montreal Neurological Inst., Dept Neurology & Neurosurgery, McGill Univ., MONTREAL, Canada H3A 3B4

An in vitro slice preparation of combined hippocampus and entorhinal cortex from adult rat was used to study the modalities of generation and propagation, and the pharmacological properties of the synchronous activity induced by 4-aminopyridine (4AP, 50μM). Simultaneous field-potential recordings were made in the entorhinal cortex, dentate gyrus, and CA3 and CA1 sectors. 4AP made three types of spontaneous activity appear. The first occurred synchronously in both entorhinal cortex and hippocampal areas and consisted of a long-lasting (0.3-1s) field potential that was reminiscent of the GABA-mediated event described in isolated hippocampal slices (*J Neurosci*, 12 (1992) 104-105). The second type was only observed in CA3 and CA1 sectors and consisted of brief (<15ms) interictal epileptiform discharges (rate = 1-2.5Hz). The third type of activity resembled ictal epileptiform discharges (duration up to 180 s) and originated in the entorhinal cortex from where it propagated to the dentate, CA3 and CA1 via the hippocampal trisynaptic loop as revealed by latency analysis and lesion experiments. Both types of epileptiform activity were blocked by the non-NMDA receptor antagonist CNQX (10μM), while the NMDA receptor antagonist CPP (10μM) only abolished entorhinal ictal discharges. Concomitant application of CNQX and CPP did not influence the occurrence of the presumably GABA-mediated potentials that were abolished by bicuculline methiodide. This study confirms that the combined hippocampal/entorhinal slice represents a suitable model for analyzing the physiopathogenesis of limbic seizures and points to the entorhinal cortex as the site of origin of an NMDA-mediated ictal discharge.

388.2

POST-ANOXIC CHANGES IN SYNAPTIC EXCITABILITY IN THE CA1 SUBFIELD OF THE RAT HIPPOCAMPUS. V. Tancredi* and M. Avoli, Dip. Med. Sper., Univ. di Roma "Tor Vergata", 00173 Rome, Italy and Montreal Neurological Institute and Department of Neurology and Neurosurgery, McGill University, MONTREAL, QC, Canada H3A 2B4

We used field-potential recording techniques to study the long-lasting changes in synaptic excitability induced by brief (<7min) episodes of anoxia in the CA1 subfield of rat hippocampal slices. Disappearance of the population spike and/or substantial reduction of the field excitatory postsynaptic potential (EPSP) induced by stratum radiatum (SR) stimuli occurred with anoxia. The amplitude of the population spike increased during re-oxygenation by 87 ± 68% (n = 35 slices) as compared to control. This post-anoxic potentiation was detected for several tens of minutes after re-oxygenation. Further anoxic episodes could induce the appearance of SR-induced epileptiform bursts, that were reduced by NMDA receptor antagonists and completely blocked by the non-NMDA antagonist CNQX. Paired-pulse stimulation protocols demonstrated that inhibitory mechanisms decreased by as much as 75% after a single episode of anoxia (n = 5 slices). Such reduction of inhibition persisted over time and became more evident as more anoxic episodes were delivered until the final appearance of epileptiform bursts. Our findings demonstrate that anoxia can induce enhancement of synaptic excitability of CA1 pyramidal cells leading to the appearance of epileptiform activity. The long-lasting changes are associated with a reduction of polysynaptic inhibitory mechanisms. Moreover, anoxia makes an NMDA-mediated component of excitatory synaptic transmission appear, which might contribute to the appearance of SR-induced epileptiform bursts. Supported by Quebec Heart and Stroke Foundation, CNR of Italy and MRC of Canada.

388.3

$[K^+]_o$ MEASUREMENTS DURING SYNCHRONOUS ACTIVITY INDUCED BY 4-AMINOPYRIDINE IN THE RAT ENTORHINAL CORTEX. M. Barbarosie, R. Köhling, A. Lücke T. Nagao, and M. Avoli*, Montreal Neurological Institute and Department of Neurology and Neurosurgery, McGill University, MONTREAL, QC, Canada H3A 2B4

The synchronous activity induced by 4-aminopyridine (4AP, 50μM) in the entorhinal cortex was analyzed with extracellular field-potential and K^+-selective recordings in an in vitro slice preparation. $[K^+]_o$ rose to 3.4-7.6 mM during a negative-going field potential that lasted 0.5-3.5 s and occurred at rates of 0.013-0.13 Hz. This event could be followed within a few hundred ms by an ictal epileptiform discharge that was associated with $[K^+]_o$ elevations of 14-17 mM. The termination of the ictal discharges was accompanied by $[K^+]_o$ undershoots. Depth-profile analysis of the field-potential and $[K^+]_o$ activity indicated that during ictal discharge the largest values were recorded in the deep layers of the entorhinal cortex. During blockade of ionotropic excitatory amino acid receptors, the negative-going potentials continued to occur and they were accompanied by $[K^+]_o$ increases of 3.6-5.8 mM. They were blocked by the $GABA_A$ receptor antagonist bicuculline methiodide or the μ-opioid receptor (D-Ala^2,N-Me-Phe^4,Gly^5-ol)-enkephalin. These findings demonstrate that the occurrence of ictal discharge in the entorhinal cortex is preceded by a GABA-mediated increase in $[K^+]_o$. As reported in the immature hippocampus (*J Neurophysiol* 70 (1993) 1018-1029), such a potential appears to be instrumental in initiating ictal activity in the entorhinal cortex of adult rat brain. Supported by MRC of Canada and Savoy Foundation.

388.4

INTRACELLULAR ACTIVITY OF RAT ENTORHINAL NEURONS DURING ICTAL-LIKE EPILEPTIFORM DISCHARGES INDUCED BY 4-AMINOPYRIDINE. V. Lopantsev* and M. Avoli, Montreal Neurological Inst., Dept Neurology & Neurosurgery, McGill University, MONTREAL, QC, Canada H3A 3B4

Spontaneously occurring ictal-like epileptiform discharges, were induced by 4-aminopyridine (4AP, 50μM) in slices of entorhinal cortex from adult rats. Conventional intracellular recordings were made from 29 neurons of the entorhinal cortex (0.2-1.3 mm from glial surface). The intracellular counterpart of the ictal epileptiform discharge consisted of a long-lasting (29-110s), large-amplitude (20-52mV) depolarization of the membrane associated with discharge of fast action potentials At resting membrane potential (RMP = -72 ± 7.1 mV) such ictal activity was often initiated by a depolarization (duration = up to 2.7 s) that corresponded to a negative-going, presumably GABA-mediated field potential and was not associated with action-potential discharge. When the RMP was depolarized with intracellular injection of current, this depolarization inverted to a hyperpolarizing potential (reversal potential = approx. -63 mV). Blockade of ionotropic excitatory amino acid receptors abolished the epileptiform discharges, while the presumably GABA-mediated potentials continued to occur. At control RMP, it consisted of a long-lasting depolarization associated with a large increase in membrane conductance. These potentials were abolished by the $GABA_A$ receptor antagonist bicuculline methiodide or the μ-opioid receptor agonist DAGO. Our findings show that entorhinal neurons generate a GABA-mediated depolarization that is due to postsynaptic activation of $GABA_A$ receptors, is resistant to antagonists of ionotropic excitatory amino acid receptors and might be instrumental in initiating ictal discharge.

388.5

SYNCHRONOUS ACTIVITY IN THE ABSENCE OF EXCITATORY AMINO ACID-MEDIATED TRANSMISSION IN THE RAT HIPPOCAMPUS IN VITRO.

T. Nagao*, R. Köhling, A. Lücke, D. Mattia and M. Avoli, Montreal Neurological Institute, Dept Neurology & Neurosurgery, McGill University, MONTREAL, QC, Canada H3A 3B4

Field-potential recordings were performed in the CA3 stratum radiatum of rat hippocampal slices during concomitant application of 4-aminopyridine, tetraethylammonium, and ionotropic excitatory amino acid (EAA) receptor antagonists. Prolonged synchronous field potentials (duration = 40.2 ± 4.5 s, n = 18 slices) occurred spontaneously and were made up of positive-negative components associated with population-spike discharges. In 80% of the slices spreading depression-like episodes also occurred (duration = 120 ± 19 s). The field-potential discharges were associated with $[K^+]_o$ elevations of 6.1 ± 0.7mM (n = 5), while during spreading depression $[K^+]_o$ increased up to 27mM. Both types of activity were blocked by the $GABA_A$ antagonist bicuculline or application of Ca^{2+}-free, high-Mg^{2+} medium. Simultaneous field potential recordings in different hippocampal areas demonstrated that the prolonged field-potential discharges occurred in a synchronous fashion. By contrast, spreading depression episodes were more frequently recorded in the CA1 than in the CA3 subfields and were never seen in the subiculum or the dentate area. Our findings indicate the presence of a novel type of prolonged field-potential discharge and spreading depression-like episodes in the rat hippocampal slice when ionotropic EAA receptors are blocked. The occurrence of this synchronous activity is presumably dependent upon the postsynaptic activation of the $GABA_A$ receptor located on neuronal and glial elements, while rises in $[K^+]_o$ and consequent redistribution processes are sufficient to make all types of synchronous activity propagate. Supported by MRC of Canada.

388.6

IMAGING SPREADING DEPRESSION IN THE RAT HIPPOCAMPAL SLICE USING INTRINSIC OPTICAL SIGNALS

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Spreading depression (SD), a pre-migraine event characterized by propagating waves of membrane depolarization and extracellular DC potential shifts across grey matter, was induced in rat hippocampal slices by superfusion of 100 μM ouabain (35°C). Increases in tissue light transmittance (T), which correlate with cell swelling, occur during SD onset and were used to map the propagation of SD. Following ouabain exposure, T increased by 30-50% in CA1 stratum oriens (OR) and spread along OR and into CA1 stratum radiatum (RAD) (6/6 slices). This wave front traversed CA1 OR with an average peak velocity of 6.7 ± 3.2 mm/min. As the optical wave passed a recording electrode in CA1 RAD, the DC potential shifted by -20 to -30 mV, consistent with a spatiotemporal correlation of optical signals and electrophysiological SD. Propagation stopped at CA3, but with a delay crossed the hippocampal fissure into dentate gyrus (n = 5/6). Increased T in CA1 RAD reversed over 0.5-1.5 min. Subsequently, T decreased below baseline in CA1 RAD/OR and increased in CA1 stratum pyramidale, indicating dendritic shrinkage and somatic swelling. Imaging of SD in real time using intrinsic optical signals will allow for a systematic study of the cellular mechanisms of SD propagation.

388.7

BRIEF PERIODS OF EPILEPTIFORM ACTIVITY INDUCE LONG-TERM POTENTIATION IN RAT HIPPOCAMPAL SLICE CULTURES.

S.M. THOMPSON, D. DEBANNE* and B.H. GÄHWILER.
Brain Research Institute, University of Zurich, 8029 Zurich Switzerland

CA1 pyramidal cells were recorded intracellularly in rat hippocampal slice cultures, and EPSPs were evoked at low frequency (0.3 Hz), with an extracellular stimulating electrode located in area CA3. A brief period of epileptiform activity was induced by bath application of the $GABA_A$ receptor antagonist bicuculline. An increase in the evoked EPSP amplitude, lasting for more than 30 minutes, was observed after complete washout of bicuculline. A similar long-lasting potentiation could be observed when epileptiform activity was produced by bath-application of magnesium-free extracellular saline, indicating that epileptiform activity itself elicits the potentiation. In addition to the bicuculline-induced potentiation of the monosynaptic EPSP, a polysynaptic excitatory component triggered by stimulation in area CA3 was unmasked. As excitatory connections are four times more probable between CA3 pyramidal cells than between CA1 cells in slice cultures, the recruitment of polysynaptic excitation could be due to potentiation of excitatory connections within area CA3. In order to test this hypothesis, pairs of monosynaptically connected pyramidal CA3 cells were recorded intracellularly. Long-lasting potentiation of EPSPs at CA3-CA3 unitary synapses was also found to result from epileptiform activity. Bicuculline-induced potentiation required NMDA-receptor activation, occluded further LTP induction, and could be reversed by 3 Hz presynaptic tetani. We conclude that short periods of epileptiform activity induce an LTP-like long-lasting potentiation of excitatory connections at CA3-CA1 and at CA3-CA3 cell synapses. The long-term modification of the balance between synaptic excitation and inhibition in favour of excitation could represent a substrate underlying the persistence of epilepsy after an initial insult.

388.8

DISINHIBITION IN RAT VENTRAL HIPPOCAMPAL SLICES CAUSES PROLONGED EPILEPTIC DISCHARGES WITH THREE DISTINCT PHASES

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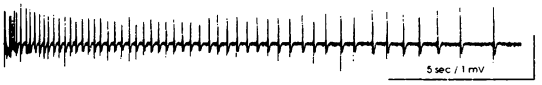
Blocking inhibitory transmission in hippocampal slices causes epileptic discharges. In standard transverse slices taken from dorsal hippocampus, pharmacological blockade of $GABA_A$-mediated inhibition with bicuculline (BIC; 10 - 30 μM) leads to brief synchronized population bursts, often followed by a series of shorter secondary bursts, and lasting a few hundred ms overall. Here we show that disinhibition and relatively high extracellular potassium (5mM) causes much longer bursting activity in ventral hippocampal slices prepared from normal male Sprague Dawley rats. BIC (10-30 μM) induced spontaneous synchronized bursts in the CA3 region. Stimulation of the perforant path evoked bursts lasting up to 40 s (Fig.) and spreading synchronously through the entire hippocampus proper. Bursts in the ventral hippocampus started with a primary burst and a short series of secondary bursts similar to those in dorsal slices. Subsequently, a long train of short tertiary bursts separated by brief intervals was generated in the CA3 region. These tertiary bursts differed from the primary and secondary bursts in shape and duration. They also were more resistant to NMDA receptor antagonists, indicating different means of discharge generation and synchronization for primary, secondary and tertiary components.



388.9

MINIMAL DISINHIBITION CAN ALTER SYNCHRONIZATION IN COMPUTER MODELS OF OLFACTORY CORTEX SLICE.

S. N. Deyo* and W. W. Lytton. Neurosimulation Laboratory, Dept. of Neurology, University of Wisconsin School of Medicine and Middleton VAMC, Madison, WI 53706.

We studied the role of $GABA_A$-mediated inhibition in switching between synchronized and desynchronized activity in a computer simulation of an endopyriform nucleus slice. In our simulations inhibitory current induced by activation of $GABA_A$ synapses produced desynchronization by delaying the AMPA/NMDA-mediated depolarization. Thus, inhibited neurons took longer to initiate bursting after glutamatergic activation. The variability of the lag to burst onset produced phase shifting and desynchronization in the population. Desynchrony occured at $GABA_A$ strengths that did not shorten burst duration and would therefore be difficult to observe physiologically. This effect was particularly pronounced in networks whose activity was maintained by spontaneously bursting cells or by tonic excitation whether intrinsic to the network or due to afferents. By contrast, intrinsic afterhyperpolarizing currents, which limited both burst length and duration of network oscillation, did not desynchronize the network. The pronounced effects of relatively subtle disinhibition on synchronization suggests a way that seemingly minor inhibition/excitation imbalances might lead to focal epilepsy.

Supported by both NINDS and VA grants.

388.10

NMDA-DEPENDENT INDUCTION OF EPILEPTIFORM ACTIVITY IN PIRIFORM CORTEX IN VITRO DOES NOT INVOLVE KINASES THAT ARE REQUIRED FOR LTP.

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Interictal-like epileptiform activity can be induced in piriform cortex in vitro by a brief period of bursting activity resulting from removal of Mg^{2+} or Cl^- from the bathing medium (WH Hoffman and LB Haberly, J Neurosci 9:206). This epileptiform activity usually persists for the duration of experiments after return to normal bathing medium. Because the mechanism of generation of this abnormal activity resembles that observed in slices taken from previously kindled animals (WH Hoffman and LB Haberly, Soc Neurosci Abs 17:511), the process of induction is being investigated to gain possible insights into temporal lobe epileptogenesis.

The present study tested the hypothesis that an LTP/LTD-like change in efficacy of excitatory or inhibitory synapses contributes to the burst-induction of epileptic activity. This hypothesis is suggested by the finding that, like NMDA-dependent LTP in piriform cortex (ED Kanter and LB Haberly, Br Res 525:175) and elsewhere, the induction of epileptic activity by bursting is dependent on the activation of NMDA receptors, but once induced, this dependence is lost. The hypothesis was tested by comparing the effects of KN-62, a relatively specific inhibitor of Ca^{2+}-CaM-KII, on the induction of LTP and epileptiform activity. LTP was assayed at synapses of the intrinsic associational fiber system in piriform cortex using extracellular recording methods as described previously (ED Kanter and LB Haberly, J Neurosci 13:2477). As reported for hippocampus (Ito et al, Neurosci Lett 121:119), synaptic potentiation induced by shock trains returned to near baseline within 1 hour in the presence of KN-62 (3 μM), whereas it remained elevated in control slices. By contrast, the induction of epileptiform activity was unaffected by KN-62. Other relatively non-specific kinase inhibitors including W-7 (10-100 μM) and H-7 (10-50 μM), also failed to block the induction of epileptiform activity, although the effects of H-7 were complicated by its blockade of $GABA_A$-mediated inhibition as previously documented in hippocampus (Arai et al, Soc Neursci Abs 15:84).

It is concluded that the changes that underlie the bursting-induction of epileptiform activity in piriform cortex in vitro do not include an NMDA-dependent LTP or other process that depends on the activation of Ca^{2+}-CaM-KII, PKC, or PKA. Supported by NS19865 from NINDS.

388.11

INTRINSIC AND SYNAPTIC RESPONSES OF NEURONS IN THE RAT PERIRHINAL CORTEX. H.J. Lennox* and D.C. McIntyre. Psychology Dept., Carleton Univ., Ottawa, Ont., Canada K1S 5B6.

The perirhinal cortex (PRh) has recently been linked to both memory and rapidly-kindled short-latency seizures. Oscillatory activity is arguably a factor in the induction and expression of both of these behaviours. Previously, we demonstrated that both the perirhinal and piriform (Pir) cortices of the rat develop and sustain prolonged oscillatory activity when a coronal slice of these structures and the adjacent amygdala nuclei is exposed to 0-Mg perfusate. The same slice preparation was used in the present study to assess the intrinsic and synaptic excitability of PRh cells. Activity in over 40 cells was recorded intracellulary in response to both briefly injected depolarizing current and to field stimulation of up to 19 slice locations. Field potentials were also recorded in the neighbourhood of the PRh cell and in dorsal Pir. Injected current evoked bursts (2-5 spikes, 4-16 ms apart) from about 1/3 of the cells. The bursts were followed by single spikes whose number increased with current intensity. Although the remaining 2/3 cells fired only single spikes during current injection, bursts were later induced in some of these cells by stimulations of the PRh, Pir, and of the lateral and basolateral (BL) nuclei. BL stimulation also elicited many of the synaptically-evoked bursts and single spikes in burst cells, and several of the larger epsp responses in both cell types. Recurrent synaptic activity was evident both intracellulary and extracellularly from the increase in interstimuli "spontaneous" events during stimulations of either type, and from recurrent responses associated with virtually all stimulation sites. While some recurrent activity was oscillatory in nature, other events occurred at less predictable and sometimes very long intervals (sec). Although recurrent activity was not generally more than pairwise-synchronous across recording sites, stimulation of the dorsal endopiriform nucleus was notably effective in eliciting large and/or recurrent activity coincident at all three recording sites. Thus, intrinsic, local and larger network activation all support the recurrent excitation of perirhinal cells.

388.12

DYE COUPLING BETWEEN RAT HIPPOCAMPAL CA1 PYRAMIDAL CELLS IN THE TETANUS TOXIN MODEL OF EPILEPSY.

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Electronic coupling via gap junctions represents a mechanism for intercellular communication, with the possibility for synchronization of neuronal discharge. The tetanus toxin chronic model of epilepsy produces epileptiform activity in the hippocampus, initially involving disinhibition (Whittington and Jefferys, *J.Physiol.* 481: 593-604, 1994). We have used intracellular injections of biocytin to examine whether there are morphological changes which could also be associated with this model of epilepsy.

Tetanus toxin (6ng protein; 12 MLD's), or it's buffer, was stereotactically and unilaterally injected under general anaesthesia into the hippocampus of male Sprague Dawley rats (250-300g). Hippocampal slices (400μm thick) were prepared after 8 weeks. Biocytin was injected into individual CA1 pyramidal cells and then visualized using the diaminobenzidine method for peroxidase.

Only 7% of biocytin injections into single cells from the buffer injected group resulted in "dye-coupled" cells. In contrast, a significantly higher number of cell impalements in the tetanus toxin treated group resulted in dye-coupled cells (63%, $P<0.007$). Half of these coupled cells were joined at the soma, and the other half appeared to be linked by dendrodendritic contacts. In the latter case, the separation of the soma was 84±24μm.

Increased electrotonic connectivity in the tetanus toxin treated group may play a role in the synchronization and maintenance of epileptiform activity. Further work is required to determine whether these dendrodendritic contacts are gap junctions.

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388.13

RHYTHMIC EXCITATORY SYNAPTIC EVENTS IN CA3 INTERNEURONS. G.J. Klapstein* and W.F. Colmers, Dept. of Pharmacology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

Neuropeptide Y potently inhibits epileptiform activity induced by several different methods in the *in vitro* rat hippocampal slice preparation. In the stimulus train induced bursting (STIB) model, trains of stimuli are delivered every 4 to 5 minutes to stratum radiatum of area CA3. CA3 pyramidal neurons exhibit clonic or tonic-clonic afterdischarges and delayed, spontaneous, interictaform bursts. Following several trains, spontaneous, rhythmic, synchronous events (SRSEs) become evident in whole-cell pyramidal cell recordings. SRSEs are inhibited by the $GABA_A$ antagonist picrotoxin (100μM), but also require glutamate, as they can also be inhibited by the AMPA receptor antagonist NBQX (1μM), but are insensitive to NPY.

Recordings from interneurons in strata oriens or radiatum of CA3 revealed excitatory events of similar frequency to the SRSEs recorded in pyramidal neurons. These are also inhibited by 1μM NBQX, and reverse near -9mV, indicating a glutamatergic origin, although they are not affected by the NMDA or metabotropic receptor antagonists, APV (50μM) or MCPG (100μM). They are not inhibited by 1μM NPY, differentiating their glutamatergic afferents from those innervating CA3 or CA1 pyramidal neurons. Their frequency is independent of membrane potential, suggesting that they are not caused by intrinsic oscillations. Their frequency is also unaffected during paroxysmal depolarization shifts, suggesting that the afferent drive may have distant origins.

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388.14

NEURONAL BURSTING ACTIVITY INDUCED BY RISING TEMPERATURE IN THE HIPPOCAMPAL SLICE. V.Y. Vasilenko, T.A. Petruchuk, R. Sankar* and R.A. Wallis, Inst. Physiol. Acad. Sci., 220072 Minsk, Belarus; and Depts. of Neur. & Ped., UCLA and Sepulveda VAMC, Sepulveda, CA 91343.

The occurrence of febrile seizures has been demonstrated to correlate with the rate of temperature rise developing during fever. The hippocampus is known to be involved in the development of seizures from a variety of etiologies. Therefore, we evaluated the effect of temperature rise upon the firing rate of CA3 pyramidal neurons in the hippocampal slice.

Single-unit activity of CA1 and CA3 neurons in the pyramidal layer of guinea pig slices was recorded extracellularly at temperatures ranging from 32 to 40°C. At a constant temperature of 38°C, 4.2 ± 0.6 S.E. spontaneous bursts/min, and 5 ± 1 impulses per burst were observed. No significant difference was seen at 32 or 40°C.

In contrast, slices given gentle warming (0.5°C/min) between 36 and 40°C displayed significant increases in firing. Spontaneous bursts/min increased to 9.6 ± 1.2 ($P<0.01$), while the number of impulses per min increased to 10 ± 2 ($P<0.05$). Cessation of warming or cooling at any temperature within 32-40°C was followed by a return of neuronal activity to original levels.

These data demonstrate the existence of hippocampal, which respond to temperature rise, but not constant temperature elevation, with increased burst activity. This increased neuronal firing with temperature rise, is similar to the occurrence of febrile seizures seen with rapid temperature elevation, and suggests that neuronal bursting in response to temperature rise may play a role in the generation of febrile seizures.

388.15

HIGH-FREQUENCY SYNCHRONOUS FIRING OF INTERNEURONS INCREASES THE EFFECTIVENESS OF GABAERGIC TRANSMISSION. M.F. Jackson, B. Esplin and R. Čapek*. Dept. of Pharmacol. & Therap., McGill Univ., Montreal, Que. H3G 1Y6.

Whole-cell recordings of monosynaptic IPSPs were made from rat CA1 pyramidal cells in order to determine the mechanism responsible for the increase in the effectiveness of GABA-mediated inhibition following high-frequency stimulation of inhibitory pathways, which we recently reported (Epilepsy Res. 16 (1993) 123-130). With excitatory amino acid receptors blocked by CNQX (10 μM) and CPP (5 μM) direct stimulation of interneurons by a single stimulus produced both fast- (f-) and slow-IPSPs (s-IPSPs). Stimulation by a train of 2 to 100 pulses at 100 Hz caused (1) an increase in both the amplitude and duration of the f- and s-IPSPs (by as much as 200% following a train of 5 pulses), as well as (2) the appearance of a depolarizing response (DR) which occurred between, and overlapped with, the two components of the IPSP. The amplitude of each component of the triphasic response increased with the number of pulses over the range of 2 to 5. Further increases in the amplitude of the f- and s-IPSP, over the range of 10 to 100 pulses, could not be observed due to the prominent increase in the DR. In fact, the DR increased to the point that it was able to cause the firing of a burst of action potentials. Both the f-IPSP and the DR were completely blocked by bicuculline methiodide (40 μM) suggesting that they were $GABA_A$ receptor-mediated. Under these conditions, the train-induced s-IPSP was isolated and showed a nearly fourfold increase in amplitude as well as a pronounced increase in duration. In separate experiments, the s-IPSP was blocked by the $GABA_B$ receptor antagonist, 2-hydroxysaclofen (200 μM). Such block resulted in a substantial increase of the DR indicating that the DR and s-IPSP occluded each other. The frequency-dependent changes in GABA-mediated transmission described here may play a role in synaptic plasticity (LTP and kindling) as well as in the propagation of seizures. (Supported by the MRC of Canada and FCAR)

388.16

Waves of synchronous neuronal activity in neonatal rat cortical slices A. Peinado* Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, NY, 10461.

We have previously demonstrated that injections of the gap junction tracer Neurobiotin into single neurons in developing neocortical slices results in labeling of upto 80 neurons in the immediate vicinity of the injected cell (Peinado et al., 1993). The pattern of labeling suggested that neurons are coupled in a syncytium that spans large areas of cortex. To test this I have analyzed spatiotemporal patterns of neuronal activity under conditions that favor spread of electrical activity via gap junctions.

Transverse slices of neocortex were made from neonatal and early postnatal rat pups (P0-P7) and stained with Fura-2 AM (Yuste and Katz, 1991). Neuronal activity, as reflected by changes in Fura-2 fluorescence, was monitored at a single excitation wavelength (380 nm) with a fast cooled integrating CCD camera. Imaged area was 350μm x 350 μm.

As early as P0, addition of K^+ channel blockers (TEA or barium) to the aCSF to enhance the flow of currents through gap junctions induced the appearance of synchronized neuronal activity occuring as waves that travel along the horizontal axis of the cortex at 500-1000 μm/sec. Waves were abolished by TTX (1μM) but not by the broad spectrum glutamate antagonist kynurenic acid (2mM) or by combined application of NMDA and non-NMDA antagonists (APV, 100μM and CNQX, 50μM). Wave duration and frequency are 5-10 sec. and 0.02-0.1 Hz, respectively. Not all cortical areas support wave-like synchronous activity.

These results are consistent with the idea that gap junctions between neurons in developing neocortex link neurons into a syncytium capable of synchronizing large numbers of neurons under conditions that reduce potassium channel activity. Enhanced gap junctional communication may be important in synchronizing activity during seizures in early development.

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388.17

INTRINSIC FIRING PATTERNS OF RAT HIPPOCAMPAL PYRAMIDAL CELLS MODULATED BY EXTRACELLULAR OSMOLALITY. R. Azouz, G. Alroy & Y. Yaari*, Dept. of Physiology, The Hebrew University Medical School, Jerusalem, Israel.

Pyramidal cells (PCs) in the hippocampus display a gradient of burst characteristics, which ranges from regular firing to spontaneous bursting (Jensen et al., *J. Neurophysiol.* 71, 831-839, 1994). Intracellular recordings in rat hippocampal slices were used to examine the modulation of these firing patterns in CA1 PCs by extracellular osmolality.

Exposing PCs to hyposmolal saline (260 mOsmol/Kg) had no effect on passive membrane properties, but the spike afterdepolarization was increased. Most regular spiking cells were converted to bursters by hyposmolal saline. Coversely, subjecting PCs to hyperosmolal saline (340 mOsmol) decreased fast spike afterhyperpolarization and afterdepolarization. Most burst-firing PCs were converted into regular spiking cells.

Burst firing persisted in normosmolal saline in which Ca^{2+} currents were suppressed by equimolar replacement of Ca^{2+} with Mn^{2+}. Hyperosmolar saline converted burst firing cells into regular spiking cells also in this condition, indicating that modulation of Ca^{2+} currents and Ca^{2+}-gated K^+ currents is not involved in this action.

Blocking most K^+ currents with TEA and Ca^{2+}-free Mn^{2+} saline induced Na^+-dependent plateau afterdepolarizing potentials. Hyposmolality prolonged the duration of the plateau potential, whereas hyperosmolal saline reduced it.

We conclude that the firing pattern of CA1 PCs are strongly modulated by extracellular osmolality. The osmotic effects may be mediated by alterations of the ratio of persistent Na^+ to K^+ currents.

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388.18

EFFECTS OF REDOX MANIPULATIONS ON LOW MAGNESIUM-INDUCED EPILEPTIFORM ACTIVITY IN IMMATURE RAT HIPPOCAMPAL SLICES. C. Wang* and F. E. Jensen, Dept. Neurol. Children's Hosp. Boston, MA 02115.

Recent studies have demonstrated that sulfhydryl residues in NMDA receptor may function as redox modulatory sites and redox manipulations of these sites can regulate NMDA-induced currents. In this study, we tested the effects of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and sodium nitroprusside (SNP), which are shown to oxidize NMDA receptor redox sites, on low magnesium-induced ictal activity in hippocampal slices from immature Long Evans rat pups (10-15 day old). Extracellular field potentials were recorded in area CA1. Bath application of the oxidizing agent DTNB (1 mM) completely blocked spontaneous ictal discharges in 9/14 slices ($p = 0.0003$) within one hr perfusion period. The effect of DTNB persisted after 30 min washout. Application of the reducing agent dithiothreitol (DTT, 1 mM) or tris-(2-carboxyethyl)phosphine (TCEP, 1 mM) reversed the effect of DTNB in 5/5 and 3/4 slices respectively. Bath application of SNP, (50 μM) inhibited ictal activity in all tested slices ($n = 8$, $p < 0.0001$) within 30 min. The effect of SNP persisted after 30 min washout. DTT (1 mM) reversed the effect of SNP in 7/8 slices. Interictal activity was seen when ictal activity was inhibited by DTNB or SNP. These results indicate that redox agents can modify epileptiform activity, possibly via an action on the NMDA receptor redox site. Redox agents may have potential in the treatment of epilepsy. (Supported by NS31718 and NS07264)

388.19

ROLE OF NONSYNAPTIC MECHANISMS IN THE SPREAD OF EPILEPTIFORM ACTIVITY IN AREA CA1 OF THE HIPPOCAMPUS. S.R. Sinha* and P. Saggau, Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

In vitro studies have shown that epileptiform activity can occur even when synaptic transmission is blocked. Thus, in addition to synaptic spread, several nonsynaptic mechanisms, including electrotonic coupling, changes in ionic concentrations and ephaptic coupling, have been postulated. We are investigating if such nonsynaptic mechanisms play a role in the spread of epileptiform activity in an *in vitro* epilepsy model where synaptic transmission remains intact: application of the K^+-channel blocker 4-aminopyridine to guinea pig transverse hippocampal slices.

We employed a technique using two optical indicators for simultaneously recording transients of membrane potential (V_m) and calcium ($[Ca^{2+}]_i$) with high spatio-temporal resolution (Sinha, et al., *J. Neurosci. Methods*, in press). The optical indicators were bath applied; therefore, both signals recorded at each location are the weighted average of pre- and postsynaptic structures. We observed that the delay between the voltage and Ca transients increased in the direction of propagation. The Ca transient consists of two temporally distinct components, presynaptic followed by the postsynaptic transient. We hypothesized that the increase in delay reflected a decreased contribution from the presynaptic component, and the occurrence of a postsynaptic Ca transient without a presynaptic transient is indicative of nonsynaptic spread. To test this hypothesis we investigated the effect of changing the osmolarity of the bathing medium on this delay during evoked epileptiform activity. A hypo-osmolar solution (- 40 mOsm) increased the delay compared to control conditions in the same slice; hypo-osmolarity shrinks the extracellular space and thus enhances the effects of changes in extracellular concentrations and of ephaptic coupling. Accordingly, a hyper-osmolar solution (+ 40 mOsm) should reduce these effects and indeed caused a slight decrease in the delay. Thus, even when synaptic transmission is intact, nonsynaptic mechanisms play a role in the spread of epileptiform activity in area CA1 of the guinea pig hippocampus.

EPILEPSY: BASIC MECHANISMS III

389.1

ABSENCE OF GRANULE CELLS DOES NOT PREVENT KAINIC ACID INDUCED PYRAMIDAL CELL DEATH IN THE CA3 AREA OF THE ADULT RAT HIPPOCAMPUS. Zs. Horváth*, G. Czéh, B. Czéh and L. Seress. Department of Physiology, University Medical School Pécs, 7643-Pécs, Hungary.

Newborn Wistar rats were X-ray irradiated. Two and three month after irradiation kainic acid were injected into the cerebral ventricles or directly into the Ammon's horn, using stereotaxic conditions. Other animals received colchicine injection into the remainding dentate gyrus and two weeks later they were also injected with kainic acid. The effect of kainic acid was also examined in slice preparations.

X-ray irradiation destroyed 85% of granule cells of the dentate gyrus. A consecutive colchicine injection resulted in a complete loss of granule cells. Under both experimental conditions a single injection of kainic acid resulted in behavioral signs of seizures and loss of CA3 pyramidal cells both in Nissl and Timm stained sections. In the slice preparations from X-ray irradiated animals, perfusion of 500 nM kainic acid induced epileptiform discharges of the CA3 pyramidal cells.

In conclusion: Loss of presynaptic kainic acid receptors does not prevent kainic acid induced epileptic activity, and the consequent pyramidal cell loss in the adult rat hippocampus. This suggests that matured kainic acid receptors at the postsynaptic site are sufficient to mediate the typical overexcitation of pyramidal cells. Therefore, not the lack of mossy fibers, but immaturity of kainic acid receptors may explain the lack of kainic acid effect in the young (1-15 day old) rats.

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389.2

NPY RECEPTORS IN THE RAT HIPPOCAMPUS AFTER KAINIC ACID-INDUCED LIMBIC SEIZURES. C. Schwarzer, C. Röder, G. Sperk*, M. Gobbi#, T. Mennini# and A. Vezzani#. Dept. of Pharmacology, Univ. Innsbruck, A-6020 Innsbruck, Austria. #Istituto di Ricerche Farmacologiche "Mario Negri", I-20157 Milan, Italy.

Changes in peptide YY (PYY) receptor binding were investigated at various intervals after kainic acid (KA, i.p., 10-12 mg/kg) induced limbic seizures. Brain slices were incubated with ^{125}I-PYY (75 pM) in the presence and absence of [Leu^{31}][Pro^{34}]NPY (100nM), representing global and Y_2 specific binding, respectivly. PYY binding consisted mainly of Y_2 sites in the hilus of the dentate gyrus and in the hippocampus proper and of Y_1 sites in the molecular layer of the dentate gyrus. Six to 24 h after KA, global PYY binding and Y_2 specific binding were markedly enhanced in the strata radiatum and oriens CA3 (maximal increase of 185% and 175% of control respectively). Seven and 30 days after KA, a reduction of these sites by up to 63% was found. In the hilus of the dentate gyrus an increase of global PYY binding by up to 400% was observed after 24 h which became attenuated to 125% above controls after 30 days. In the molecular layer of the dentate gyrus global PYY binding increased by up to 90% between 6 and 24 h after KA injection and was reduced by 37% after 30 days. These late reductions in receptor binding were prevented by injecting phenobarbital (60 mg/kg) 3 h after KA.

The early general increase in NPY receptors suggests a facilitation of NPY-mediated neurotransmission. The subsequent decrease in NPY binding may be related to seizure-induced neuronal damage and/or to increased NPY release previously demonstrated. The lasting increase in Y_2 specific receptors in the dentate hilus is consistent with their proposed localization on mossy fibers. Augmented transmission through these receptors may supress glutamate release from mossy fibers and thus may ameliorate seizure activity.

389.3

NEUROPROTECTIVE EFFECT OF BASIC FIBROBLAST GROWTH FACTOR ON SEIZURE-INDUCED BRAIN DAMAGE

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We have previously reported that chronic infusion of basic fibroblast growth factor (bFGF) during seizures reduced seizure-induced cell loss in the hippocampus. In this study, we further examined the effect of such treatment on seizure-induced long-term behavioral deficits.

Prolonged seizures were induced by i.p. injection of kainic acid (KA) in 35 day-old rats. bFGF or phosphate buffered saline (PBS) was chronically infused into the lateral ventricle 2 days before and 5 days after the induction of seizures. Treatment with bFGF or PBS did not modify the latency and duration of the acute seizures. However, rats pretreated with bFGF had significantly less behavioral deficits than rats pretreated with PBS. These results suggest that bFGF is neuroprotective against seizure-induced brain damage.

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389.4

IBOTENIC ACID-INDUCED LESIONS OF THE PONTINE RETICULAR FORMATION ATTENUATE MAXIMAL ELECTROSHOCK SEIZURES IN RATS.

M.L. Maring-Smith*, R.W. Clough[1] and R.A. Browning. Depts. of Physiology and Anatomy[1], So. Ill. Univ. Sch. of Med., Carbondale, IL 62901.

Large bilateral mechanical lesions of the pontine tegmentum involving the superior cerebellar peduncles (PCS) and the nucleus reticularis pontis oralis (RPO nucleus) are more effective in attenuating the tonic components of maximal electroshock (MES) seizures than discrete electrolytic lesions, which damage only the PCS or the RPO. Further, reversible inactivation of the RPO, via microinfusion of lidocaine HCl, attenuates MES seizures in a dose- and volume-dependent manner. As electrolytic lesions and mechanical lesions do affect, and lidocaine microinfusion may affect cell bodies and fibers of passage, the effect of ibotenic acid (IBO)-induced lesions of the RPO on MES seizures was examined. IBO, an excitatory amino acid agonist, has been reported to damage cell bodies, while sparing fibers of passage. Additionally, in order to investigate whether the total volume of pontine tegmentum damaged influences the degree of seizure attenuation, the effects of bilateral lesions produced by 0.25 or 0.5 µl of IBO were examined. Sprague-Dawley rats received MES seizure pretests (150 mA, 0.2 sec, 60 Hz, via corneal electrodes). Under anesthesia, bilateral injections into the RPO (AP 6.5 mm posterior to bregma , ± 1.5 mm lateral from the midline, 7.5 mm ventral to dura) of 0.25 or 0.5 µl of IBO (10 µg/µl) or saline, were made at a rate of 0.25 µl/min. MES testing was carried out 4, 7, 14, 21, and 28 days later. Seizure attenuation was found to correlate with the extent of damage produced. The largest lesions reduced tonic seizure severity in up to 100% of animals. However, histological examination suggested that fibers of passage within the lesions may have sustained damage. In summary, IBO-induced lesions of the RPO attenuated the tonic components of MES seizures. Whether the effect is due to intrinsic neuron death or damage to fibers of passage remains to be determined.

389.5

NGF REDUCES DNA FRAGMENTATION IN CA1 NEURONS BUT NOT ELSEWHERE AFTER SYSTEMIC KAINATE-INDUCED SEIZURES IN THE RAT.

S. W. Weiss, O. Cataltepe and A.J. Cole*. Epilepsy Research Lab, Neurology Service, Massachusetts General Hospital, and Harvard Medical School, Boston, MA, 02114.

In previous studies we have used in situ nick translation histochemistry to characterize the magnitude and distribution of DNA fragmentation in rat brain after limbic seizures induced by systemic kainate. This assay offers single cell resolution and results may be easily quantified, especially in anatomically compact structures such as hippocampus. NGF is neuroprotective in models of cerebral ischemia which may induce excitotoxic cell death. We therefore examined the effect of intraventricular infusions of NGF on the occurrence of DNA fragmentation after systemic kainate. Male SD rats (200 gm) were implanted with osmotic pumps containing NGF or 1% BSA in ACSF. NGF was infused into the lateral ventricle at a rate of 1 µg/day for 6 days. On day 3, all animals were treated with systemic kainate (15 mg/kg) and severity of resultant seizures was graded. 72 hours later animals were sacrificed and brains were processed for in situ nick translation histochemistry. Cryostat sections (11 µm) were incubated with DNA polymerase I in the presence of biotinylated dUTP, and incorporation was visualized with avidin-biotin-peroxidase histochemistry. Sections were examined by an observer blinded to treatment and staining severity was assessed. Cell counts in hippocampus were performed. Animals were stratified according to seizure severity grade. At every grade, we found significant reductions in nick translation positive cells in NGF treated animals in CA1 (paired t-test $p < .0001$ in preliminary analysis) but not in other structures. We conclude that NGF protects CA1 neurons from kainate induced DNA fragmentation. While NGF may prevent apoptosis during neuronal development, the mechanism of NGF's neuroprotective action in this model remains uncertain.

389.6

EFFECTS OF PENTOBARBITAL, SCOPOLAMINE AND MK-801 ON PILOCARPINE-INDUCED SEIZURE AND BRAIN DAMAGE.

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Intraperitoneal pilocarpine (500 mg/kg) injection induced tonic and clonic seizures for 10-20 min and electrical seizure activities persisted after the behavioral seizures in rats. Pilocarpine-induced behavioral seizure was monitored by recording the vibration and electrical activity was measured from hippocampal electrodes. The behavioral seizure was significantly suppressed by the order of pentobarbital (5 mg/kg i.p.), scopolamine (10 mg/kg i.p.) and MK-801 (0.5 mg/kg i.p.). The electrical seizure was suppressed by pentobarbital, but was enhanced by MK-801. In scopolamine-treated group the electrical seizure was suppressed but there were regular bursts. We observed pyramidal layer of CA1 and CA3 area and granule cell layer to examine the degree of the hippocampal damage. Pentobarbital, scopolamine and MK-801 protected the pilocarpine-induced hippocampal damage. These results suggest that the pilocarpine-induced brain damage may be a consequence of NMDA receptor activation which may not be related to electrical seizure and behavioral seizure.

389.7

MUSCIMOL INJECTIONS IN THE ZONA INCERTA EXACERBATE PILOCARPINE-INDUCED SEIZURES.

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The Zona Incerta (ZI), a subthalamic structure, has been proposed to play an important role in motor control mechanisms in animals and humans. Connections of the ZI to the substantia nigra (SN), entopeduncular nucleus have been demonstrated in rats. Furthermore manipulation of excitatory neurotransmission within the SN pars reticulata or entopedunular nucleus modulates the threshold for pilocarpine-induced seizures. The ZI appears to give rise to a major cortical projecting system and to receive inputs from the cortex, dorsal column nuclei, trigeminal nuclear complex and some portions of the superior culliculus. The ZI neurons may collateralize to provide GABAergic inputs to both neocortex and brain stem structures. Due to all the above mentioned structural connections it is supposed to provide an important link between ascending sensory and motor systems. Male Wistar rats were stereotaxically implanted with guide cannulae into ZI bilaterally under choral hydrate anesthesia. After a three to five days postoperative period, rats were injected with (0,5 µL/side, over a period of 2 min) saline or muscimol (100 nmoL/side) into the ZI. After another 30 min period animals were injected with Pilocarpine (PILO), either 350 mg/kg or 250 mg/Kg, i.p.. At the end of the experiments animals were deeply anesthetized, injected with 0.2 µL of a 2% Evans Blue solution via the guide cannulae, perfused with 10% formalin, and had their brains processed for histological analysis. Injection of Muscimol in the ZI reduced in 60% the latency for status epilepticus after 350 mg/Kg of Pilo. Under 250 mg/Kg of Pilo every muscimol injected rat developed SE, compared to only 40% of the control animals. Our results suggest an important role for the ZI in modulating the limbic seizures induced by PILO.

Research supported by CNPq, CAPES, FAPESP and FINEP (Brazil).

389.8

ULTRASTRUCTURAL ANALYSIS OF THE HIPPOCAMPUS FOLLOWING KAINIC ACID ADMINISTRATION IN THE RAT: EFFECTS OF AGE.

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The immature brain appears to be resistant to the neurotoxic effects of kanic acid (KA) administration and subsequent seizure activity. Light microscopic analysis of the adult brain demonstrates neural degeneration, cell loss and mossy fiber sprouting in the CA3/CA4 areas of the hippocampus following systemic KA administration. In the young animal however, these effects have not been observed

In order to determine whether previously undetected ultrastructural changes occur in the hippocampus of the young animal, we injected 14-day rat pups with a single dose of KA (5mg/kg) or vehicle. Four days later the animals were sacrificed and their brains processed for electron microscopy. The CA3/4 regions were dissected and analyzed at the ultratructural level. Preliminary data indicate that there are alterations in CA3/4 pyramidal cells in the KA treated animal. Small cytoplasmic vacuoles are found and irregular cell limits. The neuropil surrounding the cells is also changed, with slight enlargement of neuronal processes The number and characteristics of synapses appear not to be affected. Subsequent studies will determine whether these changes are the acute response to tissue insult.

389.9

ULTRASTRUCTURAL CHANGES IN THE PILOCARPINE MODEL OF CHRONIC EPILEPTIC SEIZURES. R.V.R. Duran*, R.L. Smith, E.F. Haapalainen and L.E.A.M. Mello, Depto. de Fisiologia e Depto de Morfologia, Escola Paulista de Medicina, UNIFESP, São Paulo, 04023-900 Brazil.

Assessment of the morphological changes in the epileptic condition has helped in the understanding of brain circuitry and its plasticity. Chronic spontaneous recurrent seizures were obtained by inducing *status epilepticus* (SE) in adult, male, EPM-1 Wistar rats with pilocarpine (320 mg/Kg, i.p.). More than 9 (nine) months after SE, the animals were perfused and fixed with paraformaldehyde 2% and glutaraldehyde 2.5% under anesthesia. The hippocampal subfields were dissected under light microscopy and processed for electron microscopy. Qualitative analysis of the different hippocampal layers (e.g. oriens, radiatum, etc.) revealed that changes in the neuropil were specific for each different layer. Findings included: enlarged and discontinuous (perforated) synapses; normal mitochondria (not swollen) but with asymmetrical aspects of the inner membrane; aberrant extracellular collagen deposited in the neuropil, next to pericytes; regions of the neuropil, surrounded by glial cell membranes, without necrotic features but with apoptotic motifs. Evidence of apoptosis, plastic changes of membranes (synapses and mitochondria) and collagen synthesis 9 months after the induction of SE, suggest that these events may be related to the spontaneous seizure episodes occurring in these animals. Furthermore, it suggests that epilepsy is an ongoing plastic process.

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389.10

SUPPRESSION OF PILOCARPINE-INDUCED EPILEPTIFORM ACTIVITY IN THE CA3 REGION OF RAT HIPPOCAMPUS. E.J. Hadar, Y. Yang and P.A. Rutecki*. Depts. of Neurosurgery and Neurology, Univ. of Wisconsin Medical School, Wm. S. Middleton Memorial V.A. Hospital, Madison, WI 53792

The CA3 subfield of rat hippocampal slices bathed in 7.5 mM $[K^+]_o$ & 10μM pilocarpine (pilo) displays ictal-like epileptiform activity (bursting at >2 Hz. for periods >3 sec.). Modeling studies suggest that dendritic Ca^{2+} oscillations and NMDA receptor activation lead to afterdischarges (Traub *et.al.* J.Physiol. 461:525, 1993). We tested if blockade of NMDA receptors or calcium channels altered pilo-induced ictal-like activity.

Rat hippocampal slices were incubated in 7.5 mM $[K^+]_o$ & 10 μM pilo to induce ictal-like activity. Extracellular recordings were made and the duration of ictal-like discharges and the period between them were compared before and after the addition of specific pharmacologic agents.

The NMDA antagonist DL-APV(100 μM) had no effect on ictal-like discharges. Dantrolene (100 μM), which blocks release of Ca^{2+} from intracellular stores, stopped ictal-like activity in 55% of slices. Nifedipine (1 μM), a L-type Ca^{2+} channel blocker, eliminated the ictal-like pattern in 80% of slices. N^6-Cyclopentyladenosine (CPA, 100 nM), a specific agonist of the A_1 adenosine receptor, blocked ictal-like activity in 75% of slices. Baclofen (1 μM), a $GABA_B$ receptor agonist also stopped ictal discharges in >80% of slices.

We conclude that NMDA activation is not required for generation of the ictal-like activity produced by pilocarpine. This pattern appears to be mediated in part by activation of L-type Ca^{2+} channels and can be blocked by adenosine A_1 and $GABA_B$ receptor activation. Supported by the NIH and VA research.

389.11

DIFFERENTIAL ALTERATIONS OF HIPPOCAMPAL LEVELS OF [MET5]-ENKEPHALIN AND SOMATOSTATIN AFTER KAINIC ACID TREATMENT. K. H. Zhang, X. P. Wu, J. Wang, W. Q. Zhang* and J. S. Hong,+ Dalian Medical University, Dalian, China. +NIEHS/NIH, Research Triangle Park, NC 27709.

Several studies have shown that intrahippocampal injection of enkephalins enhance hippocampal excitability and may produce both EEG and behavioral seizures. In contrast, somatostatin has been reported to have an inhibitory action on the pyramidal neurons of the hippocampus. To further study the roles of enkephalins and somatostatin in the excitability of the hippocampus, we determined the changes of these two neuropeptides after kainic acid (KA) treatment. A single injection of KA (10 mg/kg, s.c.) produced limbic seizures lasting 4-6 hours in rats. Two days after KA injection, hippocampal level of somatostatin were significantly reduced. Immunocytochemistry revealed a large loss of somatostatin-positive neurons in the hilar region. Similar to previous reports, KA treatment greatly enhanced the immunoreactivity of [Met5]- enkephalin in both the mossy fiber and perforant path terminals of the hippocampus. In addition, [Met5]-enkephalin-immunoreactive neurons were observed in the ventral hippocampus. In situ hybridization revealed an elevation of proenkephalin mRNA in both the hippocampus and entorhinal cortex of the convulsed rats. The differential changes of somatostatin and enkephalins after KA treatment may be responsible for the enhanced seizure susceptibility which is often observed in animals with a prior history of seizures.

389.12

PILOCARPINE-INDUCED SEIZURES CAUSE CHANGES IN MU AND DELTA OPIOID RECEPTOR CIRCUITRY IN THE RAT DENTATE GYRUS S.B. Bausch*, T.M. Estep and C. Chavkin Department of Pharmacology, University of Washington, Seattle, WA 98195-7280

The pilocarpine model of temporal lobe epilepsy described by Turski et al. (1983) was used to study changes in dentate gyrus circuitry induced by recurrent seizures. As previously reported by other investigators, pilocarpine-treated rats developed recurrent seizures and showed a loss of hilar neurons and mossy fiber sprouting. Mu opioid receptor immunoreactivity (-IR) was not significantly changed in the granule cell layer but was decreased by approximately 60% in the hilus. In contrast, delta opioid receptor-IR was decreased by 30-40% in both the granule cell layer and hilus. By 14 days an increase in diffuse mu opioid receptor-IR and GABA-IR was evident in the inner molecular layer of animals that had pilocarpine-induced seizures. No change in delta opioid receptor-IR was observed in the molecular layer. These data suggest sprouting of mu opioid receptor-positive GABAergic fibers.

The sensitivity to opioids was also affected by pilocarpine-induced seizures. Application of the mu opioid receptor agonist, DAMGO, caused a 40% increase in primary population spike excitability in control animals. In animals that exhibited seizures, this response to DAMGO was decreased to approximately 6% at 5-13 days post-injection of pilocarpine then recovered to control levels by 2-6 weeks post-injection. A secondary spike was generated in response to DAMGO at all time points. Application of the delta agonist, DPDPE, caused an increase in primary population spike excitability in control animals but decreased excitability in rats that had pilocarpine-induced seizures. Preliminary data showed that in the presence of bicuculline, DPDPE caused a decrease in population spike excitability in both control and pilocarpine-treated animals. These data suggest that there is a loss of a subpopulation of mu and delta opioid receptors followed by compensatory changes for mu but not delta opioid receptor circuitry. Supported by DA04123 and DA07278.

389.13

DECREASED MAGNESIUM AFFINITY OF NMDA RECEPTOR-CHANNELS UNDERLIES EPILEPTIFORM ACTIVITY IN THE KAINIC ACID-LESIONED RAT HIPPOCAMPUS. Y. Chen, J.E. Chad & H.V. Wheal* Dept. of Physiology & Pharmacology, University of Southampton, Southampton, SO16 7PX, U.K.

An enhanced NMDA component in synaptic transmission has been shown in various animal models of epilepsy, such as the kainic acid-lesioned model and the kindling model, as well as in human epileptic tissue. The reduced sensitivity of the NMDA-mediated response to Mg^{2+} has also been demonstrated (Turner & Wheal, 1991, *J. Neurosci.* **11** 2786-2794). We therefore set out to investigate the role of NMDA receptor-channels and their voltage-dependent block by Mg^{2+} in the kainic acid model of epilepsy (Chen et al., 1994, *Soc. Neurosci. Abstr.* **20**:407).

Unilateral kainic acid-lesion was carried out on adult Wistar rats (180g) by injecting kainic acid (0.5μg in 0.5μl, phosphate buffer, pH 7.4) into the left ventricle. The rats were left to recover for one or four weeks. In our recent experiments, using whole-cell patch clamp recordings from CA1 cells in both lesioned and control slices (400μm), we have demonstrated that the voltage-dependence of the NMDA receptor-channel has been altered. The calculated apparent dissociation constant of the Mg^{2+} block (K_{Mg0}) was 7.52mM for the lesioned ones (n=4), which was 4.3 times that of the control (1.76mM, n=6). The reduced voltage dependence was brought back towards the control level in the presence of higher $[Mg^{2+}]_o$ (2.6mM instead of the normal 1mM). The same concentration of Mg^{2+} was also shown to diminish the bursting activity recorded as population spikes in the CA1 area. The effect of 2.6mM $[Mg^{2+}]$ for 10min was equivalent to that of 10μM D-APV for the same time period, suggesting (1) that a large portion of the bursting activity is NMDA-mediated, and (2) the sensitivity of the NMDA component to Mg^{2+} ions.

Our results support the essential role of NMDA receptors in maintaining the epileptiform activity. The long-term (one to four weeks) reduction of the Mg^{2+} block of NMDA receptor-channels induced by the lesion process is instrumental in this role of NMDA receptors.

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389.14

LOSS OF NADPH DIAPHORASE-POSITIVE NEURONS IN THE DORSAL HIPPOCAMPAL FORMATION OF PILOCARPINE CHRONIC EPILEPTIC RATS. C. Hamani[1], L. E. A. M. Mello*[1], F. T. A.Costa [2], R. Mendez-Otero[2] Depto. de Fisiologia, Escola Paulista de Medicina UNIFESP, 04023-900 São Paulo, Brazil[1]; Instituto de Biofísica, UFRJ, 21949-900 Rio de Janeiro, Brazil[2]

Nitric oxide (NO) is a novel neuronal retrograde messenger proposed to play important roles in various CNS functions and neuronal plasticity. In the kainic acid model of epilepsy, N-nitro-L-arginine, a nitric oxide synthase inhibitor, was suggested to enhance the severity of the seizures through supression of the NO synthase activity in the vascular endothelium. In our study, adult male Wistar were injected with pilocarpine (PILO) (350 mg/kg i.p.) developing status epilepticus (SE), and following a period of approximately two weeks spontaneous recurrent seizures could be behaviorally observed. Six to nine months after induction of SE, they were sacrificed, perfused with a mixture of paraformaldehyde 0.5% and glutaraldehyde 2%, and their brains were processed for direct NADPH histochemistry. Quantitative counts of NADPH-positive neurons were made with a reticulate grid under optical microscopy for each of the subfields of the dorsal hippocampus. Compared to an age-matched control group, the PILO injected rats showed a significant decrease in NADPH-stained neurons in the hilus, superior blade of the polymorphic layer of the dentate gyrus, stratum pyramidale and radiatum in the CA3, and stratum oriens, pyramidale and radiatum in CA2 and CA1 of the dorsal hippocampal formation. Overall the reduction in NADPH-stained neurons in the epileptic animals was of 50%. No change between the two groups was seen for stratum granulosum and moleculare of the dentate gyrus, the inferior blade of the polymorphic layer of the dentate gyrus, nor for the CA3 oriens. Considering the fact that NADPH-positive neurons in the hippocampal formation are mostly GABAergic, the loss of these neurons may be associated to the epileptic condition of these animals.

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389.15

REGIONAL DISTRIBUTION AND TIME-COURSE OF CALPAIN ACTIVATION FOLLOWING KAINATE-INDUCED SEIZURE ACTIVITY IN RAT BRAIN. X. Bi*, V. Chang, G. Tocco and M. Baudry, Neurosci. Prog.,USC, Los Angeles, CA 90089

Systemic injection of kainic acid (KA) in adult rat elicits epileptic seizure activity and a pattern of neuronal pathology which exhibits several features of human temporal lobe epilepsy. KA-induced seizure activity is accompanied by the activation of calpain in limbic structures. In the present study, we evaluated the spatio-temporal activation of calpain after the onset of seizure activity by immunohistochemistry using an antibody for the selective breakdown product of spectrin (sbdp) generated by calpain-mediated proteolysis of spectrin. In addition, we compared the spatio-temporal pattern of changes in SBDP immunoreactivity with that of changes in immunoreactivity to some subunits of AMPA receptors. One hour after seizure onset, sbdp accumulation was observed in selected interneurons in stratum oriens and in the hilus of dentate gyrus. By 4 hrs after seizure onset, sbdp immunoreactivity was prominent in dendritic fields of the hippocampus as well as in neurons in thalamus and piriform cortex. By 8 hrs, sbdp immunoreactivity had disappeared from interneurons but was localized in pyramidal cell bodies in hippocampus. Intense labeling of cell bodies and dendritic fields persisted until 5 days following KA treatment. Changes in GluR subunit immunoreactivity were mirror images of those seen for sbdp. In general, increased sbdp immunoreactivity in dendritic fields was associated with decreased GluR1 immunoreactivity. However, increased sbdp immunoreactivity in neuronal perikarya was also associated with increased GluR1 immunoreactivity. These results indicate that calpain activation following seizure onset exhibits a very specific spatio-temporal pattern, with activation in restricted interneurons preceding widespread activation in pyramidal neurons. Calpain activation also precedes clear signs of neuronal pathology and could thus represent an initial trigger for neuronal pathology. Finally, the results clearly demonstrate that calpain activation results in rapid alterations in GluR subunit properties which could be involved in the development of hyperexcitability observed following seizure activity.

389.16

INTRACELLULAR CORRELATES OF SYNCHRONOUS EXCITATORY AND INHIBITORY ACTIVITY INDUCED BY CARBACHOL IN ENTORHINAL CORTEX LAYER II. **R. Klink*, C. T. Dickson and A. Alonso, Dept. Neurology & Neurosurgery, M.N.I. and McGill University, Montreal, PQ, Canada, H3A-2B4**

As shown in an accompanying comunication (Dickson and Alonso, *Soc. Neurosci.* 1995), synchronized field oscillations resembling ictal and/or inter-ictal events can be generated by bath application of the cholinomimetic carbachol (CCh:10-30μM) independantly in layer II and layer IV of the entorhinal cortex (EC). In the present study we examined the cellular and synaptic mechanisms underlying CCh-induced population oscillations in neurons from EC layer II by means of intracellular recordings in slices. We focused on layer II since it is the main origin of the perforant path and it does not contain intrinsically bursting cells; it is comprised instead of two electrophysiologically and morphologically distinct types of projection neurons: stellate cells and pyramidal-like cells (Alonso and Klink, *J. Neurophysiol 70.* 128 1993). CCh induced a sustained depolarization in both types of neurons which led to tonic firing in the pyramids and to the manifestation of the typical intrinsic subthreshold oscillations and "cluster" discharge of the stellates. Following the depolarizing responses, field oscillations (ictal-like events) developed. These were correlated with synchronous *epsp*-driven oscillatory bursting discharge in pyramids, stellates, and interneurons. Block of glutaminergic transmission with CNQX and AP-5 abolished the population oscillations and the intracellular *epsps*. However, in these conditions, "giant" *ipsps*, comprised of a fast and a slow component, were observed in the projection cells. These events were often, but not necessarily, correlated with small amplitude field events and could show periodicities of ~ 0.2Hz. Both the *ipsp*, and the field potentials were blocked by bicuculline or picrotoxin. These data suggest that CCh can induce the synchronization of GABAergic EC interneurons in the absence of excitatory amino acid transmission.

389.17

ELEVATION OF $[K^+]_o$ REVEALS ABNORMAL ACTIVITY IN THE DENTATE GYRUS OF SLICES FROM EPILEPTIC KAINATE-TREATED RATS P.R. Patrylo* and F.E. Dudek Department of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523

The kainate-treated rat is an animal model of temporal-lobe epilepsy. Rats were given either multiple kainate (IP; 5 mg/kg per hr) or saline (150 mM NaCl) injections for 5-8 hr. Approximately 1-4 months post-treatment, most kainate-treated rats started having spontaneous convulsive seizures. In normal ACSF (i.e., 1.3 mM Ca^{2+}; 3 mM K^+) most slices prepared from kainate-treated rats displayed multiple population spikes to perforant path stimulation. Hilar stimulation resulted in abnormal responses, ranging from multiple population spikes to prolonged negative field-potential shifts, in approximately 15% of slices. When $[K^+]_o$ was elevated to 6 mM, however, most slices showed prolonged negative field-potential shifts with a corresponding increase in multiple-unit activity to hilar stimulation. In control slices, hilar stimulation did not elicit prolonged negative field-potential shifts. Raising $[K^+]_o$ to 9 mM resulted in spontaneous bursts, ranging in duration from 200 ms to 20s, in some slices from the kainate-treated rats. Timm's staining revealed mossy fiber sprouting in all slices from kainate-treated rats. These results suggest that physiological changes (i.e., elevated $[K^+]_o$) can unmask new recurrent excitatory circuits in the dentate gyrus from epileptic kainate-treated rats.

Supported by NIH grant NS16683

389.18

HYPEREXCITABILITY IN THE BASOLATERAL AMYGDALA OF KAINATE-TREATED RATS. B.N. Smith* and F.E. Dudek Dept. of Anat. and Neurobiol., Colorado State Univ., Fort Collins, CO 80523.

The kainate-treated rat has been used as a model of temporal lobe epilepsy. We used a modified kainate injection protocol (see Patrylo and Dudek, this meeting) that induced spontaneously recurring seizures for over one year post-injection. The hypothesis of the present study is that neuronal excitability in the basolateral amygdala (BLA) is altered after kainate treatment. Using extracellular field potential and intracellular recordings in horizontal slices, the excitability of the BLA of kainate-treated rats was compared with that of age-matched saline-treated animals.

Resting membrane potential, input resistance, and spike amplitude were not different between cells from control and kainate-treated rats. Maximal orthodromic stimulation resulted in a single population spike in the BLA of control slices and 3 to 10 spikes (5 ± 2; mean ± SD; $p<0.001$) in slices from kainate-treated rats. In the presence of 10-30 μM bicuculline, spike number was increased to 6 ± 1 in control and 8 ± 2 in kainate-treated animals ($p>0.05$). In nominally Mg^{2+}-free perfusion medium, control responses were only slightly modified, but population spike number was increased by more than 200% in slices from kainate-treated animals. In Mg^{2+}-free solutions containing bicuculline, spontaneous ictal-like bursts and negative DC shifts were seen in most slices tested from control and treated animals. The frequency of these events, as well as spike frequency within the bursts, was greater in the BLA of kainate-treated animals.

Therefore, kainate treatment leads to increased excitability in the BLA, which may involve both decreased inhibition and increased excitation of neurons in the BLA. Supported by postdoctoral fellowship NS09289 (BNS) and NIH grant NS16683 (FED).

389.19

EVIDENCE FROM GLUTAMATE MICROSTIMULATION FOR NEW LOCAL EXCITATORY CIRCUITS IN THE DENTATE GYRUS OF KAINATE-TREATED RATS

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Hippocampal mossy fiber reorganization occurs in patients with temporal lobe epilepsy and in animal models of epilepsy. Mossy fiber sprouting has been proposed to form excitatory connections between dentate granule cells, which could lead to hyperexcitability and contribute to the generation of seizures. To test this hypothesis, we used intracellular recordings, combined with microapplication of glutamate in the granule cell layer, to determine if local excitation of granule cells could evoke an increase in the size and/or frequency of excitatory postsynaptic potentials (EPSPs) in neighboring granule cells. Slices were prepared from kainate-treated animals (n = 5) that displayed spontaneous recurrent seizures. Timm's stain revealed mossy fiber sprouting in all the slices from all the animals. In the presence of bicuculline (10 μM) and high K^+ (6 mM), electrical stimulation of the hilar region (which antidromically actived mossy fibers), evoked bursts of population spikes, negative field-potential shifts and delayed bursts in the dentate gyrus in all slices tested (n = 24). In these conditions, glutamate microdrops (20 mM, 50-100 μm in diameter) applied in the granule cell layer near the recorded cells (50-700 μm) evoked bursts of EPSPs in 20 out of 27 cells tested. Glutamate microdrops, tested in 16 cells from 5 control rats, had no effect on the size and frequency of EPSPs. These results support the hypothesis that the reorganization of mossy fibers in rats treated with kainate forms excitatory connections between granule cells and leads to seizure-like activity.

Supported by NIH grant NS16683.

390.1

PSYCHOTROPIC DRUG TREATMENT AND CONCENTRATIONS OF AMYLOID PRECURSOR-LIKE PROTEIN IN VENTRICULAR CSF OF DEPRESSED PATIENTS P.T.Francis, N.A.Clarke, L.M.Brown, M-T.Webster, A.W.Procter and D.M.Bowen (SPON Brain Research Association) Institute of Neurology, Queen Square, London, WC1N 1PJ, U.K.

We have previously shown that intractably depressed patients' psychotropic drug treatment affects the concentration of amyloid precursor protein (APP)-like immunoreactivity (APPLIR) in ventricular CSF (VCSF, Clarke et al. 1993, Neurodegeneration 2, p243-248). Thus, those patients receiving treatment with lithium and antidepressants had significantly lower VCSF concentrations of APPLIR than those receiving neither. The antibody used for quantitation, 22C11, does not distinguish between APP and amyloid precursor-like protein 2 (APLP2). Using a recently developed monoclonal antibody to APLP2 (Webster et al. 1995, Biochemical Journal, in press) we have re-examined these samples using the same paradigm. There was no significant effect of antidepressants with anticholinergic side effects or lithium on the VCSF concentrations of any of the three major high molecular weight bands identified by western blotting with the APLP2 specific antibody (Kruskal-Wallis ANOVA, $P>0.05$). This is consistent with the previous study where an APP specific antibody, 10D5, showed a greater effect of treatment conditions than the 2-fold difference demonstrated with 22C11 labelling of N-termini of APP and APLP2. This data suggests that there may be some difference in the factors controlling secretion of APP and APLP2 in humans.

390.2

THROMBIN PROMOTES SECRETION OF AMYLOID PRECURSOR PROTEIN(APP) FROM HUMAN ENDOTHELIAL CELLS J.R. Ciallella* and J.P. McGillis, Dept. Microbiology and Immunology, and Sanders Brown Center on Aging, University of Kentucky College of Medicine, Lexington, KY 40536

Alzheimer's disease (AD) is a neurodegenerative brain disorder presenting hallmark pathological lesions called neuritic and cerebrovascular plaques. The core of these plaques is comprised of a 42-43 amino acid peptide known as Aβ. This peptide is cleaved from amyloid precursor protein (APP), which is ubiquitously expressed throughout the body. The normal or pathological function for APP is not known. In this study, human umbilical vein endothelial cells (HUVEC) were used to examine the effects of thrombin, a major coagulant, on APP expression. Thrombin has been localized to senile plaques and can process purified APP. Since thrombin induces secretion of growth and coagulant factors in HUVEC, it was examined whether APP is also secreted under these conditions. HUVEC were treated with thrombin for various time periods. Cell supernatants were collected, concentrated, and immunoblotted to measure secreted APP. Thrombin specifically induced secretion of APP which peaked at approximately thirty minutes post-treatment. The secretion was protein kinase C (PKC) dependent since it was diminished by an inhibitor of PKC. Moreover, thrombin induced a marked increase in intracellular calcium levels, which was partially inhibited by a secreted form of APP. These data suggest that thrombin may be involved in secretion and accumulation of APP leading to a protective effect and\or increased amyloidogenic species.

390.3

THE EFFECTS OF HEAVY METAL IONS ON METABOLISM OF ß-AMYLOID PRECURSOR PROTEIN AND ß-AMYLOID IN CULTURED SMOOTH MUSCLE CELLS. J. Frackowiak*, B. Mazur-Kolecka, R.T. Carroll[a], D.O. Espinoza, H.M. Wisniewski. NYS Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314, [a]Parke Davis, Ann Arbor MI 48105.

Formation of ß-amyloid is suggested to be the crucial pathological event in Alzheimer's disease (AD) dementia, however the cellular mechanisms of this process remain to be elucidated. Exposure to metal ions (e.g. aluminum) has been considered as one of the environmental risk factors in AD. Recently we have developed a cell culture model to study accumulation of ß-protein and formation of ß-amyloid using isolated canine vascular smooth muscle cells [Neurosci Lett 183 (1995) 120-123; Brain Res 676 (1995) 225-230]. We applied this culture model to study the influence of heavy metals, Al^{3+}, Co^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+}, Fe^{2+}, and Fe^{3+} on secretion and accumulation of ß-peptide and on the metabolism of ß-amyloid precursor protein (ßAPP) in human and canine smooth muscle cells. Exposure to iron cations altered maturation of ßAPP and induced accumulation of ß-protein, but not its secretion. The effect on accumulation of ß-protein was time- and dose-dependent. The treatment with ferric and ferrous ions also stimulated cell metabolic activity (in MTT reduction test), increased cell protein content and slightly stimulated cell proliferation. These data indicate that both ferric and ferrous ions may play a role in the induction and acceleration of the pathological mechanisms leading to ß-amyloidogenesis in AD.

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390.4

CHOLINESTERASE INHIBITORS ALTER APP SECRETION AND APP mRNA IN RAT CEREBRAL CORTEX. E. Giacobini, F. Mori, A. Buznikov and R. Becker[1]*. Depts. of Pharmacology and [1]Psychiatry, Southern Illinois Univ. Sch. Med., P.O. Box 19230, Springfield, IL 62794-9230 USA.

Three cholinesterase inhibitors (ChEI), both reversible and irreversible, were tested for their ability to enhance the release of nonamyloidogenic APP soluble derivatives (APPs): physostigmine (PHY), heptylphysostigmine (HEP) and 2,2-dichlorovinyldimethyl phosphate (DDVP), at concentrations producing cholinesterase (ChE) inhibition ranging from 5% to 95%. All three ChEI elevated APPs release significantly above control levels. Similar increase was observed after muscarinic receptor stimulation with bethanechol (BETHA). Level of total APP RNAs in rat cortical slices did not change after incubation with BETHA, DDVP and PHY. Activation of protein kinase C with phorbol 12-myristate 13-acetate (100 nM) increased the level of total APP mRNA by 50%. Physostigmine and metrifonate administration (0.3 and 80 mg/kg s.c., 3-48 hrs) did not significantly change the levels of APP 695 mRNA and APP-KPI mRNA. On the other hand, HEP administration (5 mg/kg s.c., 3-48 hrs) decreased the level of APP-KPI mRNA 35% in rat cerebral cortex. These findings suggest that administration of ChEI to Alzheimer disease patients may have a neuroprotective effect by activating normal APP processing.

390.5

AMYLOIDOGENIC PROCESSING OF ALZHEIMER'S AMYLOID RECURSOR PROTEIN BY THE PROTEOLYTIC ACTIVITY ASSOCIATED WITH ACETYLCHOLINESTERASE: ITS MODULATION BY METAL IONS AND TACRINE. Young Hae Chong[1,2]* and Yoo-Hun Suh[2]. [1]Institute of Genetic Engineering Center, Inje University, Pusan 614-735, Korea, [2]Department of Pharmacology, College of Medicine, & Department of Molecular Biology, Neuroscience Center, Seoul National University, Seoul 110-799, Korea.

We evaluated the possible role of AChE-AP in amyloidogenic processing of APP by the Western blot analysis using Aβ specific monoclonal antibodies. Prolonged incubation of a recombinant APP770 substrate with different AChE preparations produced several amyloidogenic fragments on the immunoblots. Protease inhibitory profiles on this processing confirmed the trypsin-like serine protease activity present in AChE preparation. This observed APP processing was significantly enhanced by Ca^{2+}, Mg^{2+}, or Mn^{2+} at 2 mM concentration, accumulating a relatively stable 18 kDa carboxy terminal fragment, which could be further degraded with higher AChE concentration. Furthermore, APP processing was shown to be modulated in concentration dependent manners by physiologically available metal ions such as Ca^{2+}, Zn^{2+} or Fe^{2+}/Fe^{3+}, Al^{3+} or a tacrine, centrally active cholinesterase inhibitor, with higher concentrations increasing accumulation of APP. These observations imply that AChE and its associated proteolytic activity may be involved in the generation of potential sources of amyloidogenic peptides under certain physiological conditions. Furthermore, our data provide the direct evidence supporting that locally disturbed homeostasis of essential metal ions or imbalance of protease and protease inhibitors may result in abnormal accumulation of APP and subsequent deregulated metabolism of APP, eventually leading to amyloid deposition in AD brain. In addition, this study raises the possbility that zinc or tacrine treatment of AD patients with high dosage or in the long term may have effects on the process of amyloidogensis.

390.6

THA: A NOVEL TOOL TO STUDY THE PROCESSING OF THE AMYLOID PRECURSOR PROTEIN. **C.M. Reever*, M.P. Vitek and D.D. Flynn. Dept. of Pharmacology, Univ. of Miami School of Medicine, Miami, FL 33101 and The Picower Institute for Medical Research, Manhasset, NY 11030.**

Tacrine (THA) is one of very few drugs approved for the treatment of mild to moderate Alzheimer's disease (AD). The therapeutic efficacy of THA has been attributed to its action as a potent, centrally-active acetylcholinesterase (AChE) inhibitor elevating cortical levels of acetylcholine. While THA's purported improved clinical effects over other cholinesterase inhibitors have been attributed to its improved pharmacokinetics, THA's complex pharmacological profile may also contribute to its antidementia activity. Recent studies (Lahiri et al, *J.Neurosci.Res.* 37:777, 1994). have suggested that THA plays a role in the proteolytic processing of the amyloid precursor protein (APP) by inhibiting a cholinesterase-like protease. These findings have implications for the use of THA as a therapeutic treatment for AD, since the processing of APP leading to the progressive cerebral deposition of β-amyloid is believed to be a pathogenic event in the disease. We have further evaluated the role of THA and other cholinergic agents in the regulation of APP proteolysis. THA produced a time- (4-16 hrs) and concentration- (10-400 μM) dependent decrease in APPs secretion from IMR-32 neuroblastoma cells and Chinese Hamster Ovary (CHO) cells. The effects of THA were not mediated by esterase inhibition or via actions at the muscarinic receptor since: 1) CHO cells do not express AChE, 2) physostigmine did not show similar effects, 3) THA effects were observed both on m1 receptor-transfected and non-transfected CHO cells, and 4) THA effects were not mimicked by muscarinic antagonists or allosteric modulators. THA-mediated decreases in APPs secretion were mimicked by the lysomotropic agents, NH_4Cl and chloroquine. These findings are consistent with THA's action as a weak base and possible inhibitor of lysosomal function, and with the proposed endocytic processing within the endosomal/lysosomal compartment as a major metabolic pathway for APP.

390.7

AMYLOID PROTEIN PRECURSOR SECRETION FROM CONTROL, ALZHEIMER'S AND DOWN'S FIBROBLASTS IN BASAL CONDITION AND FOLLOWING PROTEIN KINASE C ACTIVATION.
S. Bergamaschi, L. Gasparini, C. Quaglia, M. Racchi, G. Binetti[1], S. Govoni[2] F. Battaini, A. Bianchetti[1], F. Giovetti[3] and M. Trabucchi*[1]. Inst. Pharmacol. Sci., Univ. Milano, [1]Alzheimer's Dept., Sacred Heart, Hospital of Brescia, [2]Inst. Pharmacol. Univ. Pavia, [3]Hospital Inst. of Sospiro, Italy.

Literature data show that Down patients overexpress amyloid precursor protein (APP) in various brain areas and in fibroblasts. We previously demonstrated that PKC activity and PKCα immunoreactivity are reduced in Alzheimer disease (AD) fibroblasts. Moreover, we observed in the medium of AD fibroblasts, a reduced soluble APP release both in basal conditions and following PKC activation using low phorbol-12,13-dibutyrate (PdBu) concentrations (9 and 18 nM). The present study compares PKCα immunoreactivity and APP secretion in fibroblasts cultured from ten Down, several AD and respective age-matched controls. PdBu treatment of the cells (for 2 hrs) was carried out with concentrations ranging from 4.5 to 150 nM. PKCα immunoreactivity, in soluble and particulate fractions, was not statistically different in control and Down fibroblasts (while it was lower in the soluble fraction of AD cells). The basal secretion of soluble APP was higher (+101%, P<0.001) in Down cells respect to controls (while it was lower in AD cells). In Down's fibroblasts PdBu was able to stimulate maximally APP secretion (+37%) already at low concentrations (9nM), while in control and AD fibroblasts APP release showed a dose-dependent response reaching the maximum (+79%) at 150 nM PdBu. The results indicate that in Down fibroblasts the mechanisms controlling APP release differ both in comparison with control and AD cells. (Partially supported by grants from the Regione Lombardia and the Ministry of Health)

390.8

DEHYDROEPIANDROSTERONE (DHEA) AUGMENTS AMYLOID PRECURSOR PROTEIN SECRETION IN DESENSITIZED M1-TRANSFECTED PC12 CELLS.
R. Haring, D. Gurwitz*, E. Heldman, Z. Pittel, A. Fisher and H.D. Danenberg[1]. Israel Institute for Biological Research, P.O.B. 19, Ness-Ziona 74100, and [1]Div. of Medicine, Hadassah University Hospital, Jerusalem, ISRAEL.

Secretion of amyloid precursor protein (APP) by cultured cells is coupled to several receptors including m1 muscarinic acetylcholine receptors (m1AChR), and is associated with decreased production of amyloid β-protein (βA4). Amyloid β A4 is the major component of senile plaques, a distinct neuropathological lesion in the brains of Alzheimer disease (AD) patients. Dehydroepiandrosterone (DHEA) is an abundantly secreted adrenal steroid with weak androgenic activity. Plasma levels of DHEA progressively decline with advancing age, reaching 10-20% of adulthood levels. Epidemiological studies suggest that the age-associated decline in DHEA levels is correlated with AD. We have investigated the effect of DHEA on APP processing in m1AChR-transfected PC12 cells. DHEA (0.1 μM, 24 h) did not significantly alter basal or m1AChR-stimulated (100 μM carbachol; 1 h) APP secretion. However, DHEA significantly diminished the desensitization of APP secretion in cells exposed to carbachol for 24 h. In similar experiments, basal, m1AChR-stimulated and desensitization of APP secretion were not affected by the potent glucocorticoid dexamethasone (0.1 μM, 24 h). The effect of DHEA on APP processing is probably not related to up-regulation of m1AChR or increased m1AChR-activated phosphoinositide hydrolysis since these occurred with dexamethasone but not with DHEA treatment. The elucidation of a mechanism by which DHEA affects m1AChR-stimulated APP metabolism may help in identifying events involved in the formation of amyloid deposits and the cholinergic deficiency in AD.

390.9

REGULATION OF AMYLOID PRECURSOR PROTEIN METABOLISM IN NEURONS AND ASTROCYTES BY SECOND MESSENGERS COUPLED TO METABOTROPIC GLUTAMATE RECEPTORS.
R. K. Lee*, R. J. Wurtman, A.J. Cox, K. Brown and B. Tan. Dept. Brain Cog. Sci., M.I.T, Cambridge 02139.

Metabolism of amyloid precursor protein (APP) is regulated by second messengers coupled to cell-surface receptors. We showed that in both hippocampal neurons and cortical astrocytes of fetal rats, aminocyclopentane dicarboxylic acid (ACPD; 10μM) activates metabotropic glutamate receptors (mGluR) coupled to phosphatidylinositol (PI) hydrolysis, and increases secretion of soluble APP (APPs) peptides by 3- to 4-fold.

Diacylglycerol and IP_3/calcium mobilization generated during PI hydrolysis activate protein kinase C (PKC). We now demonstrate that direct activation of PKC by the phorbol ester phorbol myristate acetate (PMA; 5μM) also increases APPs secretion by 2-3 fold relative to unstimulated controls ($p<0.05$). However, a PKC-independent pathway for regulating APP processing exists in both neurons and astrocytes since ACPD increased APPs release by ~1fold above unstimulated controls after down-regulation of PKC by chronic (24h) PMA incubation ($p<0.05$). Consistent with the presence of redundant signalling pathways, the mGluR antagonist (+)-α-methyl-4-carboxyphenylglycine (MCPG; 500μM), which suppresses mGluR-mediated PI hydrolysis ($p<0.05$), had no inhibitory effect on mGluR-mediated APPs secretion ($p>0.05$). The addition of 5μM EGTA to the incubation medium did not suppress the stimulatory effects of ACPD. Although APP proteolysis appears to be insensitive to extracellular calcium, BAPTA (5μM), which chelates intra- and extracellular calcium, was an effective inhibitor of ACPD-mediated APPs secretion ($p<0.05$).

Various mGluR subtypes can affect both intracellular cAMP and PI hydrolysis. Elevating intracellular cAMP levels by addition of either forskolin (10μM) or membrane-permeant dibutyryl cAMP (10μM) potently inhibited APPs secretion during ACPD or PMA (both 10μM) treatment. These studies suggest that aberrent neurotransmission created by either a loss of PI-coupled mGluR or an increase in receptors activating cAMP may exacerbate amyloid formation.

390.10

INTRACELLULAR DETECTION OF βA4-AMYLOID; βA4 SECRETION CAN BE MODULATED BY PHOSPHORAMIDON BUT IS NOT COUPLED TO SECRETION OF APP IN SY5Y CELLS.

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The metabolism of the amyloid precursor protein (APP) of Alzheimer's disease involves at least two pathways: one pathway includes cleavage at the α-secretase site within the βA4 sequence, releasing a secreted form (sAPP); another pathway involves cleavage at the β- and γ-secretase sites releasing full-length βA4. Studies of protein kinase C (PKC) activation have suggested a reciprocal relationship between sAPP secretion and βA4 release. Our studies show that phorbol ester activation of PKC in SY5Y human neuroblastoma cells increases sAPP secretion but has no effect on βA4 release. βA4 is produced intracellularly and subsequently secreted from these cells. Treatment with the metalloprotease inhibitor phosphoramidon (but not thiorphan) results in a 2-fold increase in βA4 recovered from the medium and an increase in βA4 recovered from cell lysates, yet does not affect sAPP secretion. Phosphoramidon appears to inhibit breakdown of βA4 after its production but prior to release as no extracellular breakdown of βA4 was detected in our system.

390.11

CELL CYCLE REGULATED APP SECRETION IN ST14A AND HIB5 CONDITIONALLY IMMORTALIZED CNS PROGENITOR CELL LINES.
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Cell cycle dependent APP phosphorylation linked to a modulation of APP metabolism (Suzuki, EMBO J., 1994) has been shown. Specifically, a reduced secretion of APP is detected in G2/M phase cells paralleled by maximal phosphorylation of APP. To ascertain whether such phenomenon occurrs also in CNS derived cells, ST14A and HiB5 cell lines, immortalized using the temperature sensitive allele of the Large T antigen from SV40, were used. Both cell lines secrete substantial amounts of APP readily detectable in the culture medium at two hours of incubation. Time course experiments demonstrated that the secretion of APP from serum deprived cells at 33°C follows a cyclic pattern. Indeed 48h after serum deprivation, the APP secretion doubled in ST14A cells compared to the 24h time point. At 72h the level of secretion sharply dropped, however it increased steadily at the following time points reaching again the maximum at 168h. Secretion from HiB5 cells followed a similar pattern. It has also been demonstrated that PKC activation stimulates APP secretion. To further characterize our cellular system, we have investigated the activation of the secretory pathway of APP metabolism through pharmacological modulation. Direct activation of PKC with 12,13 phorbol dibutyrrate (PdBu) in either cell lines stimulated APP secretion in a concentration dependent manner at 33°C. Cells incubated at 39°C are blocked in their proliferative activity as the immortalizing oncoprotein is inactivated. In these conditions the cells still respond to the phorbol ester. However the secretory effect observed was less pronounced. These findings suggest the possibility that the physiological regulation of APP metabolism may be dependent on proteins associated with the cell cycle. Alteration in these proteins may play a role in the pathogenesis of Alzheimer disease (partially supported by Alzheimer's Association PRG-94-057 to E.C.).

390.12

Aß SECRETION AND CLEARANCE IN MIXED FETAL GUINEA PIG BRAIN CULTURES.
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Amyloid ß protein (Aß), which deposits as insoluble amyloid fibrils in Alzheimer's disease (AD) brain, is secreted by normal cellular metabolism. To better understand Aß metabolism in brain, we studied Aß secretion and clearance in primary cultures from the fetal guinea pig brain. Mixed glial/neuronal cells prepared from embryonic day 30 guinea pig brain were cultured in Opti-MEMI containing 5% calf serum. Since guinea pig and human Aß have the same sequence, we were able to analyze guinea pig Aß using the same ELISAs that we previously employed to study human Aß. After plating, medium was changed every three days, with cultures maintained for as long as 18 days. The accumulation of Aß1-40, Aß1-42, and total Aß was followed by analyzing aliquots taken 4, 8, 24, 48, and 72 hours after each medium change. Aß clearance was evaluated by removing conditioned medium from the cells, adding synthetic Aß1-40 or Aß1-42, and following it by analyzing aliquots drawn at 4, 8, 24, 48, and 72 hours after Aß addition. As cultures were maintained, the amount of Aß accumulating in 3 days gradually increased with ~3 pmol/ml of Aß accumulating from day 15-18. Consistent with our previous analysis of human cell lines, CSF, and plasma, most of the Aß secreted by these cultures was Aß1-40 and ~10% was Aß1-42. Synthetic Aß1-40 and Aß1-42 were cleared from conditioned medium, although the rate of removal was much less than that observed in many human cell lines. As cultures were maintained over several weeks, the conditioned medium removed every three days showed a progressive decrease in the rate at which exogenously added Aß1-40 or Aß1-42 was cleared. These results show (i) that mixed fetal guinea pig brain cultures release large amounts of Aß1-40 and Aß1-42 and (ii) that Aß is cleared from medium conditioned by these cultures. Further study of these cultures may provide insight into the secretory and clearance mechanisms that regulate Aß concentration in brain.

390.13

β-AMYLOID$_{1-40}$ INCREASES EXPRESSION OF β-AMYLOID PRECURSOR PROTEIN IN NEURONAL HYBRID CELLS. W-D. Le*, W.J. Xie, O. Nyormoi, B.K. Ho, R.G. Smith and S.H. Appel. Department of Neurology, Baylor College of Medicine, Houston, TX 77030.

Studies of cell injury and death in Alzheimer's disease (AD) have suggested a prominent role for β-amyloid peptide (β-AP), a 40-43 amino acid peptide derived from a larger membrane glycoprotein, β-amyloid precursor protein (β-APP). Previous experiments have demonstrated that β-AP induces cytotoxicity in a neuronal hybrid cell line (MES 23.5) *in vitro*. Here, we demonstrate that accompanying β-AP induced cell injury, cell-associated β-APP can be increased up to 14 fold; while secreted forms of β-APP including c-terminal fragments and β-AP can also be significantly elevated. β-APP mRNA is increased up to 3.5 fold in 24 hours after treatment with β-AP$_{1-40}$. Application of β-APP antisense oligodeoxynucleotide reduces both cytotoxicity and β-APP expression. While 6-hydroxydopamine application or glucose deprivation cause extensive cell damage, they do not increase β-APP expression. These results suggest a selective positive feed back mechanism whereby β-AP may induce cytotoxicity and increase levels of β-APP as well as its amyloidogenic fragments which, in turn, could lead to further neuronal damage.

390.14

STUDY ON THE PROTEOLYTIC ACTIVITY OF APP α-SECRETASE: A MODEL FOR AN ENZYME ASSAY *IN VITRO*

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Several studies have demonstrated that "α secretase" cleavage is not sequence specific and requires a substrate associated with the membrane. In light of these data it is evident that screening for the "secretase" using synthetic peptide substrates might not be useful. We have approached the problem by constructing synthetic substrates for "α secretase" that can be integrated *in vitro* in membranes as the physiologic precursor. A construct encoding APP751 in pGEM3Zf(+) vector that allows *in vitro* transcription of the cDNA was used for the construction of two substrates. The first, referred to as $\beta\Delta$XB, was obtained by deleting the region included between the XhoI and BglII sites of the APP 751 cDNA. This construct transcribed and translated *in vitro* yielded a protein of 50 kDa that was integrated during translation in dog pancreas microsomal membranes in the same orientation as authentic cellular APP. A second substrate referred to as HPLAPP, was obtained by recombinant PCR technique and is a fusion construct between the entire coding region of human placental lactogen (HPL) and a C terminal region of the APP cDNA beginning 6 aminoacids N terminal to the βA4 peptide. Transcription and translation of this construct yielded a protein of 38 kDa immunoprecipitable by antibodies directed against HPL or the C terminus of APP. These substrates integrated in microsomal membranes have been used to characterize in vitro the "α secretase" processing activity of membrane fractions of COS cells and human fibroblasts, in a wide variety of conditions including detergents and protease inhibitors. Current experiments are aimed at the assessment of the specificty of the assay. (Supported by Alzheimer's Association PRG-94-135 to M.R.)

390.15

CHOLESTEROL MODULATES STABILITY AND α-SECRETASE CLEAVAGE OF AMYLOID PRECURSOR PROTEIN.

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We have examined the hypothesis that cellular levels of cholesterol modulate the processing of amyloid precursor protein (APP). Both APP and cholesterol metabolism are genetically linked to Alzheimer's disease (AD), the latter through apolipoprotein E, a lipid and cholesterol transport protein. The ApoE4 allele is associated with an increased risk of AD as well as higher plasma cholesterol levels. In the current study, methyl-β-cyclodextrin solubilized cholesterol was added to the culture media of APP 751 stably transfected HEK 293 cells. Radiolabeled APP and APP$_{sol}$, the soluble N-terminal derivative following α-secretase cleavage, were precipitated from lysates and conditioned media; the relative levels were determined by quantitative densitometry following separation by SDS-PAGE. The data show that increasing cellular cholesterol augments levels of both mature and immature APP holoprotein in a dose-dependent fashion, while dramatically reducing levels of APP$_{sol}$. The effects were specific for APP processing because similar changes did not occur in general cellular or secreted proteins. Cholesterol may act by impeding membrane fluidity, which could account for the increased levels of the membrane-spanning APP holoprotein by impeding turnover and degradation. The decreased fluidity may also account for the reduction of APP$_{sol}$ by impeding the interaction of the substrate with its protease(s). If APP$_{sol}$ were to function trophically, as suggested by other studies, the current conclusion suggests that changes in cellular cholesterol levels in Alzheimer's disease could contribute to neuronal degeneration by decreasing the production of APP$_{sol}$.

390.16

α-SECRETASE DOES NOT APPEAR TO BE GELATINASE A.

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It was suggested recently that the matrix metalloproteinase (MMP) gelatinase A (EC 3.4.24.24) may be responsible for the α-secretase-mediated cleavage of the amyloid precursor protein (APP) associated with Alzheimer's disease. We have examined the effects of two MMP inhibitors on the release of soluble APP by PC-12 cells. These inhibitors were tissue inhibitor of MMP-1 (TIMP-1) and [4-(N-Hydroxyamino)-2R-isobutyl-3S-(2-thiophenylthiomethyl) succinyl] -L-phenylalanine-N-methylamide (SB1). The latter is a small, potent hydroxamate-based inhibitor of MMP that was shown to penetrate cells. Neither TIMP-1 nor SB1 prevented the secretion of APP. Furthermore, purified gelatinase A was shown to cleave the βA4 peptide (residues 1-40) at more than one site and PC-12 cells were found to possess only small amounts of gelatinase A activity, whereas BU-17 cells (which secrete very little APP into the culture medium) were found to have much higher gelatinase activity. These results strongly suggest that α-secretase is not gelatinase A, but do not preclude a role for gelatinase A in βA4 degradation.

390.17

ARACHIDONIC ACID ACTIVATES α-SECRETASE PROCESSING OF THE AMYLOID PRECURSOR PROTEIN.

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Metabotropic glutamate receptors (mGluR) can generate second messengers which regulate APP processing. In rat hippocampus and transfected cells expressing mGluR, the selective mGluR agonist aminocyclopentane dicarboxylic acid (ACPD) stimulates phosphatidylinositol (PI) hydrolysis and arachidonic acid formation. We showed that mGluR-mediated secretion of soluble APP (APPs) peptides in hippocampal neurons is dependent on the phospholipase C/protein kinase C signal transduction cascade. Quinacrine (5μM), an inhibitor of phospholipase A$_2$, did not suppress APPs secretion in ACPD-treated neurons, suggesting that phospholipase A$_2$ does not regulate APP processing during mGluR activation.

Arachidonic acid (AA), which is liberated during ischemia and neuronal injury, mediates glutamatergic signalling in neurons. AA, in conjunction with phorbol esters, can also potentiate glutamate release from synaptic terminals. We now report that incubation of primary hippocampal neurons (derived from fetal rats) with AA (100μM; 1h) increases APPs secretion into the extracellular medium by 2-3 fold compared to unstimulated controls ($p<0.05$). This stimulatory effect is attenuated and reduced to baseline levels ($p>0.05$) by the putative mGluR antagonist, L-AP3 (250μM) or by inhibiting glutamatergic transmission at the glutamate autoreceptor with 2-amino-4- phosphonobutyrate (AP4; 100μM).

Depolarizing with KCl (30μM), or directly activating PKC with phorbol esters, promotes glutamate exocytosis and synaptic transmission and also mimics the action of AA by increasing APPs secretion ~2-fold relative to controls ($p<0.05$). Consistent with a role for arachidonic acid in modulating glutamatergic transmission, we further demonstrate that AA enhances ACPD-induced release of APPs and that this effect is blocked when glutamate is inhibited with AP4. These data show that arachidonic acid can enhance glutamatergic transmission at metabotropic receptors to increase APPs secretion.

390.18

Inhibition of βA4 production by specific modulation of β-secretase activity

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To study APP processing we expressed different APP isoforms with and without the swedish mutation and the membrane inserted C-terminal 100 residues of APP (SPA4CT) in the human neuroblastoma cell line SY5Y. We show that expression of the swedish mutation results in a significant production of the amyloidogenic intermediate A4CT which is further processed by γ-secretase leading to an overproduction of βA4. Treatment with methylamine and ammonium chloride, inhibitors interfering with intracellular transport mechanisms, inhibits β-secretase activity without influencing the physiological APP cleavage by α-secretase activity.

By expressing SPA4CT we demonstrate that secretion, but not generation, of βA4 from SPA4CT is inhibited by methylamine resulting in intracellular βA4. This is the first experimental evidence for the intracellular localisation of γ-secretase activity and βA4 generation.

391.1

BROMODEOXYURIDINE (BrdU) INCORPORATION OF CELLS IN GERBIL HIPPOCAMPUS FOLLOWING TRANSIENT GLOBAL ISCHEMIA. J. Liu*, M. Hill and F. R. Sharp. Dept. of Neurology, Univ. of California at San Francisco and SFVAMC, San Francisco, CA 94121.

Neuronal cell loss is a common event after brain injury, however, the mechanisms of which are poorly understood. The CA1 pyramidal neurons in the hippocampus have been shown to undergo selective cell death both in experimental animals and in humans after ischemic insult. Recent developments have suggested the delayed death of the CA1 pyramidal neurons after transient ischemia is apoptotic. Some apoptotic cell death may involve abortive activation of cell cycle, as shown in developing CNS and axotomized neonatal rat facial motoneurons. The current study was designed to look for proliferating cells after transient global ischemia and to determine the cell types involved. BrdU (50 mg/kg) was given i.p. daily to control gerbils and animals received an ischemic insult via bilateral occlusion of common carotid arteries for 5 and 10 minutes. The animals were sacraficed 3 days after ischemia and the brains were prepared for immunostaining. Elevated BrdU staining was seen in the hippocampus of 5 minute-ischemic gerbils, and in the cortex, striatum in addition to hippocampus of 10 minute-inchemic animals. The control gerbils showed positive staining in the of medial striatum near ventricles, and in the pyramidal layers of dentate gyrus. In situ nick-end labelingof biotinylated dUTP mediated by terminal deoxytransferase (TUNEL) confirmed the apoptotic cell death in CA1 regions in all ischemic animals tested. The origin and nature of the proliferating cells in hippocampus have yet to be determined by using cell specific markers. If neurons are incorporating BrdU in the CA1 dying neurons, this might suggest they are re-entering the cell cycle prior to an apoptotic-like cell death. The role of BrdU uptake into glia surrounding the same neurons remains to be answered.

391.2

DIFFERENTIAL DISPLAY PCR AND TRANSIENT FOREBRAIN ISCHEMIA IN THE RAT F.Kamme*, J.Babity, H.Robertson and T.Wieloch. Lab. for Exp. Brain Research, 221 85 Lund, Sweden and Dept. of Pharmacology, Dalhousie Univ., Halifax, Canada.

We have previously shown that there is a late induction of Immediate Early Genes and Tumor suppressor genes after transient forebrain ischemia prior to delayed neuronal death, predominant in vulnerable areas of the hippocampus. This is shifted temporally to an earlier timepoint by intraischemic hypothermia, which also prevents cell death. Thus, many of the genes seen to be induced late after ischemia are not unique to dying cells and may be part of adaptive responses such as a stress response or repair mechanism. In an attempt to identify genes involved in the death mechanism of CA1 neurons after 15 min. of transient forebrain ischemia in the rat, we have employed the Differential Display PCR technique. Total RNA was extracted from normothermic ischemic brains after 48 h of reperfusion and compared to RNA from hypothermic ischemic brains after 12 and 18 h of reperfusion.

On average, 1-2 bands per primer combination were found to be differentially expressed, i.e. expressed at 48 h in normothermic animals but neither in 12 or 18 h hypothermic ones. These were reamplified and sequenced directly and subcloned into a TA vector. In a number of cases direct sequencing was not possible due to the presence of several sequences contained within the same band.

391.3

TRANSIENT RETINAL ISCHEMIA LEADS TO APOPTOTIC CELL DEATH AND MAY BE AMELIORATED BY AURINTRICARBOXYLIC ACID. D.M. Rosenbaum*, P.S. Rosenbaum, D.M. Michaelson, D. H. Hall, and J.A. Kessler. Albert Einstein College of Medicine, Bronx, NY 10461.

The purpose of this study was to determine if the delayed cell loss seen after transient retinal ischemia is apoptotic and whether drugs which have been shown to inhibit neuronal apoptosis *in vitro* prevent ischemic retinal neuronal death *in vivo*. Transient retinal ischemia was produced in rats by raising IOP to greater than systemic pressure for 60 minutes. Morphologic and biochemical evidence for apoptosis was determined by electron microscopy (EM) and Southern blot analysis. Both ultrastructural and Southern blot analysis were consistent with apoptosis after 12 hours of reperfusion. We, therefore, administered aurintricarboxylic acid (an agent that has been shown to inhibit neuronal apoptosis *in vitro*) intravitreally either 24 hours prior to ischemia or in a separate set of experiments, 6 hours after ischemia, and the retinas analyzed 7 days later. The animals treated with ATA either before (100%; $p < .001$) or after (54%; $p < .01$) ischemia demonstrated significantly less ischemic damage in the inner retinal layers. There was also preservation of the retinal histoarchitecture. These data support the hypothesis that delayed cell death after transient retinal ischemia may, in part, result from apoptosis and opens new strategies aimed at preventing retinal damage from this process.

391.4

EFFECT OF TTC-909 ON DELAYED NEURONAL DEATH OF CA1 PYRAMIDAL NEURONS FOLLOWING BRIEF ISCHEMIA; AN IMMUNOCYTOCHEMICAL STUDY.

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We have previously shown that delayed neuronal death occurring in the CA1 pyramidal layer of gerbil hippocampus after brief ischemia is not necrosis but apoptosis (J. Neurosci. 15: 1001-1011, 1995). In the dying processes of the CA1 pyramidal cells, cytoplasmic alterations are largely mediated by the formation of autophagolysosomes immunopositive for lysosomal cysteine proteinases, cathepsins B, H and L, 3 days after ischemic insult, while nuclear DNA fragmentation into oligonucleosomes is demonstrated by in situ nick end labeling of dUTP (TUNEL staining) and Southern blot analysis 3 and/or 4 days later. Moreover, heterophagocytosis by microglial cells immunopositive for cystatin β (Cβ), an endogeneous cysteine proteinase inhibitor, and cathepsin H is also seen 4 to 7 days after ischemic insult. Using this neuronal death model, we examined effects of TTC-909, isocarbacyclin methyl ester incorporated in lipid microsphere, administered (i.v.) soon after ischemic insult. In some cases of gerbil brains treated with TTC-909 (500 ng/kg), invasion of Cβ-immunopositive microglial cells into the CA1 region was largely inhibited even on day 7, whereas GFAP-immunopositive astrocytes extended their cytoplasmic processes into the CA1 region as greatly as seen in those of the control brains without TTC-909 treatment. Moreover, in these brains, the CA1 neurons showing negative staining of TUNEL appeared abundantly even on day 7. The results suggest that TTC-909 may be potent in suppression of delayed neuronal death in the CA1 pyramidal layer after brief ischemia.

391.5

DELAYED INDUCTION OF IMMEDIATE EARLY GENES (IEGS) FOLLOWING TRANSIENT FOCAL CEREBRAL ISCHEMIA I. Pyykönen, R. Keinänen* and I. Koistinaho. A.I. Virtanen Institute, University of Kuopio, FIN-70211 Kuopio, Finland.

Middle cerebral artery (MCA) occlusion transiently induces IEGs in the cortex adjacent to the ischemic core as well as in subcortical structures remote from the infarction. Even though this IEG induction is thought to be mediated by spreading depression, the regulation of IEG induction in focal brain ischemia is not well understood. In this study we compared the temporal and regional patterns of c-fos induction following 30 and 90 minutes of MCA occlusion. MCA occlusion in halothane-anesthetized rats was produced using an intraluminal suture without craniotomy. The rats were anesthetized and decapitated 0 h, 1 h , 4 h, 12 h, 24 h, 3 or 7 days following 30 min or 90 min of ischemia. The brains were removed and processed for in situ hybridization. In MCA territories adjacent to the ischemic core and in the hippocampus and substantia nigra the expression of c-fos, jun-B and NGFI-A peaked within one hour following the ischemia. The expression of IEG mRNAs was back to control levels 12-24 h following 30-min ischemia and 3 days following 90-min ischemia. There was no or only minor IEG induction in the infarct core within 24 h following the insult. However, a delayed IEG expression occured in the infarct core 3-7 days following 30-min ischemia but not following 90-min ischemia. Emulsion-coated sections hybridized with IEG probes showed grains throughout the core, but more distinctly over a few neuronal cell bodies. The early IEG expression is likely to be induced in neurons by spreading depression and is therefore stronger following a long-lasting ischemia when more glutamate is released to stimulate NMDA receptors. A delayed IEG expression in the infarct core following 30-min of ischemia may be required for regeneration of the surviving neurons.

391.6

DNA-FRAGMENTATION, INDUCTION OF IEGs, p53 AND WAF1 IN HYPOGLYCEMIC COMA IN RAT. M.Ferrand-Drake*, P. Stubberöd, G. Tomasevic, F. Kamme, M. Shamloo, T. Wieloch. Lab. for Experimental Brain Research, University Hospital, Lund, 221 85 Sweden.

Using *in situ* hybridisation we studied the expression of the Immediate Early Genes (IEGs) (*c-fos*, *fosB*, *c-jun*, *junD* and *fra-2*) and *p53* and *WAF1* following hypoglycemic coma in the rat. All of the IEGs studied were induced after hypoglycemia. A peculiarity is seen with the induction of *c-fos*;it is induced already at the end of the hypoglycemic period in the tip of Dentate Gyrus(DG), which exhibits necrosis already at this timepoint. *c-jun*, *jun*D and *fra-2* are maintained at a high level until 6 h of recovery and then progressively decline. In the vulnerable CA1 region, however, expression is elevated until 48 h of recovery. *c-fos* declines by 3 h but is reinduced later on in areas that are susceptible to damage, i.e. the tip of DG, subiculum and parts of CA1. We propose that *c-fos* is reinduced in vulnerable areas, perhaps due to activation of microglia, and that *c-jun*, *junD* and *fra-2* are expressed persistently in neurons as a reaction to stress. We also found induction of these genes in the synovial membrane overlying CA1 and the distribution of labelling corresponded to the distribution of damage seen in the CA1 underneath.

Additionally we studied *p53* and *WAF1* which are induced in CA1 at 24 h and not at all in the DG. We therefore suggest that in some vulnerable areas after a hypoglycemic insult, *p53* and *WAF1* are induced as an indication of DNA damage.

As a measure of cell death, DNA-fragmentation was detected by the TUNEL-method and eletrophoresis and appeared at the end of the hypoglycemic period in the tip of DG, at 24 h. in CA1 and in cortex.

391.7

CONSECUTIVE EXPRESSION OF AP1 COMPLEX IN THE RAT HIPPOCAMPUS AFTER TRANSIENT CEREBRAL ISCHEMIA.
Bing-Ren Hu* and Tadeusz Wieloch, Lab. for Exp. Brain Res., University Hospital, 221 85 Lund, Sweden.

The AP-1 DNA binding activity and the alterations in its Fos and Jun protein components, were studied in the rat hippocampus after transient forebrain ischemia. The AP-1 complex in the nuclear extract was markedly induced at 4 hrs and was still elevated at 24 hrs of reperfusion. Expression was observed both in CA1 region and in CA3/dentate gyrus (DG) area, albeit with different intensities. Nearly all of members of the Fos and Jun families were expressed in the AP-1 complex at 4 hours of reperfusion. By 24 hrs of reperfusion, c-Fos/Jun B disappeared while Fras/Fos-B and c-Jun/Jun-D were still present. On brain sections, immunoreactivity of Fras first appeared at 1 hour, was strong at 4–6 hrs and was still elevated at 24 hrs of reperfusion in DG neurons. Induction of Fras was delayed 2 hrs in pyramidal neurons. Expression of c-Jun was temporally dissociated between CA1 and DG neurons, i.e. stronger in the DG neurons than in CA1 neurons before 24 hrs and then strong in CA1 but not present in DG neurons after 24 hrs of reperfusion. Immunoblots confirmed that c-Fos was transiently induced at 4 hrs of reperfusion. The dissociated c-Jun expression between CA1 and CA3/DG neurons was also observed on Western blots. The data demonstrate a dynamic change in AP1 complex formation presumably reflecting activation of different intracellular signalling pathways, which may alter gene programs causing long lasting effects or/and neuronal damage.

391.8

HYPOXIA-INDUCIBLE FACTOR 1 (HIF-1) AS A MEDIATOR OF ADAPTIVE RESPONSES TO HYPOXIA IN NG108 CELLS
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The central nervous system displays high vulnerability to hypoxic insult. Little is known about the way neuronal cells respond to hypoxic stress. Recently a hypoxia-inducible DNA binding activity was identified in erythropoietin (EPO) producing cells. The transcription factor responsible for this activity has been named hypoxia-inducible factor 1 (HIF-1) and binds to a region upstream from the EPO gene. Using a double-stranded radiolabelled DNA probe corresponding to the specific HIF binding element in the EPO gene, we monitored the induction of HIF-1 in the EPO producing cell line Hep3B and non-EPO producing cell line NG108 with the gel-shift assay. Exposure of both cell lines to 1% O_2 induced HIF-1 activity within 4 hours as compared to normoxic conditions (20% O_2). In both cell lines hypoxic induction of HIF-1 activity was inhibited by a 2 hour prior exposure to the general protein kinase inhibitor 2-aminopurine (2-AP). These results suggest a role of HIF-1 as a mediator of adaptive responses to hypoxia in neuronal cells and implicate protein phosphorylation in the transduction of hypoxic signals.

391.9

EXPRESSION OF PROLIFERATING CELL NUCLEAR ANTIGEN IN THE POSTISCHEMIC RAT HIPPOCAMPUS.
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Proliferating cell nuclear antigen (PCNA) is an important auxiliary factor in DNA replication, and is also required for DNA excision repair. PCNA's replicative - but not it's reparative - function is inhibited by p21/WAF1. We used *in situ* hybridization to study the expression of PCNA mRNA following transient (15 min) global normo- (37° C) and hypothermic (33° C) cerebral ischemia in the rat. PCNA protein expression was studied by means of immunocytochemistry. In the normothermic situation, PCNA mRNA and protein were induced after 2 hrs of recircualtion in the resistant dentate gyrus subfield only. We have previously shown that these neurons express p21/WAF1 mRNA at this timepoint, which indicates that DNA-reparative processes might be activated early during recirculation in resistant neurons. No PCNA induction was detected in the sensitive CA1 neurons following normothermic ischemia. Intra-ischemic hypothermia is known to confer protection against neuronal damage. Following hypothermic ischemia, PCNA mRNA was also induced in the CA1 and CA3 regions after 24 hrs of recirculation. We have previously shown that the hypothermic CA1 neurons express p21/WAF1 mRNA after 18-24 hrs of recirculation. The induction of PCNA in the hypothermic CA1 subfield indicates that DNA-reparative functions play an important role in the neuronal defense against hypoxic damage.

391.10

EXPRESSION OF Myc AND p53 FOLLOWING TRANSIENT GLOBAL ISCHEMIA
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The proto-oncogene *c-myc* and the tumor suppressor gene p53 encode proteins that function as transcriptional regulating factors which govern cell proliferation, differentiation, and apoptosis. The objective of this study was to determine whether the protein products of these genes are expressed in neurons that undergo delayed neuronal death following transient global ischemia. Four vessel occlusion was produced in male Wistar rats by cautery of the vertebral arteries and clamping of the common carotid arteries for 20 min. Animals were perfused transcardially 2, 12, 24, and 48 h following recirculation. Immunohistochemical staining revealed a dramatic increase in both mutant and wild type p53-like immunoreactivity exclusively in vulnerable CA1 neurons of the hippocampus 48 h after the ischemic insult. Myc-like immunoreactivity was also elevated in the majority of CA1 neurons at this time. The close temporal correlation between these changes in gene expression, and the loss of CA1 neurons suggest that alterations in Myc and p53 expression may contribute to the delayed neuronal death of CA1 neurons following transient forebrain ischemia. (Supported by a grant from the Heart and Stroke Foundation of Canada.)

391.11

EXPRESSION OF p53 AND CYCLIN G IN NUCLEI OF CELLS UNDERGOING ISCHAEMIA-INDUCED APOPTOSIS IN THE RAT BRAIN
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Recent evidence suggests that some forms of ischaemia induced cell death may occur through apoptosis. We have investigated the gene-products which may be involved in a cell death program, using a rat model of focal cerebral ischaemia.

Ischaemia was induced by permanent occlusion of the left middle cerebral artery. Internucleosomal DNA fragmentation was studied 24 h after ischaemia using a method of terminal d-UTP nick end labelling (TUNEL) on paraffin sections. Adjacent sections were immunocytochemically stained for the tumor suppressor protein p-53 and one of its transcriptional targets, cyclin G. Nuclear morphology was revealed by counterstaining with methyl green.

In the ischaemic core area, involving part of the striatum and cerebral cortex, a high density of TUNEL-positive cells were found 24 h post-ischaemia. Less than 20% of the TUNEL-stained nuclei had an apoptotic morphology and these were mainly located in the ventrolateral part of the affected striatum and cerebral cortex. P-53 and Cyclin G immunoreactivities were present in all nuclei with an apoptotic morphology. In addition, immunoreactivity for cyclin G was found in small-sized nuclei, presumably non-neuronal, with the highest density in the corpus callosum followed by the striatum and cerebral cortex in both the ipsi- and contralateral hemispheres.

The present study demonstrates that focal ischaemia induces internucleosomal DNA fragmentation associated with both apoptotic and necrotic cell death. The presence of cyclin G in all apoptotic cells indicates a possible relationship between the expression of genes involved in the regulation of the cell cycle and apoptotic cell death. Further studies will identify the cell types expressing cyclin G and characterise the temporal expression of related genes involved in ischaemia-induced programmed cell death.

391.12

GADD45 MRNA IS PERSISTENTLY EXPRESSED IN VULNERABLE NEURONS FOLLOWING GLOBAL CEREBRAL ISCHEMIA IN THE RAT.
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Growth arrest and DNA damage-inducible (GADD) genes are novel genes that encode at least five acidic proteins that are induced by growth arrest signals, DNA-damage and other genotoxic stresses. Although the precise function of GADD gene products is unclear, they have been suggested to be important regulatory factors for growth control and apoptosis in proliferating cells. Recently one of this gene family, GADD45, has been found to be induced in the rat brain by UV radiation, suggesting that this gene may also be inducible in postmitotic cells by DNA damage. Therefore, we studied the mRNA expression of GADD45 in the brain following global ischemia.

Adult rats were subjected to 15 min of incomplete global ischemia by means of four-vessel occlusion. The brains were taken at a variety of time points during the reperfusion period and processed for in situ hybridization using 35S-labeled GADD45 antisense oligonucleotide probe, for TUNEL staining (24h and 48h) and for cresyl violate staining (72h). The specificity of the oligonucleotide probe was verified with northern analysis.

Elevation of GADD45 mRNA was detectable in CA1 neurons and DG granule cells at 2h. By 4-8h, intensive GADD45 mRNA induction was shown throughout the hippocampal formation, the caudate putamen, cortex, and the thalamus. By 24-48h, elevation of GADD45 mRNA was detected only in CA1 neurons, coinciding with the distribution of positive TUNEL staining. By 72h, GADD45 mRNA signal was lost in CA1 neurons, coinciding with histological cell death.

These data show that transcriptional expression of GADD45 can be induced in rat brain neurons following transient global ischemia. This gene was transiently expressed in non-vulnerable neuron populations but was persistently expressed in vulnerable CA1 neurons. These data also suggest that GADD45 may have a role in regulating neuronal responses to DNA damage and subsequent apoptotic cell death. Whether GADD45 is induced alone or concurrently induced with other GADD members is currently under investigation.

391.13

THE EXPRESSION OF CYSTATIN C, A CYSTEINE PROTEASE INHIBITOR, IN THE RAT HIPPOCAMPUS FOLLOWING TRANSIENT FOREBRAIN ISCHEMIA. D. Palm*, N. Knuckey, M. Primiano, S. Cascio, C. Johanson; Dept. Clin. Neurosci., Program in Neurosurg., Brown Univ. & RI Hospital, Providence, RI 02902

Recent immunohistochemical studies have demonstrated a temporal profile of the cysteine protease inhibitor, cystatin C, in degenerating rat hippocampal neurons following 10 min of transient forebrain ischemia (TFI). We have also demonstrated that cystatin C immunoreactivity (CC-IR) coincides with the density of neuronal damage following a mild to severe ischemic insult. However, the origin of cystatin C in the degenerating hippocampus following TFI is unknown. To address this issue, northern blot hybridization analysis was performed to examine cystatin C mRNA concentrations in rat hippocampus at 3, 7 and 14 days following TFI. The two-vessel occlusion model with hypotension for 10 min was used to induce TFI. Total hippocampal RNA was isolated from frozen tissue using the standard guanidinium thiocynate method and 10 µg aliquots of RNA were separated by gel electrophoresis and transferred to Nytran. Cystatin C cDNA provided by Dr. Gerhard Schreiber, was used to probe the filters under stringent hybridization and washing conditions. Our results demonstrate that hippocampal cystatin C mRNA concentrations are identical in normal and sham animals and in 3, 7 and 14 days following TFI. These results indicate that cystatin C is produced in the hippocampus following TFI. Also, the elevated levels of cystatin C visualized after TFI by immunohistochemical analysis may therefore require post-transcriptional regulation.

Supported by funds from R.I. Hospital and Brown Univ. Fellowship (D.E.P.)

391.14

IN SITU HYBRIDIZATION STUDIES OF ß-ACTIN GENE EXPRESSION IN RAT BRAIN TISSUE FOLLOWING ISCHEMIC INJURY AND REPERFUSION. J. P. Ray*, P. Britton, X.-C. M. Lu, F. C. Tortella and J.R. Dave. Division of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307.

In an effort to identify the ischemic "penumbra", the viable (and potentially salvageable) but compromised area of neuronal tissue circumscribing the core infarct, we have examined the distribution of several markers (including *c-fos* and *hsp-70* gene) of cellular viability in infarcted rat brains using the *in situ* hybridization technique. Here we describe the patterns of ß-actin mRNA in brains which have received 2 hr of intraluminal middle cerebral artery occlusion (MCAO) and 24 hr of reperfusion. MCAO was achieved by advancing a 3-0 monofilament nylon suture into the internal carotid artery of male Sprague-Dawley rats (270-330 g). After 2 hr of complete MCAO the filament was retracted to allow for reperfusion. Large infarcts (volume approximately 20% of the contralateral hemisphere) were routinely obtained with this technique. In comparison to the contralateral hemisphere, infarcted brain regions exhibited only very low levels of ß-actin mRNA. However, regions adjacent to the primary lesion exhibited consistently elevated ß-actin mRNA. This elevated expression of ß-actin gene in brain regions adjacent to the lesioned tissue may be due to a precompensatory state of neuronal function following MCAO insult. Thus, the region over-expressing the ß-actin gene may represent a true penumbra sensitive to neuroprotective therapies.

391.15

EXPRESSION OF CYTOSKELETAL PROTEINS IN HYPOTHERMIC FOREBRAIN ISCHEMIA. K. Kumar*, A.T. Evans and X. Wu, Department of Pathology, Michigan State Univ., E. Lansing, MI 48824.

The expression of β-actin mRNA was examined in hypothermic ischemic animals and compared to that in normothermic ischemic animals. Mongolian gerbils were subjected to forebrain ischemia by bilateral carotid occlusion of 10 min at 30°C followed by normothermic reperfusion for 1 h, 6 h, 1 d, 3 d, 2 weeks, and 1 month. The expression of β-actin mRNA was determined in hypothermic control and postischemic (PI) animals subjected to intraischemic hypothermia (n = 3 in each gp.) by using *in situ* hybridization. In the case of normothermic ischemic brains, there was decrease in the expression of β-actin in the CA1 region beginning by 6 h PI, clearly diminished by 3 d, only faintly visible by 7 d, and completely absent by 1 month PI (shown by us previously). In contrast, the hypothermic ischemic brains showed no significant decline in its expression in the CA1 region. The expression was preserved in the CA1 region in all except one hypothermic ischemic animal in the 2 week PI period. These results indicate that hypothermia leads to preservation of the expression of a cytoskeletal protein, β-actin, in a selectively vulnerable region of the brain following ischemia.

391.16

ALTERATIONS OF β-TUBULIN AND MICROTUBULE-ASSOCIATED PROTEIN IMMUNOSTAINING INDICATE AXONAL PATHOLOGY IN RESPONSE TO FOCAL CEREBRAL ISCHEMIA IN VIVO. D. Dewar*, M. Fitzpatrick & D. Dawson. Wellcome Surgical Institute, University of Glasgow, Glasgow, G61 1QH, U.K

Despite the clinical importance of white matter damage resulting from cerebral ischemia, axonal pathophysiology has been relatively neglected in comparison with that of neuronal perikarya. This study examined the axonal cytoskeleton following focal cerebral ischemia in vivo by means of immunohistochemical staining of β-tubulin and microtubule-associated proteins (MAPs).

All experiments were performed in physiologically monitored, mechanically ventilated, halothane anesthetised rats. Permanent focal ischemia was induced by occlusion of one middle cerebral artery (MCA). Sham controls had the artery exposed but not occluded. Two or 6 hours later the brains were perfused fixed and processed for MAP1A, MAP5 and β-tubulin immunohistochemistry. In white matter tracts within MCA territory (corpus callosum and fiber bundles within the caudate nucleus) there was increased β-tubulin, MAP1A and MAP5 immunoreactivity compared to shams. The white matter staining in MCA-occluded rats had a more granular appearance than the shams. The changes in immunostaining of all antibodies were more pronounced at 6 compared to 2h of MCA occlusion. Axons with an irregular, beaded profile were detected with all three antibodies. This morphological appearance has been suggested to represent the early stages of axonal pathology which precede axotomy. Alterations in microtubule proteins indicate the development of axonal pathology relatively early in response to focal cerebral ischemia in vivo.

391.17

REDUCED EXPRESSION OF NA^+-DEPENDENT PHOSPHATE COTRANSPORTER mRNA PRECEDES LOSS OF HIPPOCAMPAL CA1 PYRAMIDAL NEURONS FOLLOWING TRANSIENT GLOBAL ISCHEMIA IN THE RAT. B. Ni*, D. Stephenson, X. Wu, J. A. Clemens and S. M. Paul. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

The brain concentration of high-energy organic phosphates is frequently used as a measure of tissue viability and, more specifically, as an index of mitochondrial function after transient ischemia. We have recently cloned a brain-specific Na^+-dependent phosphate (Pi) cotransporter (rBNP1) which is specifically expressed in pyramidal and granule neurons of the cerebral cortex and hippocampus (Ni et al., Proc. Natl. Acad. Sci. 91:5607-5611, 1994) and have found that Na^+-dependent ^{32}Pi uptake in cultured rat fetal cortical neurons correlates positively with intracellular energy charge (Glinn et al., J. Neurochem, in press). Using *in situ* hybridization histochemistry, we now report that expression of rBNP1 mRNA is markedly reduced in hippocampal CA1 pyramidal neurons by 22 hrs and completely abolished by 72 hrs after 4-V occlusion. No decrease in rBNP1 mRNA was observed in other regions of the brain. Cell staining with H/E, immunostaining with a MAP2 antibody as well as cellular morphology analysis with DIC microscopy reveal that CA1 pyramidal neurons are morphologically intact at 22 hrs postischemia but begin to degenerate by 72 hrs; consistent with the delayed neuronal death of CA1 neurons in the transient ischemia model. Our findings indicate that a decrease in Pi import may precede cell death of CA1 hippocampal neurons induced by transient global ischemia and may, in part, account for the reduction in high energy organic phosphates which occur in delayed neuronal death.

391.18

STRIATAL DOPAMINE RECEPTOR GENE EXPRESSION FOLLOWING TRANSIENT GLOBAL ISCHEMIA. M.Y.-T. Globus*, J. Singer, J. Rabe, D. Mash, R. Busto, I. Valdes, and M.D. Ginsberg. Cerebral Vascular Disease Research Center, Department of Neurology, University of Miami, School of Medicine, Miami, FL, 33101.

We have previously demonstrated that ischemia leads to a selective loss of D1 receptors, linking the detrimental role of dopamine (DA) to a D1-mediated process. To determine whether the changes in DA receptors are due to alteration in mRNA synthesis, we evaluated the effects of ischemia on striatal DA D-1 and D-2 receptor gene expression by in situ hybridization. Animals were subjected to 20 min of 2-vessel occlusion combined with hypotension, followed by 24h (n=6) or 7 days (n=6) of reperfusion. Adjacent sections were probed for D-1 and D-2 receptor mRNA using ^{35}S-riboprobes. Negative and positive controls were included using sense and antisense probes, and regional hybridization signals were analyzed autoradiographically. In the dorsolateral striatum, reductions in D1 (% reduction from controls±SD; 62%±39, $p<0.01$ by ANOVA) and D2 (63%±20, $p<0.01$) mRNA were detected following 24h of reperfusion. After 7 days of recirculation, D1 mRNA remained low (73%±24, $p<0.01$) while D2 mRNA normalized. In the medioventral striatum, a 29%±18 and 37%±8 ($p<0.01$) reduction in D1 mRNA were observed at 24h and 7 days of reperfusion, respectively. D2 mRNA demonstrated a 43%±13 ($p<0.01$) reduction at 24h, while no significant changes were observed 7 days following ischemia. These results indicate that ischemia leads to a sustained inhibition of striatal D1 gene expression, while only a transient decrease in D2 mRNA was observed. These results could provide an explanation for the selective loss of D1 receptor observed following ischemia.

391.19

WORSENING OF FOCAL CEREBRAL ISCHEMIC DAMAGE IN TRANSGENIC MICE OVEREXPRESSING AMYLOID PRECURSOR PROTEIN F. Zhang[1]*, K.K. Hsiao[1], L. Mucke[2] and C. Iadecola[1], [1]Department of Neurology, Univ. of Minnesota, Minneapolis, MN 55455; [2]Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037

Cerebral ischemia may activate the amyloidogenic pathway for processing of amyloid precursor protein (APP), resulting in accumulation of the neurotoxic Aß peptide (J. Biol. Chem. 269, 15253, 1994). In this study we used transgenic (Tg) mice expressing APP to determine whether excess APP, and presumably Aß, can influence the outcome of cerebral ischemia. The middle cerebral artery was occluded in Tg C57B16xSJL (B6xSJL) mice expressing human APP_{751} ($HuAPP_{751}$) and in Tg FVB/N mice overexpressing $HuAPP_{695}$. Both APP transgenes contained a mutation (V717I) associated with familial Alzheimer's disease. One day after ischemia, infarct volume was determined in thionin-stained sections by computer-assisted planimetry. In non-Tg FVB/N (n=16) and B6xSJL (n=9) mice neocortical infarct volume was 27.6±2.0 and 24.7±1.5mm^3, respectively. Cortical infarct volume increased by 43% in $HuAPP_{751}$ (35.2±2.5mm^3; n=9; $p<0.05$) and by 30% in $HuAPP_{695}$ (36.0±3.0mm^3; n=7; $p<0.05$). The larger infarct volume in APP transgenics was not due to an increase in ischemic edema because tissue swelling, measured by the method of Lin et al. (Stroke 24, 117, 1993), did not differ between Tg and non-Tg mice. Thus APP Tg mice have larger infarcts than non-Tg controls. Although we did not rule out the possibility that the greater damage is due to transgene-dependent vascular factors resulting in more severe ischemia, the data provide evidence that expression of APP worsens the outcome of focal cerebral ischemia. These findings suggest the possibility that cerebral ischemia induces alternative processing of APP resulting in increased levels of Aß, a peptide that may worsen brain damage by impairing calcium homeostasis in the ischemic penumbra.

(Supported by AHA and NIH)

ISCHEMIA: GLUTAMATE

392.1

NMDA CHANNEL ACTIVITIES IN RAT CA1 HIPPOCAMPAL NEURONS FOLLOWING A BRIEF HYPOXIC-HYPOGLYCAEMIC CHALLENGE IN BRAIN SLICES. L Zhang*, P Miu & JH Eubanks, Playfair Neuroscience Unit, Toronto Hospital, Departments of Medicine (Neurology) and Surgery (Neurosurgery), Bloorview Epilepsy Program, University of Toronto, 399 Bathurst Street, Toronto, Canada M5T 2S8.

We have demonstrated that a brief hypoxic-hypoglycaemic (H-H) challenge in brain slices causes an up-regulation of NMDA NR2C gene expression in hippocampus and cerebral cortex. In the present study, we examined the possible functional correlation of NR2C gene induction in H-H challenged CA1 neurons. Slices were exposed for 3 min to an external medium which contained 10 mM sucrose rather than glucose and was aerated with 95% N_2-5% CO_2 rather than 95% O_2-5% CO_2. In control slices, which were maintained at 34°C for more than 12 hours, CA1 neurons showed synaptic field potentials and basic membrane properties comparable to those previously reported, with the exception of a decreased sAHP. Recorded in the cell-attached mode, NMDA channel activities in these neurons were dominated by a main conductance level of 60-70 pS. In slices examined at 9-12 hours following the H-H challenge, CA1 neurons exhibited decreased synaptic filed potentials, but no apparent change in their membrane properties in comparison with those in control slices. Interestingly, in these challenged neurons, we consistently observed a population of subconductance opening in the range of 35-40 pS. The appearance of this subconductance level could be abolished by removing Ca^{2+} from the recording pipette, thereby implicating dependency on external Ca^{2+}.

We hypothesize that the subconductance level may reflect the functional expression of newly induced NMDA channels following the H-H. Supported by the MRC and Heart and Stroke Foundation of Canada.

392.2

BOTH APOPTOSIS AND EXCITOTOXIC NECROSIS CONTRIBUTE TO INFARCTION INDUCED BY FOCAL CEREBRAL ISCHEMIA. C. Du*, R. Hu, C.A. Csernansky, X.Z. Liu, C.Y. Hsu and D.W. Choi.. Dept. of Neurology and Center for the Study of Nervous System Injury, Washington Univ. School of Medicine, St. Louis, MO 63110.

The temporal evolution of cerebral infarction was examined in rats subjected to transient occlusion of both common carotid arteries and the right middle cerebral artery. Consistent with other studies, the extent of tissue infarction after severe (90 min) ischemia was predictable after 6 hr and fully developed after 1 day (compared with 14 d). After mild (30 min) ischemia, no cortical infarction or histological evidence of injury was present by 1 d. However, modest infarction was evident after 3 d, and by 14 d infarction volume was comparable to that induced by the 90 min insult. 3 d after such mild ischemia, TUNEL staining and DNA gel electrophoresis revealed prominent evidence of internucleosomal DNA fragmentation in neurons destined to die; furthermore, progression to infarction at 14 d could be attenuated by pretreatment with 1 mg/kg i.p. cycloheximide.

In addition, combination treatment with the NMDA antagonist, dextrorphan, and cycloheximide produced an additive protective effects against infarction induced by severe transient ischemia. Administration of either 30 mg/kg dextrorphan or 0.5 mg/kg cycloheximide, i.p. 15 min before 90 min ischemia, reduced acute infarct volume by about 65%. When effective concentrations of both drugs were given, infarct volume was reduced by 87% as measured 14 d later. Taken together, present observations support the idea that infarction induced by transient focal ischemia occurs both by excitotoxicity, and by apoptosis dependent on new protein synthesis. Combined therapies directed at both mechanisms may be more effective than therapies directed at either mechanism alone. Supported by NIH NINDS grant NS 32636 (DWC).

392.3

MODULATION OF NMDA-MEDIATED EXCITOTOXICITY BY GABA UPTAKE INHIBITORS OR BENZODIAZEPINES IN CORTICAL CELL CULTURE. J.K. Muir* and D.W. Choi. Dept. of Neurology and the Center for the Study of Nervous System Injury, Washington Univ. Sch. of Med., St. Louis, MO 63110.

We have previously reported that the $GABA_A$ agonist, muscimol, attenuated excitotoxic neuronal death, but exacerbated the neuronal death induced by oxygen-glucose deprivation (Soc. Neurosci. Abstr. 20:182, 1994). Here we examined whether these observations would extend logically to GABA uptake inhibitor and benzodiazepine drugs.

Murine cortical cell cultures containing both neurons and glia were exposed for 24 hr to NMDA (15 µM), AMPA (10 µM) or kainate (40 µM) with or without drugs of interest. The GABA uptake inhibitors, guvacine or nipecotic acid (10-1000 µM), both attenuated the neuronal death induced by 24 hr exposure to NMDA, but not AMPA or kainate. These drugs are not acting as NMDA receptor antagonists since they do not alter ^{45}calcium uptake stimulated by a 5 min exposure to 200 µM NMDA. The benzodiazepine chlordiazepoxide (1-100 µM) alone had no effect on neuronal injury induced by 5 min exposure to 100-300 µM NMDA, but potentiated the protective effect of 10 µM muscimol in the same paradigm. Both GABA uptake inhibitors and chlordiazepoxide potentiated the neuronal death induced by a 40-55 min period of oxygen-glucose deprivation. These observations add further support to the idea that activation of $GABA_A$ receptors may be paradoxically harmful to hypoxic cortical neurons, despite anti-excitotoxic effects on normoxic neurons.

Supported by NIH NINDS grant NS 32636 (DWC).

392.4

EFFECTS OF MK 801, 7-CHLOROKYNURENIC ACID AND GYKI 52466 ON STRIATAL DOPAMINE RELEASE DURING ISCHAEMIA. C.C. Toner & J.A. Stamford*, *Anaesthetics Unit (Neurotransmission Laboratory), London Hospital Medical College, London E11BB, UK.*

Massive release of neurotransmitters, including dopamine (DA), is thought to play a central role in neuronal death during acute cerebral ischaemia. Since DA is under presynaptic glutamatergic control, we investigated the effects of MK 801, 7-chlorokynurenic acid (7-CK) and GYKI 52466, antagonists at the NMDA phencyclidine binding site, glycine site and AMPA receptor respectively. Rat striatal slices were superfused (400ml/h) with artificial cerebrospinal fluid (ACSF) at 34°C. "Ischaemia" was mimicked by removal of O_2 and reduction in glucose concentration from 4 to 2mM. The latency (T_{on}), rate ($\Delta_{[DA]}$) and maximum level of DA release (DA_{max}) were monitored by fast cyclic voltammetry. Control (means ± s.e.m, n=8) values were 156±5s (T_{on}), 8.3±2.2µM/s ($\Delta_{[DA]}$) and 84±12µM (DA_{max}). MK 801 (10µM) significantly prolonged T_{on} (to 197±10s, $P<0.01$), reduced $\Delta_{[DA]}$ (to 1.6±0.4µM/s, $P<0.05$) and decreased DA_{max} (to 60±7µM, $P<0.05$), (n=8). 7-CK (50µM) and GYKI 52466 (25µM) had no effect on any parameter. These data suggest that NMDA but not AMPA receptors are involved in ischaemia-induced DA release and, surprisingly, that the glycine site does not appear to modulate NMDA receptor function in this model.

392.5

KETAMINE ANTAGONIZES HYPOXIA-INDUCED DOPAMINE RELEASE IN RAT STRIATUM. Y. Wang*, A.L.Chiou, J.C. Lin. Dept. of Pharmacology, Nat'l Defense Med. Ctr., Taipei, Taiwan 100.

The purpose of this study is to investigate the hypothesis that ketamine, a non-competitive antagonist of the NMDA receptor, attenuates hypoxia-induced striatal dopamine release *in vivo*. High-speed chronoamperometric recording techniques, using Nafion-coated carbon fiber electrodes, were used to evaluate extracellular dopamine (DA) concentration in the striatum. KCl and DA were locally applied directly to the striatum of urethane-anesthetized Sprague-Dawley rats. These anesthetized animals were paralyzed with d-tubocurarine and connected to a respirator to allow controlled respiration. Systemic concentrations of oxygen and carbon dioxide were altered by changing the partial pressure of O_2, CO_2, N_2 of inspired air and the rate of the respirator. Our data indicates that lowering the respiratory rate from 90 to 20 times/min for 5 minutes, in room air, caused a decrease in blood O_2 while increasing the CO_2 concentration. We also found that lowering the respiratory rates potentiated K^+-induced DA release but not DA clearance in the striatum. In an attempt to induce hypercapnia, the room air was replaced with high CO_2 -containing air, and this change resulted in increased blood CO_2 levels without lowering O_2 concentration. The hypercapnia did not alter K^+-induced DA release in the striatum. Next, we attempted to simulate anoxic hypoxia in the absence of hypercapnia. Respiration with pure N_2 for 30 sec resulted in lowering blood O_2 without increasing CO_2 levels. Both basal and K^+-evoked DA release were increased during N_2 -induced anoxic hypoxia, suggesting that transient hypoxia facilitates DA release in the striatum. We also found that systemic application of ketamine antagonized hypoxia-induced electrochemical responses. These data suggest that the increase in DA release *in vivo* during short term hypoxia may probably be mediated through NMDA receptors.

392.6

NEUROPROTECTIVE EFFECT OF DEXTROMETHORPHAN FOLLOWING TRANSIENT, BUT NOT PERMANENT, FOCAL CEREBRAL ISCHEMIA IN THE RAT. P. Britton, X.-C.M. Lu, [1]D. Lloyd, [1]S. Gribben, [1]J. Loats, and F. Tortella*. Walter Reed Army Inst. Res., Washington, DC 20307, [1]Loats Associates, Westminster, MD 21158.

Dextromethorphan (DM) has been observed to afford neuroprotection in a variety of *in vitro* and *in vivo* experimental models of CNS injury. In the present study we have evaluated the neuroprotective activity of DM following both transient and permanent focal cerebral ischemia in the rat. Middle cerebral artery occlusion (MCAO) was achieved by advancing a 3-0 monofilament nylon suture up the internal carotid artery of male Sprague-Dawley rats (270-330 g, n=4-7/group). In rats subjected to transient focal ischemia, the endovascular suture was retracted to allow reperfusion 2 hours after MCAO. Body temperature was maintained normothermic throughout stroke surgery and reperfusion procedures. Animals were dosed s.c with 20 mg/kg DM at 0.5, 1, 2, 4, and 6 hours post occlusion. Following 24 hours of permanent occlusion or reperfusion, animals were sacrificed and their brains removed for analysis of infarct volume using TTC staining and computer-assisted image analysis. As a result of transient MCAO, vehicle treated rats exhibited a total infarct volume of 203.3 ± 32.6 mm^3. DM treatment produced a 61% reduction in infarct volume to 78.7 ± 12.8 mm^3. In contrast, permanent MCAO not only produced a larger infarct volume (406.3 ± 43.5 mm^3) when compared to corresponding transient MCAO values, but also one which was not significantly reduced in size by treatment with DM (313.8 ± 58 mm^3). Infarcted hemispheric oedema was not different in vehicle treated rats following transient or permanent MCAO and was not significantly reduced by DM in either group. In summary, the reduction in infarcted tissue volume produced by treatment with DM in our model of transient focal cerebral ischemia provides further support for the *in vivo* neuroprotective activity of this compound. Importantly, the severity of injury resulting from permanent MCAO may ultimately underlie the lack of protective effect of DM, thereby questioning the ability of this model to effectively evaluate the activity of putative neuroprotectants.

392.7

CHARACTERIZATION OF MORPHINANS HIGHLY PROTECTIVE IN PERMANENT MIDDLE CEREBRAL ARTERY OCCLUSION (MCAO) IN RATS. G. Fischer, A. Bourson, J.A. Kemp*, H.P. Lorez, V. Mutel and G. Trube. Preclinical Research, F. Hoffmann La-Roche, 4002 Basel, Switzerland

A series of dextrorotary morphinans was tested in 3H-MK 801 binding with rat brain membranes. They showed different affinities for the NMDA receptor open-channel blocker binding site and were further characterized *in vitro* with protection of cultured rat cortical neurons against glutamate toxicity and electrophysiology on recombinant NMDA receptors expressed in Xenopus oocytes. *In vivo* tests included seizures in mice (audiogenic seizures, i.c.v. NMDA-induced convulsions) as well as permanent focal ischemia (MCAO) in rats. A high correlation was found between affinity in binding experiments and protection against glutamate toxicity as well as blockade of NMDA-induced currents. However, although anticonvulsant effects were seen at comparable doses for all compounds, only the most potent ones were found to be neuroprotective in MCAO given post-occlusion. Ro 24-6173 was neuroprotective at a dose that had only slight effects on motorbehaviour and was without cardiovascular or vacuole-inducing effects in cingulate-retrosplenial cortex.

392.8

EFFECT OF IFENOPRODIL ON INFARCT SIZE, BLOOD-BRAIN BARRIER BREAKDOWN, AND EDEMA FORMATION AFTER FOCAL CEREBRAL ISCHEMIA IN THE CAT. M. K. Başkaya, A. M. Rao, D. Donaldson, M. R. Prasad, R. J. Dempsey*. Division of Neurosurgery, Department of Surgery, University of Kentucky Medical Center, Lexington, Kentucky, 40536-0084.

The effect of the glutamate N-methyl-D-aspartate (NMDA) receptor antagonist Ifenoprodil in reducing infarct area has been examined in anesthetized cats, with drug (n=7) or saline (n=7) treatment being initiated 5 min after the induction of cerebral ischemia. Focal ischemia was produced by permanent occlusion of right middle cerebral artery, and animals were sacrificed 6 h later. Local cerebral blood flows in ischemic, penumbra, and contralateral brain regions were measured during the experimental procedure. Ifenoprodil reduced the total area of infarct (51.7±3.9 vs 29.6±7.3). Infarct area was expressed as a percentage of right hemisphere area (mean±SE). Although Ifenoprodil decreased the brain edema (1.028±0.02 vs 1.035±0.02), it attenuated the Evans blue extravasation, an indicator of blood-brain barrier breakdown, in only one brain section, but not in all brain sections. There was no significant difference in cerebral blood flow measurements between control and drug treated groups. Results from this study suggest that Ifenoprodil has a tissue sparing effects and this is not related to its vasodilatory or vasoconstrictor effects. It is likely that its neuroprotective effect may be related to its ability to antagonize NMDA receptors.

392.9

CNS 1102 PROTECTS AGAINST BRAIN DAMAGE DUE TO HYPOXIC ISCHEMIA (HI) IN THE NEONATAL RAT. S. Wang, D. Zhou*, J. B. Fischer, A. G. Knapp and W. F. Holt. Pharmacology Department, Cambridge NeuroScience, Inc., Cambridge, MA 02139.

Perinatal asphyxia/ischemia is a frequent cause of neurological disorders in human neonates. Such deficits incurred during birth may produce permanent brain damage. Presently, there is no effective treatment for perinatal ischemia. The excitatory amino acid glutamate has been postulated to be an important mediator of focal ischemic brain damage in adult mammals. This suggested a possible role for glutamate in promoting ischemic damage in neonatal mammals, particularly since NMDA receptor density is at its zenith in neonates. Thus we evaluated a neuroprotective role for the noncompetitive NMDA antagonist, CNS 1102 (currently in Phase II clinical trials for traumatic brain injury and stroke) in a neonatal rat model of hypoxic ischemia (HI)-induced brain damage. The left common carotid artery of 7-8 day old rats was ligated, and the animals were then exposed to 7.8% O_2 for 1.5 hours in an incubator at 37°C. Two days later the animals were sacrificed, and their brain removed and cut into four 2 mm thick slices prior to staining with TTC (triphenyltetrazolium chloride). Image analysis of brain slices indicated that HI caused measurable infarction (Mean±SEM =18.4±3.3 mm^3) in approximately 85% of the 22 control animal brains evaluated. Treatment of rat pups with CNS 1102 by intraperitoneal injection immediately before and after HI resulted in about 40% reduction in the incidence of infarction ($p<0.05$) at a 2 X 3 µmol/kg drug dose (i.e. 2 X ~1 mg/kg; n=21) and approximately 90% reduction ($p<0.001$) at 2 X 10 µmol/kg drug dose (n=21). Infarcts were observed in only 52% and 10% of the HI rat pups treated with CNS 1102 at 3 and 10 µmol/kg, respectively. The results suggest that glutamate mediates HI-induced brain damage in neonatal rats and that the NMDA ion-channel blocker CNS 1102 can protect against such lesions.

392.10

PROTECTION OF RODENTS AGAINST GLOBAL ISCHEMIA BY FPL 15896AR. E.F. Cregan, A.S. Howell, M.L. Stagnitto, R.J. Murray, J.A. Miller* and G.C. Palmer. Fisons Pharmaceuticals, Box 1710, Rochester, NY 14603.

FPL 15896AR[+] exhibits moderate uncompetitive antagonism of the NMDA receptor (IC_{50} to displace MK801 binding - 1.3 µM), as well as sigma-1 receptor affinity (IC_{50} to displace [+]pentazocine binding = 4.5 µM). Moreover, FPL 15896AR possesses antiepileptic activity (Knowles et al., FASEB J. 9:A394, 1995) and reduces infarct damage in rodent models of focal ischemia (Murray et al., Med. Chem. Soc. 209: MEDI 074, 1995). In the present study FPL 15896AR treatment prolonged survival of rodents exposed to hypoxia: The ED_{50}s (mg/kg) to extend survival in mice were: i.v. = 21; p.o. = 36.7. FPL 15896AR (24 mg/kg) likewise extended the time to loss of righting reflex and survival in rats. When rats were subjected to a period of 20 min of global ischemia (4-vessel occlusion technique) followed by dosing of FPL 15896AR (24 mg/kg, po at reflow and b.i.d. for 7 days), CA1 neuronal damage along the septotemporal hippocampus axis was significantly reduced. Experiments are in progress to evaluate efficacy of the compound in other global models as well as the MCA suture occlusion model of focal ischemia.

392.11

HU-211, A NOVEL NON-COMPETITIVE NMDA ANTAGONIST, REDUCES FOCAL ISCHEMIC BRAIN DAMAGE IN THE RATS.

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HU-211 is a non-psychotropic cannabinoid analog which has been shown to act as a functional N-methyl-D-aspartate (NMDA) receptor blocker. We have investigated the neuroprotective efficacy of HU-211 following transient middle cerebral artery occlusion (MCAo) in rats.We modified the method of Zea Longa et al., 1989 to study focal cerebral ischemia.

Male Wistar rats were anesthetized with halothane and subjected to 90 min of temporary MCAo by retrograde insertion of an intraluminal nylon suture coated by polylysine (polycationic polymer),through the external carotid artery into the internal carotid artery and MCA. The drug (HU-211 in cosolvent, 4 mg/kg, i.v.) or vehicle was administered in a blinded fashion 70 min after onset of MCAo. The neurological status was evaluated during occlusion (60 min) and 3 days after MCAo. Three days after MCAo, the rats were perfusion-fixed and infarct volumes were determined.

HU-211 significantly improved the neurological score compared to vehicle 3 days after MCAo (1.71±0.28 and 4.07±0.47 respectively). Treatment with HU-211 significantly reduced infarct size (55.52±10.01 vs. 121.58±28.9 mm^2) and brain edema (2.61±1.33 vs. 6.66±1.24 %) compared to vehicle rats.

These results demonstrate the neuroprotective ability of HU-211 against focal cerebral ischemia as judged by neurological score, infarct size and brain edema. We have found also that the modified technique of MCAo is reliable, effective and produced consistent results.

392.12

NEUROPROTECTIVE EFFECTS OF A NOVEL NMDA GLYCINE SITE ANTAGONIST, SM-18400, IN THE RAT MODEL OF CEREBRAL ISCHEMIA.

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SM-18400((S)-9-chloro-5-[p-aminomethyl-o-(carboxy-methoxy) phenyl-carbamoylmethyl]-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione hydrochloride trihydrate) has high affinity and selectivity for NMDA receptor associated glycine sites in vitro. In the current study, we investigated the neuroprotective effects of SM-18400 in both a global and focal ischemia model in rats. In the global ischemic model, SHRs were subjected to a permanent occlusion of the bilateral common carotid artery. SM-18400 was injected i.v. immediately and 2 hrs after the occlusion. The results showed that the ischemic insult produced severe brain edema and death. SM-18400 inhibited edema as measured by the reduction of water content in the forebrain when measured 3 hrs after the occlusion. SM-18400 also improved the survival rate at 24 hrs after the occlusion. In the focal ischemic model, SD rats received a transient left middle cerebral artery occlusion by the insertion of a nylon suture in the proximal anterior cerebral artery simultaneously with a permanent occlusion of the left common carotid artery. Starting 1 hr after the occlusion, SM-18400 was administered i.v. (bolus + infusion) for 25 hrs. SM-18400 dose-dependently reduced the infarction volume in the cerebral hemisphere. These findings show that the novel glycine antagonist, SM-18400, has protective effects against both global and focal ischemic brain injury. The present study strongly suggests a high therapeutic potential of SM-18400 in the treatment of stroke.

392.13

A MECHANISM FOR ISCHEMIC DELAYED NEURONAL DEATH BASED ON ALTERED METABOTROPIC GLUTAMATE RECEPTOR (mGluR) TRANSDUCTION PATHWAYS AND APOPTOSIS.

LJ Martin*, FE Sieber and RJ Traystman. Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

Cerebral ischemia causes selective delayed neuronal death (DND). We propose a cascade for DND in which postischemic alterations in signal transduction pathways that modulate intracellular Ca^{2+} predispose neurons to apoptosis. Using a global incomplete cerebral ischemia model, we tested the hypothesis that DND has characteristics of apoptosis and that ischemia promotes in neurons that undergo DND an aberrant expression of mGluRs and their molecular effectors. Compression ischemia was induced (20 min) in dogs (n=11), decreasing cerebral blood flow by 80%. Nonischemic dogs (n=4) served as controls. After 7 days recovery, brains were prepared for neuropathology, TUNEL, immunocytochemistry (ICC) and histochemistry (ischemia, n=9; control, n=2) or for immunoblotting (ischemia, n=3; control, n=2). Cerebellum was used as a regional model for DND. Purkinje cells (70 ± 7% [M ± SE]) showed evidence for DND. By TUNEL, Purkinje and granule cells undergo nuclear DNA fragmentation postischemia, and ICC showed that expression of the apoptotic protein Bax is upregulated postischemia in these neurons. By ICC, mGluR1α and phospholipase Cβ (PLCβ) expression is increased relative to controls. Granule cells which do not express mGluR1 in controls are enriched in mGluR1 postischemia. Areas with increased mGluR/PLC expression showed elevated Ca^{2+}-ATPase activity. Brain regions nonvulnerable to DND did not show increased mGluR1α/PLCβ expression. Immunoblots confirmed that expression of Bax and membrane fraction mGluR and PLCβ was elevated relative to controls. We conclude that selective DND is associated with postischemic changes in mGluR-coupled transduction mechanisms that regulate intracellular Ca^{2+} and that abnormalities in these pathways may precondition neurons for apoptosis.

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392.14

NEONATAL ASPHYXIC BRAIN DAMAGE IS SYSTEM PREFERENTIAL.

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We used a piglet model of asphyxic cardiac arrest to test the hypothesis that neonatal brain shows system selective vulnerability to hypoxia-ischemia that is related to regional metabolism and ameliorated by AMPA receptor blockade with NBQX. Piglets (1-week-old) were exposed to 30 min hypoxia (arterial O_2 saturation 30%), 5 min room air (O_2 saturation 65%), and 7 min airway occlusion (O_2 saturation 5%). NBQX (n=10) or vehicle (n=10) was given (30 mg/kg,iv) before airway occlusion and during early recovery (15 mg/kg). Shams received (n=2) or did not receive (n=5) NBQX. Most asphyxic piglets seized after 24 h and received diazepam. After 4 days, brains were prepared for neuropathology, morphometry, and histochemical assays for cytochrome oxidase (CO) and Na^+,K^+ ATPase activities to evaluate regional damage and metabolic abnormalities. Asphyxic-vehicle and -NBQX piglets had 30% and 70% reductions in viable parietal cortical neurons, respectively. Neuron loss in hippocampus, not differing among asphyxic cohorts, was 10% (CA1) and 50% (CA3). Injury distribution was selective in asphyxic-vehicle piglets, with damage to somatosensory cortex and thalamus, putamen, and nigra reticulata. In asphyxic-NBQX piglets, damage was augmented and expanded into additional regions. In shams, CO and ATPase were higher (ca. 30%) in vulnerable regions relative to less vulnerable regions. Postasphyxic changes in CO and ATPase were regional, with reductions (40-50%) in vulnerable regions but increases (30-40%) in less vulnerable areas. NBQX worsened postasphyxic metabolic deficits. This neonatal asphyxic encephalopathy is consistent with combined hypoxic-ischemic and seizure-related insults that preferentially damage somatosensory systems in which regional metabolism and connectivity are possible determinants. Asphyxic injury is potentiated by AMPA receptor blockade which may alter adaptive synaptic and metabolic plasticity. (Supported by NS20020)

392.15

AMPA PARTIALLY PROTECTS THE CEREBRAL CORTEX FROM BRIEF LOCAL ISCHEMIA.

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Topically applied NMDA has been shown to abolish cortical neuronal electrical activity. This effect of NMDA is prevented by a preceding application of AMPA. Since activation of NMDA receptors plays an important role in ischemia-induced neuronal damage, we set out to establish whether AMPA could reduce this damage. Male Sprague Dawley rats were anesthetised with urethane. Cerebral ischemia was induced by ligation of one common carotid artery followed by clipping of the ipsilateral middle cerebral artery (MCA) for 5-10 mins. The forepaw was stimulated and the somatosensory evoked potentials (SEPs) recorded. MCA occlusion caused a gradual decrease in the amplitude of the SEPs. The time taken (T) for the eventual abolition of the SEPs was used as an estimate of the effects of compounds applied to the cortex. Topical application of AMPA (25-100 μM) for 10 mins prior to and during MCA occlusion caused a slight increase in the amplitude of the SEPs, and increased the time taken for the response to be abolished. This effect occurred in a dose dependent manner. AMPA at 25μM, 50μM and 100μM produced T values of 129±14s (n=7), 154±12s (n=13) and 217±32s (n=9) [mean±sem]; compared with control values of 99±12s, 116±10s and 121±15s respectively. A Wilcoxon test showed the effects of AMPA at 50μM and 100μM to be significant ($p<0.01$). The effects of 50μM AMPA were prevented by a preceding application of CNQX (10μM). Higher concentrations of AMPA abolished the SEPs. In comparison, MK801 (4mg/Kg i.p) totally prevented the abolition of SEPs by the ischemia (n=5). The protective effect of AMPA may be due to desensitization of the receptors.

392.16

METABOTROPIC-GLUTAMATE RECEPTOR ACTIVATION DOES NOT CAUSE ANOXIC HYPERPOLARIZATION OF HIPPOCAMPAL NEURONS

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Brief anoxia (2-3 min) induces a characteristic G_K-mediated hyperpolarization and conductance increase in CA1 neurons. The depression of the anoxic response by internal EGTA (Erdemli and Krnjević (1994) Proc. Can. Fed. Biol. Soc. p:123) and by some blockers of internal Ca^{2+} release (Belousov et al J.Physiol. 1995 in press) indicated that internal Ca^{2+} release is a major element in the anoxic response. In this study, we tested an agonist (t-ACPD) and antagonists (MCPG and L-AP3) of metabotropic glutamate receptors (mGLUR) on anoxic responses of CA1 neurons recorded with patch electrodes filled with $KMeSO_4$, in slices kept at 33°. In 6 cells clamped at -45 mV, t-ACPD (50 μM) caused a decrease in input conductance (G_N, by 31.4±6.9 %) and an inward shift in holding current (ΔI_H=-72±20 pA). In these cells, 8 anoxic trials (2 min) raised G_N by 8.4±1.5 nS and shifted I_H outward by 281±32 pA; and t-ACPD had no significant effect on anoxic ΔG_N (6.2±1.4 nS) and ΔI_H (254±57 pA). In another 4 cells, MCPG (0.5 mM) was also ineffective: the anoxic changes were 105±65.2 % for ΔG_N and 273±27 pA for ΔI_H in control runs and 130±102 % and 155±117 pA in the presence of MCPG. L-AP3 (1 mM) also had no effect on anoxic responses: in control runs, anoxic G_N increased by 106±65.2 % and ΔI_H was 273±27 pA; in the presence of L-AP3, the corresponding ΔG_N was 130.5±10.1 % and ΔI_H=155±116.9 pA.

These preliminary results suggest that Ca^{2+} release from mGLUR-activated internal stores is not responsible for the hyperpolarization of CA1 neurons induced by brief anoxia.

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392.17

ONSET AND DURATION OF OCCLUSION EVOKED GLU AND DA RELEASE IN THE RAT BRAIN USING THE REAL-TIME MONITORING METHOD. Y. Watanabe[1], H. Uchino[2], A. Ishiki[2], T. Nishimura[3], T. Nishimori[3] and T. Shibuya[1,3]. 1) Dept of Phramacology, Tokyo Medical College, Tokyo 160, Japan. 2) Dept of Anesthesiology, Tokyo Medical College, Tokyo 160, Japan. 3) Environmental Biological Life Science Research Center Inc., Shiga 528, Japan. 4) Dept of Biomed Sci, Sch of Med, Univ of IL at Rockford, U.S.A.

It has been documented that the tolerance of neuronal change induced by repeated occlusion is seen in the post-synaptic neuron. The changes in the pre-synaptic neuron, however, are still unknown. In this experiment, we measured the onset and duration of the increases of glutamic acid (Glu) and dopamine (DA) levels in the rat caudate putamen after ischemia, using the microdialysis biosensor (for Glu) and fast cyclic voltammetry (for DA). Two types of ischemic animal models were used. One was the two vessel occlusion plus exsanguination treated normoglycemic model, and the other was the streptozotocine induced hyperglycemic ischemia model. In the normoglycemic ischemia model, the levels of Glu and DA were raised within one minute of the first occlusion, and these levels increased during ischemia. In addition, unlike the previous reports, the increases of Glu and DA levels evoked by ischemia were not transient. The ischemia evoked Glu release was much faster than DA release. The magnitude of these increases seen in normoglycemia was much less than those seen in hyperglycemia. After re-perfusion, the inflated levels of both transmitters were gradually decreased. Moreover, repeated occlusion delayed the onset of transmitter release, although the total quantity of neurotransmitters remained unchanged. These results suggest that neuronal activities in pre-synaptic neurons are influenced by repeated occlusion, and that serum glucose levels are related to the resultant quantity of Glu and DA release.

ISCHEMIA: NITRIC OXIDE AND OTHER TRANSMITTERS

393.1

SPONTANEOUSLY HYPERTENSIVE RATS HAVE DECREASED CEREBRAL NITRIC OXIDE ASSAYED BY ELECTRON SPIN RESONANCE. Neal J. Sondheimer , Mark E. Mullins, Yanming F. Wang*, and Stuart A. Lipton. Dept. of Neurology, Children's Hospital, Prog. in Neurosci., Harvard Med. Schl., Boston, MA 02115; Dept. of Chemistry, Harvard Univ., Cambridge, MA 02138.

Electron spin resonance (ESR) spectroscopy was used to monitor nitric oxide ($NO^{\bullet}$) levels in rodent cerebral cortex under various conditions. Male spontaneously hypertensive rats (SHR, n = 4) weighing 300-320 g or control rats [Wistar-Kyoto (WKY) or Sprague-Dawley (SD), n = 16], were injected parenterally with the $NO^{\bullet}$-specific spin trap, iron/diethyldithiocarbamate [Fe(II)/DETC] under chloral hydrate sedation. Within 30 min, a filament was threaded into the lumen of the middle cerebral artery to produce transient occlusion (Longa et al., *Stroke* 1989), and the animals were sacrificed 10 min later. Cortical tissue was then harvested and analyzed by ESR for $NO^{\bullet}$; basal levels of $NO^{\bullet}$ were determined on unoperated rats 40 min after Fe(II)/DETC injection. We found that basal $NO^{\bullet}$ production in SHR was dramatically lower than in the cortex of WKY or SD rats ($P < 0.05$ by ANOVA). We also observed that the induction of focal ischemia in SHR (n = 6) did not significantly elevate $NO^{\bullet}$, whereas SD rats displayed >100% increase in $NO^{\bullet}$ compared with basal levels (n = 12). Brain nitric oxide synthase activity is reportedly normal in SHR (Clavier et al., *Stroke* 1994). However, it has been hypothesized that there is increased activity of superoxide anion ($O_2^{\bullet-}$) in SHR (Nakazono et al., *PNAS* 1991). In theory, this could account for the decrement in $NO^{\bullet}$ since $O_2^{\bullet-}$ and $NO^{\bullet}$ react to form peroxynitrite ($ONOO^-$). This reaction would decrease $NO^{\bullet}$ and potentially contribute to neuronal damage in these animals.

393.2

INDUCTION OF NEURONAL NITRIC OXIDE FOLLOWING HYPOTHERMIC CIRCULATORY ARREST IN A CANINE SURVIVAL MODEL M.V.Brock, C.J. Lowenstein, M.A. Lange, M.V. Johnston*, W.A. Baumgartner, M.E. Blue. The Johns Hopkins Medical Institutions, Baltimore, MD 21287

Permanent neurological injuries after hypothermic circulatory arrest (HCA) are problems in cardiac surgery occurring in approximately 12% of children and 15% of adults. The cause of this neurotoxicity is unknown and there is no treatment.

We have developed a canine survival model of HCA that closely simulates clinical practice. We have previously shown that this model produces a consistent neurologic deficit and histopathological pattern of selective neuronal death involving neurotransmitter excitotoxicity and changes in glutamate specific receptors. Adult male hound dogs (n=12) were exsanguinated using cardiopulmonary bypass (CPB) and subjected to 2 hours of HCA at a brain temperature of 18°C. The animals were then slowly reperfused to normothermia, separated from CPB, survived for 6, 18 and 72 hours, and compared with controls (n=6) who did not undergo HCA.

In canine striatal homogenates, the expression of neuronal nitric oxide synthase (nNOS) increases significantly at 6 and 18 hours after reperfusion (340% and 500% respectively; $p<0.05$), but returns to baseline by 72 hours. Concomitant with this increased enzymatic activity is a 7-fold accumulation of nitric oxide (NO) metabolites in the serum ($p<0.05$) 4 hours after reperfusion which also returns to baseline by 3 days. Furthermore, immunocytochemical staining with nNOS specific monoclonal antibody (Transduction Labs) reveals marked augmentation of nNOS expression 6-18 hours after HCA that is attenuated by 72 hours. Dark field analysis demonstrates localization of nNOS to neuronal processes with the development of a widespread, dense plexus of these NOS fibers throughout the neocortex 6-18 hours after HCA.

We conclude that excitatory neurotransmitter mediated neuronal death during HCA involves a significant increase in nNOS expression in neuronal processes in the brain and that this leads to widespread augmented NO production. Supported by NIH-RO1NS 21238

393.3

THE ROLE OF ATP AND NITRIC OXIDE IN HYPOXIA-INDUCED POTENTIATION IN THE RAT HIPPOCAMPUS. M. Lyubkin, D. M. Durand, M. A. Haxhiu, K. E. Ward, T. S. Whittingham*. Departments of Biomedical Engineering, Medicine, Neuroscience, Neurosurgery, Case Western Reserve University, Cleveland, OH 44106.

Recently, it was demonstrated that the pharmacologically isolated N-methyl-D-asparate (NMDA)-mediated responses in the CA1 region of the rat hippocampus were significantly potentiated for at least one hour by a (1-3 min) hypoxic-aglycemic episode. Little is known about the mechanisms responsible for the induction and maintenance of this novel form of potentiation. In the present study, we investigated the role of ATP and nitric oxide (NO) in hypoxia-induced potentiation using extracellular recordings in the CA1 region of the rat hippocampus. Hippocampal slices were taken from Sprague Dawley rats and placed in an interface chamber containing normal ACSF solution. 0%O_2-hypoxia was induced by replacing the 5%CO_2, 95% O_2 gas mixture with 5%CO_2-95%N_2 for 2 min. Significant potentiation of the slope of the dendritic EPSP was found in normal ACSF fluid (69% ±23 SEM, $P<0.01$, n=10). One hour pre-incubation in creatine (25mM) rich ACSF inhibited hypoxia-induced potentiation even in the presence of picrotoxin (10μM). L-arginine, a substrate of NO, also blocked hypoxia-induced potentiation at 50μm but not at 25μm. These data indicate that 1) low levels of ATP are in part responsible for the induction of hypoxia-induced potentiation; 2) creatine blockade of hypoxia-induced potentiation is not mediated by GABA-ergic inhibition and; 3) that nitric oxide may play a concentration-dependent role in the induction and maintenance of hypoxia-induced potentiation.
Supported by NIH grant #HL25830.

393.4

NEITHER L-ARGININE NOR L-NAME ALTER NEUROLOGIC OUTCOME FOLLOWING GLOBAL ISCHEMIA IN CAT J.R. Kirsch, A. Bhardwaj, L.J. Martin, D. Hanley*, R.J. Traystman. Dept. of Anesth. Crit. Care Med. Johns Hopkins Med.Inst. Balt. MD 21287

We tested the hypothesis that inhibition of nitric oxide (NO) synthase with N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME) would improve neurologic outcome and L-arginine (L-ARG) would worsen neurologic outcome following transient global ischemia. During halothane anesthesia cats were mechanically ventilated. A heat lamp was used to maintain esophageal temperature at 38.0±0.5°C in all groups at baseline, during ischemia and the immediate period (30 min) of reperfusion. Cats (n=6 each group) were treated with IV saline, L-NAME (5 mg/kg or 10 mg/kg) or L-ARG (300 mg/kg) 30 min prior to onset of ischemia. Ischemia was produced for 10 min by tightening ligatures around the left subclavian and brachiocephalic arteries with hemorrhagic hypotension to a mean arterial pressure of 50. Time to isoelectric EEG was similar between groups (saline 26±11; L-NAME-5 15±4; L-NAME-10 36±27; L-ARG 22±7 sec). At 30 min of reperfusion cats in the L-ARG group were administered an additional 300 mg/kg IV. After wound closure all cats emerged from anesthesia and were extubated. At 72 hrs reperfusion neurologic deficit score was similar between groups (saline 38±25; L-NAME-5 52±15; L-NAME-10 47±18; L-ARG 40±30). Likewise, severity of neurodegeneration in CA1 hippocampus and cerebellum was similar in all groups. Because L-NAME and L-ARG do not affect neurologic, and possibly neuropathologic, outcomes we conclude that NO does not appear to be important in the mechanism of brain injury following global ischemia. Supported by NS 20020.

393.5

NOS INHIBITION INDUCES BILATERAL NEUROBEHAVIORAL DEFICITS AFTER NON-OCCLUSIVE COMMON CAROTID ARTERY THROMBOSIS IN RATS. N.E. Alexis*, W.D. Dietrich, R. Prado, E. Green, and B.D. Watson. Departments of Neurology and Psychology, University of Miami School of Medicine, Miami, FL

Photochemically induced common carotid artery thrombosis (CCAT) leads to transient neurobehavioral deficits. Because the role of nitric oxide in the pathophysiology of thromboembolic stroke has not been investigated, we determined the neurobehavioral consequences of NOS inhibition following CCAT. Rats were assigned into 4 groups. Group 1 (n=12) underwent right CCAT. Group 2 (n=12) served as sham-operated controls. Group 3 (n=8) underwent right CCAT and were immediately given 15 mg/kg L-NAME i.v. Group 4 (n=8) served as CCAT controls but were administered 15 mg/kg L-NAME. Beginning 24 hr after injury, rats were tested daily for 3 days using a battery of 3 standard sensorimotor tasks. Statistical analysis was performed using the Kruskal-Wallis nonparametric test. Although no differences across groups in postural reflex scores were observed, differences existed in beam balance scores among both CCAT groups and shams ($p < 0.05$). Elicited forelimb placing data indicated that the CCAT/L-NAME group exhibited consistent bilateral deficits which were significantly greater and more reproducible than in the nontreated CCAT group. In addition to the anticipated contralateral placing deficits, ipsilateral placing scores were 0.2 ± 0.2, 1.0 ± 0.5 and 3.2 ± 0.6 in the sham, CCAT, and CCAT/L-NAME groups, respectively ($p < 0.01$). In this thromboembolic model, acute NOS inhibition may aggravate neurobehavioral outcome by increasing platelet capture and thus intensifying brain ischemia.

393.6

NG-NITRO L-ARGININE METHYLESTER (L-NAME) REDUCES CORTICAL INFARCT AND NECROTIC DAMAGE BUT NOT APOPTOTIC CELL LOSS. C. Charriaut-Marlangue[1], I. Margaill[2], R.J. Walsh*, M. Plotkine[2] and Y. Ben-Ari[1], Université René Descartes, Paris V - [1]INSERM U29, 123 bd de Port-royal, 75014 Paris. [2]4 avenue de l'observatoire, 75006 Paris. France.

Activation of apoptosis has been suggested to play a role in pathological situation in the adult central nervous system, namely after global ischemia as demonstrated by oligonucleosomal degradation of genomic DNA and protection with protein synthesis inhibitors (J. Neurochem, 1993, 61, 1973). Since nitric oxide (NO) plays a role in neuronal cell death, we have examined the effects of L-NAME on the DNA fragmentation damages in the striatum and cortex produced by a transient MCA occlusion in the rat brain. One hour MCA and CCAs occlusion induced oligonucleosomal fragments that are recognized as the characteristic DNA ladder at 18 h recirculation associated with a slight random DNA fragmentation. The random DNA cleavage was predominant at 48 h. Using PFGE, we have detected initial stages of DNA fragmentation (300 and 50 kbp) which occurred already at 6 h reperfusion whereas they were not detectable on conventional gel. A significant number of cells in the striatum, cortex, and islands of Calleja die with the characteristics of apoptosis (chromatin condensation, nucleus segmentation and apoptotic bodies) as detected by DNA nick-end labeling (TUNEL staining) preferentially in the penumbra, i.e. in cells mainly located to the inner borders of the necrotic volume. In rats treated with L-NAME (3 mg/kg, i.p., 5 min and 3 hours following MCA occlusion), a 24 % ($P<0.01$) reduction of cortical infarct volume was found at 24 h. However, DNA fragmentation at 18 h recirculation, in the cortex and striatum, was also observed but laddering was more conspicuous. In addition, scattered apoptotic nuclei were always present in these regions as well as in islands of Calleja. In contrast, the number of necrotic cells decreased in the ischemic core as shown with the TUNEL staining. L-NAME, which significantly decreased the cortical infarct, decreases the necrotic component but not the apoptotic component of cell death.

393.7

NITRIC OXIDE INHIBITORS ATTENUATE CA1 DEGENERATION INDUCED BY ISCHEMIC CONDITIONS IN RAT HIPPOCAMPAL SLICES A.M. Benz, Y. Izumi, D.B. Clifford* and C.F. Zorumski
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In rat hippocampal slices, we previously observed that nitric oxide synthase (NOS) inhibitors attenuate neuronal damage produced by N-methyl-D-aspartate (NMDA), suggesting a role for NO in excitotoxic injury (Izumi et al. Neurosci Lett, 135:227,1992). Despite evidence implicating excitotoxic processes in stroke, the role of NO in ischemic brain damage remains unclear. We examined the involvement of NO in ischemic damage using hippocampal slices prepared form 30 day old albino rats and exposure to 20 min of oxygen/glucose deprivation (ischemia) followed by 90 min postincubation in oxygen and glucose containing media. Damage in the CA1 region was rated on a 0 (intact) to 4 (severe neuronal damage) scale by a rater blind to the experimental condition. Control slices exposed to ischemia were rated as 2.8 ± 0.5 (N=9). L-N^G-monomethylarginine (L-NMMA), a non-selective NOS, inhibitor, partially attenuated ischemic damage in CA1 at concentrations of 30- 300 μM. At 300 μM L-NMMA, slices were rated as 1.3 ± 0.5 (N=8). A specific inhibitor of brain NOS, 7-nitroindazole (30 μM), was also effective against ischemic degeneration (0.7 ± 0.3, N=5). These results suggest that activation of brain NOS is involved in ischemic degeneration in the CA1 region.

Supported by the Diabetes Research and Training Center, AG05681 and AG11355.

393.8

NOS INHIBITION ATTENUATES KCN-INDUCED "EXCITOTOXIC" LESIONS AND EXCITATORY AMINO ACID RELEASE G.S. Neal, W.J. Nicklas* and G. D. Zeevalk. Dept. of Neurology, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, N.J. 08854.

Pathological production of nitric oxide (NO) during hypoxia has been linked to activation of nitric oxide synthase (NOS) upon prolonged activation of glutamate receptors (excitotoxicity). This association led to the hypothesis that ischemia-induced CNS damage, in part, is mediated by pathophysiological effects of NO. In these studies, 450 μm transverse hippocampal slices from male rats were used to investigate the ability of N^G-methyl-L-arginine (100μM NMA), a NOS inhibitor, to attenuate efflux of EAAs and associated neuronal damage induced by chemical hypoxia (2.5mM KCN). Histology and amino acid release were used as indices of excitotoxicity. To investigate the temporal effects of NOS inhibition on EAA release and glutamatergic receptor activation, we treated slices with KCN in the presence or absence of NMA for 5, 10, 20 or 30 min. At 5 min there was rounding of CA1 neurons that did not coincide with increased EAA efflux, but was substantially attenuated by NMA or a NMDA antagonist . At 10 min there was increased EAA efflux, moderate CA1 and CA4 damage, and mild CA3 damage. At 20 to 30 min, in addition to increased EAA efflux, all hippocampal regions appeared severely damaged. NMA substantially decreased EAA efflux. NMA or a combination of NMDA and non-NMDA antagonists afforded neuronal protection during 10, 20 or 30 min KCN exposure. These data support our previous studies which suggest that when energy stores are compromised, changes in the physiological state of the NMDA receptor, rather than increases in extracellular EAAs may be responsible for early receptor activation. At later times, increased EAA efflux may correlate with non-NMDA receptor activation. Moreover, these data suggest that NO augments the formation of "excitotoxic" lesions and concomitant EAA efflux.

393.9

NITRIC OXIDE SYNTHASE INHIBITION, ADENOSINE RECEPTOR BLOCKADE, AND BLOOD FLOW AFTER RETINAL ISCHEMIA. S. Roth*, P. Ostwald, I. Goldstein, A. Pachnanda, S. Park, A. Moshfeghi. Dept of Anesthesia and Critical Care, Univ of Chicago, Chicago, IL 60637

We found previously that hyperemia follows a 60 min period of retinal ischemia in cats. To determine the mechanisms responsible, we examined the effects of nitric oxide synthase (NOS) inhibition and adenosine (ADO) receptor blockade in our ischemia model using measurements of retinal blood flow and the electroretinogram (ERG). Cats were anesthetized with chloralose and divided into four groups. In all groups, one eye was subjected to 60 min of ischemia by raising intraocular pressure above systolic arterial pressure. Group 1 received 30 mg/kg L-NG-nitro-arginine-methylester (L-NAME), group 2 intravitreal 8-SPT (ADO receptor antagonist), group 3 combined L-NAME and 8-SPT, and group 4 was a saline control. Five minutes after the end of ischemia, blood flow in the retina, choroid, and iris/ciliary body was measured using injections of radioactively labeled microspheres. ERG studies were carried out before, during, and 1, 2, 3 and 4 hours after ischemia ended. Basal retinal blood flow was decreased by L-NAME, but not by 8-SPT . Postischemic retinal hyperemia was eliminated by 8-SPT, but not by L-NAME, and slightly decreased by combined L-NAME/8SPT. ERG a- and b-wave recoveries were not altered by L-NAME or by 8-SPT; L-NAME infusion significantly decreased b-wave amplitude. Adenosine appears to be the primary regulator of increased blood flow after retinal ischemia. The interaction of ADO and NO after ischemia deserves further study.

Study supported by NIH grant RO1EY10343

393.10

EFFECTS OF ADENOSINE ON NEURONAL DAMAGE PRODUCED BY INTRASPINAL OR INTRATHECAL INJECTION OF THE NITRIC OXIDE SYNTHASE (NOS) INHIBITOR L-NAME R.P. Yezierski*, R. Buchanan, S. Koch, H. Belmont, and A. Sanchez, The Miami Project and Depts. of Neurology and Neurological Surgery, University of Miami, Miami, FL 33136.

Intraspinal or intrathecal injections of L-NAME produce dose-dependent damage of spinal neurons. This damage is thought to result from a reduction in basal levels of nitric oxide (NO) producing an ischemic reaction secondary to vasoconstriction and reduced spinal cord blood flow (SCBF). The putative cellular mechanism of L-NAME induced neuronal damage may involve the release of glutamate, a substance known to have a role in excitotoxicity. Adenosine, a vasoactive agent, has been reported to have neuroprotective properties due to its actions as a vasodilator and inhibitory effects on glutamate release. Together these results led to the hypothesis that adenosine should exhibit neuroprotective effects against neuronal injury induced by inhibitors of NO synthesis. Intraspinal (0.3-0.6μl) or intrathecal (5-10μl) injections of L-NAME (50-250mM), adenosine (10-50mM) or L-NAME+adenosine were made at spinal levels T10-L5 in adult rats. After 48 hour survival periods spinal segments containing injection sites or those at the tip of intrathecal cannula were cut on a freezing microtome and stained with cresyl violet. The rostrocaudal extent of neuronal loss and the area of gray matter damage were evaluated with light microscopy and/or image analysis. The present results confirm previous observations of dose-dependent L-NAME damage of spinal neurons. Additionally, control injections of adenosine showed no effects, while there was a significant reduction in neuronal damage with injections of L-NAME+adenosine. These data suggest that adenosine may have neuroprotective effects against injury induced vasoconstriction and reduced SCBF resulting from reduced tissue levels of NO. Supported by NS28059 and The Miami Project.

393.11

WITHDRAWN

393.12

ADENOSINE RE-UPTAKE INHIBITORS ARE NEUROPROTECTIVE.
S.L. Moon*, L. Signor, R.A. Myers, F. Matos, L. Lombardo, J. Hibbard, A. Ortiz, S. Reid, and N. Balasubramanian[1]. CNS Biology and [1]Central Chemistry, Bristol-Myers Squibb, Wallingford CT, 06492.

Adenosine, acting as an endogenous neuroprotectant, inhibits the release of neurotransmitters including glutamate, and this effect is mediated by adenosine (A) receptors. Presynaptically, A_1 receptor activation hyperpolarizes the membrane, and postsynaptically adenosine stabilizes the membrane. However, in the brain, adenosine is rapidly removed from the extracellular space by a re-uptake mechanism. The therapeutic potential of adenosine receptor activation has been investigated previously, mainly through the use of A_1 agonists. We have investigated adenosine re-uptake inhibitors (ARIs) as neuroprotective agents; unlike A_1 agonists, their functional effect could be greater at the site of injury, *i.e.*, where the adenosine level has been greatly elevated. Through the use of ARIs, a high concentration of extracellular adenosine could be sustained, and thereby potentiate adenosine's neuroprotective action.

When administered 1 hr. after bilateral carotid occlusion in the gerbil, our novel ARIs significantly decreased hippocampal damage. A single dose given at 2 hr. post-insult reduced infarct size after permanent middle cerebral artery occlusion in the spontaneously hypertensive rat by as much as 29% and improved neurologic outcome. In the mouse, pretreatment with ARIs completely blocked seizures induced by electroshock. Through the use of *in vivo* micro-dialysis, we have shown that a systemically delivered ARI increased extracellular striatal adenosine for > 12 hr. in the rat after anoxic insult. ARIs induced hypotension in the dog. We have blocked this hypotension by co-administering 8-(*p*-sulfophenyl)theophylline, a polar A_1 antagonist that poorly penetrates brain.

393.13

ADENOSINE RECEPTOR ANTAGONISTS REDUCE $[K^+]_e$ INCREASE AS A RESULT OF OXYGEN AND GLUCOSE DEPRIVATION IN THE RAT HIPPOCAMPUS *IN VITRO*.
M.D.R. Croning, T.S.C. Zetterström, T.W. Stone* and N.R. Newberry. Oxford University-SmithKline Beecham Centre for Applied Neuropsychobiology, University Department of Clinical Pharmacology, Radcliffe Infirmary, Oxford, OX2 6HE, England, U.K. and *Department of Pharmacology, University of Glasgow, G12 8QQ, Scotland, U.K.

In the CA1 region of the hippocampal slice preparation, oxygen or oxygen+glucose deprivation is known to cause an increase in the extracellular K^+ concentration ($[K^+]_e$). We have investigated whether adenosine receptors might have a modulatory role in this increase by studying the effects of adenosine receptor antagonists.

Submerged rat hippocampal slices were superfused with aCSF (3 mM K^+, 5 mM glucose) at 30 °C. $[K^+]_e$ was measured in the CA1 stratum pyramidale using valinomycin-based K^+-selective microelectrodes. Slices were subjected to a single insult: either oxygen or oxygen+glucose deprivation with or without antagonist pretreatment (30 min).

Oxygen deprivation (5 min) resulted in small changes in $[K^+]_e$, reaching 4-5 mM from the basal level of 3 mM. Pretreatment with theophylline (100 µM) or 8-cyclopentyl-1,3-dipropylxanthine (100 nM) reduced this increase by ca. 50%.

Oxygen+glucose deprivation (10-25 min) resulted in a biphasic increase in $[K^+]_e$; the initial increase was gradual (phase 1) reaching 7-12 mM in 6-8 min then the rate of rise increased sharply (phase 2) reaching 20-30 mM over the next 1-3 min. Both antagonists reduced the phase 1 increase in $[K^+]_e$ by ca. 40% and delayed the onset of the phase 2 increase without affecting its magnitude.

In conclusion, these experiments suggest that adenosine A_1 receptors may contribute to the increase in $[K^+]_e$ in the CA1 pyramidal cell layer as a result of oxygen or oxygen-glucose deprivation.

393.14

POST-ISCHEMIC CHANGES IN $GABA_A$ RECEPTOR α1 SUBUNIT EXPRESSION IN CA1 HIPPOCAMPUS: REVERSAL BY DIAZEPAM.
J.R. Inglefield*, C.A.Wilson, A.E. Barrier, P.J. Chase, K.S. Jones and R.D. Schwartz-Bloom. Dept. Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710

Following cerebral ischemia, hippocampal area CA1 pyramidal cells degenerate over 2-4 days, while the interneurons are spared. At the same time, $GABA_A$ receptor binding decreases within area CA1 dendritic fields. Here we examined the expression of $GABA_A$ receptor α1 subunits on vulnerable CA1 pyramidal cells and on interneurons up to 35 days after 5 min bilateral carotid occlusion in the Mongolian gerbil. Beginning at 3-4 days and continuing up to 35 days after ischemia, there were degenerative changes in α1-immunoreactive (IR) CA1 pyramidal cell dendrites (beading, followed by loss). At the same time, the number of α1-positive CA1 interneuron soma increased progressively over the 35 day survival period. Many area CA1 α1-IR interneurons also developed beaded dendrites by day 3, and had reduced dendritic extensions later. The α1-subunit expression was neuron-specific, and no changes were observed in area CA2-3 or dentate gyrus at any time. Subsequently, we compared α1 subunit expression with the degree of protection of CA1 pyramidal cells 3-5 weeks after treatment with diazepam (DZ) (10 mg/kg, 30 + 90 min post-ischemia) or the GABA uptake inhibitor, tiagabine (TG; 45 mg/kg, 10 min post-ischemia). These drugs protect CA1 pyramidal cells when assessed 4-7 days after ischemia (Schwartz *et al.*, Brain Res. 647:153, 1994; Inglefield *et al.*, submitted). Three weeks after ischemia, TG protected only 23±9% of these cells, while DZ protected 71±11% 5 weeks after ischemia (see poster by Schwartz-Bloom *et al.*). Similarly, DZ markedly attenuated the ischemia-induced increase in α1-IR interneurons and the pyramidal cell dendritic beading 35 days later. However, while such changes in α1-IR were prevented in TG-treated gerbils at 4 days, TG failed to prevent this pathology 3 weeks post-ischemia. We conclude that 1) there are pathologic changes in $GABA_A$ receptor phenotype in both ischemia-sensitive and -resistant hippocampal neurons, and 2) that DZ is superior to TG in preventing these changes in both pyramidal cells and interneurons long-term. Supported by NS28791, NS07274, and American Heart Assoc.

393.15

DIAZEPAM PRODUCES SUSTAINED NEUROPROTECTION IN GERBIL HIPPOCAMPUS & PREVENTS ISCHEMIA-INDUCED WORKING MEMORY DEFICITS.
Rochelle D. Schwartz-Bloom*, Peter D. Chase, Janet Ko and Edward D. Levin. Depts. Pharmacology & Psychiatry, Duke University Medical Center, Durham, NC 27710.

Previously we showed that acute injection of diazepam following transient cerebral ischemia in both gerbils and rats produces substantial neuroprotection of the hippocampus 4-7 days later (Brain Res. 647:153-160, 1994; J. Neurosci. 15:529-539, 1995). In the present study we determined if neuroprotection by diazepam is long-lasting and if diazepam also prevents ischemia-induced working memory deficits. Gerbils were trained on an 8 arm radial maze for 20 sessions, when they reached asymptotic performance for choice accuracy. They were subjected to 5 min bilateral carotid occlusion and then injected 30 and 90 min later with diazepam (10 mg/kg). Four weeks later, they were retrained on the radial arm maze for 5 days; the following week they were sacrificed for histological analysis. Transient cerebral ischemia produced significant deficits in working memory tasks measured 4 weeks later ($p<0.0001$ in 3 different measures of choice accuracy). Diazepam completely prevented the ischemia-induced working memory deficits ($p<0.0001$). In the same gerbils, diazepam produced complete neuroprotection of area CA1 pyramidal neurons in 3/8 gerbils, partial protection in 3/8 gerbils and little protection in 2/8 gerbils. The degree of neuroprotection was the same at 5 weeks post-ischemia compared to 7 days post-ischemia. We conclude that enhancement of GABA neurotransmission with a benzodiazepine provides long-lasting neuroprotection in the hippocampus and, more importantly, it prevents memory deficits associated with cerebral ischemia.

Work supported by NIH grant NS28791 and the American Heart Association.

393.16

SUPPRESSION OF $GABA_A$-MEDIATED CURRENTS BY METABOLIC INHIBITION IN RAT HIPPOCAMPAL NEURONS CORRELATES WITH ELEVATED $[Ca^{2+}]_i$.
Alex V. Nowicky* and Michael R. Duchen. Physiology Dept. University College London, Gower St, London, U.K. WC1E 6BT.

Ischemia or hypoglycemia are associated with the development of seizures. Hypoxia also causes: i) failure of GABA-mediated IPSPs before failure of glutamate-mediated EPSPs in brain slices and ii) a rise in $[Ca^{2+}]_i$ in isolated hippocampal cells (Nowicky & Duchen, (1994) J. Physiol., 475P, 148). As $GABA_A$-mediated Cl^- currents may be depressed by a rise in $[Ca^{2+}]_i$ (Inoue et al (1986) Nature, 324:156-58, and Stelzer et al (1988) Science, 241:339-41), we have examined the relationship between metabolic inhibition, $[Ca^{2+}]_i$ and $GABA_A$-mediated currents in isolated hippocampal neurons.

Perforated patch clamp recordings using amphotericin B were obtained from CA1 neurons freshly dissociated from hippocampal slices of 15-25day old rats. GABA (10µM) was applied repeatedly near the soma by pressure ejection, and elicited an inward current at a holding potential of -50mV. The current reversed at E_{Cl} and was blocked by the $GABA_A$ antagonist, bicuculline (100µM). No run-down of the current was seen for periods of up to 1hour. Both uncoupling mitochondrial oxidative phosphorylation with 1µM FCCP or inhibition of the respiratory chain with 2mM NaCN for 2-5 min reversibly reduced the mean peak current by 66%±6.7% (mean± sem, n=6). In a further series of experiments, changes in $[Ca^{2+}]_i$ and GABA current were measured simultaneously in cells loaded with fura-2 (AM). The reduction in the GABA current showed a clear temporal correlation with the rise in $[Ca^{2+}]_i$. These data suggest that impaired mitochondrial function may lead to a reduction of $GABA_A$- mediated Cl^- conductance triggered by a $[Ca^{2+}]_i$-dependent postsynaptic regulatory mechanism.

(supported by Action Research)

393.17

DIFFERENT DISTRIBUTION OF GABAERGIC COMPONENTS BETWEEN CA1 AND CA3 REGION IN RAT HIPPOCAMPUS Yao, Z.B.*, Li, Z.C. and Xu, Z.C. Dept. of Neurology, University of Tennessee, Memphis. Memphis TN 38163

CA1 pyramidal cells in hippocampus degenerate while cells in CA3 region survive following transient forebrain ischemia. Excitotoxic effect has been hypothesized as the cause of this selective neuronal lost. Disturbance in the balance between excitation and inhibition may contribute to the hyperactivity of the vulnarable neurons. To examine the morphological basis of the above hypothesis, we compare the GABAergic components between CA1 and CA3 region in rat hippocampus using quantitative light (LM) and electron microscopy (EM).

Male Wistar rats (230-350 gm) were perfused transcardially with 2% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer. Vibratome sections (50μM) were processed for GABA immunocytochemistry using monoclonal antibodies (Chemicon). Sections for EM were postfixed in 0.5% osmium tetroxide for 1 hr and then embedded in Aradite. Ultrathin section were cut and observed with a JEOL 1200 electron microscope.

Under LM, GABA positive somata and puncta scattered in all layers of hippocampus. GABA positive neurons exhibited a variety of sizes and shapes. Quantitative analysis showed that the ratio of GABA positive neurons to pyramidal neurons was 1:12 in CA1/subiculum area and 1:9 in CA2/CA3 area (n=7, P<0.01). In stratum pyramidale, GABA positive puncta appeared as pericellular baskets around cell bodies. The number of GABA positive puncta around each soma in CA1 was 7.05±2.06 (n=204) and 10.01±3.02 in CA3 (n=199, P<0.01). The number of puncta per 10 μm pericellular membrane was compared. It was 1.71±0.05 in CA1 and 2.03±0.05 in CA3 region respectively (P<0.01). EM observation revealed that these GABA positive puncta were axon terminals. They formed symmetrical synapses on pyramidal cell bodies and their dendrites. The present study suggested that CA1 pyramidal neurons may receive less inhibition than CA3 neurons, which may partially contribute to the selective neuronal damage following transient ischemia.

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393.18

HALOTHANE EFFECT ON ISCHEMIC OUTCOME AND TEMPERATURE PATTERN FOLLOWING TRANSIENT FOREBRAIN ISCHEMIA IN RATS Ren, Y.B*. and Xu, Z.C. Dept. of Neurology, University of Tennessee, Memphis. Memphis TN 38163

Anesthetics and brain temperature have great effects on neuronal injury following transient forebrain ischemia. Using ischemic depolarization (ID) as an indication of complete ischemia, the present study investigated the temporal threshold of severe CA1 cell damage under halothane anesthesia and the pattern of brain and body temperature change 6 days after ischemia.

Male adult Wistar rats were fasted overnight and anesthetized with 1-2% halothane. Severe forebrain ischemia was induced using 4-vessel occlusion method. ID was recorded using electrodes filled with 3 M KCl. Brain temperature were maintained at 37°C during and shortly after ischemia. Brain and body temperature were monitored continuously for 6 days. The extent of CA1 cell damage was evaluated on Hematoxylin-Eosin staining sections 6 days after reperfusion.

Under halothane anesthesia, 13 min ID (~15 min ischemia) was required to produce complete CA1 cell damage. The brain temperature dropped to approximately 36° C within 2 h after recirculation then rose to about 38.3° C within 30 h. The brain temperature gradually returned to around 37.5° C 40 h after reperfusion and fluctuated between 37° and 38° C with a diurnal rhythm. Same pattern was observed in sham-operated animals but the temperature was about 0.5°C lower than that in ischemic animals during the first 21 h of reperfusion (P<0.05). The brain temperature was 0.5-1.0° C lower than body temperature at the first 58 h of reperfusion in ischemic animals (P<0.05), at the first 20 h of reperfusion in sham-operated animals (P<0.05). No significant difference was found in control animals. Our data suggests that halothane anesthesia attenuates neuronal damage in rat hippocampus following ischemia. Ischemic insult delays brain temperature recovery following ischemia. Halothane anesthesia also contributes to the delay of postischemic brain temperature recovery.

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393.19

EFFECT OF DEXMEDETOMIDINE ON HIPPOCAMPAL STRUCTURES FOLLOWING TRANSIENT ISCHEMIA IN GERBIL **J. Sivenius*, J. Kuhmonen, R. Miettinen, P.J. Riekkinen Sr, J. Pokorny, A Haapalinna. Dept. of Neurology and A.I.Virtanen Institute, University of Kuopio, Kuopio, Finland.**

Dexmedetomidine is an agonist of presynaptic α_2-adrenergic receptors that is shown to inhibit the synaptic release of norepinephrine in a variety of noradrenergic neurons. There is biochemical evidence that norepinephrine potentiates the actions of excitatory amino acids in the hippocampus. Neuroprotective effects of an α_2 agonist dexmedetomidine were investigated in a gerbil transient cerebral ischemia model, involving bilateral carotid occlusion of 5 min in ether inhalated normothermic animals. Dexmedetomidine was administered subcutaneously in four different dose regimens (8 animals in each group): 3 μg/kg or 30 μg/kg 30 min before the induction of ischemia, and the same dosages given 3 hours after the ischemia induction. In addition, animals of each group received a corresponding dose 3, 12, 24 and 48 hours after ischemia. One group of ischemic animals received saline only. After one week the animals were killed and the brains fixed by transcardiac perfusion. Delayed neuronal damage of cells of sectors CA1, CA3 and hilus was evaluated after silver staining with counting damaged cells in an area of 1 mm^3, and the data were analyzed by Mann-Whitney U-test. Most effective dose regimen was 3μg/kg given before the induction of ischemia. Mean number of damaged cells in comparison with saline treated animals was 9.1% in CA3 ($p<0.01$), 10% in hilus ($p<0.01$) and 51% in CA1 ($p<0.05$), respectively. These data demonstrate that dexmedetomidine effectively prevents ischemic damage when given before the induction of ischemia in the model of transient forebrain ischemia. The reason for more potent effect on CA3 and hilar neurons than on CA1 neurons may be due to different density of α_2 receptors in these structures.

ISCHEMIA: OXIDATIVE INJURY

394.1

FREE RADICAL PRODUCTION AFTER FOREBRAIN ISCHEMIA IN THE RAT, AS MEASURED WITH THE SALICYLATE-HYDROXYLATION METHOD: INFLUENCE OF BODY TEMPERATURE. K. Pahlmark*, B. K. Siesjö. Lab for Exp Brain Research, University of Lund, Sweden.

Salicylate hydroxylation rates, assumed to reflect hydroxyl radical (•OH) formation, were measured in control rats, as well as in those subjected to 15 min of forebrain ischemia, and allowed recirculation periods of 5 and 15 min at body and brain temperatures of 33°C, 37°C and 39°C. HPLC was used to measure the formation of 2,5-dihydroxybenzoic acid (DHBA) in samples from neocortex, hippocampus, and caudoputamen. Hydroxylation of salicylic acid in control animals was similar in neocortex and hippocampus, and lower in caudoputamen. Results obtained at body temperatures of 37°C and 39°C showed similar hydroxylation rates after 5 and 15 min of recirculation, but at 33°C an increased hydroxylation was seen only after 5 min in the examined regions.

The results demonstrated that, in contrast to gerbils, rats do not show a higher rate of postischemic •OH production in the hippocampus than in the neocortex. Furthermore, although temperature affects the basal rate of benzoic acid hydroxylation, it does not influence the production of •OH during the first 5 min of recirculation. However, since an increased hydroxylation was not observed after 15 min in the 33°C group, hypothermia seems to shorten the period of postischemic hydroxyl radical production.

394.2

BRAIN LIPID OXIDATION FOLLOWING CARDIAC ARREST AND RESUSCITATION. G. Fiskum*, Y. Liu and R.E. Rosenthal Depts. of Biochemistry and Molec. Bio. and Emergency Medicine, George Washington Univ. School of Medicine, Washington, D.C. 20037

This study tested the hypothesis that high ventilatory [O_2]s during and following resuscitation (RE) after 10 min canine cardiac arrest (CA) worsen neurological outcome and result in high levels of brain lipid oxidation. The mean neurological deficit scores (0=normal, 100=brain dead) measured at 23 hr after RE were 45.1±3.6 s.e. for animals given 21% O_2 during RE and 58.3±3.8 for animals given 100% O_2 during and for 1 hr after RE ($p<.05$). HPLC and mass spectrometry measurements of oxidized lipids in frontal cortex samples taken 24 hr post-RE showed increased levels of 13-hydroxyoctadecadienoic acid (HODE) for hyperoxic animals (5.0±0.8 μg wet wt.) compared to normoxic dogs (2.3±0.2, $p<.05$) and compared to non-ischemic control samples (1.6±0.2, $p<.05$). Total oxidized lipids in hyperoxic animals was twice as high in the striatum than in the cortex and hippocampus with the striatum also displaying the largest increase (300%) compared to non-ischemic controls. These results support the results of Zwemer *et al.* (*Resusc* 27:159-170, 1994) indicating that 100% inspired O_2 during and following RE exacerbates delayed neurological injury and provide new evidence that high [O_2] may be neurotoxic following global cerebral ischemia through its potentiation of free-radical dependent lipid oxidation. (Supp. by the Emergency Med. Foundation and NS 34152)

394.3

PARABANIC ACID FOR MONITORING OF OXYGEN RADICAL REACTIONS IN THE ACUTELY INJURED HUMAN BRAIN. L. Hillered* and L. Persson. Departments of Clinical Chemistry and Neurosurgery, Uppsala University Hospital, Uppsala, Sweden.

Oxygen radicals are currently thought to play an important role in the pathophysiology of acute brain injuries, including stroke and trauma. There is a great need for methods by which oxygen radical activity can be measured *in vivo*. The endogenous scavenger uric acid, the end product of purine metabolism in man, is oxidized to mainly allantoin and parabanic acid when exposed to highly reactive radical species *in vitro*[1,2].

We used microdialysis[3] to harvest allantoin and parabanic acid in the frontal cortex of neurointensive care patients after aneurysmal subarachnoid hemorrhage. Dialysate samples were collected 24h/d during 7-8 days and ananalyzed for lactate, pyruvate, allantoin and parabanic acid by HPLC.

Clinical events involving secondary ischemia (as evidenced by increased lactate/pyruvate ratios), eventually leading to structural damage, were associated with a dramatic elevation of the dialysate level of parabanic acid, whereas allantoin showed less robust changes. In one patient with an uneventful clinical course without signs of secondary ischemia parabanic acid levels remained low.

In conclusion, the results support the involvement of highly reactive oxygen radical species in human cerebral ischemia. Parabanic acid appears to be an important marker of free radical reactions *in vivo* and may be used to monitor increased free radical activity and to evaluate pharmacological therapy with radical scavengers in acute brain injuries.

1. Kaur & Halliwell, Chem-Biol Interactions, 1990; 73:235-247.
2. Hicks et al, Free Rad Res Comms, 1993; 18:337-351.
3. Persson & Hillered, J Neurosurg, 1992; 76:72-80.

394.4

DIFFERENTIATION OF NEUROBLASTOMA 104 CELLS INCREASES SUSCEPTIBILITY TO PEROXIDATIVE INSULT. J.A. Rafols*, B.J. O'Neil, S. Alousi, and B.C. White. Departments of Anatomy/Cell Biology and Emergency Medicine, Wayne State Univ., School of Medicine, Detroit, MI 48201.

Undifferentiated (UNDIFF) and differentiated (DIFF; 1mM each dibutryl cAMP and theophylline) NB104 cell cultures were exposed to 50 nM cumene hydroperoxide added to the media containing 3 μM$FeSO_4$ for 2,4 and 6 hours. Reactions were stopped along the time course with 1 mM DETAPAC. To examine the extent and progression of subcellular organelle damage, controls (unexposed) and exposed cultures were fixed with a mixture of aldehydes, embedded in 1.5% agarose, post fixed with OsO_4, and processed for electron microscopy. Control DIFF cells developed a lobulated nucleus with patches of heterochromatin, and unlike that of UNDIFF cells, the cytoplasm contained many RER and Golgi cisternae, as well as a dense feltwork of intermediate filaments. Following 2 hrs of peroxidative insult many DIFF and UNDIFF cells showed vesiculation of Golgi cisternae and increased numbers of lysosomes and membranous whorls. Large vacuole containing filaments and microvesicles or RER and polysomal debris were observed, many in close proximity to the plasmalemma. By 6 hrs most DIFF cells observed had shrunken nuclei with dense heterochromatin, electron dense cytoplasm, and extruded cytoplasmic debris between adjacent cells; all signs of apoptosis. In contrast UNDIFF cells had electron lucent cytoplasm with intact organelles, although a few showed advanced stages of degeneration. The results indicate that the membranes of the Golgi and RER are early targets of peroxidative insult. They also suggest that DIFF cells undergo earlier and more severe subcellular damage than UNDIFF cells. Further characterization of this model may advance our knowledge of selective vulnerability in neurons. (Supported by NIH grant NS01585 and the Detroit Neurotrauma Institute at Wayne State University).

394.5

CALCIUM-INDUCED INCREASE IN THE OXIDATIVE METABOLISM OF CEREBELLAR NEURONS DISSOCIATED FROM THE RATS. Y. Oyama*, T. Ueha and L. Chikahisa. Lab. of Cell Signaling, Fac. of Integrated Arts and Sciences, The University of Tokushima, Tokushima 770, Japan.

Excessive Ca influx into neurons plays a key role in neuronal death during brain ischemia although the cellular events resulting from excessive Ca influx are not fully elucidated. We examined the effect of ionomycin on the oxidative metabolism (the formation of reactive oxygen species) of rat cerebellar neurons using a flow cytometer and 2',7'-dichlorofluorescin (DCF) diacetate, an indicator for intracellular H_2O_2. Ionomycin increased the intensity of DCF fluorescence, but not under the external Ca-free condition. Time-course for ionomycin in increasing the intracellular Ca concentration ([Ca]i) was more rapid than that in augmenting DCF fluorescence. Furthermore, there was a good correlation between the augmented DCF fluorescence and the increased [Ca]i. Therefore, the excessive Ca influx may lead to a great increase in oxidative metabolism.

394.6

EFFECT OF GINKGO BILOBA EXTRACT ON CALCIUM-INDUCED INCREASE IN THE OXIDATIVE METABOLISM OF RAT CEREBELLAR NEURON. L. Chikahisa*, E. Okazaki and Y. Oyama. Lab. of Cell Signaling, Faculty of Integrated Arts and Sciences, The University of Tokushima, Tokushima 770, Japan.

Excessive Ca influx into neurons results in an increased formation of reactive oxygen species which are involved in ischemic brain damage. We examined the effect of Ginkgo biloba extract (EGb) on the oxidative metabolism of rat cerebellar neurons using 2',7'-dichlorofluorescin (DCF) diacetate, a fluorescent indicator for intracellular H_2O_2. Incubation with EGb at concentrations of 0.3 μg/ml or higher (up to 3 μg/ml) decreased the DCF fluorescence of resting neurons in a dose-dependent manner. EGb also reduced the ionomycin-induced increase in DCF fluorescence without affecting the intracellular Ca concentration. Quercetin, a constituent of EGb, at concentrations of 30 nM or more resembled the EGb. Therefore, the flavonoid constituents in EGb may be partly responsible for the protective actions of EGb in the brain neurons subjected to ischemia or hypoxia.

394.7

MUTAGENESIS and NEURODEGENERATION AFTER CEREBRAL ISCHEMIA. JK Cui, YY He, CY Hsu*and PK Liu. Div. Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, TX 77030 and Dept. of Neurology, Washington University St. Louis, MO 63110

Hydroxyl radicals that are increased in the brain due to cardiac arrest, stroke or head injury are known to be toxic and mutagenic in a variety of testing systems. To determine DNA damaging effects caused by cerebral ischemia in mice, we measured mutation frequency of the reporter *lac* I gene in the λ phage from the Big Blue transgenic mice. Cerebral ischemia was induced for 30 min, followed by variable periods of reperfusion (expression time). The λ phage was rescued from brain genomic DNA using in vitro packaging extract and E. coli SCS-8. After plating in the presence of X-gal (70 mg/plate), mutants in this reporter gene were selected on the basis of exhibiting blue plaques from the non-mutant clear plaques. The background frequency was 2×10^{-5} (n=3), and the frequency after ischemia was increased from 2×10^{-5} (at 1/4 h, n=3) to 8×10^{-5} (at 8 h, n=7) and 6×10^{-5} (at 24 h, n=3). Base substitutions (4/4) were found in the *lac* I mutants from the sham-operated mice, but deletions (10/26), base substitutions (13/26) and insertions (3/26) were found in ischemia-induced *lac* I mutants. DNA fragmentation as detected using the Apop Taq (TUNEL) kit did not occur on 1 day (n=4), but occurred in samples on 2-3 days (n=4) after ischemia. The results supported the hypothesis that the genome instability can be initiated by cerebral ischemia, and may be linked to neuronal degeneration. (Sponsored by the Vivian L. Smith Foundation for Restorative Neurology, Baylor College of Medicine, Houston, Texas)

394.8

POSTISCHEMIC, SYSTEMIC ADMINISTRATION OF MODIFIED SUPEROXIDE DISMUTASE PREVENTS HIPPOCAMPAL CA1 NEURODEGENERATION IN RAT GLOBAL CEREBRAL ISCHEMIA. T.M. Wengenack*, G.L. Curran, and J.F. Poduslo. Molec. Neurobiol. Lab., Mayo Clinic and Foundation, Rochester, MN 55905.

Free radicals are believed to play an important role in cerebral ischemia. Free radical scavenging enzymes such as superoxide dismutase (SOD) have shown neuroprotective effects in animal models of cerebral ischemia. Due to the low blood-brain barrier (BBB) permeability and short plasma half-life (~4 min) of SOD, experiments using native enzyme have had limited success, only with very high doses. Thus far, various modifications to increase the half-life or permeability of SOD have resulted in limited neuroprotective effects. Furthermore, none of these studies have demonstrated neuroprotection with postischemic administration. Permeability of native (nSOD) and modified SOD (mSOD) across the BBB was determined using an I.V. bolus injection technique. Transient, global cerebral ischemia was produced in male Wistar rats for 12 min using the four-vessel occlusion method. Animals were dosed (I.V.) 1.5 min after reperfusion with either saline, nSOD (5,000 or 10,000 U/kg), or mSOD (5,000 U/kg) twice daily for 3 days. Neuroprotective effects on hippocampal CA1 pyramidal cells were then assessed using a standard neuropathological rating scale (0 = 0%, 1 = 25%, 2 = 50%, 3 = 75-100% damage). The permeability coefficient-surface area product (PS) at the hippocampal BBB was $1.68 \pm 0.10 \times 10^{-6}$ ml/g/s ($\bar{x} \pm$ SEM) for nSOD. The PS increased fivefold to $8.93 \pm 0.74 \times 10^{-6}$ ml/g/s for mSOD. Histologically, saline-treated animals had total CA1 damage (2.99 ± 0.01). Native SOD did not show any neuroprotective effects at the doses tested (5,000 U/kg: 2.86 ± 0.15, 10,000 U/kg: 2.57 ± 0.43). Modified SOD, however, resulted in significantly less CA1 damage (1.48 ± 0.36; Mann-Whitney U-test: $p<0.02$). Modification of SOD and other antioxidant enzymes, as well as growth factors, resulting in increased permeability across the BBB may provide a useful tool for the systemic delivery of therapeutic proteins in neurodegenerative disorders.

394.9

ANTIOXIDANT NEUROPROTECTION FOLLOWING ANOXIA IN HIPPOCAMPAL SLICES. T.J. Sick*, P.L. Mumford, M. Rosenthal and M. A. Pérez-Pinzón. Dept. of Neurology, U. of Miami Sch. of Medicine, Miami, Fl. 33101

Evidence indicates that damage may occur not only during brain ischemia but also during reperfusion afterward. Our studies in rat cerebral cortex showed that reperfusion after global ischemia (10-60 min duration) was characterized by hyperoxidation of the electron carriers of the mitochondrial respiratory chain. Recovery of evoked potentials was improved by respiring these animals with a slightly 'hypoxic' gas mixture which also ameliorated post-ischemic mitochondrial hyperoxidation (PIMHo). When rats were respired with 'hyperoxic' gas mixtures during reperfusion after global ischemia, PIMHo was increased and electrical recovery was lessened. Mitochondrial hyperoxidation (particularly hyperoxidation of NAD) also characterized reoxygenation following anoxia in hippocampal slices. Present goals were to determine if antioxidants could suppress PIMHo and improve electrical recovery after anoxia in hippocampal slices which were reoxygenated for 60 min after 2 min of anoxia-induced 'anoxic depolarization' (AD). Ascorbate did not influence the reduction/oxidation (redox) status of NAD which became approx 30% hyperoxidized after anoxia. However, ascorbate significantly improved evoked potential recovery. We conclude that at least one antioxidant (ascorbate) improved electrical recovery after anoxia in slices but through mechanisms not apparent in the redox state of NAD.

394.10

IN VITRO AND *IN VIVO* NEUROPROTECTIVE EFFECTS OF CYCLIC NITRONE ANTIOXIDANTS. C.E. Thomas, D.F. Ohlweiler, D.R. McCarty, J.V. Ficorilli, H.C. Cheng, P.A. Chmielewski and M.P. Johnson*. Marion Merrell Dow Research Institute, Cerebrovascular Biology, Cincinnati, OH, 45215.

The nitrone spin trap α–phenyl-*tert*-butylnitrone (PBN) has been shown to provide neuroprotection in models of acute and chronic neurodegeneration, presumably via trapping of reactive oxygen-derived free radicals. We have synthesized a series of novel cyclic nitrone spin traps and examined them for radical trapping activity *in vitro* and for neuroprotective effects in animal models of stroke. The cyclic nitrones were all found to be active inhibitors of iron-dependent lipid peroxidation with the most potent compound, MDL 104,342, being 550-fold more active than PBN. Likewise, the compounds prevented both lipid peroxidation and the loss of viability resulting from treatment of cerebellar granule cells in culture with ferrous iron. Using electron spin resonance (ESR) spectroscopy, we determined that MDL 101,002 was a more efficient hydroxyl (•OH) and superoxide anion radical trap than was PBN. The α-hydroxy nitroxide adduct derived from trapping of •OH was more stable than that of PBN ($t_{1/2}$ of 5 min vs < 1 min) suggesting it to be a superior ESR reagent. In the 3-vessel occlusion model of stroke in the Wistar rat, treatment with a bolus plus infusion dose of MDL 101,002 (75 mg/kg + 270 mg/kg/6 hr) provided substantial protection (~95% reduction in infarct size). Similarly, in a permanent model of ischemia in the spontaneously hypertensive rat, MDL 101,002 provided greater than 50% protection vs. control animals when administered as a single bolus dose (100 mg/kg). These data indicate that cyclic nitrone spin traps such as MDL 101,002 have improved radical trapping efficacy relative to PBN and may be useful agents for treatment of neurodegenerative diseases.

394.11

•EFFECTIVENESS OF ANTIOXIDANTS AGAINST OXIDATION OF CIS-PARINARIC ACID ENRICHED LIPOSOMES. • M.R. Emerson, F.E. Samson*, T.L. Pazdernik; Smith Research Bldg., Dept. of Pharm., Tox., & Ther.,Univ. of Kansas Med. Center, Kansas City, KS 66160.

• Free radical damage by reactive oxygen species, is implicated in several neuropathologies. Transition metals (Fe,Cu) may be dislocated into the extracellular fluid during brain trauma, ischemia, etc. and then may catalyze the formation of oxygen radicals by the Fenton reaction. This leads to a lipid peroxidation cascade, membrane damage and loss of cellular integrity. A membrane model of a liposome preparation enriched with cis-parinaric acid was used to study protection against lipid peroxidation from an aqueous metal catalyzed radical generating system of ascorbate(330 µM), copper (1.0 µM) and H_2O_2(10 mM). Oxidative decay of cis-parinaric acid was measured fluorometrically. Antioxidant effectiveness of alpha-tocopherol(0.1-10 µM), lactate(1.0-10 mM) and U-74389F(1.0-10 µM) was studied. Alpha-tocopherol as low as 0.1 µM gave a 50% reduction in oxidized cis-parinaric acid, but increases up to 10 µM did not go above 65% protection. U-74389F was less potent than alpha-tocopherol. Lactate, an aqueous oxidizable substrate, provided a maximum of 20-40% protection even at a concentration of 10 mM. This data suggests that formation of hydroxyl radicals in solution is not as important as site specific oxidation within the lipid membrane. Thus, the effectiveness of antioxidants against lipid peroxidation depends critically on their location and insertion into lipid bilayers.

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394.12

DEHYDROEPIANDROSTERONE (DHEA) AND HYDROXYL FREE RADICAL (°OH) IN ISCHEMIC GERBIL BRAIN. B. Delbarre*, G. Delbarre and F. Calinon, Faculté de Médecine, 37032 Tours, France.

DHEA, a first neurosteroid, was introduced by Beaulieu to study aging. Some compounds, analogs to neurosteroids, were investigated in cerebral ischemia. Delbarre et al. show that °OH increased with age during brain injury. A study was undertaken to show the relation between DHEA and °OH. Four groups of 7 adult gerbils were used : One sham operated (SO), and three (1 control and 2 DHEA, 3 or 10 mg/kg, i.p., 30 min. before occlusion) were subjected to unilateral left carotid occlusion (60 min. followed by 30 min. reperfusion).°OH was determined in left hemisphere by HPLC/Electrochemical (salicylate as scavenger) according to Floyd method (Floyd, R. A., et al. *Free Rad. Biol.Med.* 2:13-18,1986).

		SO	Control	10mg/kg	3mg/kg
°OH	Mean	0.170	2.910	0.492	0.248
	± sem	0.025	0.303	0.081	0.059
		-	***	NS	NS

Results were given in ng/mg of proteins. Student Newman-Keuls test versus Control group. n = 7. p≥0.05 NS - p<0.001 ***.

Results show that °OH is significantly increased in ischemic group. DHEA (3 and 10 mg/kg) reverses this increase. The low dose is more effective. Our study shows the importance of the doses of DHEA for maintaining the normal activity of the brain.

The currently pharmacological action of DHEA may be reconsidered.

394.13

LEVELS OF IRON DURING INCOMPLETE GLOBAL ISCHEMIA. P.D. Hurn*, D.C. Lipscomb, L.K. Gorman ,R.J. Traystman, Dept. of Anesthesiology and Critical Care Medicine, Johns Hopkins Medical Institutions, Baltimore, MD 21287

We hypothesized that during global cerebral ischemia with high density tissue acidosis, levels of low molecular weight (LMW) Fe^{2+} are increased and potentially catalyze free radical-mediated oxidant injury via Fenton-Haber chemistry. Pentobarbital-fentanyl anesthetized dogs received intravenous dextrose (plasma glucose ≅500 mg/dl) followed by an infusion of artificial cerebrospinal fluid into the lateral ventricle to reduce cerebral perfusion pressure (10 mm Hg for 30 minutes). Cerebral energy metabolites and intracellular pH were monitored by magnetic resonance spectroscopy. After ischemia, the brain was perfused with ice-cold phosphate buffered saline solution, and many different brain areas were sampled. Iron levels (total and LMW Fe^{2+}) were determined by the rapid colorimetric micromethod (W. Fish , 1988). With hyperglycemic ischemia, intracellular pH decreased from 7.1-7.2 to 5.9-6.1, and phosphocreatine and ATP decreased to 0% and <50% of control values, respectively. In cortical regions, hippocampus, caudate, and thalamus, LMW Fe^{2+} was increased in ischemic animals as compared to sham-operated controls. There were no differences in the cerebellum and other brain stem structures. These regional differences may reflect the basal localization of iron which is to a large extent in the cortex and hippocampus. These results suggest that global incomplete ischemia when accompanied by exaggerated tissue acidosis results in a regionally specific pattern of increased LMW Fe^{2+} that may play a role in oxidative injury in these areas. (Supported by NR03521, NS20020)

394.14

SEVERE HYPOXIA IN PC12 CELLS INCREASES MITOCHONDRIAL MEMBRANE POTENTIALS WHILE MARKEDLY ELEVATING $[Ca^{2+}]_i$ H. Zhang, R.C. Carson, H M.Thomas III and G.E.Gibson*, Cornell University Medical School at Burke Medical Research Inst. White Plains, NY 10605.

Studies with isolated mitochondria and synaptosomes suggest that reduced oxygen or high calcium diminishes the mitochondrial membrane potential, indicating an abnormal oxidative state. To study the effect of anoxia on mitochondrial oxidative state in intact cells, the relation of $[Ca^{2+}]_i$ (measured with fura-2) to mitochondrial membrane potentials (measured with TMRE) was tested in NGF-differentiated PC12 cells during anoxia with or without glucose. By the use of pre-tonometered buffers, the pO_2 on the microscope stage was changed from 140 ± 2 to 2.5 ± 0.7 mm Hg (mean ± SE). In the presence of glucose, 30 min of anoxia increased $[Ca^{2+}]_i$ in the growth cones (from 102 ± 2 to 424 ± 196 nM) and cell bodies (from 78 ± 7 to 502 ± 171 nM). The $[Ca^{2+}]_i$ then gradually returned toward the basal value. K^+ depolarization after 5 min of anoxia greatly increased $[Ca^{2+}]_i$ in growth cones (1126 ± 171 nM) and cell bodies (>5000 nM). In similarly treated cells, anoxia increased the mitochondrial membrane potentials in the growth cones (from -108 ± 2 to -152 mV) and cell bodies (from -125 ± 1 to -158 ± 2 mV). The absence of glucose reduced the mitochondrial membrane potentials but the pattern of response to anoxia was similar; the mitochondrial membrane potential increased in the growth cones (from -65 ± 7 to -82 ± 1 mV) and cell bodies (from -103 ± 1 to -118 ± 1 mV). Thus, in intact cells anoxia raised $[Ca^{2+}]_i$ as expected, and, in contrast to results with fractionated cells, increased the mitochondrial membrane potentials. The elevated potentials indicate an abnormal oxidative state in response to anoxia, which does not resemble the effect of cyanide, but parallels that of oligomycin, an inhibitor of both ATP synthesis and uncoupler-stimulated ATP hydrolysis.

395.1

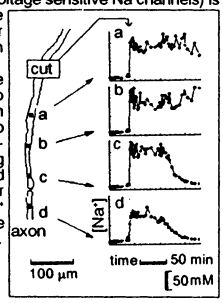
VOLTAGE-SENSITIVE CALCIUM CHANNELS MEDIATE CALCIUM ENTRY INTO MAMMALIAN SYMPATHETIC NEURONS FOLLOWING AXOTOMY. R. Sattler, I. Fayaz, C.H. Tator, M. Hafner, M.C. Wallace*, and M. Tymianski, *U. of Toronto, Canada & Tech. University of Mannheim, Germany.*

Some mechanisms linking axonal injury and neurological dysfunction are thought to be associated with a rise in free intracellular Ca^{2+} concentration ($[Ca^{2+}]i$). We studied the effects of axotomy on $[Ca^{2+}]i$ in axons and cell bodies of cultured superior cervical ganglion (SCG) neurons using the fluorescent Ca^{2+} indicator fluo-3 and a laser-scanning confocal microscope. Axotomy produced a rapid $[Ca^{2+}]i$ rise in the cell soma which preceded any $[Ca^{2+}]i$ rise in the axon (n = 30), suggesting a role for membrane depolarization, and voltage sensitive Ca^{2+} channels (VSCCs). Inhibiting membrane depolarization by bath-applying 140mM potassium prior to axotomy prevented any significant calcium rise from occurring (n = 9; Mann-Whitney rank-sum test, $p<0.001$). However, axotomy-induced depolarization did not require the activation of conventional sodium channels sensitive to tetrodotoxin (TTX) , a Na+ channel blocker, as there was no difference between the calcium rise observed following axotomy in control solution or in the presence of TTX (n = 15; p = 0.265). To test whether axotomy-induced Ca^{2+} influx also occurred via Ca^{2+} conductances other than conventional VSCCs, we blocked Ca^{2+} channels during the axotomy using nimodipine and ω-conotoxin GVIA , blockers of L-type and N-type Ca^{2+} channels, respectively. Ca^{2+} channel blockade significantly attenuated axotomy induced $[Ca^{2+}]i$ increases both in the cell soma and in axons (n=21, $p<0.001$). These data contradict the intuitive hypothesis that Ca^{2+} entry following mechanical axotomy occurs via non-specific influx pathways produced by cell-membrane disruption. The results provide the first evidence in mammalian neurons that immediate, traumatically-induced, increases in neuronal $[Ca^{2+}]i$ occur through well-defined pathways which are amenable to pharmacological manipulation.

395.2

POST-TRANSECTION CHANGES IN AXOPLASMIC SODIUM ION CONCENTRATION IN VERTEBRATE PERIPHERAL MYELINATED AXONS. G.David*, J.N. Barrett and E.F. Barrett, Dept. of Physiology & Biophysics, Univ. of Miami Med. Sch., P.O Box 016430, Miami, FL 33101.

To investigate the effects of axonal transection on the axoplasmic concentration of Na^+ ($[Na^+]_n$) we obtained ratiometric estimates of $[Na^+]_n$ from SBFI-injected motor axons innervating the ceratomandibularis muscle of lizards (Anolis sagrei). Two phases were observed following transection in normal lizard saline ($[Na^+]_{out}$ 160 mM): 1. Increase in $[Na^+]_n$, spreading from the transection site along the axon and usually reaching $[Na^+]_n$ within 1-10 min at distances of up to ~800 μm from the cut end (Fig. a-d). This phase could not be mimicked by depolarizing the axons with high bath $[K^+]$, was resistant to TTX, but was abolished if axons were transected in bath solutions in which $[Na^+]$ was replaced with $[K^+]$, indicating that Na^+ entry through the cut end (but not through voltage sensitive Na channels) is the major contributor to the increased $[Na^+]_n$. The time course of this phase could be accounted for by Na^+ diffussing in the axoplasm with a diffusion coefficient of ~$1*10^{-5}$ cm^2/sec.



2. Recovery of $[Na^+]_n$, starting 5-50 min after the transection, during which $[Na^+]_n$ decreases to resting levels in all imaged regions of the axon (Fig. c,d) except a 5-200 μm segment adjacent to the transection site (Fig. a,b). This re-establishment of normal $[Na^+]_n$ may be initiated by resealing of the axon membrane (e.g., between sites b and c in Fig.). $[Na^+]_n$ recovery along the axon is faster than expected by diffusional redistribution of Na^+ along the axon, suggesting a contribution of active Na-removal mechanisms, such as Na/K pumps.

Supported by NS 12404

395.3

4-AMINOPYRIDINE AFFECTS CONDUCTION IN INJURED SPINAL CORD AXONS *IN VITRO* AT CLINICALLY RELEVANT CONCENTRATIONS. Riyi Shi*, W. H. Ferrell III, J. E. Gadzia and A. R. Blight, Division of Neurosurgery, Univ. of North Carolina, Chapel Hill, NC 27599.

Previous studies have shown restoration of action potential conduction in chronically injured spinal cord axons *in vitro* in the presence of 0.1 - 1 mM concentrations of the potassium channel blocking drug 4-aminopyridine (4-AP). Clinical studies have shown improvements in function in human subjects with chronic spinal cord injury using systemic doses that give rise to peak serum concentrations of 1-2 μM 4-AP. The question arises whether the clinical effects of the drug seen in spinal cord injury and multiple sclerosis are due to improved safety factor for conduction in myelinated axons, to enhanced synaptic transmission or a combination of the two. Data from rat central axons demyelinated with ethidium bromide indicate that the effective dose for restoration of conduction is too high (>100 μM) to be achieved clinically, and this has been interpreted as suggesting the clinical effects of the drug are principally or exclusively on synaptic transmission (Felts & Smith, *Ann.Neurol.* 36:454, 1994). We examined the dose-response characteristics of 4-AP in isolated normal and traumatically injured guinea pig spinal cord, using a sucrose-gap technique for recording conduction in strips of white matter superfused with oxygenated Krebs solution. In contrast to data from chemical demyelination in rats, this preparation showed evidence of enhanced conduction through both acutely (<6 hr) and chronically (>4 month) injured axons with a threshold around 1 μM and a peak effect at 10 μM 4-AP. These low concentrations had no effect on normal spinal cord tissue. These data, together with parallel histopathology, are interpreted as support for the concept that clinical response to 4-AP, at least in trauma, may involve significant enhancement of safety factor for action potential conduction in partially demyelinated or poorly remyelinated nerve fibers. (Supported by a grant from the Canadian Spinal Research Organization and by grant NS21122 from NIH, NINDS)

395.4

AXONAL INJURY AND NEURONAL STRESS AFTER UNILATERAL SOMATOSENSORY CORTEX CONTUSION INJURY. A.A. Dunn-Meynell* & B.E. Levin, Neurology Svc., VA Med. Ctr., E. Orange, NJ 07018 and Dept. Neurosciences, New Jersey Medical Sch., Newark NJ 07103.

To characterize the effects of unilateral traumatic injury of the somatosensory cortex, c-fos immunoreactivity (IR) was used to identify neuronal stress and 68kd neurofilament revealed axonal injury. A pneumatically powered cylinder (5mm diameter tip) was driven 3 mm deep into the exposed somatosensory cortex of anesthetized adult male SD rats to produce a unilateral contusion. Immunocytochemistry was examined after 3hr (c-fos) or 24 hr (68kd) survival.

The injury produced severe disruption of the impact site, without gross damage to subcortical structures. Both c-fos IR and axonal injury was seen in structures close to and underlying the injury including ipsilateral cortex, lateral thalamus (ventroposterior, ventrolateral, and reticular nuclei), dorsal striatum, hippocampus and internal capsule. Axonal injury was sometimes present but always sparse in more remote structures (including ipsilateral cerebellum, superior colliculus, and deep mesencephalic nuclei), and was rarely seen in the intact hemisphere. However, c-fos was induced in discrete structures remote from the injury including, the entire cortex and the subthalamic nucleus ipsilaterally, and bilaterally in some medial thalamic nuclei (e.g. centrolateral, paraventricular, centromedial), cerebellar white (but not grey) matter, and brainstem. c-fos IR seemed mainly confined to neurons as addition of GFAP IR showed few double labelled cells.

Therefore, axonal injury was largely a function of distance from injury site, whilst neuronal stress tended to be seen in discrete brain nuclei adjacent to and remote from the injury site.

Supported by the Research Service of the Dept. of Veterans Affairs.

395.5

PATTERN OF AXONAL DEGENERATION IN THE BRAIN AFTER TRAUMATIC BRAIN INJURY (TBI) OF RAT SENSORIMOTOR CORTEX. J.S. Soblosky*, Murray A. Matthews[1], D.A. Chorney, June F. Davidson, L.L. Colgin and M.E. Carey, Neurotrauma Res. Lab., Dept. Neurosurgery, and [1]Dept. of Anatomy, LSU Medical Cntr, New Orleans, LA 70112

Isoflurane-anesthetized rats were injured on the right side fore- and hindlimb sensorimotor cortex by a piston impact depressing the dura 1mm @ 5 M/sec. We have previously shown that this TBI results in unilateral long lasting fore- and hindlimb deficits. We determined the pattern of subsequent axonal degeneration in the brain using a silver staining method. Eight weeks after TBI there was extensive axonal degeneration 1) around the injury site with many degenerating fibers crossing the corpus callosum and entering the contralateral sensorimotor cortex; 2) in the ipsilateral dorsolateral and medial striatal areas; 3) in the ventrobasal, ventromedial, ventroposterolateral, ventroposteromedial, ventrolateral, and posterior nuclei of the ipsilateral thalamus; 4) in the cerebellum occurring bilaterally around the lateral, medial and interpositus nuclei. Degenerating fibers extended well into the folia terminating predominately in the granule cell layer. These results: 1. suggest that significant transsynaptic axonal degeneration occurs after TBI; 2. may aid in determining brain areas involved in neuronal reorganization leading to functional recovery after TBI.

395.6

CALCIUM DEPENDENCE OF MEMBRANE RESEALING IN TRANSECTED MYELINATED RAT AXONS. M.J. Howard, G. David, and J.N. Barrett*, Dept. of Physiology and Biophysics, Univ. of Miami School of Medicine, Miami, FL 33136.

Calcium has been implicated in promoting the resealing of damaged cell membranes in a number of different cell types, both in vivo and in vitro. We examined the Ca^{2+}-dependence of the resealing process in transected rat dorsal root and sciatic nerve axons in vivo, using a dye-exclusion assay to estimate resealing. When spinal roots where transected in physiological saline containing no added Ca^{2+} (free $[Ca^{2+}]$ estimated to be <5μM), the percentage of axons that resealed increased with time from 13% when dye was added 15 minutes following the lesion, to 16% at 30 minutes, 27% at 60 minutes and 41% at 120 minutes. In physiological saline containing 2 mM Ca^{2+}, resealing was 45% at 15 minutes, 47% at 30 minutes, 70% at 60 minutes and 86% at 120 minutes. At each time point, resealing was significantly higher in the 2 mM Ca^{2+} saline ($P>0.001$, SNK, Tukey-Kramer test, Dunn's test). Similar experiments done on the sciatic nerve showed resealing to be 60% at 30 minutes and 62% at 60 minutes in the low $[Ca^{2+}]$ solution. In 2 mM Ca^{2+}, resealing was 72% at 30 minutes and 86% at 60 minutes. Again, resealing was enhanced by 2 mM Ca^{2+} ($P>0.01$, SNK, Dunn's test).

Based on these findings, we hypothesize that there are Ca^{2+}-dependent processes that contribute to the overall resealing mechanism in myelinated rat axons. Additionally, the data suggest that the resealing process in the spinal roots may be slower and have a different Ca^{2+} dependence than peripheral nerve.

395.7

EARLY CYTOKINE INDUCTION FOLLOWING CNS TRAUMA. J.A. Olschowka*, B. Harvey, J. Merola, K. Sakai[1], and J.T. Hansen. Dept. of Neurobiology & Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642 and [1]Dept. of Neurol. Surgery, Okayama Univ. Med. Sch, Okayama, Japan.

A hallmark of CNS injury is the rapid appearance of inflammatory cells, phagocytosis of damaged or dead cells, and the hypertrophy and proliferation of astrocytes. These events are mediated by proinflammatory cytokines and include molecules such as IL-1ß, IL-6, and TNFα. Repair of the CNS injury site depends principally on the important interplay between these inflammatory cytokines, glial derived neurotrophic factors, and endogenous CNS cells responsible for removing cellular debris and facilitating tissue homeostasis. To experimentally address the roles of these molecules, rats received a unilateral stab injury to their parietal cortex. Two, 6, 12 and 24 hours postlesion, tissue was collected for determination of cytokine mRNA levels using quantitative RT-PCR. IL-1ß mRNA levels were seen to be increased by 2 hrs and to remain elevated at 24 hrs in the lesioned cortex. Immunocytochemical staining for IL-1ß at 6 hrs postlesion demonstrated a few faint cortical microglial (?) cells. More marked staining was observed in cells within the underlying corpus callosum, presumably astrocytes. At 12 hrs, the staining intensity was increased. Additional staining was also observed in the contralateral corpus callosum and in areas surrounding the lateral ventricles. Double labeling techniques to determine the cell types expressing IL-1ß are currently underway, as well as studies of other cytokines. In conclusion, there appears to be a rapid induction of cytokines following CNS trauma that may play a role in its remodeling. Supported by PHS NS25778 (JH) and NS29400 (JO).

395.8

INCREASES OF CALPAIN I SPECIFIC CYTOSKELETON BREAK DOWN PRODUCTS FURTHER IMPLICATE CALPAIN PROTEOLYSIS FOLLOWING TRAUMATIC BRAIN INJURY (TBI). R.M.Posmantur*, A.Kampfl, S.J.Liu, G.Clifton, R.L.Hayes. Department of Neurosurgery, UT Health Science Center-Houston, Texas, USA.

Previous quantitative Western blot analyses have demonstrated the loss of neurofilament 68 (NF68) and neurofilament 200 (NF200) protein subunits following lateral cortical impact injury. Loss of NF68 in cortical homogenates was associated with the concomitant increase of low molecular weight immunopositive break down products (BDP's) suggesting calpain mediated proteolysis of the neuronal cytoskeleton persisting for at least 24 hrs after injury (Posmantur et al., J Neurotrauma 11: 533-545, 1994). Recent immunohistochemistry studies examining corresponding morphopathology have found diffuse process injury (DPI) preferentially occurring in dendrites rather than axons at early time points following injury (Posmantur et al; J Neur Exp Neurol, submitted). To further examine the role of calpain mediated proteolysis 24 hrs after TBI, we used Western blots to examine BDP's both for NF68 and alpha spectrin using a polyclonal antibody (Robert-Lewis et al., J Neurosci 14: 3934-3944, 1994) specifically recognizing calpain 1 mediated spectrin BDP's. Spectrin BDP's increased 374% in cortex ipsilateral to injury ($p \leq 0.004$) and increased 178% in the contralateral cortex ($p \leq 0.042$) compared to sham injured levels. Other comparisons indicated that spectrin was more vulnerable than NF68 to calpain mediated proteolysis. BDP's for spectrin but not NF68 were detected in the contralateral cortex. In addition, BDP's for spectrin but not NF 68 were detected bilaterally in the cortices of animals surgically prepared for injury (bilateral craniotomies) but not injured. Our ongoing examinations of cytoskeleton proteolysis employing *in vivo* and *in vitro* models of TBI have also provided evidence that specific anti-proteolytic compounds may provide a therapeutic approach to treating these protein losses (e.g. Kampfl et al., this meeting). (Supported by NIH grants PO1 NS 31998 and RO1 NS 21458)

395.9

FOCAL OPTIC NEUROPATHY PRODUCED BY ELEVATED INTRAOCULAR PRESSURE IN A RAT MODEL OF GLAUCOMA. E.C. Johnson*[1], B.G. Gold[2], C.G. Moore[1] and J.C. Morrison[1]. Casey Eye Institute[1], Center for Research on Occupational and Experimental Toxicology and Dept. of Cell Biology and Anatomy[2], Oregon Health Sciences University, Portland, OR 97201.

Glaucoma, a major cause of blindness, is characterized by regional optic nerve fiber susceptibility to elevated intraocular pressure (↑IOP). The mechanism whereby ↑IOP leads to axonal degeneration is unknown. A rat model of glaucoma has been created by unilateral sclerosis of the aqueous humor outflow pathways to produce sustained ↑IOP. This study examines, by light and transmission electron microscopy, the characteristics of optic neuropathy in this model.

After 1 to 65 days of mean ↑IOP of 4-30 mmHg (relative to fellow untreated eyes), rats were perfused with 5% glutaraldehyde, optic nerve samples embedded in plastic, 1µm sections cut and stained with toluidine blue. Mean ↑IOP of <10 mmHg for 3-34 days produced focal optic nerve lesions characterized by axonal swellings, axonal degeneration and myelin debris. Similar lesions were observed in nerves with ↑IOP of 10-20 mmHg for <3 weeks. Digitization (on micrographs at 620x) of damaged areas revealed lesions comprising 0.5% to 10.4% of the total neural area, with 70% of the lesions located in the superior temporal quadrant. ↑IOP of 10-20 mmHg for >3 weeks or >20 mmHg for >1 week resulted in total involvement of the optic nerve, with occasional morphologically normal axons. Ultrastructurally, axonal swellings demonstrated a central neurofilamentous core with peripherally segregated accumulations of membranous vesicles and microtubules.

This rat model reproduces the variability in optic nerve axon susceptibility to ↑IOP observed in human glaucoma. Although the mechanism leading to development of these structural alterations is unknown, the presence of neurofilamentous and membranous axonal swellings suggests underlying axonal transport deficits in the pathogenesis of ↑IOP neuropathy.

NEUROMUSCULAR DISEASES: MOTONEURONS

396.1

DISTRIBUTION AND LEVELS OF INSULIN-LIKE GROWTH FACTOR (IGF-I AND IGF-II) AND INSULIN RECEPTOR BINDING SITES IN THE SPINAL CORDS OF AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS. C. Krieger[1]*, S. Doré[2], S. Kar[2], and R. Quirion[2]. Univ. British Columbia, Vancouver, B.C. V6T 1Z3[1], Douglas Hosp. Res. Ctr, McGill University, Montreal, Qc. Canada, H4H 1R3[2].

IGF-I, IGF-II and insulin are structurally related peptides with a wide spectrum of functions. These trophic factors are important for neuronal survival and the maintenance of the integrity of the organism. ALS is characterized by the progressive degeneration of motoneurons. Recently, reports have been provided to suggest a role for various neurotrophic factors, including IGFs, in the maintenance and survival of motoneurons, potentially opening new therapeutic approaches toward the treatment of ALS. Additionally, it was shown that the apparent density of IGF type I receptor is increased in ALS cords (Adem et al. Mol. Neurobiol. 9:225, 1994). However, the status of other related receptor populations such as the IGF-II and insulin receptors remains to be established in ALS. The main objective of the present study was to compare the anatomical distribution of ^{125}I-IGF-I, ^{125}I-IGF-II and ^{125}I-insulin binding sites in the human spinal cord, and their possible alterations in ALS. Twenty µm lumbar spinal cord sections were obtained from three controls and three ALS cases and processed for quantitative receptor autoradiography as described earlier (Kar et al. J. Comp. Neurol. 333:375, 1993) using 25pM of each radioligand. Specific ^{125}I-IGF-II binding sites were most abundant in the normal human spinal cord followed by ^{125}I-IGF-I and ^{125}I-insulin sites. For all these ligands, the highest amounts of binding were located around the central canal/lamina X area > dorsal horn > intermediate zones > ventral horn > white matter. Unlabelled insulin (10µM) was found to compete for ^{125}I-IGF-I and ^{125}I-IGF-II binding sites suggesting that these sites represent genuine receptors and not IGF binding proteins (IGFBPs) which are generally believed to be devoid of affinity for insulin. As reported earlier, highly significant increases in the levels of ^{125}I-IGF-I binding sites were apparent in the ALS lumbar cord being particularly evident around the central canal as well as the dorsal and ventral horns, the later showing extensive neuronal degeneration in ALS. In contrast, no significant difference in ^{125}I-insulin binding was observed while ^{125}I-IGF-II labeling was significantly increased only in the dorsal horn and white matter areas of the ALS lumbar cord. Hence, the apparent levels of ^{125}I-IGF-I, ^{125}I-IGF-II and ^{125}I-insulin binding sites are differently altered in ALS cords. The distribution of IGF receptors in the normal spinal cord and changes in ALS cords may be of relevance for future therapeutic strategies.

396.2

DIFFERENTIAL ACTIONS OF BCL-2 AND CILIARY NEUROTROPHIC FACTOR ON A GENETIC MOUSE MODEL WITH NEUROMUSCULAR DYSFUNCTION

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Cell death is a major event in the developing nervous system and in certain pathological states such as neurodegenerative diseases. In vertebrates it has been shown that the proto-oncogene, bcl-2, can act as a natural repressor of apoptosis in the nervous system.

To determine whether the protein *Bcl-2* could prevent the disease progression in an animal model which mimics a human motor neuron disease, we have used a genetic mouse model with neuromuscular dysfunction called *pmn* because of its progressive motor neuronopathy. The *pmn* mice show degeneration in motor nerves and motoneuron cell bodies associated with a severe muscle wasting leading to death. After crossing them with Bcl-2 overexpressing mice, we observed a rescue of the facial motoneurons with a preservation of the soma size. However, Bcl-2 did not prevent degeneration of motor nerve fibers nor increase the life-span of the *pmn* mice. In contrast, treatment of *pmn* mice with polymer-encapsulated cells releasing ciliary neurotrophic factor (CNTF) improved their life-span. Therefore, we suggest that divergent pathways exist for neuronal survival versus fiber outgrowth and that CNTF can act on both pathways whereas Bcl-2 acts more specifically on neuronal survival.

396.3

EFFECT OF GDNF ADMINISTRATION IN AN ANIMAL MODEL OF MOTONEURON DISEASE. Y. Sagot[*,♥], S.A. Tan[$], J. Hamming[#], D. Muller[*], A.C. Kato[♥] and P. Aebischer[$]. [*]Dept of Pharmacology and [♥] Div. of Clinical Neuromuscular Research, Geneva University, Switzerland; [$]Gene Therapy Center & Division of Surgical Research, Lausanne University, Switzerland; [#]CytoTherapeutics Inc., Providence, RI, USA.

Glial cell line-derived neurotrophic factor (GDNF), a member of the TGF-β superfamily, has been shown to be a very potent neurotrophic factor that enhances survival of various neuronal cell types including motoneurons. In order to assess its therapeutic potential in treating neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), we treated *pmn* mice (a mutant mouse model displaying motoneuron degeneration) with encapsulated GDNF-secreting cells. Effects of GDNF treatment on pmn/pmn mice were compared with previous results obtained with CNTF.

In contrast to CNTF, GDNF could not significantly improve the motor function or increase the life-span of *pmn* mice. However, counts of facial motoneurons revealed that in GDNF-treated mice, the motoneuron loss was significantly reduced as compared to untreated pmn mice. Surprisingly, axonal counts revealed that the GDNF action on nerve degeneration was negligeable as compared to CNTF. Therefore GDNF and CNTF are equally potent in rescuing motoneuron cell bodies whereas GDNF is less efficient in preventing or compensating for nerve degeneration in pmn mice. This difference between CNTF and GDNF in their biological action may be relevant for clinicals trials in neurodegenerative diseases.

396.4

THE EFFECTS OF PROLONGED CO-ADMINISTRATION OF CNTF AND BDNF IN WOBBLER MICE. B. Klinkosz, H.Mitsumoto. Neurology & Neuroscience, Cleveland Clinic, Cleveland, Ohio 44195, J.M. Cedarbaum, V. Wong, R.M. Lindsay Regeneron Pharmaceut. Tarrytown, NY

Systemic co-administration of CNTF/BDNF for 4 weeks arrests the progression of wobbler mouse motor neuron disease (MND)(Science 265,1107,94). To investigate the effects of an extended CNTF/BDNF treatment, we administered subcutaneously, recombinant human CNTF (1mg/kg, 3/week) and BDNF (5mg/kg, 3/week) alternately in a randomized, blinded, placebo (vehicle)-controlled fashion. Eleven wobbler mice received CNTF/BDNF for the entire 8 weeks -Grp A; 10 mice received CNTF/BDNF for the first 4 weeks and vehicle for the second 4 weeks-Grp B; Grp C (8 mice) received vehicle alone for 8 weeks. 6 animals died in Grp A (between week 2 and 7) and 2 animals in Grp B (in week 2), and none in Grp C. Body weight decreased more in Grp A and Grp B (both P< .01) than in Grp C early on. Mice that died in Grp A weighed less than those which survived (P<.02 at week 3). The average grip strength did not decline in Grp A but rather increased from baseline in 60% of animals. Grp B started losing strength after week 4, but was still stronger (P<.0001) than Grp C, which lost nearly 100% strength by week 6. Running speed was faster in Grp A (P<.001) than in Grp C; in Grp B it was similar to Grp A but slowed after week 4. Semiquantitative paw abnormality and walking patterns (grade 1, minimum; grade 4, maximum deficits) deteriorated slightly in Grp A (60% remained in grade 1 by week 8), whereas Grp B only deteriorated after week 4 (40% in grades 3+4 by week 8). 100% of Grp C were in grade 4 by week 8. This study not only confirms our previous observation that 4-week administration of CNTF/BDNF clinically arrests wobbler mouse MND, but also shows that combination treatment sustains the synergistic effect of these neurotrophic factors as long as treatment was continued.

396.5

CLINICAL AND HISTOLOGICAL FEATURES OF THE NZB-ELICITED WOBBLER ("NEW") MOUSE HYBRID. T. Ishiyama, B. Klinkosz, E.P. Pioro* and H. Mitsumoto. Depts of Neurology & Neuroscience, Cleveland Clinic Fdn, Cleveland OH 44195.

The wobbler (wr) mouse is an extensively studied animal model of motor neuron disease (MND) but its molecular basis is still unknown. However, the wr gene is closely linked to the pseudogene glutamine synthetase (*gln*s) on mouse chromosome 11 allowing linkage analysis. Recently, Junier et al., (J. Neurosci., 1994) used PCR to genetically identify homozygous wr mice from a new cross-bred strain (C57BL/6J wr x New Zealand Black) which carries 2 different sized *gln*s microsatellites. The purpose of our study was to characterize this NZB-elicited wobbler ("new") mouse and compare it with the original wr that we have extensively used. Weekly assessments of various parameters were made in new and original wr mice beginning at 2 weeks of age. Body weight was slightly greater in new mice. Paw position and walking abnormalities (both grade 1) developed by 4 weeks and worsened with age, particularly in new mice. Grip strength in new mice (n=11) was significantly less (p<0.001) than in wr mice from the onset of disease at 4 weeks. Running speed showed a similar trend. Most wr and new mice survived beyond 6 months. Histological study of both groups at 8 weeks of age showed vacuolar degeneration in cervical cord anterior horn cells and no difference in numbers of axons in C5 and C6 ventral roots. Thus histological features were similar in the new and wr mice although new mice were more affected clinically. Genetically identifiable new mice will be useful for further studies in this MND. (We thank F. Rieger & A. Ait-Ikhlef, INSERM, France for *gln*s primers and helpful discussion.)

396.6

ABNORMAL DNA REPAIR IN PATIENTS WITH ALS. Z.L. Olkowski[1] and J. Rosenfeld[2]. Depts of Radiation Oncology[1] and Neurology[2], Emory University School of Medicine, Atlanta, GA 30322

DNA injury and compensatory repair mechanisms are frequent, processes found in all eukaryotic cells. Among the most common DNA injury, the formation of abasic sites (AP), results from the removal of a purine base. This common injury is normally repaired by an endogenous repair enzyme, AP-endonuclease which is among the most abundant DNA repair enzymes. DNA injury is accelerated in the presence of reactive oxygen species (ROS), implicated in many neurodegenerative conditions; if DNA injury is unopposed by DNA repair, cell injury or death will result.

ROS have been implicated in the pathogenesis of many neurodegenerative diseases. In a search for etiologic factors contributing to neurodegenerative disease, we have examined the quantity and activity of AP-endonuclease in patients with familial and sporadic ALS compared to unaffected control patients.

AP-endonuclease activity is significantly decreased in lymphocytes from patients with sporadic ALS (decr. 53%, n=3) and familial ALS (decr. 31%, n=2) when compared to healthy controls (n=3). Furthermore, initial sequencing revealed an abnormal 5' c-DNA insertion. Complete gene sequencing as well as analysis of other neurodegenerative disorders will be presented.

This abnormality in AP-endonuclease, a major DNA repair enzyme, results in an increased number of unrepaired AP sites and may be reflected in dystrophic cell changes and/or cell death. Such abnormalities may have primary importance in the pathophysiology of ALS as well as other neurodegenerative diseases.

396.7

ACTIVATION OF PROTEIN KINASE C HAS MULTIPLE EFFECTS ON NEUROFILAMENTS OF MOTOR NEURONS. M. M. Doroudchi* and H. D. Durham. Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada, H3A 2B4.

Characteristic responses of motor neurons to injury include accumulation of neurofilaments in perikarya and proximal axons and apparent increase in the phosphorylation of C-terminal domains of neurofilament proteins. Experiments were conducted in dissociated cultures of murine embryonic spinal cord to determine if activation of Protein Kinase C (PKC) would induce such changes in mammalian motor neurons. PKC was activated by: (1) exposure of cultures to 10 nM 12-O-tetradecoanoyl phorbol 13-acetate (TPA); (2) microinjection of 1 mM dioctanoylglycerol (DiC_8, DAG) directly into perikarya of motor neurons; (3) addition of 10 μM DiC_8 to the culture medium. Within 1 to 4 hrs, fragmentation and disassembly of the neurofilament network was observed in neuronal perikarya by indirect immunocytochemistry. 1 - 7 days following treatment, motor neurons were observed with extensions of perikaryal cytoplasm or massive enlargements of proximal processes, both containing intact neurofilament networks. Over 3 -12 days, there was a gradual increase in the number of motor neuronal perikarya immunoreactive with antibodies specific for neurofilaments phosphorylated at C-terminal domains. These results demonstrate that activation of PKC leads to different immediate and long term effects on neurofilaments of motor neurons. These changes resemble alterations in shape and in neurofilament phosphorylation occurring in motor neurons following injury.

396.8

TRANSGENIC ANIMAL MODEL OF AMYOTROPHIC LATERAL SCLEROSIS: ENHANCED RELEASE OF ACETYLCHOLINE FROM THE MOTOR NERVE TERMINALS. Yong I. Kim* and Mark E. Gurney. Departments of Biomedical Engineering and Neurology, University of Virginia, Charlottesville, VA 22908 and Department of Cell and Molecular Biology, Northwestern University , Chicago, IL 60611.

The transgenic mice that express a human Cu,Zn superoxide dismutase (SOD) mutation are a plausible animal model of amyotrophic lateral sclerosis (ALS) in familial form (*Science* **264:** 1772-1775, 1994). To explore the effects of the SOD mutation on the motor nerve terminal (MNT) function, we studied neuromuscular transmission in three different groups of animals: (i) mice with the mutated human SOD gene (herein called "G1H"); (ii) mice with the wild-type human SOD gene (N29) for use as a control; and (iii) normal untreated (wild-type, wt) animals. Phrenic nerve-diaphragm muscles were exposed to 1.2 mM $[Ca^{2+}]_o$ and 10 mM $[Mg^{2+}]_o$ solution and the number of ACh quanta released per motor nerve impulse was measured using the direct method. In 75 to 113 days old G1H animals, quantal content (**m**) of the neurally-evoked end-plate potentials (EPPs) was 1.86 ± 0.14 (mean ± SEM, n = 16 animals), significantly increased (*P<0.001*) from the values found in the corresponding control N29 mice (1.14 ± 0.09, n = 11) or wt animals. The EPP quantal content in 54 to 65 days old G1H mice, however, did not differ significantly from either N29 or wt animals. The frequency and amplitude of the spontaneously occurring miniature end-plate potentials (MEPPs) were not significantly different between the three groups of animals.

We suggest two possible explanations for this finding. (1) calcium entry into the MNT, presumably mediated by the voltage-dependent calcium channels, is significantly increased in G1H mice; and/or (2) increase in **m** may represent the effect secondary to the motor nerve sprouting, a known morphological feature in G1H mice (Supported by NINDS and the Muscular Dystrophy Association).

396.9

IRON IN SPINAL MOTOR NEURONS IN AMYOTROPHIC LATERAL SCLEROSIS (ALS). E.J. Kasarskis*, M. Lovell, X. Bi. Dept. Neurology, VA and Univ. Kentucky Medical Centers, Lexington KY 40511.

Free radicals have been implicated in the death of spinal motor neurons in ALS. Iron (Fe) participates in the generation of the hydroxyl free radicals from hydrogen peroxide. In a previous study, we found that total Fe was increased nearly two-fold in cervical motor neurons from ALS patients compared to matched controls using Laser Microprobe Mass Spectrometry (In press, J. Neurol Sci., 1995).

In the present work, we have examined Fe distribution within motor neurons in paraffin embedded spinal cord from 10 ALS and 10 matched controls. Non-heme Fe was visualized using Perl's reaction enhanced with DAB and silver. The distribution of total Fe was mapped using an Fe-specific chelating agent coupled to rhodamine.

Iron was distributed heterogeneously in control motor neurons with the highest apparent concentration in the perinuclear cytoplasm. Iron was also visualized in the pia, endothelial cells, and glia. Although Fe distribution was similar in ALS, degenerating and some large motor neurons were intensely stained for Fe which had a homogeneous cytoplasmic distribution.

These studies indicate that Fe is present in spinal motor neurons and may be available to catalyze the generation of hydroxyl free radicals within the cells subject to ALS-type degeneration. (Supported by the VA Research Service).

396.10

PHARMACOLOGICAL ACTION OF RILUZOLE ON ION CHANNELS OF EXCITABLE MEMBRANES. Hyung C. Kim, Taick S. Nam, Anna M. Preta, Joseph J. Pancrazio* and Yong I. Kim. Departments of Biomedical Engineering, Neurology and Anesthesiology, University of Virginia School of Medicine, Charlottesville, VA 22908.

In a recent investigation by Bensimon *et al.* (*N. Eng. J. Med.* **330:** 585-591, 1994), *riluzole* (2-amino- 6-trifluoromethoxy benzothiazole), a newly synthesized antiglutamate drug, significantly increased survival time of amyotrophic lateral sclerosis (ALS) patients. However, the pharmacological mechanism underlying the therapeutic action of riluzole is not clearly understood. The present study is aimed at determining the pharmacological action of riluzole in modifying the function of voltage-dependent Ca^{2+} and Na^+ channels.

Riluzole was applied to bovine adrenal chromaffin cells at concentrations of 1, 3, 10, 30, 55, 100, 200, and 400 μM and whole-cell patch-clamp measurements of voltage-dependent calcium (I_{Ca}) and sodium (I_{Na}) currents were performed. The drug caused a dose-dependent blockage of the function of these ion channels. In the range of concentrations from 1 to 30 μM, riluzole produced a slight to moderate effect on I_{Ca}; at 30 μM, peak I_{Ca} was reduced by 12% (n=77 cells) and the steady-state I_{Ca} by 27% (n=81) compared to the control. The steady-state component of the current was consistently more inhibited than the peak at all concentrations studied. With a 200 μM concentration, nearly complete inhibition of I_{Ca} and I_{Na} was observed, which fell by 93 and 72% (n=27), respectively, from the control.

These findings suggest that riluzole blocks the function of L- or N-type Ca^{2+} channels and Na^+ channels. (Supported by a research grant from the Muscular Dystrophy Association).

396.11

PHARMACOLOGICAL ACTION OF RILUZOLE ON CHOLINERGIC TRANSMITTER RELEASE. Michael P. Viglione*, J. Mark Longacher, Tanja M. Pieters and Yong I. Kim Departments of Biomedical Engineering and Neurology, University of Virginia School of Medicine, Charlottesville, VA 22908.

Recent studies have shown that *riluzole* (2-amino-6-trifluoromethoxy benzothiazole) increased survival rate of amyotrophic lateral sclerosis (ALS) patients. To explore the possible therapeutic mechanism of this drug, we determined the pre- and post-junctional effects of riluzole on cholinergic neuromuscular transmission.

Experiments were conducted to establish a comprehensive dose-reponse relationship from 1 to 400 μM. When riluzole up to 100 μM was applied to mouse phrenic nerve-diaphragm muscle preparations in normal Ringer's solution, the miniature end-plate potential (MEPP) frequency was relatively unchanged. At 100 to 400 μM, however, MEPP frequency was significantly increased ($p<0.05$) by 82 to 226% (n=21-38 fibers) compared to control fibers. Pretreatment with ω-agatoxin IVA (40 nM), a specific P-type calcium channel blocker, did not compromise the riluzole-induced enhancement of MEPP discharge. Using muscles bathed in 1.6 mM Ca^{2+}/10 mM Mg^{2+} Ringer, spontaneous MEPPs and nerve-evoked end-plate potentials (EPPs) were recorded and quantal content (m) was estimated. Riluzole produced no consistent change in m; however, at higher concentrations (>100 μM), some motor end-plates exhibited a complete failure of EPPs. Similarly, in more than 35% of fibers exposed to 140 μM riluzole, indirectly and directly elicited muscle fiber action potentials (APs) failed to occur.

These findings suggest that (1) riluzole does not interfere with the function of P-type calcium channels at the neuromuscular junction; and (2) the blockade of evoked EPPs occurs at high concentrations of riluzole as a result of the failure in the production and propagation of APs. (Supported by a research grant from the Muscular Dystrophy Association).

396.12

NEW GENE TRANSFER FOR MOTOR NEURON DISEASES AND TRAUMA. F.F. Finiels*, M. Gimenez Y Ribotta, M. Barkats, M.L. Samolyk, J.J. Robert, A. Privat, F. Revah and J. Mallet, UMR C9923 CNRS-Rhône-Poulenc Rorer-Gencell F-91198 Gif-sur-Yvette and U 336 INSERM, F-34095 Montpellier, France.

Several neurotrophic factors are candidates for the therapy of motor neuron degenerative diseases such as Amyotrophic Lateral Sclerosis (ALS), Kennedy's disease and Spinal Muscular Atrophy (SMA). However, there is no efficient, safe, and practicable administration route which hampers the clinical use of these potentially therapeutic agents. We show that specific and high yield gene transfer into motor neurons can be obtained by peripheral intramuscular injections of recombinant adenoviruses. These vectors are retrogradely transported from muscular motor units to motor neuron cell bodies. Gene transfer can thus be specifically targeted to particular regions of the spinal cord by appropriate choice of the injected muscle. The efficiency of gene transfer is high, with more than 50 % of the motor neurons afferent to the injected muscle taking up the transgene. This new therapeutic protocol allows specific targeting of motor neurons without lesioning the spinal cord, and should avoid undesirable side effects associated with systemic administration of therapeutic factors.

396.13

SERUM AUTO-ANTIBODIES OTHER THAN ANTI-GM-1 IN THE PATHOGENESIS OF PROGRESSIVE MUSCULAR ATROPHY. R. Atkinson, C. Williams, W.K. Engel and C.A. Miller*. Univ. So. Cal. Sch. of Med., Dept. of Pathology, Los Angeles, CA 90033.

Some patients with progressive muscular atrophy (PMA), a lower motorneuron disease, have circulating immunoglobulins that bind to surfaces of alpha-motorneuron somas and proximal dendrites. In most of those cases, circulating antibodies to GM-1 ganglioside were demonstrated. We previously found that sera from three PMA patients also identified a 400kDa chondroitin sulfate proteoglycan (CSP-400) on Western blots. To determine the localization of this antigen, serum from one PMA patient with circulating anti-GM-1 antibody was absorbed by GM-1 bound to octyl-sepharose. The eluate (GM-1 fraction) and the non-bound fraction were tested for immunoreactivity on cryostat sections of normal human lumbar spinal cord. The eluate showed diffuse spinal cord grey matter localization. The non-bound serum fraction showed only motorneuron immunoreactivity, with distinct perineuronal and proximal dendritic staining, including circular profiles. Anti-cholera toxin, which binds specifically to GM-1 ganglioside only diffusely stained the neuropil. Thus, this patient's perineuronal immunoreactivity: a) seems to be the result of an autoantibody other than that one specific for GM-1 ganglioside, and b) may be involved in the pathogenesis of lower motorneuron disease.

396.14

UPTAKE OF IMMUNOGLOBULIN G FROM AMYOTROPHIC LATERAL SCLEROSIS(ALS) PATIENTS BY MOTOR NERVE TERMINALS IN THE MICE. S.A. Fratantoni*, S.E. Starkstein*and O.D. Uchitel*. FLENI e *Inst. de Biologia Celular y Neurociencias Dr. E.D.P. De Robertis, Fac. de Medicina, Univ. de Buenos Aires, 1211, Buenos Aires, Argentina.

Animal studies have established that there is a normal motor nerve terminal uptake, retrograde axonal transport, and soma accumulation of immunoglobulin G (IgG). This process could be implicated in neuromuscular diseases. The passive transfer of dysfunction in the neuromuscular transmission treated with ALS-IgG supports an autoimmune etiopathogenia for ALS (*PNAS vol 88: 647-651; Neurology 42: 2175-2180*). In the present study, we investigated in mice motoneuron somas, the presence of human ALS and control IgGs applied on to the motor nerve terminals. We applied the IgGs from 6 ALS patients and 6 controls by subcutaneous injections on the facial nerve terminals of the Levator auris longus muscle. After repetitive injections (10 per week, 1mg of IgG each) the brainstem containing the facial nucleus was serially cut into slices and immunoprocessed to detect human IgG. The immunoreactive neuronal somata were quantitatively evaluated with a computarized image analyzer. In all cases the number of motoneurons remained constant and they were inmunoreactive in different degrees for human IgG. Motoneuron labeling was more intense in the ipsilateral facial nucleus compared with the contralateral side for all IgG tested. The difference between ipsi and contralateral side was more pronounced in all the ALS-IgG treated animals. Furthermore, in the ALS IgG treated mice the ipsilateral labeling was significantly higher than the ipsilateral control IgG treated mice ($P< 0.001$). Therefore, our results are compatible with the concept that motoneurons differentially take up, transport or accumulate ALS-IgG with respect to control IgG. Uptake of pathogenic antibodies by motoneuron terminals may play a role in the pathogenesis of motoneuron disease. Supported by Muscular Dystrophy Association.

396.15

BRAIN FREE RADICAL SCAVENGING ENZYME ACTIVITIES IN AMYOTROPHIC LATERAL SCLEROSIS. D. Donaldson[1], M.W., Jakowec[1], A.B. Naini[1]*, S. Kish[2] and S. Przedborski[1]. [1]Dept. Neurol., Columbia University, New York, NY, 10032 and [2]Clarke Instit. of Psychiatry, Toronto, Ontario, Canada.

Amyotrophic lateral sclerosis (ALS) is a fatal paralytic disorders of unknown cause. Recent evidence has implicated the role of free radicals in the death of motor neurons in this disease. To further investigate this hypothesis, we have measured the activity of the main free radical scavenging enzymes copper/zinc-superoxide dismutase (SOD-1), manganese-superoxide dismutase (SOD-2), catalase, and glutathione peroxidase (GPx) in post-mortem samples from nine patients with ALS and from nine controls. We examined samples from the motor area of the cerebral cortex, a regions affected in ALS, and from the cerebellar cortex, a region not affected in ALS. The two groups did not differ in age or post-mortem delay. In the cerebral cortex motor area from ALS patients, GPx activity measured by an indirect spectrophotometric assay (0.46 ± 0.08 μmol/min/mg protein; [mean ± SEM]) was reduced significantly as compared to controls (0.76 ± 0.08 μmol/min/mg protein). The homospecific activity of GPx determined by ELISA was significantly reduced in ALS by 44% of the control values. In contrast, neither GPx activity or its homospecific activity was significantly altered in the cerebellar cortex from ALS as compared to controls. SOD-1, SOD-2 (corrected for citrate synthase or not corrected), and catalase were not significantly altered in the cerebral cortex motor area or cerebellar cortex in ALS samples. This study indicates that in ALS GPx enzymatic activity is reduced in brain regions affected in this disease and thus suggests that this finding may be relevant to the physiopathology of ALS.

396.16

SUPEROXIDE DISMUTASE AND AXOTOMY J. Rosenfeld and M. Crutcher*
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Endogenous antioxidant proteins, such as superoxide dismutase (SOD), are present in all cells in response to volatile free radical molecules. Many disparate complex neurodegenerative conditions have been related to intracellular oxidative stress. The purpose of this study is to investigate a simple model of neuronal injury on the expression of SOD. Using reversible and nonreversible axotomy, Mn-SOD and Cu/Zn-SOD are studied by immunocytochemistry and quantitative western blot analysis at multiple time points during neuronal recovery or degeneration.

After crush (reversible) or transection (non-reversible) lesion of the sciatic nerve both dorsal root ganglia and ventral horn motor neurons are evaluated. By 12 days following lesion, Mn-SOD reveals a marked increase in immunoreactivity in the dorsal root ganglia (DRG) along with a detectible increase on immunoblot compared to unoperated controls. Increased immunoreactivity appears greater in the transected nerve. By 60 days, the increased immunoreactivity subsided in the DRG following both lesions. Motor neurons did not display the same enhanced immunoreactivity, however, preliminary results from quantitative immuno blots suggest an increase of at least 25% in Mn-SOD by 24 days after reversible lesion. Data correlating quantitative western blots, immunocytochemistry and cellular morphology will be presented.

These data suggest that after axotomy, the simplest of neuronal injuries, Mn-SOD increases its expression presumably in response to intracellular reactive oxygen species. This increase may be greatest in the DRG and following complete nerve transection. This model lends itself to subsequent study of therapeutic agents with potential antioxidant effects.

396.17

INHIBITION OF ANTAGONIST MUSCLES DURING AUTOMATIC POSTURAL PERTURBATIONS FOLLOWING UPPER MOTOR NEURON (UMN) DAMAGE IN HUMANS. P.M.Diedrich, T. Matsumoto, J.A. McMillan*, and C.T. Leonard. Motor Control Research Laboratory, The University of Montana, Missoula, MT,59812 USA.

Individuals with UMN damage are unable to sufficiently inhibit the soleus muscle alpha motoneuron pool, as measured by H-reflex testing, during attempts at voluntary ankle dorsiflexion. Automatic reactions are typically not controlled directly by cortical projections. Rather, they are triggered by proprioceptive and vestibular input. The present study compared soleus muscle alpha motoneuron inhibition during activations of the tibialis anterior (TA) muscle initiated voluntarily or elicited by automatic postural perturbations. Although there were exceptions, individuals with UMN damage did not inhibit antagonist alpha motoneuron pools during automatic perturbations. Although tonic effects of descending cortical projections cannot be ruled out, it appears unlikely that the abnormal cocontraction associated with UMN damage can be explained solely by simultaneous activation of agonists and antagonists by descending cortical projections.

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400.1

POSITRON EMISSION TOMOGRAPHY, APOLIPOPROTEIN E EPSILON 4 HOMOZYGOSITY, AND THE GENETIC RISK FOR ALZHEIMER'S DISEASE. E.M. Reiman*, R.J. Caselli, S. Minoshima, S.N. Thibodeau, D. Bandy, J. Lawrence, and K. Chen. Univ. Arizona, Mayo Clinic Scottsdale, Mayo Clinic Rochester, Univ. Michigan, and the Samaritan PET Center, Good Samaritan Regional Medical Center, Phoenix, AZ 85006.

Positron emission tomographic (PET) measurements of the cerebral metabolic rate for glucose (CMRgl) were used to compare individuals with two copies of the apolipoprotein E epsilon 4 (APOE-ε4) allele to those with no copies of this allele prior to the age of risk for late-onset Alzheimer's disease (AD).

Advertisements were used to recruit 235 50-65 year-old volunteers with a family history of probable AD. Two had the ε2/2, 19 had the ε2/3, 5 had the ε2/4, 124 had the ε3/3, 73 had the ε3/4, and 12 had the ε4/4 genotype.

Eleven subjects with the ε4/4 genotype (mean age 55) and 22 age- and gender-matched control subjects (mean age 56) had neurological and psychiatric evaluations, a neuropsychological test battery, a T1-weighted volumetric magnetic resonance image (MRI), and PET measurements of CMRgl as they rested quietly with their eyes closed. An automated algorithm previously used to characterize posterior parietal and posterior cingulate abnormalities in patients with probable AD was used to investigate group differences in regional measurements and asymmetries.

In comparison to the control group, the APOE-ε4/4 group had significant, bilateral reductions in posterior cingulate and prefrontal CMRgl, nonsignificant reductions in parietal CMRgl, no differences in whole brain CMRgl, and no significant memory or naming impairments.

This study provides preclinical evidence that the APOE-ε4 allele is a risk factor for the development of AD. It demonstrates brain regions that are altered prior to the onset of illness, illustrates the value of studying APOE-ε4 homozygotes prior to the age of risk, and suggests PET's potential to rapidly test future AD prevention drugs.

400.2

APOLIPOPROTEIN E4 AND AMYLOID ANGIOPATHY-ASSOCIATED INTRACEREBRAL HEMORRHAGE. S.M. Greenberg*, G.W. Rebeck, J.P.G. Vonsattel, T. Gomez-Isla, B.T. Hyman. Dept. of Neurology, Mass. General Hosp. and Brain Tissue Res. Center, McLean Hosp., Harvard Med. School, Boston, MA 02114

Cerebral amyloid angiopathy (CAA) is characterized by deposition of the amyloid ß-peptide in cerebral arterioles and, in severe cases, intracerebral hemorrhage. We tested whether the apolipoprotein E4 (apoE4) allele was associated with 1) pathologic severity of CAA and 2) the presence and age of onset of intracerebral hemorrhage. ApoE genotype was determined without knowledge of pathology for 93 postmortem cases systematically graded for severity of CAA and for 20 patients with CAA-associated intracerebral hemorrhage. We found a significant and independent effect of apoE genotype in both cohorts. In the pathologic cases, the presence of apoE4 increased the odds ratio for moderate or severe CAA by 2.9-fold relative to cases without E4; two copies of apoE4 increased the odds ratio 13.1-fold. This effect was independent of senile plaque density. In the cohort of CAA-associated cerebral hemorrhages, apoE4 allele frequency was 0.38, significantly greater than the control frequency of 0.14 ($p<0.0005$). Among cases in which full clinical history was available ($n=11$), average age at the time of first hemorrhage was significantly less in the presence of apoE4 (70.0 yrs) than in its absence (81.1 yrs; $p<0.05$). Our results suggest that apoE4 is a risk factor for both CAA and CAA-associated intracerebral hemorrhage.

400.3

DISTRIBUTION OF APOE-IMMUNOREACTIVE NEURONS IN CONTROL AND AD BRAINS. G. Einstein*, P. Bautista, V. Patel, M. Kenna, L. Melone, R. Fader, K. Karson, A. Saunders, C. Hulette, A. Roses, and D. Schmechel. Depts. of Neurobiology and Neurology, Duke U. Med. Cent., Durham, NC 27710.

The genotype of apolipoprotein E is a susceptibility factor in familial and sporadic, late-onset Alzheimer's disease (AD). However, the relationship of the protein (apoE) to the primary pathology of AD, neuronal degeneration and death, is unknown. Since apoE is present in cortical neurons in both control and AD brains we reasoned that if apoE in neurons was related to pathogenic processes in AD its distribution would match the distribution of neuronal death in AD established by Braak and Braak (1991) and be coincident with other markers of AD pathology. To test this hypothesis we examined in detail, across neocortical areas 17, 18, and posterior inferior temporal cortex (IT), the relationship of apoE-immunoreactive neurons (apoE-IRn) to tau, GFAP, and A4 in both AD and control brains of known APOE genotype and cognitive status. Tissue was collected at autopsies performed no longer than 8 hours postmortem, placed in 4% paraformaldehyde in 0.1M phosphate buffer, fixed for 24 hours, vibratome sectioned at 40 µm, and stained consecutively for Tau, GFAP, A4, and Nissl. We found that apoE-IRn were (1) present in both control and AD brains but were more intensely stained in AD than controls; (2) present rarely in area 17, occasionally in area 18, and regularly in IT; (3) correlated in AD with dense gliosis and tau-IR but not with plaque formation. Finding that the distribution of apoE-IRn follows the staging of the progression of AD and is correlated with markers of AD pathology suggests that apoE plays a role in the pathological process of AD. The presence of apoE in aged control brains as well as in AD suggests that its presence marks a general response to stress. Whether its presence is harmful or protective is as yet to be determined and may depend on genotype. Supported by AG-05128 and the John A. Hartford Foundation.

400.4

NEURONAL APOLIPOPROTEIN E RECEPTOR, LRP, IN THE HUMAN CENTRAL NERVOUS SYSTEM SYNAPTIC NERVE TERMINAL. T.W. Kim*, M. Di Figlia, W. Wasco, G.W. Rebeck, B.T. Hyman, D.K. Strickland, R.E. Tanzi. Dept. of Neurology, Massachusetts General Hospital, MA 02129 and Biochemistry Lab., American Red Cross, Rockville, MD 20855.

Apolipoprotein E (apoE) has been implicated in neuronal plasticity and regeneration following peripheral and CNS injury. Recent studies indicate that the Apo-e4 allele greatly increases the risk of late-onset sporadic and familial Alzheimer's disease (AD). Moreover, apoE knock-out mice display significant synaptic loss and structural alterations, indicating that neuronal uptake of apoE may be critical to maintain structural integrity of the synapse. Among members of the low-density lipoprotein (LDL) receptor family, LDL receptor-related protein (LRP) is the only identified major neuronal receptor for apoE. LRP binds and mediates the internalization of a diverse array of ligands, such as α2-macroglobulin and plasminogen activator. LRP immunoreactivity has been demonstrated in plaques, where its multiple LRP ligands are also found, as well as in reactive astrocytes in the AD brain. We now report that LRP is significantly enriched in isolated synaptic nerve terminals from the human cerebral cortex. LRP is localized sub-synaptically to synaptic plasma membrane but was not detectable in the postsynaptic density or light membrane (LP2) fractions. The synaptic localization and the multiple ligand binding properties of LRP suggest that apoE may be taken up directly in the synaptic terminals, raising the possibility that dysfunction of lipoprotein metabolism and protease clearance at the synapses may lead to the loss of synaptic integrity or to synaptic degeneration that is characteristic of AD.

400.5

INFLUENCE OF APOE GENOTYPE ON DEVELOPING COGNITIVE IMPAIRMENT IN THE GENERAL POPULATION. B.T. Hyman*, T. Gomez-Isla, M. Briggs, H. Chung, S. Nichols, F. Kohout and R. Wallace. Neurology, Mass. Gen. Hosp, Boston, MA 02114 & Depts. Prevent Med and Periodontics, Univ. Iowa, Iowa City, IA 52242.

ApoE e4 (E4) is over-represented, and E2 under-represented, in clinic populations of Alzheimer disease (AD) patients and in late onset familial AD (fAD). It has been suggested that E4/4 genotype leads to nearly inevitable development of dementia by age 80 in fAD (Corder et al, 1994). To evaluate the degree of E2 or E4 associated increased risk for cognitive impairment in the general population, we studied participants in the Iowa 65+ Rural Health Study. This 10 year, population based epidemiological study screened multiple social and medical factors, including cognitive function screening tests, in essentially all individuals over the age of 65 in 2 rural counties. At year 7 (FU-6), 1899 blood samples were obtained. ApoE allele frequencies were: E2 0.09; E3 0.76; E4 0.14. Longitudinal analysis showed that after controlling for demographic variables, participants with any E4 were more likely to have lower FU-10 delayed recall scores ($p=0.002$) and those with any E2 were likely to have better delayed recall scores ($p=0.017$) than those with other genotypes. Cross-sectional analysis at FU-6 showed that E4 was 1.8X more common in the lowest 25% of verbal delayed recall scores ($p<0.01$), and 0.5X as common in the top 25% ($p<0.01$). Nonetheless, 11 of 34 E4/4's (age 77.2 ± 3.8 yrs) scored above the mean in verbal recall, including 2 in the top 10th percentile. Thus E4 and E2 have opposing influences on risk of developing cognitive impairment with increasing age. However, the magnitude of this influence is not as large in the general population as predicted from fAD or clinic-based samples.

400.6

Form and function of apoE-containing lipoproteins in the CNS. G.W. Rebeck*, H. Chung, A. Mendez, J.H. Growdon and B.T. Hyman. The Neurology Service and Cardiac Unit of the Massachusetts General Hospital, Boston, MA.

Genetic data have indicated that apolipoprotein E (apoE) plays a role in the pathophysiology of Alzheimer's disease. We analyzed apoE in human CSF to determine the normal function of apoE in the CNS. ApoE is present on high density lipoproteins (HDL) in CSF. We isolated lipoproteins from post-mortem CSF of seven AD patients by ultracentrifugation. CSF HDL consisted of 38% protein, 34% phospholipid, 13% free cholesterol and 15% cholesterol esters. The protein component of CSF HDL was almost entirely apoE and apoAI. In order to test whether these particles could deliver lipids to cells, we radioactively iodinated ApoE3-containing CSF HDL and incubated them with fibroblast and PC12 cells in culture. CSF HDL were taken up and degraded in a dose dependent manner, with significant uptake occuring at physiologically relavent concentrations (1-10 ug/ml). As comparison, plasma HDL were not taken up by cells in culture. We analyzed which receptors might mediate CSF HDL uptake by examining fibroblasts that expressed the LDL receptor and the LDL receptor-related protein (LRP) and fibroblasts that expressed only the LDL receptor. These two cell lines did not differ in CSF HDL uptake, suggesting that LRP did not affect metabolism of CSF HDL in fibroblasts.

These data are the first showing a function of the CSF lipoprotein and address how apoE complexes are cleared from CSF.

400.7

IN VITRO INTERACTIONS OF APOLIPOPROTEIN E ISOFORMS WITH TAU W.J. Strittmatter* and V. deSerrano Bryan Alzheimer's Disease Research Center, Duke University Medical Center, Durham, NC, 2771

The apolipoprotein E4 (apoE4) allele is a susceptibility gene for late-onset Alzheimer's disease. ApoE is found in neurofibrillary tangle bearing neurons. Neurofibrillary tangles are formed from the hyperphosphorylated microtubule associated protein tau. Previous studies showed that apoE3, and not apoE4, irreversibly bound tau, and synthetic peptides representing each of the four microtubule-binding domains of tau. Reversible interactions of the apoE isoforms with tau are being studied with plasmon resonance technology. Both apoE3 and apoE4 reversibly bind tau with similar time and concentration dependence. Binding is inhibited by peptides of each of the microtubule binding domains of tau. These observations support a two-step interaction of apoE with tau: reversible and non-isoform specific, followed by irreversible, isoform-specific binding. Interactions of apoE with tau may regulate intraneuronal tau metabolism in Alzheimer's disease and alter the rate of formation of neurofibrillary tangles.

400.8

EXPRESSION OF HUMAN APOLIPOPROTEIN E IN RODENT BRAIN VIA AN ADENO-ASSOCIATED VIRUS VECTOR. M.G. Kaplitt*,[1,2], J.Smith[1], F. Ferrari[3], R. J. Samulski[3], D.W. Pfaff[1], M.J. During[4], S. Gandy[2] and J. Breslow[1]. [1]The Rockefeller University, New York, NY 10021;[2]Cornell University Medical College, New York, NY 10021;[3]U of N. Carolina, Chapel Hill, NC 27599; 4Yale Univ. School of Medicine, New Haven, CT 06520.

We report the first expression of human apolipoprotein E (Apo E) in the rodent brain using an adeno-associated virus (AAV) vector. Although transgenic mice have been created which either express no endogenous apoE or overexpress human apoE, to date expression of human apoE has not been reported in the rodent brain. Previously we have demonstrated that an AAV vector can safely and stably transfer and express foreign genes in the rodent and primate brain, and therapeutic improvement has been achieved in animal models of Parkinson's disease. We have now created AAV vectors containing human ApoE2 or ApoE4 isoforms under the control of the human cytomegalovirus promoter. Following packaging of AAV-ApoE, expression of both isoforms was demonstrated in a tissue culture cell line (293T) using a goat-anti human ApoE antibody. Purified AAV-ApoE2 vector was then unilaterally injected into cerebral cortex and hippocampus of the rat brain. After 3 days, cells expressing human apoE were observed within these regions, while there was a complete absence of positive cells on the contralateral hemisphere which had been injected with a control vector expressing the lacZ gene. Studies examining long-term expression and gene transfer into Apo E-deficient mice are currently in progress. Given recent advances in the development of animal models of Alzheimer's disease, these results present the opportunity to examine whether specific ApoE isoforms promote or prevent neuropathology and suggest the possibility of a novel gene therapy approach to this disorder.

400.9

THE INHIBITION OF NEURITE OUTGROWTH BY APOLIPOPROTEIN E4 IS MEDIATED THROUGH THE LOW DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN. B. P. Nathan,* K.-C. Chang, S. Bellosta, R. W. Mahley,†‡ and R. E. Pitas‡. Gladstone Institute of Cardiovascular Disease, Cardiovascular Research Institute, Departments of †Medicine and ‡Pathology, University of California, San Francisco, CA 94141-9100

Apolipoprotein (apo) E4 inhibits neurite outgrowth from murine neuroblastoma (Neuro-2a) cells. The inhibitory effect of apoE4 was observed only in the presence of lipoproteins and was blocked by a monoclonal antibody to the lipoprotein receptor–binding domain of apoE, suggesting that the interaction of apoE with lipoprotein receptors is important in eliciting the effect. In the present study, we treated cells with reagents that inhibit binding and uptake of lipoproteins by the low density lipoprotein (LDL) receptor–related protein (LRP), but not by the LDL receptor, and examined the effect on apoE4-mediated inhibition of neurite outgrowth. Neuro-2a cells were preincubated for 1 hour in serum-free medium alone or in medium containing either heparinase (10 units/ml) and chlorate (30 mM), or the receptor-associated protein (RAP) (5 μg/ml). Heparinase and chlorate deplete cell-surface heparan sulfate proteoglycans that bind apoE-enriched lipoproteins prior to internalization via the LRP, whereas the RAP competitively inhibits the binding of lipoproteins to the LRP. Following preincubation, β-migrating very low density lipoproteins (β-VLDL) (40 μg cholesterol/ml) and apoE4 (30 μg/ml) were added to the medium, and the cells were incubated for 24 hours in the presence of the inhibitors. After this incubation, inhibitors were re-added to the medium, and the cells were incubated for an additional 24 hours. Heparinase and chlorate or RAP alone, in the absence of β-VLDL and apoE4, did not have any effect on neurite outgrowth. Consistent with our previous studies, apoE4 in the presence of β-VLDL alone inhibited neurite outgrowth. However, when cells were treated with heparinase and chlorate or with RAP, the inhibitory effect of apoE4 with β-VLDL on neurite outgrowth was abolished. The data suggest that binding of apoE4-enriched lipoproteins by the LRP is necessary for the inhibitory effect on neurite outgrowth.

400.10

LRP MEDIATES APOLIPOPROTEIN E-DEPENDENT NEURITE OUTGROWTH IN A CNS-DERIVED NEURONAL CELL LINE. D. M. Holtzman*[1], R. E. Pitas[2], J. Kilbridge[3], Y. Sun[1], B. Nathan[2], R. W. Mahley[2], G. Bu[1], A. L. Schwarz[1]. Washington University[1], St. Louis, MO, 63110, Gladstone Institute[2], UCSF, San Francisco, CA, 94141, UCSF[3], San Francsico, CA, 94143.

The ε4 allele of apolipoprotein E (apoE) is a major risk factor for Alzheimer disease (AD) suggesting that apoE may directly influence neurons in the aging brain. Recent data suggest that apoE-containing lipoproteins can influence neurite-outgrowth in an isoform-specific fashion. The neuronal mediators of apoE effects have not been clarified. Herein, we show that in a CNS-derived neuronal cell line (GT1-1 trk9), apoE3 but not apoE4 increases neurite extension. The effect of apoE3 required the presence of lipoproteins (ß-VLDL) and was blocked at low nM concentrations by purified 39-kDa protein which regulates ligand binding to the low density lipoprotein receptor-related protein (LRP). Anti-LRP antibody also completely abolished the neurite promoting effect of apoE3. Understanding isoform specific cell biological processes mediated by apoE-LRP interactions in CNS neurons may provide insight into AD pathogenesis.

400.11

DNA DAMAGE IN ALZHEIMER'S DISEASE: COLOCALIZATION WITH C-JUN IMMUNOREACTIVITY, AND EFFECT OF BRAIN AREA AND POSTMORTEM DELAY. Aileen J. Anderson*, Joseph H. Su and Carl W. Cotman. Brain Aging and Dementia Institute. University of California, Irvine. Irvine, CA 92717-4550.

Neurons in Alzheimer's disease (AD) exhibit terminal deoxynucleotidyl transferase (TdT) labeling for DNA strand breaks with a distribution suggestive of apoptosis in many cells (Su et al., 1994). We have previously shown that immunoreactivity for the immediate early gene c-Jun is elevated in AD, and found in association with neuronal pathology (Anderson et al., 1994). In addition, cultured neurons undergoing β-amyloid-mediated apoptosis exhibit a selective and prolonged induction of c-Jun (Anderson et al., in press). Consequently, we examined whether c-Jun is associated with DNA damage in AD tissue by double-labeling for DNA damage and c-Jun in 8 AD and 5 control cases. We observed a strong colocalization of these markers, supporting a relationship between the expression of c-Jun and neuronal risk and/or cell death. In order to examine the relationship of TdT labeling to brain area, we compared TdT labeling in the hippocampal formation and cerebellum of 5 individuals with AD. As would be predicted if the DNA damage detected with TdT is associated specifically with AD-related neuronal risk/cell loss, these results revealed low to absent TdT labeling the the cerebellum. Gel electrophoresis of the genomic DNA from 4 AD and 4 control cases revealed no evidence for either an apoptotic or necrotic mechanism of cell death in AD. Although the above studies were conducted in tissues with very short postmortem delay (PMD), we also examined the effect of PMD on TdT labeling. In contrast to recent reports, our results suggest that PMD does affect TdT labeling at later timepoints.

400.12

CANDIDATE GENES FOR THE MAJOR EARLY ONSET FAMILIAL ALZHEIMER'S DISEASE GENE ON CHROMOSOME 14 INCLUDING A NOVEL PROTEASE. R.E.Tanzi*, D.M.Romano, W.Pettingell, P. Jondro, S.Schmidt, A.C.Crowley, J.Murrell, A.Buckler, K.Felsenstein, W.Wasco. Genetics and Aging Unit, Mass. General Hospital, Charlestown, MA; Bristol Myers-Squibb, Wallingford, CT.

The gene defect responsible for over 80% of familial Alzheimer's disease (*FAD3*) lies in an approximately 3 million bp region on chromosome 14 between markers *D14S61* and *D14S289*. In this region, we have assessed a number of candidate genes including the E2k component of α-ketoglutarate dehydrogenase (*DLST*), transforming growth factor β3 (*TGFB3*), and latent transforming growth factor-beta binding protein (*LTBP-2*). Of these genes, only *TGFB3* has been excluded. We have also isolated full-length cDNAs for a novel protease using "trapped exons" from the candidate region. This gene is ubiquitously expressed, differentially spliced, and has been shown to be active in its ability to cleave APP. We are currently in the process of searching for pathogenic mutations in this gene.

BETA-AMYLOID: NEUROTOXICITY

401.1

EXPRESSION OF MEMBRANE ATTACK COMPLEX CONTRIBUTES TO THE INCREASED β-AMYLOID PROTEIN INDUCED NEURONAL CELL DEATH BY INTERRUPTION OF COMPLEMENT INHIBITOR CD59. Y. Shen*, T. Sullivan, S. Magnuson, K. Shiosaki and C.W. Lin Neuroscience Department, Abbott Laboratories, Abbott Park, Illinois 60064, USA

Evidence that β-amyloid protein (Aβ) is detected in normal brain tissues and cultures, and that synthetic Aβ is not highly neurotoxic, suggests Aβ alone may not be sufficient to cause Alzheimer's disease (AD). In addition to Aβ, focal immunoreactivity for various complement components has been found in senile plaques, tangles and dystrophic neurites in AD brains. Recently, we reported that complement activation causes neuronal cell death, and this degenerative process is regulated by homologous restriction (*Brain Res.* 671:282,1995). Furthermore, blockade of complement inhibitor CD59 (endogenous inhibitor of reactive lysis) resulted in increased vulnerability of differentiated SH-SY5Y human neuroblastoma cells to Aβ toxicity (*Neurosci. Abstr.* #676.10, 1994). This evidence has led to our hypothesis that interruption of CD59 increases neuronal vulnerability to Aβ by activating endogenous complement components and forming membrane attack complexes (MAC) on the cell membranes. To test this hypothesis, we were able to utilize techniques of reverse transcriptase polymerase chain reaction (RT-PCR) and PCR-Southern hybridization to detect the expression of message for the major complement components (C1q, C3, C4, C5, C6, C7, C8 and C9) in SH-SY5Y cells as well as in human brains. The results indicate that SH-SY5Y cells and human brains are able to synthesize the major complement components required to form MAC. Immunocytochemical studies demonstrated the pregence of MAC on SH-SY5Y cells after treatment with Aβ or human serum in combination with either CD59 antibody or the enzyme which cleaves complement inhibitors on cell membranes (PI-specific phospholipase, PI-PLC). The results suggest that in SH-SY5Y cells, Aβ can activate complement cascade, and that MAC can be detected following depletion of CD59. MAC observed in senile plaque may be a consequence of Aβ action on neurons with dysfunctional endogenous complement inhibitors.

401.2

COAGULATION FACTOR XIa CLEAVAGE OF THE AMYLOID ß-PROTEIN (Aß) ALTERS ITS PATHOLOGICAL PROPERTIES. Susan M. Saporito-Irwin* and William E. Van Nostrand. Department of Microbiology and Molecular Genetics, College of Medicine, University of California, Irvine, CA 92717-4025.

Deposition of Aß in senile plaques in the neuropil and in the walls of cerebral blood vessels is one the hallmark features of Alzheimer's disease. Aß is proteolytically derived from its transmembrane parent protein, the amyloid ß-protein precursor (AßPP). AßPP is a multidomain protein which can be translated from alternatively spliced transcripts from a single gene on chromosome 21. The major mRNA species encode proteins of 695, 751 and 770 amino acids. The latter two isoforms contain a 56 amino acid domain that is homologous to Kunitz-type serine protease inhibitors (KPI). Recently, we have shown that $Aß_{1-42}$ induces cellular degeneration which is accompanied by a striking increase in its precursor, AßPP, in cultured human cerebrovascular smooth muscle cells. Here we show that coagulation factor XIa, a target protease for inhibition by the KPI domain of AßPP, proteolytically cleaves Aß between residues Arg^5 and His^6 of the peptide. Proteolytic processing of $Aß_{1-42}$ at this site abolishes its ability to induce cellular degeneration and increased levels of cellular AßPP in the cultured cerebrovascular smooth muscle cells. These results suggest that the amino terminus of $Aß_{1-42}$ is important for eliciting pathologic changes in these cells. In addition, these findings indicate that the KPI domain of AßPP can regulate the activity of proteases that can process and alter the pathologic and possible physiologic properties of Aß.

401.3

AMYLOID ß PROTEIN TOXICITY AND ACTIVATED GLUCOCORTICOID RECEPTORS IN HIPPOCAMPAL NEURONS

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Amyloid ß protein (Aß), which accumulates in the central nervous system plaques, the characteristic feature of Alzheimer's disease (AD), is cytotoxic to clonal neuronal cells as well as to primary neurons. Addition of Aß to cultured neurons initially induces accumulation of hydrogen peroxide and finally results in lipid peroxidation and cell death. Dramatic cell death occurs in the hippocampus of AD patients. Since hippocampal neurons express high levels of glucocorticoid receptors, and the activation of these receptors has been proposed to exacerbate neuronal injury in the rat brain following ischemia and other metabolic insults, it was investigated if activated glucocorticoid receptors play also a role in Aß induced cell death. HT22 and HN10e, two clonal mouse hippocampal cell lines express endogeneous glucocorticoid receptors as shown by functional transactivation assays, western blot analysis, and immunocytochemistry. These cells are sensitive to Aß: 63 ± 8 % of HT22, 78 ± 5 % of HN10e, and 30 ± 6% of primary neurons are surviving a 24 h exposure to $A\beta_{25-35}$ as measured by MTT and trypan blue exclusion assays. When these hippocampal cells are preexposed to 10^{-5} to 10^{-9} M corticosterone or dexamethasone, cell death is further increased by up to 20 %. Cell death induced by the excitotoxin glutamate, which generates oxidative stress in a non receptor mediated fashion in HT22 cells, is also increased by up to 40 % after a 24 h exposure to 1 mM glutamate when endogeneous glucocorticoid receptors have been activated. These data show that the activation of glucocorticoid receptors may increase oxidative injury induced by glutamate as well as by Aß in hippocampal neurons.

401.4

MEMBRANE INTERACTIONS AND TOXICITY OF ALZHEIMER'S ß-AMYLOID.

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ß-amyloid is a 39-43 amino acid peptide derived from an integral membrane glycoprotein precursor, the amyloid precursor protein. Deposits of ß-amyloid peptide accumulate in Alzheimer's disease (AD) brain, but their precise contribution to the subsequent neuropathology is presently unclear. A primary role for ß-amyloid in AD pathology was suggested by reports that ß-amyloid was directly toxic to neurons in vitro and in-vivo. However contradictory observations regarding the neurotoxic effects of ß-amyloid have been reported, and the mechanism by which these peptides cause toxicity have not been adequately explained. In this work synthetic peptides to ß-amyloid 1-40 and 1-42 and their fragments (1-28, 10-20, 25-35 and scrambled sequences) were used to study their interaction with a membrane bilayer system. In such a system, two halves of a closed membrane bilayer may respond differently to various perturbations while remaining coupled to one another. The same fragments were also used in a tissue culture system to study their effect on differentiated neurons and on undifferentiated cells. The extent of membrane damage was measured by lactate dehydrogenase released into the media. The work identifies precise regions of ß-amyloid that interact with the membrane in a closed bilayer system and in cultured cells and suggests mechanisms involved in the interaction.

401.5

ALZHEIMER'S DISEASE β-AMYLOID PEPTIDE 25-35 IS LOCATED IN THE MEMBRANE HYDROCARBON CORE: X-RAY DIFFRACTION ANALYSIS.

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Neurosciences Research Center, Allegheny Campus, Medical College of Pennsylvania and Hahnemann University, Pittsburgh, PA 15212.

Previous studies have demonstrated that Alzheimer's disease β-amyloid (β/A4) 25-35 contributes to neurotoxicity by modulating the activity of membrane-bound proteins, including calcium channels. Small angle x-ray diffraction was used to explore the structural interactions of β/A4 (25-35) with membrane lipid bilayers. Multilamellar vesicles composed of 1-palmitoyl-2-oleoyl lecithin (POPC) were mixed with β/A4 (25-35) at varying concentrations in HEPES buffer (2.0 mM NaCl, pH 7.3). At 37°C, the *d*-space of control POPC membrane bilayers was 51.4 Å and the intrabilayer headgroup separation was 39 Å. One-dimensional electron density profiles generated from the data demonstrated that addition of β/A4 (25-35) produced a broad, concentration-dependent increase in electron density 0-12 Å from the center of the membrane bilayer. Increases in electron density associated with the interbilayer water space were not observed. These data provide direct evidence for a strong hydrophobic association of β/A4 (25-35) with the membrane hydrocarbon core as opposed to an electrostatic membrane surface interaction. These interactions of β/A4 (25-35) with the lipid bilayer hydrocarbon core may induce conformational changes in the structure of membrane-bound proteins, which could contribute to perturbations in ion channel activity.

401.6

ß-AMYLOID PEPTIDE MODULATES CALCIUM CURRENTS IN hNT CELLS.

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hNT cells are produced from NT2 teratocarcinoma precursor cells by differentiation with retinoic acid. Whole cell patch clamp recordings under our conditions indicate that calcium currents in these cells are 30-80% of the dihydropyridine-sensitive type. We find that external application of the ß-AP_{1-40} peptide at 140 μM causes a rapid and reversible increase in inward calcium current in most hNT cells. However, low concentrations of the peptide [20-50μM] caused a rapid decrease in calcium current, usually reversible. The effect of the ß-AP_{1-40} peptide might well be the initial step in the neurotoxic response of neuronal cells to high concentrations of ß-AP_{1-40} [Yankner, et al., Science, 1990, 250:279-282], since this would interfere with the ability of neuronal cells to maintain normal calcium homeostasis.

401.7

TEA SENSITIVITY OF HIPPOCAMPAL K^+ CHANNELS IS REDUCED IN β-AMYLOID INJECTED RATS.

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We have recently shown that β-amyloid, the main component of Alzheimer's Disease (AD) plaques induces some of the changes consistently observed in fibroblasts from AD patients, namely alterations in K^+ channels and reductions of Cp20 (a memory-associated GTP binding protein). Nevertheless, it remained important to establish whether or not the alterations observed in peripheral tissue (particularly those mimicked by β-amyloid treatment), could take place in brain cells, the main target of the disease process. Adult rats were cannulated and injected with 100 μM β-amyloid or ringer solution for three consecutive days. Hippocampal cells were acutely dissociated 24 h after the last injection and conventional patch-clamp techniques were used to assess K^+ channel function. Overall, the number of patches with observable K^+ channels in the β-amyloid treated group was somewhat reduced (62%) as compared to active patches in controls (85%). A large conductance K^+ channel (≈ 180 pS) showed significant differences in TEA sensitivity in cells from control and β-amyloid treated animals. The activity of this channel was eliminated or greatly reduced by TEA (50-100 mM) in cells from ringer injected animals (N= 9 rats), while it was preserved in all cells from β-amyloid treated animals (N= 7 rats), $p < 0.01$ (contingency table). This reduced TEA sensitivity has been previously observed in fibroblasts and olfactory neuroblasts from AD patients. The frequency of appearance of this K^+ channel was also reduced in treated cells (60%) as compared to 82% in control patches. These preliminary observations provide evidence that alterations in K^+ function/regulation do occur in brain cells, and they can be induced by β-amyloid treatment.

401.8

NITRIC OXIDE MEDIATES A COMPONENT OF ß-AMYLOID NEUROTOXICITY IN PRIMARY CORTICAL NEURONAL CULTURES.

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Derangements in glutamate neurotransmission have been implicated in several neurodegenerative disorders including Alzheimer's disease (AD). AD is characterized by progressive neurodegeneration of unknown etiology, but may be associated with the abnormal metabolism or deposition of the ß-amyloid protein. In primary neuronal cultures fragments of the ß-amyloid protein (ß-amyloid 1-40 and ß-amyloid 25-35) when preaggregated, are directly toxic to neurons with cell death occurring within 12 hours. The soluble peptides enhance glutamate induced neurotoxicity predominantly through activation of the N-methyl-D-aspartate (NMDA) receptor subtype of glutamate receptors. NMDA receptor activation causes an influx of calcium which binds calmodulin and activates neuronal nitric oxide (NO) synthase to convert L-arginine to citrulline and NO. NO has many roles in the central nervous system as a messenger molecule, however, when generated in excess NO can be neurotoxic. Inhibitors of NOS and scavengers of superoxide anion provide neuroprotection against ß-amyloid and NMDA induced neurotoxicity but not against the neurotoxicity induced by preaggregated ß-amyloid. Thus NO may play a role in neurotoxicity induced by the soluble ß-amyloid peptide. It is possible that neuronally derived NO contributes to the neurodegeneration associated with AD.

401.9

ABERRANT PROCESSING OF AMYLOID PRECURSOR PROTEIN IN DOWN'S SYNDROME ASTROCYTES.

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The development of Alzheimer's disease in individuals with Down's syndrome (DS) has been attributed to overexpression of amyloid precursor protein (APP) due to increased gene dosage. To examine the processing of APP in the DS brain, we have established primary astrocyte cultures from DS and control cortex. DS and control astrocytes were similarly viable in culture. Metabolic labeling showed increased holo-APP in DS astrocytes relative to matched controls (214±40% of control, $P<0.05$). Despite increased APP, the level of soluble APP in the medium was decreased in DS astrocytes (60±2% of control, $P<0.01$), a finding that was replicated in cultures derived from 4 different DS brains. Pulse-chase labeling showed that the rate of APP secretion was markedly decreased in DS astrocytes. Holo-APP was synthesized at higher levels in DS astrocytes, but in addition showed increased stability relative to controls, correlating with decreased proteolytic degradation. This was accompanied by increased levels of the potentially amyloidogenic 11.5 kD C-terminal fragment of APP. These results suggest that APP processing is altered in DS astrocytes, and raise the possibility that an APP processing defect independent of APP overexpression may contribute to the development of Alzheimer's disease in adults with DS.

401.10

β-AMYLOID PEPTIDES WITH AMINO-TERMINAL DELETIONS EXHIBIT NEUROTOXICITY AND INCREASED AGGREGATION *IN VITRO*.

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β-amyloid protein (Aβ) isolated from senile plaques exhibits substantial COOH- and NH_3-terminal heterogeneity. Since COOH-terminal heterogeneity significantly affects Aβ structure/activity relationships, we examined whether NH_3-terminal heterogeneity also affects Aβ bioactivity and/or aggregation. To address this issue, we synthesized a series of peptides with progressively shortened NH_3-termini (initial residues at positions β1, β4, β8, β12, and β17) and COOH-termini extending to residue β40 or β42. Peptides were assayed for *i)* neurotoxicity in primary neuron cultures, *ii)* aggregation levels by sedimentation analysis, *iii)* secondary structure by circular dichroism, and *iv)* fibrillar structure by electron microscopy. Consistent with our previous observations, we found that all aggregating Aβ peptides induced significant neurotoxicity *in vitro*. More importantly, we report that Aβ peptides with NH_3-terminal deletions exhibit enhanced aggregation levels relative to full-length species. This trend of increased aggregation with decreased NH_3-terminus length was observed in peptides terminating both at β40 and at β42, although the β42 peptides showed higher overall sedimentation levels. Peptides exhibiting significant aggregation were characterized by predominant β-sheet structure and fibrillar morphology. The increased ability of Aβ peptides with NH_3-terminal deletions to aggregate into β-sheet fibrils suggests that these Aβ species may initiate and/or nucleate pathological Aβ deposition and predict that events which promote generation of Aβ NH_3-terminal heterogeneity represent potential risk factors for Alzheimer's disease.

401.11

CHRYSAMINE G, A CONGO RED ANALOGUE WHICH ENTERS THE BRAIN, PREVENTS Aβ-INDUCED TOXICITY IN CELL CULTURE.

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Considerable data has accumulated to suggest that, at least in cell culture, beta-amyloid (Aβ) is toxic to a variety of cell types. This toxicity is dependent on aggregation into beta-sheet fibrils. Congo red is a histologic dye that binds to a wide variety of amyloid deposits, all of which have in common a beta-sheet fibril structure (Klunk et al., J.Histochem.Cytochem. 1989;**37**:1273). Recently, Congo red has been shown to prevent Aβ-induced toxicity in primary rat hippocampal culture (Lorenzo and Yankner, PNAS 1994;**91**:12243) and rat PC12 cells (Pollack et al., Neurosci. Lett. 1995;**184**:113). However, Congo red is a highly acidic di-sulfonic acid which does not readily enter the brain. We have previously reported the development of Chrysamine G (CG), a carboxylic acid analogue of Congo red which partitions well into normal mouse brain (Klunk et al., Neurobiol. Aging 1994;**15**:691). In this study, we show that CG, like Congo red, can prevent Aβ-induced toxicity in rat PC12 cells and a human small cell lung carcinoma cell line with neuroendocrine properties (SHP-77). In both cell lines, CG and Congo red prevented Aβ-induced toxicity at concentrations similar to their IC_{50} for binding to synthetic Aβ in vitro. These results suggest that CG cay be useful in the **treatment** of Alzheimer's disease.

401.12

POTENCY OF CONGO RED NEUROPROTECTION AGAINST AMYLOID-ß NEUROTOXICITY VARIES WITH Aß PEPTIDE LOT.

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Peptide conformation and aggregation state of amyloid-ß (Aß) appear to be important determinants for neurotoxicity. For example, "fresh" peptide lots of Aß with minimal structure have modest to moderate neurotoxic activity at 50µM, but little toxicity at 25µM unless "aged" for a period of days. In contrast, "pre-aged" lots of Aß already have some structure and show maximal toxicity at 25µM without aging. To further address these variables, we have compared the potency of Congo Red (CR) in affording neuroprotection against various lots of Aß. Mature, primary rat hippcampal cultures were co-treated with synthetic Aß(1-40) and CR. Cell viability was assessed 3-4 days later by an LDH release assay. As observed by others, near equimolar concentrations of CR (5-10µM) were required to block neurotoxicity of 25µM "pre-aged" lots of Aß peptide. In contrast, CR at a 100 fold lower concentration (50-100nM) provided complete protection in cultures treated with 50µM "fresh" Aß lots. Moreover, brief alkaline-treatment of Aß converted the toxicity profile of a pre-aged" peptide into that of a "fresh" peptide with the concomitant shift from micromolar to nanomolar CR protection. These data suggest that maturation of Aß into a neurotoxic species occurs via a multi-step process and that early on, the initiating Aß species may be present at low nanomolar concentrations.

PEPTIDE RECEPTOR STRUCTURE AND FUNCTION I

402.1

Characterization of neuropeptide Y (NPY) receptors in discrete rat brain areas: subtype-related differences in affinity and sensitivity to ions and guanine nucleotides

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NPY receptors [NPY-Rs] were characterized in microdissected hypothalamic and cortical areas of rat brain employing both subtype-nonselective and subtype-specific agonists. Synaptosomal fractions contained >=90% of specific binding sites for either of the NPY-R subtypes. NPY receptors from all areas studied showed essentially homogenous high-affinity binding of a Y-1 subtype- selective NPY analog, (Pro34)hPYY, with Kdiss range of 80-150 pM. High-affinity Y-2 subtype binding of [125I]-human PYY(3-36) (hPYY(3-36) was especially strong in circumventricular anterior hypothalamus. The Y-2 binding could be resolved into a high-affinity component (Kdiss <=10 pM), and a highly variable lower-affinity component which could be augmented by preincubation. The Y-1 subtype binding of (Pro34)hPYY and (Leu31,Pro34) hPYY was much more sensitive than Y-2 binding of hPYY(3-36) to inhibition by Ca2+ removal, by increases in [Na+] (affinity decrease of up to 5 pM/mM Na+), by nonionic chaotropes (urea), and by disulfide bond destabilizers (benextramine, dithiothreitol). The Y-1 binding was strongly inhibited by all guanosine (but not adenosine) di- and triphosphates tested. Particulates from circumventricular hypothalamic areas and from piriform cortex showed a considerable Y-2 receptor activation by G-protein inactivator guanosine-5'-O-(2-thiodiphosphate) (GDPbetaS), and also by GDP and GTP, but not by the corresponding adenine nucleotides. Y-2 receptor binding in all areas was <=30% inhibited by unhydrolyzable G-protein agonist guanosine-5'-O-(3' -thiotriphosphate (GTPgammaS), or by selective chelation of Ca2+. The binding of subtype-nonselective NPY-R agonists [125I]hNPY and [125I]pPYY to membranes from areas with predominance of Y-1 subtype was much more sensitive to ionic and nucleotidic inhibitors than the binding of these ligands to particles from Y-2 subtype-enriched areas, indicating that the observed differences in inhibition profiles generalize to receptor subtypes. Thus, the binding activity of Y-1 subtype NPY-Rs depends directly on association with G-protein(s), and is strongly modulated by changes in concentration of common ions. The activity of Y-2 receptors shows little direct dependence on ions or nucleotides, and could be mainly regulated through interactions with effector proteins and changes in receptor organization (microaggregation or clustering) and synaptic availability (sequestration, internalization, and extrusion).

402.2

INHIBITION OF SYMPATHETIC VASOCONSTRICTION BY THE NEUROPEPTIDE Y -Y1 RECEPTOR ANTAGONIST BIBP 3226.

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The non-peptide neuropeptide Y (NPY) - Y1 receptor antagonist BIBP 3226 (Rudolf et al., Eur. J. Pharmacol., 1994, 271, R11-R13) specifically displaced ^{125}I-NPY binding with IC50 value of about 10 nM in pig hypothalamus and renal arteries (Y1 receptors) with minor effects in the spleen (Y2 receptor). In anesthetized pigs in vivo BIBP 3226 inhibited the vasoconstrictor effects of a Y1 receptor agonist without influencing the responses to a Y2 receptor agonist, noradrenaline, α,β-methylene ATP or angiotensin II. A major reduction (about 50%) of the long-lasting vasoconstrictor responses to sympathetic nerve stimulation at high frequency (40 impulses at 20 Hz) in nasal mucosa and hind limb of control pigs occurred after BIBP 3226 (1 mg/kg). BIBP 3226 (1 mg/kg) markedly reduced (by 55-70%) the vascular response (total integrated blood flow reduction) evoked by sympathetic nerve stimulation at high frequency (40 impulses at 20 Hz) in spleen, kidney, nasal mucosa and hind limb of reserpinized pigs lacking noradrenaline. Furthermore, the maximal amplitude of the vasoconstriction was reduced especially in the kidney. BIBP 3226 did not influence stimulation-evoked NPY release. It is concluded that BIBP 3226 can act as a selective Y1 receptor antagonist in the pig. A role of endogenous NPY for the long-lasting duration of sympathetic vasoconstrictor effects in nasal mucosa and hind limb of pigs is highly suggested.

402.3

EXPRESSION CLONING AND PHARMACOLOGICAL CHARACTERIZATION OF A HUMAN HIPPOCAMPAL NPY/PYY Y2 RECEPTOR SUBTYPE. C. Gerald*, M.W. Walker, J.A. Bard, C. He, Z. Shaposhnik, P.J.-J. Vaysse, T.A. Branchek and R.L. Weinshank. Synaptic Pharmaceutical Corporation, Paramus, NJ 07652

Neuropeptide Y (NPY) and related peptides elicit a diverse range of important physiological activities through activation of membrane bound receptors. Previous functional and binding data support the existence of multiple receptor subtypes including Y1, "atypical" Y1, Y2, Y3 and PP. To date, only the rat, human and various other species homologs of the Y1 receptor subtype cDNAs have been cloned and characterized. We now report the expression cloning and full pharmacological characterization of a human hippocampal cDNA encoding a Y2 receptor subtype. Protein sequence analysis reveals that this Y2 receptor is a member of the G protein-coupled superfamily. When compared to the Y1 subtype, the Y2 receptor displays an amino acid identity of only 31% overall and 41% in the seven membrane spanning regions. ^{125}I-PYY bound specifically to membranes from COS-7 cells transiently transfected with the cDNA expression clone. Upon extensive pharmacological characterization, the receptor encoded by this cDNA clone exhibits features of a Y2 receptor, including 1) binding affinity for PYY equal to or greater than that for NPY, 2) relative insensitivity to N-terminal deletion of NPY/PYY and 3) low tolerance for modification of Gln^{34} in NPY and NPY-like peptides. Northern blot analysis reveals a broad distribution of the Y2 mRNA in human brain. RT-PCR analysis yielded positive signals in most human peripheral tissues tested except in the atrium of the heart, the liver and the uterus. Southern blot analysis is consistent with the human genome containing a single Y2 receptor gene. The isolation of a human Y2 receptor allows, for the first time, the ability to develop subtype specific ligands and will greatly facilitate the analysis of NPY-mediated physiological processes.

402.4

CLONING AND CHARACTERIZATION OF A NOVEL HUMAN NEUROPEPTIDE Y/PEPTIDE YY/PANCREATIC PEPTIDE RECEPTOR SUBTYPE (Y4). J.A. Bard*, M.W. Walker, T.A. Branchek and R.L. Weinshank. Synaptic Pharmaceutical Corporation, Paramus, NJ 07652.

The pancreatic polypeptide (PP) family of polypeptides contains NPY, PYY and PP, all consisting of 36 amino acids and shown to influence a range of physiological parameters, including food intake, psychomotor activity, central endocrine secretion and vasoactivity in the cardiovascular system. To date, only one receptor within this family, Y1, has been cloned. Here, we describe the cloning of a new member of this family, designated Y4, obtained from homology screening of a human placental genomic library, using transmembrane (TM) probes derived from the Y1 gene. The Y4 gene encodes for a predicted protein of 375 amino acids and contains several conserved structural features/residues found among the members of the neuropeptide family, including the greatest homology to the cloned human/rat/mouse Y1 gene (42% overall; 55% within TM's). Y4-transiently transfected COS-7 cells displayed high affinity binding of the PP family agonist [^{125}I]PYY(porcine), with a K_d=0.11 nM (B_{max}=1.4 pmol/mg protein), and similar high affinity to three other PP family agonists (human): NPY, PYY and PP (K_i=2.2nM, 0.87nM, and 0.06nM, respectively). Pharmacological characterization of Y4 indicated a rank order of potency of the PP family of peptides, including selective truncated peptides, to be PP>[Leu^{31},Pro^{34}]NPY=PYY>NPY>NPY_{2-36}>NPY_{13-36}>NPY_{18-36}, NPY_{20-36}, and $NPY_{free\ acid}$. Additionally, activation of the human Y4 with PP results in decreased cAMP accumulation, very likely through inhibition of adenylate cyclase, as well as increased calcium mobilization. Y4 mRNA was detected by RT-PCR in human total brain, coronary artery, and ileum, suggesting a potential role for Y4 receptors in CNS function, cardiovascular regulation, and gastrointestinal physiology.

402.5

BINDING OF NPY, PYY AND PP ANALOGS TO CLONED HUMAN Y2 AND Y4 RECEPTORS M.W. Walker, C. Gerald, J.A. Bard, P.J.-J. Vaysse, W.E. Heydorn*, R.L. Weinshank and T.A. Branchek. Synaptic Pharmaceutical Corporation, Paramus, NJ 07652.

Neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP) are members of the pancreatic polypeptide family. These peptides function as neurotransmitters and hormones through activation of membrane bound receptors. Pharmacological data support the existence of at least 5 receptor subtypes, known as Y1 (previously cloned from vertebrates), Y2, Y3, PP and Y1-like "feeding" receptors. We recently isolated human cDNAs encoding two novel Y-type receptors by expression and homology cloning techniques (Gerald et al., Bard et al., Soc. Neurosci. Abstr. 1995). Each cDNA was transiently expressed in COS-7 cells and evaluated in radioligand binding assays. The receptor clones were classified as Y2 and Y4 subtypes based on peptide binding profiles. The Y2 receptor bound porcine [125I]-PYY (Kd = 0.07 nM) and human pancreatic polypeptides in rank order: PYY (Ki = 0.4 nM) > NPY (Ki = 0.7 nM) >> PP (Ki > 1000 nM). The Y2 also bound C-terminal fragments PYY3-36 (Ki = 0.7 nM), PYY13-36 (Ki = 1.5 nM), NPY2-36 (Ki = 2.0 nM), and NPY20-36 (Ki = 3.6 nM), plus the Y2-selective cyclic disulfide analog C2-NPY (Ki = 3.5 nM). Y2 binding was disrupted by replacement of Gln34 in NPY or PYY with Pro, as in [Pro34]-PYY (Ki > 300 nM). This binding profile matches that for the pharmacologically defined Y2 subtype. The Y4 receptor bound porcine [125I]-PYY (Kd = 0.11 nM) and human pancreatic polypeptides in rank order: PP (Ki = 0.06 nM) > PYY (Ki = 0.9 nM) > NPY (Ki = 2.2 nM). Y4 affinity for PYY or NPY analogs could be enhanced by inserting PP residues into positions 31 and 34. Ki = 0.1 nM for [Pro34]-PYY and 1.1 nM for [Leu31,Pro34]-NPY. PP2-36 was well tolerated (Ki = 0.06 nM), but further N-terminal deletion was disruptive (Ki = 39 nM for PP13-36). The Y4 binding profile resembles that of the pharmacologically defined PP receptor. We propose the name "Y4" to account for the narrow range of Ki values for all human pancreatic polypeptides, and to extend the Y-type nomenclature established for Y1, Y2 and Y3 receptors.

402.6

SPECIES AND NK1 VERSUS NK2 TACHYKININ RECEPTOR SPECIFICITIES OF PERHYDROISOINDOLES. V. Fardin, L. Pradier*, A. Morgat, S.M. Moussaoui, J. Menager, A. Carruette, J.F. Sabuco, S. Grisoni, J.L. Malleron, M. Tabart, J.F. Peyronel and C. Garret, Rhône-Poulenc Rorer S.A., CRVA, CNS Research Program, 94400 Vitry-sur-Seine, France.

Following the discovery of RP 67580, a selective non-peptide Substance P antagonist having preferential affinity for the rat and mouse NK1 receptor, chemical modifications in the perhydroisoindole series led to the design of new sub-classes of NK1 antagonists with high affinity for the human receptor. Thus, RPR 100893, a triarylperhydroisoindolol derivative, potently inhibits the binding of ^{3}H-SP to the NK1 receptor in guinea-pig, dog, monkey and human tissues. In contrast to RP 67580, it has low affinity for the rat and mouse NK1 receptors. Following molecular modelling analysis of RPR 100893, we synthesized new compounds without the gemdiphenyl moiety that retain affinity and selectivity for the human NK1 receptor. For example, RPR 107880 displayed a Ki of 4 nM for the NK1 receptor of IM9 cells. RPR 100893 and RPR 107880 potently antagonize SP-evoked inositol phosphate responses in human cells and contractions induced by SP in the guinea-pig ileum. *In vivo*, as *in vitro*, these two compounds were more potent in the guinea-pig than in the rat, as revealed by their inhibition of plasma extravasation induced by septide in peripheral tissues. In addition, triarylperhydroisoindolediols with high affinity for the human NK2 receptor could also be obtained from this chemical series (ex: RPR 106145 - Ki = 16 nM). Here again, marked variations in affinity between species were observed since RPR 106145 was a poor inhibitor of the binding of ^{125}I-NK A to NK2 receptors in rat duodenum. Finally, the absolute configuration of the heterobicyclic core and the stereochemistry of substituents of perhydroisoindoles are crucial to the species and NK1 *vs* NK2 specificity.

402.7

TACHYKININ NK-3 RECEPTOR MECHANISMS IN GUINEA PIG ILEUM. E. Burcher*, C.J. Mussap, I.E. Apostolopoulos and S.K. Syed, Sch. Physiol. Pharmacol., Univ. of New South Wales, NSW 2052, Australia.

We recently found that [^{125}I]-Bolton-Hunter (BH) scyliorhinin II (SCYII) labelled autoradiographically the myenteric plexus of guinea pig ileum, but in membranes, characterization to a definitive tachykinin receptor type was not possible (Mussap & Burcher, Naun-Sch Arch Pharmacol 350, 301, 1994). Here, we have used binding and functional techniques to further investigate the receptors interacting with SCYII, its non-radioactive BH derivatives (mono-BHSCYII and di-BHSCYII) and other analogues and antagonists including SR 142801 (NK-3 antagonist). In homogenates of ileum containing longitudinal muscle and myenteric plexus only, binding of 100 pM [^{125}I]-BHSCYII was inhibited by SR 142801 ≥ senktide ≥ di-BHSCYII ≥ SCYII ≃ mono-BHSCYII > eledoisin > neuropeptide γ >> neuropeptide K > [Sar^{9},Met(O$_2$)^{11}]-SP ≥ [pGlu^{6},Pro^{9}]-SP(6-11) (septide). CP 96345 and SR 48968 were ineffective competitors. This indicates binding to a definitive NK-3 site, in contrast to our earlier anomolous binding pattern. [^{125}I]-BHSCYII also bound with high affinity to brain homogenates; SR 142801 was a much weaker competitor in rat compared with guinea pig brain. In segments of ileum suspended longitudinally in Krebs solution, these agonists typically produced both an initial fast response and a slower sustained contractile response. Initial but not late responses to mono-BHSCYII, di-BHSCYII, [Sar^{9},Met(O$_2$)^{11}]-SP and septide were inhibited by atropine and phenoxybenzamine (both 1 μM), whereas late but not initial responses were inhibited by 1 μM CP 99994. SR 142801 (0.1 μM) partially inhibited responses to senktide. SCYII and mono-BHSCYII. Both techniques demonstrate the existence of NK-3 receptors in guinea pig ileum myenteric neurons. However the functional studies illustrate the complexity of tachykinin contractile mechanisms.

402.8

NATURAL ZINC-SITE IN THE TACHYKININ NK$_3$ RECEPTOR MODULATION OF AGONIST BINDING POPULATION.

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Background: High affinity metal-ion sites are constructed by introducing His residues at positions *i* and *i+4* at the outer part of TM-V in 7TM receptors (Nature, 1995, **374**:74-77). In the NK-3 receptor, two His residues are naturally found in such a structure. **Methods and results**: HisV:01 (His^{194}) was substituted with Ala and receptors were probed for ligand binding ([^{125}I]His^{3}-MePhe^{7}-neurokinin B) and inositolphosphate turnover. On both wild-type and mutant receptor Zn^{2+} acted only as an 'antagonist' when applied in millimolar conc. However, on the wildtype receptor in contrast to the H194A-NK$_3$ receptor the dose-response curve for Zn^{2+} was biphasic as the metal-ion increased NKB binding between 10^{-6} and 10^{-4} mol/l. Competition binding curves in the presence of variable Zn^{2+} cons. demonstrated that it increases the Bmax without affecting the Ki. The two His residues are conserved in the NK$_3$-like receptor (NK$_{3R}$), which originally was cloned as a putative opiate receptor. A similar biphasic (stimulatory - inhibitory) dose-repsonse curve is found for Zn^{2+} in the NMDA glutamate receptor. **Conclusion**: A metal-ion site is located at the border between extracellular loop-2 and TM-V in the NK$_3$ receptor; coordination of physiological concentrations zinc-ions at this site increases the binding of the natural ligand, NKB, by increasing the Bmax without altering the Ki.

402.9

CONSTRUCTION OF AN ANTAGONISTIC ZINC-SWITCH IN THE KAPPA OPIATE RECEPTOR.

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Background: The kappa opiate receptor was chosen as target for introduction of a metal-ion switch due to the availability of a diversity of high affinity and selective ligands. **Method**: Based on a metal-ion site previously constructed in the binding site for a non-peptide antagonist in the tachykinin NK-1 receptor (Nature, 1995, **374**:74-77), histidyl residues were introduced in the rat kappa-opiate receptor. **Results**: His residues at positions V:01 (Asp^{223}), V:05 (Lys^{227}), and VI:24 (Ala^{298}) alone and in combination had no direct effect on the binding of radioligands (antagonists ^{3}H-DIP and agonist ^{3}H-CI977). However, zinc-ions in a dose-dependent manner prevented binding of both agonist and antagonists ligands with an apparent affinity for the metal-ion which gradually was built up to 0.5×10^{-7} mol/l (a 1000 fold increase in zinc-ion sensitivity). The zinc-site was relatively specific for Zn^{2+} versus Cu^{2+} (only 10-fold increase in affinity). **Conclusion**: A high affinity metal-ion binding site consisting of three histidyl residues has been introduced in the kappa-opiate receptor without altering ligand-binding characteristics; however, coordination of zinc-ions at this silent 'metal-ion switch' between the outer segments of TM-V and VI prevents ligand binding to the receptor. The direct transfer of this metal-ion site from a tachykinin to an opiate receptor indicate that the overall arrangements of the seven-helical bundle is common among 7TM receptors of the rhodopsin family.

402.10

METAL-ION SITES AS STRUCTURAL AND FUNCTIONAL PROBES OF HELIX-HELIX INTERACTIONS IN 7TM-RECEPTORS

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Background: A high affinity antagonistic zinc-site, consiting of two His residues at the top of TM-V and one at the top of TM-VI, was previously built into the binding site for a non-peptide antagonist in the tachykinin NK-1 receptor (Nature, 1995, **374**:74-77). **Methods & Results**: The possible involement of other residues in the receptor structure (including His residues located further down in TM-VI and in TM-III) in the coordination of the zinc-ion was excluded by Ala substitutions. Details of the antagonistic property of the zinc-ion was studied by analysis of inositolphosphate turnover. The initial zinc-site was expanded to reach out to TM-III by systematic His substitutions at this location. The helical connectivity and overall arrangement of the seven-helical bundle was probed by additional metal-ion sites around the central TM-III column. The metal-ion sites also give functional informations; for example, a zinc-site around the top of TM-II prevents binding of agonist, substance P, but not binding of non-peptide antagonist, CP96,345. Based on the results from the tachykinin receptors, similar metal-ion sites were constructed in the beta-adrenergic receptor to test the general importance of the observations. **Conclusion**: High affinity metal-ion sites can be introduced at various sites in 7TM, G-protein coupled receptors whereby the overall helical arrangement and the helix-helix interactions can be probed. These metal-ion sites impose important triangular distance and angular constraints on the molecular models of 7TM receptors.

402.11

CENTRAL HYPOTHYROIDISM CAUSED BY INACTIVATING MUTATIONS IN THE THYROTROPIN-RELEASING HORMONE RECEPTOR (*TRHR*) GENE.

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Central hypothyroidism, characterized by insufficient thyrotropin (TSH) production resulting in low circulating levels of thyroid hormones (T_3, T_4), is rare and has various etiologies. We have investigated a 12 year-old boy who had been treated with T_4 since 9 years of age for central hypothyroidism of unknown origin. His two brothers (10 and 15 years old) and unrelated parents were also investigated. The propositus, after one month off therapy, had a T_4 concentration of 42 nmol/L (normal 58-148) and a TSH concentration of 2.2 mIU/L (normal 0.1 - 5.0). TRH 200 µg iv induced no change in either plasma TSH or prolactin concentrations. His two brothers and both parents had normal plasma T_4 and TSH concentrations and normal TSH and prolactin responses to TRH. Genomic DNA was isolated from peripheral-blood leucocytes and the coding regions of the *TRHR* gene directly sequenced. Different mutations were detected in each of the propositus *TRHR* gene alleles. In one allele, identified as maternal, cytosine at position 49 was replaced by thymidine resulting in a premature stop codon. In the paternal allele, codons 343-351 were deleted and guanine at position 352 was replaced by adenine, resulting in the substitution of alanine by threonine at position 118. These mutations created new restriction sites that allowed us to haplotype the family and thus determine that the older brother had inherited the mutated maternal allele. Hormonal responses to TRH clearly show the presence of both mutated alleles is necessary and sufficient to induce refractoriness to TRH. These results indicate that central hypothyroidism may be due to inactivating mutations of the TRHR; the incidence of such mutations in the general population is under investigation.

402.12

IDENTIFICATION OF RESIDUES IMPLICATED IN THE BINDING AND INTERNALIZATION OF THE RAT NEUROTENSIN RECEPTOR.

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We modified by site directed mutagenesis the cDNA encoding the rat neurotensin (NT) receptor and examined the binding and internalization properties of the mutant receptors after their transfection into COS-7 cells. The mutation of charged residues located in the first extracellular loop (Glu124, Asp139 and Arg143) and in the sixth transmembrane domain (TM) (Arg327-Arg328) totally abolished ^{125}I-NT binding. Confocal and electron microscopic immunocytochemical studies of mutated transfectants tagged with an immunogenic peptide sequence indicated that this loss of binding was not due to altered receptor expression or to a lack of insertion of the receptor into the plasma membrane as both the wild type and mutated forms of the receptor showed identical sub-cellular localizations. We therefore conclude from these results that charged residues located in the first extracellular loop and in the sixth TM of the rat NT receptor are implicated in the recognition of NT.

Deletion of the C-terminal tail of the NT receptor distal to the Trp391 residue did not affect ^{125}I-NT binding but resulted in a 90% loss in the capacity of the cells to internalize receptor-ligand complexes. Sequential deletions of Ser and Thr clusters in this region indicated that the cluster closest to the C-terminus was responsible for this effect. Within this cluster, combined point mutations of Thr422 and Tyr424 residues were sufficient to almost completely abolish ligand internalization. Confocal microscopy confirmed that cells transfected with the wild type receptor internalized a fluorescent analog of NT in a temperature dependent fashion, whereas cells transfected with a receptor truncated at the carboxyl terminus only showed a clustering of the fluorescent peptide at the cell surface. We conclude from these results that the Thr422 and Tyr424 residues are critical for NT induced receptor internalization.

402.13

PACAP HYBRID: A NEW ANTAGONIST FOR PACAP RECEPTOR SPLICE VARIANTS.

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Pituitary adenylate cyclase activating peptide (PACAP) receptor is a 27 amino acid peptide with sequence homology to vasoactive intestinal peptide (VIP). Distinct receptors for PACAP and VIP have been identified and the PACAP receptor is a G-protein coupled receptor with splice variants (basic, hip, hopl and hip/hopl) at the third cytosolic domain. Here the effects of a new PACAP receptor antagonist, neurotensin$^{6-11}$PACAP$^{7-27}$, PACAPhybrid, on PACAP receptor splice variants was investigated. All PACAP receptor splice variants bound PACAP with high affinity (IC_{50} = 10 nM) and VIP with low affinity (IC_{50} = >1000 nM). PACAP, 100 nM, transiently increased c-fos, c-jun and c-myc gene expression after 1 hour. Surprisingly the "hopl" PACAP receptor variant strongly, the "hip/hopl" variant moderately and the "basic" as well as "hip" variant weakly increased nuclear oncogene mRNA. Similar results were observed for cAMP and phosphatidylinositol turnover. PACAPhybrid (IC_{50} = 100 nM) was more potent than PACAP(6-38) (IC_{50} = 200 nM) at inhibiting ^{125}I-PACAP binding to the stably tranfected NIH 3T3 cells. PACAPhybrid, 1 µM, inhibited the increase in nuclear oncogene mRNA caused by PACAP. These data indicate that PACAPhybrid antagonizes PACAP receptor splice variants.

402.14

MOLECULAR CLONING AND EXPRESSION OF THE RAT TYPE I GALANIN RECEPTOR.

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Galanin is a 29-amino acid neuropeptide with multiple physiologic activities, including inhibition of insulin secretion from pancreatic ß-cells and stimulation of fat ingestion via hypothalamic receptors. We report the isolation of cDNAs encoding the rat galanin receptor using expression- and polymerase chain reaction (PCR)-based approaches. Three independent clones isolated from rat hypothalamic libraries encoded cDNAs that span identical protein-coding sequences. The predicted sequence of the rat galanin receptor is 87% identical to the human type I galanin receptor. Hydrophobicity analysis revealed that, like the human receptor, the rat galanin receptor has 7 transmembrane domains. Local homology analysis revealed interspecies sequence similarities of >90% for transmembrane (TM) domains 1-4, between 80% and 90% for TM 5-7, and >90% for extracellular domains 1 and 2. This strong sequence conservation is consistent with the similar ligand-binding characteristics of the predominant galanin receptors in rat and human brain. Intracellular domains 1-3 also exhibited >90% similarity, suggesting that the two receptors utilize similar intracellular signaling mechanisms. Northern blot analysis revealed two mRNA species of approximately 7.5 and 4 kbp, respectively. High levels of galanin receptor expression were observed in whole brain and hypothalamus. Studies of normal, castrated and ovariectomized rats demonstrate that in contrast to the regulation of galanin itself, galanin receptor gene expression is strongly *inhibited* by sex steroid hormones.

402.15

IDENTIFICATION OF A NOVEL RECEPTOR SELECTIVE FOR PARATHYROID HORMONE WHICH IS EXPRESSED IN THE BRAIN: THE PTH2 RECEPTOR. T. B. Usdin*, É. Mezey, and T. I. Bonner. Lab. of Cell Biol., NIMH, and Clin. Neurosci.Branch, NINDS (É.M.), Bethesda MD 20892-4090.

PTH (parathyroid hormone) regulation of peripheral mineral homeostasis is well studied, but little is known regarding the role of PTH in the brain. Authentic PTH has been reported in the hypothalamus of several species. PTH acts at the PTH/PTHrP receptor which does not discriminate between PTH and PTHrP (parathyroid hormone-related peptide, née hypercalcemia of malignancy factor). PTHrP and the PTH/PTHrP receptor are widely distributed in the brain making identification of PTH-specific effects difficult. Using a homology based approach we identified a cDNA encoding a novel PTH receptor which we call the PTH2 receptor. It is a G-protein coupled receptor which stimulates cAMP accumulation, and is a member of the secretin receptor family. Its similarity to previously known receptors ranges from 60% amino-acid sequence identity with the PTH/PTHrP receptor to 30% identity with the CRF receptor. The PTH2 receptor is activated by PTH and not PTHrP. The PTH2 receptor is quite limited in distribution and its mRNA is particularly abundant in the hippocampus and the bed nucleus of the stria terminalis. This receptor should facilitate investigation of the CNS role of PTH.

402.16

EXPRESSION OF THE GONADOTROPIN RELEASING HORMONE (GNRH) RECEPTOR IN THE BACULOVIRUS/INSECT CELL (Sf9) SYSTEM. J.D. Neill*, J.C. Sellers and L.W. Duck. Dept. of Physiology and Biophysics, Schools of Medicine and Dentistry, Univ. of Alabama at Birmingham, AL 35294.

The GnRH-receptor is expressed on the gonadotrope cells of the anterior pituitary; it binds the hypothalamic neuropeptide GnRH and stimulates the release of the gonadotropic hormones, FSH and LH. Molecular cloning and nucleotide sequencing of its cDNA revealed a 327 amino acid protein having a 7-transmembrane topology. Previously we reported that antibodies to receptor peptide segments failed to bind the native protein; thus we constructed a GnRH-receptor cDNA encoding an epitope tag (HA-1 antigen) at the amino terminus which is recognized by a commercially available antibody. Though this approach permits study of the heterologously expressed receptor, it does not allow detection of the native receptor. In the present study, we expressed the GnRH-receptor in the baculovirus/insect cell (Sf9) system; the epitope-tagged GnRH-receptor cDNA was used so that the expressed receptor could be immunoaffinity purified. The epitope tagged GnRH-receptor expressed in Sf9 cells is functional, having an affinity of 1.4 nM (IC_{50}) for ^{125}I-D-Ala^6-des-Gly^{10}-GnRH; when expressed in COS-1 cells it has an affinity of 0.47 nM (IC_{50}). In Western blots, the epitope tagged GnRH-receptor in Sf9 cells is detected as a band at about 65 kDa which is similar with two bands at 67 kDa and 57 kDa found in COS-1 cells. Since the primary molecular weight of the GnRH-receptor is 37 kDa and the relative molecular mass of the endogenous receptor is reported to be 50-60 kDa, these results suggest that the Sf9 cells glycosylate the receptor to a slightly greater extent than do pituitary cells. The availability of large amounts of purified GnRH-receptor should permit production of antibodies that detect the native receptor and also allow studies of putative homodimerization of the GnRH-receptor molecule.

CARDIOVASCULAR REGULATION: SPINALMEDULLARY MECHANISMS

403.1

EVIDENCE FOR AT1-MEDIATED SYMPATHOINHIBITORY AND SYMPATHOEXCITATORY ROLE OF BRAINSTEM ANGIOTENSIN IN CONSCIOUS RABBITS. GA Head, RD Bendle, SC Malpas, Y Hirooka# P. Potts# and RAL Dampney#* Baker Medical Research Institute, Prahran, Victoria and #Department of Physiology, University of Sydney, N.S.W., Australia.

The present study examined the cardiovascular effects of fourth ventricular (4V) administration of angiotensin II (Ang II) to conscious rabbits and the contribution of AT1 or AT2 receptors by treatment with losartan or PD123319 respectively. Ang II (1 to 125 pmol) into the 4V caused a maximum increase in mean arterial pressure (MAP) of 17.3 ± 1.5 mmHg (n= 8). Losartan (0.001 to 10 μg) caused a dose-dependent blockade of the pressor effect of Ang II, with complete blockade produced by 10 μg. This effect lasted for at least 2 hours. PD123319 (0.1 to 1000 μg) or vehicle had no effect on the Ang II pressor response. Following a 60 minute infusion of Ang II into the 4V of conscious rabbits and subsequent immunohistochemical staining for c-Fos protein, regions such as the rostral ventrolateral medulla pressor area and caudal ventrolateral medulla depressor area were heavily stained as were other regions such as the nucleus of the solitary tract and A5 area. In another 8 conscious rabbits implanted with 4V catheters and an electrode for recording renal sympathetic nerve activity (RSNA), the effect of losartan on the baroreceptor reflexes was examined. Baroreflex curves were determined by ramp increases and decreases in MAP using i.v. phenylephrine and nitroprusside before and after 10 μg of losartan or vehicle, each on a separate day. Losartan did not alter MAP but increased resting RSNA (67± 21%) and the range of the RSNA baroreflex curve by 46 ± 15%. Vehicle had no significant effects. The effect of losartan in conscious rabbits suggests that the net effect of endogenous Ang II is a tonic inhibition of sympathetic vasomotor tone in contrast to exogenous Ang II which increases blood pressure and RSNA. Both of these actions are mediated by AT1 receptors.

403.2

NEURAL MECHANISMS UNDERLYING THE CENTRALLY INITIATED PRESSOR RESPONSE ELICITED BY ANGIOTENSIN II IN RATS: A STUDY *IN VIVO*. M.-K. Sun* & D.J. Reis. Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

Much interest has been raised as to the role of angiotensin II (Ang II) in generation and maintenance of hypertension. Its centrally initiated cardiovascular responses have been proposed to depend on responses of neurons in either (a) the area postrema or subfornical organ; (b) the rostroventrolateral reticular nucleus (RVL) of the medulla oblongata to increase arterial pressure or (c) the caudal ventrolateral medulla or the nucleus tractus solitarius to decrease arterial pressure. We investigated whether a rapid activation of RVL-spinal vasomotor neurons underlies the Ang II-induced central pressor response. Ang II (1-100 nmol), applied intracisternally (i.c.), dose-dependently and dramatically increased arterial pressure. The pressor response was neither preceded by any detectable excitatory responses of the singly identified RVL-spinal vasomotor neurons with slow and fast-conducting axons nor increased discharges of sympathetic nerves, but abolished by [Sar^1,Thr^8]-Ang II (100 nmol, i.c.), an Ang II receptor antagonist. Both types of RVL vasomotor neurons and sympathetic nerves were actually inhibited, due to their baroreflex sensitivity. The Ang II-induced pressor response was attenuated neither by a ganglionic blocker hexamethonium (250 mg, i.v.) nor by a complete cervical transection of the spinal cord (with i.v. infusion of L-phenylephrine to maintain arterial pressure to control level) but largely attenuated by [β-Mercapto-β,β-cyclopentamethylenepropinyl^1,-O-Et-Tyr^2,Val^4,Arg^8]-vasopressin (1 mg, i.v.), a vasopressin antagonist. We conclude that (a) responses of RVL-spinal vasomotor and sympathetic neurons do not underlie the Ang II-induced central pressor response and (b) the pressor response depends on release of vasopressin into the circulation.

403.3

EXCITATION OF C1 NEURONS IN ROSTRAL VETROLATERAL MEDULLA (RVLM) BY ANGIOTENSIN II IS DUE TO CLOSURE OF A PERSISTENT K^+ CONDUCTANCE. Yu-Wen Li* and P. G. Guyenet. Dept. Pharmacology, Univ. of Virginia, Charlottesville, VA 22908.

The RVLM is rich in angiotensin II (Ang II) receptors. The distribution of Ang II receptors overlaps with C1 cells. Activation of these receptors in RVLM increases sympathetic outflow and blood pressure. Fluorescent latex microspheres were injected into the thoracic spinal cord of neonatal rats (1-3 day-old). After 3-10 days, retrogradely labeled neurons in the RVLM were visualized by epifluorescence and recorded in cell-attached extracellular and/or whole-cell patch-clamp mode. Neurons were labeled with Lucifer Yellow. Tyrosine hydroxylase (TH) immunohistochemistry was later performed to identify phenotype of recorded cells. Sixty of 67 bulbospinal RVLM neurons were spontaneously active (2.7±0.2 spikes/s), with 'resting' membrane potential of -55±0.5 mV and input resistance of 857±59 MΩ. Ang II (1 μM) increased firing rate to 250% of control in 28 of 39 neurons tested in cell-attached recordings. In current-clamp, Ang II depolarized the cells (+6±0.7 mV, n=35) and increased input resistance (+21±3%, n=20). In voltage-clamp, Ang II induced an inward current (9.1±1.2 pA, n=15) at a holding potential around -50 mV. Ang II-evoked current was voltage-independent in the range of -40 to -120 mV, and had a reversal potential close to EK. The Ang II effect was abolished by the AT1 receptor antagonist losartan (3 μM), but persisted in the presence of low Ca^+/high Mg^+ solution or TTX. Ang II-sensitive neurons were inhibited by α2-adrenoceptor agonists (12/15). Ang II activated 24 of 26 TH-ir neurons. In conclusion, Ang II excites bulbospinal C1 cells in the RVLM, at least in part, by closing a persistent K^+ conductance. Supported by NIH (HL 28785) and AHA.

403.4

K^+ CURRENTS IN SPONTANEOUSLY ACTIVE AND SILENT RVLM NEURONS OF IMMATURE RAT BRAINSTEM SLICES. N. J. Dun* and T. Miyazaki.** Dept. of Anat. & Neurobiol., Medical College of Ohio, Toledo, OH 43614. **Present address: Dept. of Physiol., Tokyo Medical College, Tokyo, Japan.

Coronal 500 μm brainstem slices were prepared from 8-12 day old rats. Whole-cell patch-clamp recordings were made from rostro-ventrolateral medulla (RVLM) neurons. At the resting membrane potential of about -60 mV, two types of RVLM neurons were noted: spontaneously active and silent neurons. The types of K^+ currents present in these two groups of neurons were the focus of these studies. The resting membrane potential and input resistance among these two types of neurons were not statistically different. Tetrodotoxin (TTX, 0.5 μM) abolished the spontaneous discharges. In the presence of TTX, depolarizing step commands from the holding potential of -60 mV evoked in all RVLM neurons a TEA-sensitive outward current. The outward current had a mean reversal potential of -87.8 mV. All RVLM neurons exhibited a 4-aminopyridine (1 mM)-sensitive A-type current and a Cs^+-sensitive H-type current. A Ca^{2+}-activated K^+ current was present in most of the spontaneously active and silent RVLM neurons. The results show that these two types of RVLM neurons seem to have the same complement of K^+ currents and suggest that the spontaneous activity may be a network-related event. (Supported by NIH Grant HL51314).

403.5

VENTRAL MEDULLARY SURFACE ACTIVITY CHANGES DURING MOMENTARY BLOOD PRESSURE VARIATION WITHIN SLEEP STATES. R.M. Harper*, D.M. Rector, G. Poe, H.V. Forster, P.J. Ohtake, L.G. Pan, T.F. Lowry and D. Gozal. Dept. of Anatomy and Cell Biology, UCLA, Los Angeles, CA 90095; Dept. of Physiology, Medical College of Wisconsin and Zablocki VA Hospital, Milwaukee, WI 53226.

We examined activity changes on the rostral ventral medullary surface (VMS) during sleep in a chronic goat preparation with imaging procedures. Under sterile surgery, five adult goats were instrumented with electrodes to monitor sleep physiology, arterial and venous cannulae to assess blood pressure, a tracheostomy to assess ventilatory measures, and a miniaturized video camera coupled to a coherent fiber probe and a narrow bandwidth illuminator (660 nm) to measure VMS activity. The probes were placed over a rostral VMS region which elicited ventilatory and blood pressure depression when cooled. Arterial pressure and 320 x 100 pixel images were collected at 1 Hz during an all-night sleep recording, and arterial pressure values were cross-correlated with overall image reflectance. Pronounced episodic activity transients occurred on the VMS, particularly during REM sleep and waking. Phasic arterial pressure variation during REM sleep followed VMS activity changes in a closely-coupled fashion (1-3 sec), while blood pressure changes during quiet sleep and waking followed image changes with more substantial or variable lags. We speculate that (1) momentary changes in VMS activity play a role in initiating large blood pressure transients during REM sleep, and (2) the timing of VMS activity changes to blood pressure transients during some states over others suggests a more prominent role for the VMS in modulating blood pressure in particular states. *(Supported by HL-22418, NIDR DE-07212, and USPHS-25739; G.P. is supported by a Howard Hughes predoctoral fellowship).*

403.6

DIRECT PROJECTIONS FROM A VASODEPRESSOR AREA IN THE MEDULLARY GIGANTOCELLULAR RETICULAR NUCLEUS ONTO SYMPATHOADRENAL PREGANGLIONIC NEURONS IN THE THORACIC SPINAL CORD. S.A. Aicher*, D.J. Reis, and T.A. Milner. Dept. of Neurol. & Neurosci., Cornell Univ. Med. College, NY, NY 10021.

A vasodepressor area within the caudal ventral medullary gigantocellular reticular nucleus, the gigantocellular depressor area (**GiDA**), is topographically distinct from other vasoactive sites in the rostral ventromedial medullary reticular formation (RVM) and the GiDA innervates the thoracic spinal cord (Aicher, et al., *Neurosci*, **60**: 761-779). We investigated whether these efferent fibers monosynaptically contact retrogradely-labeled sympathoadrenal preganglionic neurons of the thoracic spinal cord. The anterograde tracer biotintylated dextran amine (BDA) was iontophoresed into the GiDA of rats and the retrograde tracer Fluoro-Gold was injected into medulla of the ipsilateral adrenal gland. Fourteen days later, rats were anesthetized and perfused with acrolein and paraformaldehyde. Sections of the thoracic spinal cord (T7-T12) were processed for immuno-gold localization of Fluoro-Gold retrograde transport and peroxidase localization of anterogradely transported BDA. BDA injection sites were confined to the GiDA. In the thoracic spinal cord, BDA-labeled axons were both myelinated and unmyelinated. BDA-labeled terminals contained small, clear vesicles and formed symmetric synaptic contacts primarily on large and small dendrites. Some of the somata and large dendritic targets of BDA terminals contained immuno-gold reaction product associated with lysosomes and multivesicular bodies in the cytoplasm indicating they were retrogradely-labeled from the adrenal medulla. We conclude that GiDA neurons project directly onto sympathoadrenal preganglionic neurons in the thoracic spinal cord. The pathway may represent a direct medullary reticulospinal sympathoinhibitory pathway. (Support: NIH #HL18974; Amer. Heart Assoc. Grant-in-Aid to SAA).

403.7

EFFECT OF SPINAL CORD INJURY ON CARDIOVASCULAR NEURONS IN THE ROSTROVENTROLATERAL MEDULLA. A.V. Krassioukov[1]* & M.G. Fehlings[2]. [1]John P. Robarts Research. Inst. & Dept. of Physiol. Univ. of Western Ontario., London, Ont., Canada, N6A 5K8, and [2]Playfair Neuroscience Unit, The Toronto Hospital Research Inst., 399 Bathurst St., Toronto, Ont., Canada M5T 2S8.

Deranged cardiovascular control in patients with paraplegia or quadriplegia can be directly linked to abnormalities within the autonomic nervous system. However, mechanisms for changes in central control of the cardiovascular system after spinal cord injury (SCI) have not been investigated. We studied changes within cardiovascular neurons in the rostroventrolateral medulla (RVLM) after compression SCI. Neurons in the RVLM has been shown to be most critical for cardiovascular control. Numbers of RVLM neurons surviving after SCI in rats were identified by retrograde labelling with Fluoro Gold after T6 introduction and counts were performed eight weeks after different degrees of SCI (20g, 35g and 50 g compression) of the cord at T1. The RVLM neurons were counted in four control and nine cord-injured rats. The numbers of labelled RVLM neurons in control rats were 296±18; the numbers in cord-injured rats were markedly reduced and 144±17 neurons were found after 20g compression, 24±6 neurons after 35g compression and finally 9±1 neurons after 50g compression. These neurons must have retained an intact bulbospinal axon after the compression injury. A rim of subpial white matter usually is preserved after SCI in humans and in the rat model of compression injury. These tracts can be in the dorsolateral funiculus, the region of most descending sympathetic vasomotor pathways to the SPNs. These surviving bulbospinal RVLM neurons also were characterized by their expression of the enzyme phenylethanolamine-N-methyltransferase. They must project to spinal sympathetic neurons caudal to the site of injury and potentially could be a source of new innervation to other sympathetic neurons in these spinal segments. (Support: Ontario Heart & Stroke Foundation)

403.8

RENAL SYMPATHETIC ACTIVITY IN CONSCIOUS SPINAL INJURED RATS. D.N. Maiorov*, L.C. Weaver and A.V. Krassioukov. John P. Robarts Research. Inst. & Dept. of Physiol. Univ. of Western Ontario., London, Ont., N6A 5K8, Canada.

Episodic hypertension in quadriplegics and high paraplegics can be induced by spinally-mediated reflex increases in sympathetic nerve activity in response to visceral stimuli. However, direct evidence for a neurogenic origin of autonomic dysreflexia does not exist. To evaluate the role of sympathetic activity in dysreflexia we studied the responses of mean arterial pressure (MAP), heart rate (HR) and renal nerve activity (RNA) to colon distension in unanesthetized rats 1-4 days after thoracic spinal cord transection (SCT). One day after SCT MAP and RNA were significantly decreased from that recorded in the 11 intact, pentobarbital anesthetized rats. In the ensuing days MAP partially recovered, whereas RNA declined further approaching levels indistinguishable from noise by day 4.

	Before SCT (under anesthesia)	After SCT (conscious) 24h(n=8)	48h(n=4)	72h(n=3)	96h(n=3)
MAP (mmHg)	93±4	69±7	77±6	77±12	97±3
HR (bpm)	422±18	390±34	390±4	395±48	365±66
RNA ($\mu V \cdot s$)	16±2	9±4	10±4	7±4	3±3

Colon distension elevated MAP by 31±7 mmHg and RNA by 33±5 $\mu V \cdot s$ on day 1 after SCT. On day 2 of testing, MAP and RNA increased by 20±6 mmHg and 32±13 $\mu V \cdot s$, respectively. On day 3 colon distension increased MAP by 22±7 mmHg and RNA by 18±5 $\mu V \cdot s$. On day 4 these responses did not differ significantly from those on day 3. On all four days of testing ganglionic blockade with hexamethonium abolished the responses in MAP and reduced the responses in RNA by 85±5%. These results demonstrated a) the absence of baseline RNA in awake rats 3-4 days after SCT, b) the neurogenic origin of autonomic dysreflexia in cord-injured rats.

403.9

MODULATION OF THE FREQUENCY AND AMPLITUDE COMPONENTS OF SYMPATHETIC ACTIVITY BY BAROREFLEXES AND CHEMOREFLEXES IN CONSCIOUS RABBITS. S.C. Malpas, J.H. Ricketts, R.D. Bendle, G.A. Head, K. Sheppard. Baker Medical Research Institute, Prahran, Victoria 3181, Australia.

Sympathetic discharges (SND) exhibit two distinct components, the frequency at which discharges occur and their relative amplitude (reflecting the number of activated nerve fibres within each burst). These may respond independently to different afferent inputs indicating separate central controlling processes. We examined the response in the frequency and amplitude of renal SND to baroreceptor and chemoreceptor activation in 8 conscious rabbits. Rabbits breathed either room air or a hypoxic gas mixture for 10 min, baroreflexes were then stimulated by ramp increases and then decreases in arterial pressure using i.v. phenylephrine (0.5mg/ml) and nitroprusside (1mg/ml) (total arterial pressure range induced 80mmHg). Hypoxia significantly increased the resting frequency, prior to baroreflex modifications, from 1.94±0.33 to 2.82±0.31 discharges per second and amplitude of SND 21±7%, although arterial pressure was unchanged. The baroreflex curve for the frequency of SND under normoxia was sigmiodal. The gain of this curve was significantly increased by hypoxia although not the range of frequencies occurring. The arterial pressure range over which these changes occurred was unaltered by hypoxia. The baroreflex curve for the amplitude of SND was also sigmoidal occurring over a similar arterial pressure range as the frequency curve. However under hypoxia the range of the amplitude curve was increased 81±3% ($p<0.05$), but the gain unaltered. These results indicate that baroreceptor afferents affect both the frequency and amplitude of SND similarly but when combined with chemoreceptor stimulation they display different responses. These findings support the hypothesis that there are independent controlling processes for the frequency and amplitude components of SND.

403.10

INTERCONNECTIONS OF CAUDAL VENTROLATERAL MEDULLARY (CVLM) AND RAPHE (R) NEURONS WITH ACTIVITY CORRELATED TO THE 10-HZ RHYTHM IN SYMPATHETIC NERVE DISCHARGE (SND) OF URETHANE-ANESTHETIZED CATS. S.M. Barman*, H.S. Orer, and G.L. Gebber. Dept. Pharmacol. & Toxicol., Michigan State Univ., E. Lansing, MI 48824.

We used spike-triggered averaging to identify CVLM and R neurons with activity correlated to the 10-Hz rhythm in SND. Nineteen of 47 CVLM neurons were antidromically activated by R stimulation. The longest onset latency (OL) of antidromic activation (AA) was 19.9±2.8 ms, a value similar to the difference (17 ms on the average) in firing times of CVLM and R neurons during the 10-Hz slow wave in SND. These responses likely reflected activation of axonal branches since the OL was decreased dramatically by moving the stimulating microelectrode within the R or by using suprathreshold current. The axons of CVLM neurons likely terminated on and excited R neurons since: 1) CVLM neurons could not be activated by stimulation of sites lateral to the midline, contralateral to the recording site; thus their axons did not cross the midline. 2) AA of some CVLM neurons was limited to stimulation of sites in one track through the midline; thus, their axons did not project to more rostral or caudal portions of the medulla. 3) 37% of R neurons were synaptically excited by CVLM stimulation (minimum OL: 18.1±2.6 ms). Eight of 41 R neurons were antidromically activated by CVLM stimulation. The OL of AA was decreased by moving the stimulating microelectrode within the CVLM or by using suprathreshold current. The axons of R neurons likely terminated in the CVLM since: 1) Higher current was needed to activate them from sites 0.5 mm further lateral or rostral. 2) 32% of the CVLM neurons were synaptically excited by R stimulation (minimum OL: 18.3±4.2 ms). These data raise the possibility that interconnections of CVLM and R neurons contribute to the expression of the 10-Hz rhythm in SND. (Supported by NIH grants HL-33266 and HL-13187.)

403.11

GABAergic and Hypothalamic involvement in the 10 Hz Sympathetic Rhythm. ME Clement* and RB McCall. The Upjohn Company, Kalamazoo, MI.

The neurotransmitters GABA and glycine have been implicated in inhibitory neurotransmission in a number of central nervous pathways. Microinjection of these compounds into the caudal depressor area or the rostral pressor area of the ventrolateral medulla increases or decreases sympathetic outflow, respectively. Administration of antagonists to these neurotransmitters increases sympathetic nerve discharge. Recent experiments in our laboratory have investigated the effects of the glycinergic antagonist strychnine and the GABAergic antagonist picrotoxin on the frequency distribution of SND. While strychnine caused an overall increase in SND, picrotoxin selectively increased the power in the 10 Hz rhythm. Picrotoxin also caused a rightward shift of the 10 Hz band in the power spectrum. Thus the current study implicates a role for the two endogenous inhibitory neurotransmitters GABA and glycine in the modulation of the frequency spectra of SND. GABA in particular seems to be involved in the intrinsic circuitry regulating the central oscillator responsible for the generation of 10 Hz activity, while glycine appears to have a more general affect on power frequency spectra of SND.

Previous investigators have reported modulation of sympathetic nerve discharge by a hypothalamic GABAergic mechanism. The current study examined the coherence between field potential activity recorded from the hypothalamic area and sympathetic nerve activity recorded from the inferior cardiac nerve. Low doses (0.1-0.3 mg/kg) of picrotoxin increased the coherence in the 10 Hz band, while higher doses (1 mg/kg) increased the coherence in the lower frequencies. The 10 Hz rhythm is not dependent on the hypothalamus however, indicating the 10 Hz activity in the hypothalamus and the inferior cardiac nerve share an input from a common generator.

403.12

BICOHERENCE ANALYSIS OF SYMPATHETIC NERVE DISCHARGE (SND). G.L. Gebber* and Y. Paitel. Dept. Pharmacol./Tox., Michigan State Univ., E. Lansing, MI 48824

The bicoherence function (normalized bispectrum) was used to test for nonlinear quadratic coupling (i.e. phase-locking) of the cardiac-related and 10-Hz rhythms (baroreceptor-innervated) or ~5-Hz and 10-Hz rhythms (baroreceptor-denervated) in SND of urethane-anesthetized cats. Such higher-order power spectra identify coupling of frequency triples where the third frequency is the sum of the other two. Recordings were made from the inferior cardiac nerve. We found that the cardiac-related and 10-Hz or ~5-Hz and 10-Hz rhythms always were nonlinearly coupled when the higher frequency component was a multiple of the lower. This suggests stable phase-locking of the central generators responsible for these rhythmic components of SND. The presence of stable 2:1 or 3:1 phase-locking was confirmed by plotting the time evolution of voltage changes in the 10-Hz band of SND against those of the lower frequencies (selectively filtered). Such orbital plots were "stationary." The bicoherence function also showed coupling of the low and high frequency rhythms in some cases when they were not harmonically related. Since the orbital plots were "non-stationary", such cases of nonlinear coupling might reflect unstable phase-locking (i.e., relative coordination) of the low and high frequency rhythm generators or convergence of their outputs onto follower circuits. Either of these models could account for the modulated frequencies, $f_1 \pm f_2$. The conditions leading to the nonlinear coupling of the generators of the low and high frequency rhythms in SND remain to be defined. (Supported by NIH grant HL-13187.)

403.13

NICOTINIC RECEPTORS ENHANCE SPONTANEOUS GABAERGIC SYNAPTIC CURRENTS IN RAT DORSAL MOTOR NUCLEUS OF THE VAGUS (DMV) NEURONS . M. Bertolino*, K.J. Kellar and R. Gillis Department of Pharmacology, Georgetown University School of Medicine, Washington DC 20007.

Spontaneous synaptic currents were studied in thin (200 μm) coronal slices of rat brainstem containing the DMV region.. The effect of nicotine was investigated by measuring the frequency of the spontaneous events before and after bath application of the agonist using the patch clamp technique. Nicotine (1-30 μM) increased the frequency of the synaptic currents in a dose-dependent way, but it failed to activate a postsynaptic current. The effect was observed also when epibatidine (0.1-10 nM) and cytisine .(10 μM), two potent nicotinic agonists, were applied. In the presence of the nicotinic receptor antagonist mecamylamine (20 μM), the effect of 3 μM nicotine on the frequency was decreased by 50 %. Tetrodotoxin (1 μM) completely blocked the effect of 3 μM nicotine. To identify the neurotransmitter involved in the increased frequency, we tested blockers of the GABA and glutamate receptors. The effect of 3 μM nicotine was blocked by 25 μM bicuculline, a GABA antagonist; in contrast, it was not affected by 1 mM kynurenic acid, a glutamate antagonist . These data suggest the existence of presynaptic nicotinic receptors that increase GABA release in a tetrodotoxin-sensitive manner. These findings are similar to recent results showing that nicotinic receptors may mediate GABA release in the interpeduncular nucleus (Léna et al. J. Neurosci. 13:2680-2688, 1993). This effect of the nicotinic receptors on the GABA release in the DMV may have an important role in the control of gastrointestinal motility.

403.14

NEUROCHEMICAL CHARACTERIZATION OF DORSAL VAGAL COMPLEX NEURONS RECEIVING INPUT FROM GASTRIC TENSION RECEPTORS IN RATS. A.E. Willing D.A. York*, H.-R. Berthoud, Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, Louisiana, 70808.

Important negative feedback information from gastrointestinal mechano- and chemoreceptors relevant to the control of digestive processes and food intake reaches the brain via vagal afferents that terminate in the nucleus of the solitary tract (nts) and dorsal motor nucleus (dmnX). To characterize the neurochemical identity of second order nts and dmnX neurons receiving input from gastric tension receptors, we measured c-fos induction in combination with catecholamine enzyme immunocytochemistry. A latex balloon was inserted into the fundic stomach of awake, freely moving male SD rats through a previously implanted chronic gastric fistula. The stomach was distended in a pattern that mimicked normal ingestion as described by Fraser & Davison (AJP 268, 1995). The large majority of neurons with c-fos positive nuclei were concentrated at the level of the area postrema, within the medial nts (mnts, 44±3 neurons/40 μm-section), commissural nts (15±1), dorsomedial nts (13±2), interstitial and intermediate nts (13±2), and dmnX (28±1). Sham-control manipulation resulted in few labeled cell nuclei (mnts: 5±2/section). The distribution of tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH) positive perikarya was similar, with the highest numbers in mnts and dmnX. The dmnX contained the highest proportion of gastric distension activated neurons with TH (13.1±2.3%) and DBH (12.3±7.6%), followed by the mnts: TH (7.8±2.9%), DBH (7.3±7.2%).These results suggest that nts noradrenergic and/or adrenergic neurons are involved in the dissemination of gastric tension cues to higher brain areas and in the dmnX they are involved in gastric tension dependent vago-vagal reflexes. Supported by NIH grant DK 47348.

403.15

EVIDENCE FOR OPPOSING EFFECTS OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) ON GASTRIC MOTOR FUNCTION IN THE DORSAL VAGAL COMPLEX (DVC) AND NUCLEUS RAPHE OBSCURUS (nRO) OF THE RAT. Z.K. Krowicki* and P.J. Hornby, Dept. of Pharmacology, Louisiana State University Medical Center, New Orleans, LA 70112.

Specific binding sites for VIP and/or VIP-immunoreactive fibers have been found in the DVC and raphe nuclei. These findings prompted us to investigate the effect of VIP (0.1 - 100 pmol; 30 nl) and vehicle (30 nl) in the DVC and nRO of α-chloralose-anesthetized rats on gastric motor function, heart rate (HR), and blood pressure (MBP). Peak changes in intragastric pressure (IGP) and areas of the response (ARIGP) as well as changes in pyloric (PMMI) and greater curvature (GCMMI) minute motility indexes, HR, and MBP vs. preinjection values in response to VIP at a dose of 10 pmol are shown as means ± SE for 5 - 6 animals.

	DVC	nRO
Peak IGP (cm H_2O)	+5.92 ± 1.54*	-1.75 ± 0.21*
ARIGP (cm^2)	+1.98 ± 0.41*	-2.09 ± 0.56*
PMMI	+2.88 ± 0.61*	-4.07 ± 1.12*
GCMMI	+ 1.12 ± 0.57	-1.36 ± 0.52*
HR (bpm)	- 33 ± 15	0 ± 0
MBP (mm Hg)	+13 ± 4*	+5 (median)

* P<0.05 vs. corresponding vehicle (Student-Newman-Keuls test)

Activation of VIP receptors in the DVC increases, whereas microinjection of VIP into the nRO decreases gastric tone and inhibits gastric motility. These data show for the first time that the same neuropeptide may produce opposite gastric motor responses on its microinjection into different medullary nuclei. Supported by PHS grant DK42714.

404.1

Anti-oscillatory effects of adenosine in the thalamus. Daniel Ulrich* and John R. Huguenard, Dept.of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford CA.

Adenosine has well established depressant effects in the central nervous system with both pre- and postsynaptic actions. We examined the effects of exogenously applied adenosine on thalamic network activity and on evoked synaptic responses in vitro. Thalamic oscillations were studied in acute slices by extracellular multi-unit recordings in the ventrobasal complex (VB) of the rat somatosensory thalamus and the adjacent GABAergic nucleus reticularis thalami (nRt). Phasic oscillations (3 - 4 Hz) in VB and nRt were evoked by extracellular stimulation of cortico - thalamic fibres. Adenosine (50 μM) reversibly decreased the total number of spikes within these oscillations in VB to (mean ± s.e.m) 46 ± 14 % of control (n = 5 experiments) and in nRt to 53 ± 9 %. In addition, adenosine reduced the duration of these oscillations without disrupting their periodicity. In a similar preparation, evoked monosynaptic inhibitory (ipscs) and excitatory (epscs) synaptic currents were recorded by whole cell patch clamp recordings. In both VB and nRt cells, adenosine decreased ipscs to (mean ± s.e.m) 33 ± 8 % (n = 7) of control and to 39 ± 7 % (n = 8), respectively. Epscs in nRt elicited by stimulation of thalamo-cortical and corticothalamic axons in VB were reduced to 16.7 ± 7.4 % (n = 5) of control. With both excitatory and inhibitory synaptic responses, the adenosine effect was accompanied by an increase in failures of synaptic transmission indicating a presynaptic site of action and was reversed by the purinergic A1 receptor antagonist 8-cyclopentyltheophylline (CPT, 1 μM).

We conclude that unlike in hippocampus where presynaptic effects of adenosine are restricted to epscs, in the thalamus both epscs and ipscs are reduced. These multiple effects of adenosine contribute to robust depressant effects on thalamic network activity. (Supported by NINDS NS 06477, the Pimley Research Fund and the Swiss National Science Foundation.)

404.2

CHOLECYSTOKININ MODULATES INTRATHALAMIC OSCILLATIONS. C.L. Cox*, J.R. Huguenard and D.A. Prince, Dept. Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA 94305.

The reciprocal synaptic connectivity between the thalamic reticular nucleus (nRt) and dorsal thalamus is implicated in intrathalamic rhythms associated with slow-wave sleep and pathophysiological conditions such as absence epilepsy. As cholecystokinin (CCK) is localized in thalamocortical and/or corticothalamic neurons, and has been found to selectively depolarize nRt neurons by suppressing a linear K_{leak} conductance, we have examined CCK actions on intrathalamic oscillations.

Simultaneous extracellular multiple-unit recordings from nRt and ventrobasal nucleus (VB) were obtained from *in vitro* thalamic slices of young rats (P10-20). Electrical stimulation of nRt evoked oscillatory activity (2-3.5 Hz) involving both nuclei. CCK (10-1000 nM, bath applied) produced dose-dependent effects upon the oscillations. High [CCK](>100 nM) shortened the oscillations (n=5 slices), whereas lower [CCK](10-100 nM) prolonged them (n=7).

Current- and voltage-clamp recordings using whole cell patch techniques were employed to test the hypothesis that CCK-mediated actions on synaptic potentials contribute to modification of oscillatory activity. Stimulation of nRt evoked multiple-peak, burst-like IPSCs in VB neurons. CCK (>400 nM) reversibly reduced IPSC amplitude and eliminated the multiple-peak shape. In contrast, low [CCK](<100 nM) did not modify the IPSCs. EPSPs in nRt neurons evoked by stimulation of VB or internal capsule were usually suppressed during the depolarization induced by high [CCK](>100 nM); however, low [CCK] had a mixed effect upon the EPSPs (40% facilitated, 60% suppressed).

Our findings indicate that CCK exerts a fine level of control over these intrathalamic rhythmic activities. At low concentrations, CCK depolarizes nRt neurons towards spike threshold without changing their spike discharge mode, thus increasing the probability of burst discharge from nRt neurons. As indicated by reduction of the multiple-peak IPSCs in VB, high [CCK] depolarizes nRt neurons, shifting these cells from burst- to single-spike mode, thereby reducing their phasic inhibitory output. The reduced inhibition onto VB neurons decreases the probability of burst discharge from these cells which, in turn, dampens the oscillatory activity. Reduction of the EPSPs, presumably due to dendritic depolarization by CCK, would also contribute to dampening of the oscillation; however in some cells, the EPSP is enhanced, likely a result of increased input resistance produced by CCK, and thus may contribute to facilitation of the oscillation. Supported by NIH grants NS06477 and NS07280, and the Pimley Training Fund.

404.3

GABAergic autoinhibition in the thalamus John R. Huguenard* and Daniel Ulrich. Dept. Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA.

Presynaptic inhibition of GABAergic synapses in the thalamus was studied in thin slices of rats at postnatal age P9 - P14 by whole cell patch clamp recordings with Cs-gluconate in the pipette. Monosynaptic inhibitory synaptic currents (ipscs) were elicited by focal stimulation (1.5 - 2x threshold) of axons and recorded in relay cells of the ventrobasal complex (VB) of the somatosensory thalamus as well as in GABAergic neurons of the nucleus reticularis thalami (nRt). The ipscs were reversibly blocked by bicuculline methiodide (10 μM) and had a reversal potential close to the expected chloride equilibrium potential. (R)-Baclofen (10 μM) decreased ipscs in relay cells to (mean ± s.e.m) 11.8 ± 2.6 % of control (n = 11 cells) and in nRt cells to 10.8 ± 1.6 % (n = 12). In both nuclei, the reduction of ipscs was reversed (> 60 %) by the $GABA_B$ receptor antagonist CGP 35348 (10 μM). In contrast, Phaclofen (1 mM) and 2-Hydroxysaclofen (0.5 mM) were less potent (< 40 %) in reversing the Baclofen induced reduction of ipscs. In the same cell types, Baclofen also decreased the frequency of TTX resistant miniature ipscs (mipscs) in VB to 39 ± 8 % of control (n = 8) and in nRt to 69 ± 16 % (n = 6). However, in both cell types, the cumulative amplitude distributions of mipscs remained unaltered by Baclofen (KS test, Q > 0.01) indicating that the postsynaptic sensitivity to GABA was unaltered.

We conclude that both evoked and spontaneous release of GABA from inhibitory neurons of nRt in the thalamus is inhibited by presynaptic $GABA_B$ receptors. The potential role for autoinhibition in regulating thalamic network activity will be discussed. (Supported by NINDS NS 06477, the Pimley Research Fund and the Swiss National Science Foundation.)

404.4

SHORT-TERM SYNAPTIC ENHANCEMENT PREDICTS LONG-TERM POTENTIATION IN NEOCORTEX. B.W. Connors* and M.A. Castro-Alamancos. Department of Neuroscience, Brown University, Providence RI 02912.

Pathways of the cerebral cortex vary in their ability to generate short- and long-term forms of synaptic enhancement. Induction of NMDA receptor-dependent long-term potentiation (LTP) depends upon adequate postsynaptic activation during the conditioning period. We tested the hypothesis that short-term forms of enhancement, which operate during conditioning, might influence a pathway's ability to generate LTP. Using rat slices *in vitro*, theta-burst stimuli applied to layer IV reliably induced long-term potentiation (LTP) in the upper layers of the somatosensory (SI) cortex, but not in motor (MI) cortex (Castro-Alamancos et al., *J. Neurosci.*, in press). In SI cortex, synaptic responses were strongly enhanced during theta-burst conditioning; in contrast, short-term enhancement was very weak in MI. Both short-term enhancement and LTP were abolished by AP5, an antagonist of NMDA receptors. The magnitude of short-term enhancement during conditioning stimulation was strongly correlated with the magnitude of LTP across slices from both cortical areas. Pharmacologically isolated IPSPs were slightly depressed during theta-burst stimulation, to a similar extent in the two cortical areas. Thus, frequency sensitivity of inhibition does not explain the strong enhancement in SI. Pharmacologically isolated NMDA receptor-dependent EPSPs showed strong short-term enhancement in SI, but not in MI, indicating that enhancement reflects an increase in NMDA receptor-mediated currents. Short-term synaptic enhancement per se was not enough to induce LTP, because one theta-burst sequence produced strong enhancement, followed only by short-term potentiation without LTP; three theta-burst sequences reliably induced LTP. We suggest that, 1) the capacity for short-term synaptic enhancement may vary widely in different neocortical pathways and areas, 2) short-term synaptic enhancement promotes the induction of long-term potentiation, 3) variations in short-term enhancement may account for some of the variability of LTP induction in neocortex. Supported by the NIH and ONR.

404.5

MECHANISMS OF THE AUGMENTING RESPONSE IN THALAMOCORTICAL PATHWAYS. M.A. Castro-Alamancos* and B.W. Connors. Department of Neuroscience, Brown University, Providence RI 02912.

During the classic "augmenting response", thalamus-evoked potentials in neocortex increase during paired stimuli or trains of between 5 and 20 Hz. We investigated the temporal, spatial and mechanistic properties of potentials in the frontoparietal cortex evoked from the ventrolateral (VL) and ventroposterior lateral (VPL) nuclei in the ketamine-anesthetized rat. Stimulation of VL produced strong augmenting responses, but repetitive stimulation of VPL yielded only depression. Current-source density analysis of VPL responses showed a primary current sink in layers III-IV, while VL produced a primary current sink in layer V. Inactivation of the thalamus with GABA or kynurenic acid did not affect the augmenting response. Recordings from a grid of 30 cortical sites showed that the augmenting response was generated in the projection field of the VL afferents and then spread into adjacent cortex. Intracellular recordings *in vivo* and *in vitro* revealed a population of intrinsically bursting cells in layer V that have I_H and I_T currents. A thalamic stimulus induced a small, brief EPSP followed by a strong, protracted IPSP in these cells; a second stimulus delivered during the IPSP induced a strong burst intracellularly and a concomitant augmenting response in the extracellular field potential. Blockade of the IPSP with low doses of $GABA_A$ receptor antagonists (but not $GABA_B$ antagonists), or local application of Ni^{2+} (a blocker of I_T) suppressed the augmenting response. In slices *in vitro*, thalamocortical EPSPs did not generate paired-pulse facilitation. The results indicate that the augmenting response is generated within the cortex when thalamic axons from a layer V-projecting nucleus produce a strong hyperpolarizing IPSP that deinactivates I_T in a subpopulation of layer V bursting cells. A subsequent thalamic EPSP that occurs during the hyperpolarization can then evoke a rebound Ca^{2+}-dependent depolarization that triggers a burst of action potentials; the spread of this enhanced activity to other cortical neurons completes the augmenting response. Supported by the NIH and ONR.

404.6

BASAL FOREBRAIN TRANSPLANTS AMELIORATE THE EFFECTS OF BASAL FOREBRAIN LESIONS IN RAT BARREL CORTEX. O. Rahimi,* G. Hill III, L.L. Porter, S.L. Juliano. Anatomy & Neuroscience, USUHS, Bethesda, MD.

Acetylcholine (ACh) is implicated in mediating functions including cortical arousal, learning and memory, and synaptic plasticity. Recent studies also demonstrate that neocortical depletion of ACh in rodents results in reduced 2-deoxyglucose uptake in response to whisker stimulation. Transplants of embryonic basal forebrain, however, restore stimulus-evoked activity toward normal. To verify previous observations that cholinergic depletion resulted in decreased stimulus-evoked activity, electrophysiological recordings were performed on rats that received unilateral basal forebrain lesions. A cortical whisker representation was selected and an electrode advanced while manually stimulating the whisker. As single units responded to the stimulation, a switching device was attached to the whisker and displaced in an upward direction for a fixed duration and amplitude. Unit responses were recorded over 30 stimulus trials for corresponding whiskers in both hemispheres. The response magnitude of each neuron was measured as spikes/stimulus during a fixed window of 8-50 msec post-trigger epoch. In the ACh-depleted hemisphere, the evoked response magnitude was significantly lower as compared to normal (unpaired t test, $p<0.01$). To assess the ability of embryonic basal forebrain transplants to ameliorate the effect of basal forebrain lesions, the above recording procedure was used to assess neuronal activity in rats that received basal forebrain lesions followed by embryonic basal forebrain transplants. The evoked response magnitude, recording from neurons in matched barrels in both hemispheres, in the grafted animals was dramatically improved compared to the lesioned animals. These results suggest that ACh plays a significant role in mediating neuronal activity and its modulatory control of cortical function.

404.7

SENSITIVITY OF SI CORTICAL INTRINSIC SIGNALS TO MODE AND PARAMETERS OF NATURAL SKIN STIMULATION. M. Tommerdahl*, O. Favorov, and B. Whitsel. Departments of Biomedical Engineering and Physiology, University of North Carolina, Chapel Hill, NC 27599.

Stimulus-evoked intrinsic signals in SI of cats and monkeys were recorded in order to characterize the cortical response to three different modes of skin stimulation: brushing, vibrotactile and thermal. In anesthetized cats and monkeys, a recording chamber containing an optical window was mounted over the contralateral forelimb region of SI. The cortex was illuminated with near infrared light (840 nm), and images were obtained (slow scan CCD camera) before and during periods of tactile stimulation. Averaged stimulus - nostimulus difference images were examined to detect the effects of changes in stimulus parameters (e.g., location, frequency, amplitude, direction of motion, temperature). A discrete vibrotactile skin stimulus initially evoked a relatively homogenous decrease in IR reflectance within an extensive SI region. As time of exposure to the stimulus increased (5-30 secs), however, a (modular) pattern of activation emerged, usually within 3-10 secs of stimulus onset. Brushing and thermal stimuli to the same skin site also evoked reflectance changes within a large SI region, but the patterns obtained with the different stimuli were substantially different. Following the optical imaging session, extracellular single unit recordings were obtained from areas selected on the basis of stimulus-evoked reflectance values. The results indicate that regions of stimulus-evoked decreases in IR reflectance correspond to regions in which stimuli enhance single neuron spike discharge, and that regions of stimulus-evoked increases in IR reflectance correspond to regions in which stimuli inhibit single neuron spike discharge. The intrinsic signal patterns were reproducible when the same stimulus conditions were used, and while the IR reflectance patterns evoked in the same SI region by different stimuli to the same skin site could be very different, all patterns shared common features. Supported by NIH NINDS R29 NS32358 and the Whitehall Foundation.

404.8

EVIDENCES FROM EXPERIMENTS AND COMPUTER MODELLING INDICATE A NOVEL MECHANISM FOR SIGNAL PROCESSING. M.T.Huber*§, M. Petersen§§, H.A.Braun and K.Voigt. § Department of Psychiatry, Univ. Marburg, R.Bultmannstr.; Institute of Physiology, Univ. Marburg, Deutschhausstr. 1-2, D-35037 Marburg; §§ Dept. of Physiology, Univ. Würzburg, D-97070 Würzburg, Germany.

Neuronal oscillations are of great interest and also controversy because of their possible functional implications for neuronal information processing. Here we present a simple computer model which bases on findings on ionic conductances found in certain cortical neurons and also incorporates noise to account for stochastic features. We show that the model remarkably well can reproduce findings from certain cortical neurons. Moreover we show own experimental work from two another and very different systems: spinal ganglion cells and shark electroreceptors, both of which clearly exhibit oscillatory activity very similar to those found in the cortical cells. We demonstrate that with the given set of "cortical" ionic conductances the experimental results from the ganglion cells and electroreceptors can be reproduced to great detail by the model. We present evidence that all the three systems might make use of oscillations and noise in principally the same way although they obviously have to deal with very different input modalities. The key finding is, that differental encoding of stimuli can occur because the noisy oscillator can be modulated in its firing probability per oscillation cycle or in its oscillation frequency depending on the stimulus modality. We conclude that neuronal oscillations per se can be advantageous for information processing at different levels of the nervous system. In this context noise is an essential feature for encoding with oscillations.

404.9

UTILIZATION OF TEMPORALLY-ENCODED VS. SPATIALLY-ENCODED INFORMATION DURING THE PERFORMANCE OF A TACTILE DISCRIMINATION TASK E. Ahissar* and E. Gamzu, Dept. of Neurobiology, The Weizmann Institute, Rehovot, Israel

Whether the finger velocity, and thus the temporal information, is of any importance in tactile identification is still controversial. Naive subjects were required to indicate which of two gratings, each presented to a different hand, is more dense. Spatial frequencies (SFs) spanned the range between 0.2 to 0.8 bars/mm. Finger velocities (V) were measured with a resolution of 0.1 mm / 40 ms. We compared the correlation between decision and spatial information to the correlation between decision and temporal information. The decision was more similar to the temporal information than to the spatial information, especially in the more difficult range of SFs (0.4 - 0.8 bars/mm). The tactile temporal frequency deviates from V*SF as the stimulus intervals become close in size to the diameters of the receptive fields (aliasing). We have simulated two possible neuronal mechanisms operating on the predicted neuronal input: A low-pass filter (LPF) and a phase-locked loop (PLL). The outcome of both mechanisms showed better fitness with the decision than the temporal frequency per se within certain regimes of receptive field sizes. This fitness is better as the task becomes more difficult. We conclude that temporal information plays a significant role in tactile texture processing and that this information is further processed by central "temporally oriented" mechanisms. These conclusions are further supported by the following: at SFs around 0.7 bars/mm there is a consistent reversal of perception leading to a confusion between the higher and lower SFs. This reversal can be fully explained by the PLL mechanism. Supported by: MJHF Germany and USA-Israel BSF.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION III

405.1

AN INCREASE IN HYPOTHALAMIC NEU (HER-2) PROTOONCOGENE RECEPTOR GENE EXPRESSION ACCOMPANIES THE INITIATION OF FEMALE PUBERTY. Y.J. Ma*, D.F. Hill, M.E. Costa and S.R. Ojeda, Div. Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.

Activation of epidermal growth factor receptors (EGFR) has been implicated as one of the mechanisms by which transforming growth factor alpha (TGFα) produced by astroglial cells facilitates the increase in LHRH secretion that leads to the onset of female puberty. However, other members of the EGF family may also be involved in the central control of sexual maturation. *Neu* differentiation factors (NDFs) are EGF-related proteins that interact with an EGFR-related receptor known as *neu* or HER-2. In the present experiments we found that the genes for both NDF and *neu* are expressed in hypothalamic astrocytes. Like TGFα, NDFβ2 (a NDF isoform found in brain) effected the release of prostaglandin E_2 (a LHRH secretagogue) from hypothalamic astrocytes, indicating that activation of *neu* or EGFR results in at least one common cellular response. The abundance of *neu* mRNA in the female rat hypothalamus increases markedly at the time of puberty, reaching maximal values at the time of the first preovulatory surge of gonadotropins. These changes may be partially attributed to ovarian steroids, as evidenced by the increase in *neu* mRNA induced by the sequential administration of estradiol and progesterone to ovariectomized immature rats. In contrast to the peripubertal changes in *neu* mRNA levels, hypothalamic NDF mRNA content was exceedingly low and did not change at puberty, suggesting that activation of *neu* receptors at puberty may involve mechanisms other than increased ligand availability. Exposure of hypothalamic astrocytes to either TGFα or NDF resulted in *neu* tyrosine phosphorylation indicating that TGFα, which does not bind to *neu*, transactivates *neu* receptors via EGFR. Thus, the NDF/*neu* receptor signaling system in the neuroendocrine brain appears to function tightly coupled to that of TGFα/EGFR during female sexual development. Supported by NIH grants HD25123, HD07133, HD18185 and RR00163.

405.2

ESTROGEN AND PROGESTERONE RECEPTOR EXPRESSION AT PUBERTY IN NEUROENDOCRINE AND RELATED NEURONS OF THE FEMALE MONKEY HYPOTHALAMUS. P.C. Goldsmith* and K.K. Thind. Reproductive Endocrinology Center, Univ. California Sch. of Med., San Francisco, CA 94143-0556.

Nuclear expression of estrogen receptors (ER) and progesterone receptors (PR) connotes transcriptional regulation by these gonadal steroids. In hypothalamic neurons of monkeys, ER and PR expression at puberty coincides with activation of pituitary function. To identify neuroendocrine (NEU) neurons affecting the pituitary directly, we microinjected retrograde tracer into the median eminence of pubertal female monkeys. To detect ER and PR expression, we immunostained vibratome sections for ER (mouse IgG C-314) or PR (rabbit IgG C-20, Santa Cruz Biotech) using DAB. Retrograde label was visible in the cytoplasm of NEU neurons after immunostaining. In the *supraoptic nucleus (SON)*, nearly all NEU neurons had ER+PR, a few were NEU or had ER only, but no neurons had PR alone. Lateral to the *SON*, all NEU neurons had ER but not PR. In the *paraventricular nucleus (PVN)*, ER neurons >> NEU+ER (half also had cytoplasmic ER), while very few were NEU+PR or had PR alone. Dorsal to *PVN*, NEU neurons >> NEU+PR or PR neurons, while ventral to *PVN*, the three groups appeared equal. In the anterior *periventricular zone (PVZ)*, all NEU neurons had ER+PR. In the more posterior *PVZ*, all NEU neurons had PR and most had ER, but ventrally half were NEU+ER and half had ER alone. In the *arcuate (ARC) region and adjacent PVZ*, virtually all NEU neurons had PR. In the *premammillary PVZ*, ER neurons >> NEU neurons, but laterally the few NEU neurons all had ER. Our data indicate that in pubertal female monkeys, nearly all NEU neurons in the *SON* express ER+PR, most in the *PVN* express ER, and all in the *ARC* express PR. In the *PVZ* anteriorly, NEU neurons express PR+ER, posteriorly, PR, and more laterally, ER. This expression of ER/PR in NEU and related neurons correlates with changes in neural control of hormone secretion occurring at puberty. Supported by HD10907 and HD11979.

405.3

A "WINDOW" FOR THYROID HORMONE ACTION IN REGULATING SEASONAL REPRODUCTION IN SHEEP L. A. Thrun, G. E. Dahl and F. J. Karsch*. Repro. Sci. Prog., Dept. of Biology, and Dept. of Physiol., Univ. of Michigan, Ann Arbor, MI 48109.

Thyroid hormones are essential for the neuroendocrine changes that lead to anestrus in the ewe. Previous studies involving thyroidectomy and timed thyroxine (T4) replacement suggest that thyroid hormones only need to be present late in the breeding season for anestrus to develop. This study tested the hypothesis that there is a limited "window" of time, late in the breeding season, when thyroid hormones must be present for progression of the neuroendocrine changes that lead to anestrus. Two approaches were used: 1) ewes were thyroidectomized (THX) late in the breeding season (mid-Dec.), just prior to the postulated "window" and 2) groups of 6-7 ewes were THX early in the breeding season (Oct.) and later received a physiological replacement of T4 at selected times during the hypothesized "window" (Jan.-Mar.; either 30 or 90 days treatment). The effect of these treatments on anestrus was determined by monitoring changes in reproductive neuroendocrine activity. For this purpose, ewes were ovariectomized and received estradiol implants. In this animal model, serum LH decreases 10-20 fold marking the onset of anestrus. Serum levels of LH and total T4 were monitored in twice weekly samples. In the first approach (THX just prior to the postulated "window"), LH remained elevated well beyond the natural transition to anestrus, indicating that ewes were deprived thyroid hormones during a time critical to development of anestrus. In the second approach (THX early in the breeding season followed by selected timed T4 replacement), LH remained elevated in ewes given 30 days of T4 during the hypothesized "window." In contrast, LH fell around day 80 in 5 of 7 ewes receiving the 90-day T4 replacement, indicating that thyroid hormones during this period were sufficient for development of anestrus. We conclude, therefore, that thyroid hormones influence seasonal changes in reproduction in the ewe only during a restricted "window" of about 80 days late in the breeding season to promote the neuroendocrine changes that lead to anestrus. (Supported by NIH HD18337 and MH10506; USDA 37203-0760).

405.4

CIRCULATING INHIBIN CONCENTRATIONS INCREASE PRIOR TO THE DECLINE IN PERIPHERAL FSH LEVELS IN SHORT PHOTOPERIOD-EXPOSED FEMALE SYRIAN HAMSTERS. M. E. McAsey and B. Benson*. Dept. of Cell Biology and Anatomy, The University of Arizona, Tucson, AZ 85724.

Female hamsters transferred from long photoperiod (LP) to short photoperiod (SP) exhibit a decline in circulating FSH concentrations and cessation of estrous cyclicity. Because inhibin may regulate FSH synthesis and secretion and hence follicle recruitment and maturation, we hypothesized that inhibin contributes to the suppression of serum levels of FSH during SP exposure and the onset of anestrus. Adult LSH/SsLak hamsters were maintained in LP (L14:D10 h) and monitored for regular four-day estrous cycles. Hamsters were transferred to SP (L8:D16 h) on estrus and groups of animals killed by rapid decapitation at either 1600 h on proestrus or 0800 h on estrus during each of five consecutive estrous cycles after transfer; groups of LP control animals were killed at comparable times. Additionally, groups of anestrous females were killed at 0800 or 1600 h 12 days after the last observed estrous discharge. One ovary from each animal was used for inhibin RIA; contralateral ovaries were reserved for inhibin mRNA analysis or processed for morphometry. Compared to estrous LP controls, serum FSH levels increased significantly ($p < 0.05$) during the first two cycles in SP then declined to concentrations that, at the fifth cycle of SP, were significantly ($p < 0.05$) lower than control values. Serum inhibin levels increased significantly by the third SP estrous cycle and remained elevated in anestrous animals. Regression analysis revealed an inverse correlation between serum inhibin and FSH levels on estrus ($p < 0.001$) but not on proestrus. Ovarian inhibin concentrations were significantly ($p < 0.05$) decreased only in ovaries from anestrous females when compared to those of LP controls. Relative levels of inhibin α- and βA-subunit mRNAs were lower in ovaries from SP proestrous and anestrous females when compared to those from proestrous LP controls, but elevated in ovaries from SP estrous and anestrous females when compared to those from estrous LP controls. The absence of antral follicles on estrus in the last two cycles of SP suggests that the increase in circulating inhibin and inhibin mRNAs is not from antral follicles which failed to ovulate but may be derived from the hyperplastic interstitium. The inverse relationship observed between inhibin and FSH suggests that inhibin may exert a regulatory role in suppressing FSH concentrations during SP-induced anestrus in hamsters. Supported in part by NIH grant #HD19521.

405.5

FSH SECRETION FROM PERIFUSED OVINE PITUITARY CELLS CHANGES MARKEDLY WITH CHANGING INHIBIN / ACTIVIN TONE. Vasantha Padmanabhan*, Judy Van Cleeff, Paul A. Fayreau, and A. Rees Midgley Jr. Department of Pediatrics and the Reproductive Sciences Program, University of Michigan, Ann Arbor, MI 48109.

Previous studies with dispersed ovine anterior pituitary cells have shown that i) pituitary FSH secretion remains elevated or increases with time during long-term perifusion, ii) activin (A) potentiates FSH release minimally (~20%), iii) follistatin (F) suppresses FSH release, and iv) F pretreatment markedly enhances subsequent FSH responses to A (Padmanabhan et al., 77th Annual Meetings of the Endocrine Society, 1995, Washington DC). Because F is an A-binding protein, we postulated that the suppressive effects of F on FSH secretion are mediated via blockade of endogenous pituitary A action. If this were true, by virtue of inhibin (I)'s ability to compete for A receptor, the effects of I on FSH secretion should be similar to that seen with F. Two million ovine anterior pituitary cells were loaded immediately after dispersion into each of four, microperifusion chambers along with SoloHill glass-coated microcarrier beads and perifused at a flow rate of 35 mL/min. Hourly pulses of GnRH (1 nM for 4 min) were administered throughout the course of the study by means of computer-driven syringe pumps. The experimental paradigm consisted sequentially of 16h stabilization, 4h baseline, 20h I pre-treatment and 24h treatment (medium control, I, A or A+I). Recombinant human I (10 ng/ml) decreased FSH secretion ~30%. Removal of I during treatment caused FSH to rebound to values approximating initial baseline secretion. Activin (10 ng/ml) increased FSH secretion markedly (~2-fold), and this stimulatory effect was blocked by I co-treatment. These results suggest that: i) suppressive effects of I, like F, may be mediated via blockade of endogenous pituitary A action and ii) changes in pituitary A and I tone may be major contributing factors in the control of FSH secretion. Supported by NIH grants U54 HD 29184 & P30 HD 18258.

405.6

THE IN-VIVO RELEASE OF NOREPINEPHRINE IN THE MEDIAL PREOPTIC AREA OF STEROID-PRIMED OVARIECTOMIZED RATS Sheba M. J. MohanKumar, P. S. MohanKumar, Deryl L. Troyer*, and S. K. Quadri, Department of Anatomy and Physiology, Kansas State University, Manhattan, KS 66506.

The afternoon of proestrus in young rats is marked by a surge in the release of luteinizing hormone (LH). This change in LH release is believed to be under the control of neurotransmitters, particularly the hypothalamic catecholamines. Norepinephrine (NE) is generally believed to have a stimulatory role in LH secretion. Recently we reported that, concomitant with the LH surge, NE release increases progressively during the afternoon of proestrus. The purpose of the present study was to investigate if a similar change in NE release accompanies the LH surge in steroid-primed ovariectomized rats. Female Sprague-Dawley rats (4-5 months) were implanted with a push-pull cannula in the medial preoptic area and ovariectomized bilaterally. On the seventh day after ovariectomy, they were injected with estrogen (30 μg/100 μl oil; s.c.) at 1000 hrs. On the ninth day they were injected with progesterone (2 mg/100 μl oil; s.c.) at 1000 hrs and subjected to push-pull perfusion. Perfusate samples were collected at the rate of 10 μl/min every 30 min from 1130-1730 hrs. They were analyzed for NE concentrations using high performance liquid chromatography with electrochemical detection. NE release was 6.9±1.4 pg/min at 1430 hrs but increased significantly ($p<0.05$) to 16.2±3.9 pg/min at about the time of the LH surge (1630 hrs) and then declined to 12.0±1.9 pg/min at 1730 hrs. It is concluded that this increase in NE release plays a role in bringing about the surge in LH in estrogen and progesterone primed ovariectomized rats.

405.7

ROLE OF GONADAL STEROID INDUCED CHANGES IN VENTROMEDIAL NUCLEUS OXYTOCIN RECEPTORS IN THE PREOVULATORY LH SURGE IN THE CYCLING FEMALE RAT. C. A. Johnston*, J. Gelineau-van Waes and V. Hardy. School of Pharmacy, Univ. of Montana, Missoula, MT 59812-1075.

Central oxytocin (OT) plays a physiologically important, stimulatory role in the expression of the preovulatory LH surge which is highly dependent on chnges in plasma gonadal steroids (Neurosci. Lett. 120: 256, 1990). We have recently demonstrated changes in OT receptor mRNA expression in the ventromedial hypothalamic nucleus (VMN) throughout the estrous cycle which correlate temporally with the ability of centrally administered OT to stimulate LH secretion (Bale et al., J. Neurosci., In Press). The present study evaluated the physiological relevance of the changes in VMN OT receptor expression on the preovulatory LH surge by administering 200 ng/ul antisense oligonucleotide to the OT receptor into the VMN via stereotaxically placed, bilateral, 26 guage stainless steel cannulae in order to determine whether preventing synthesis of OT receptors in the VMN on proestrus prevents the LH surge. The preliminary data suggest that changes in OT receptor expression in the VMN may be critical to OT's stimulatory influence on plasma LH. This work was supported by NIH grant, MH48228.

405.8

α_1-ADRENERGIC RECEPTORS MEDIATE INOSITOL PHOSPHATE METABOLISM, AND PROSTAGLANDIN E_2 AND LHRH SECRETION FROM IMMORTALIZED HYPOTHALAMIC NEURONS. S.M. Kreda[1], M.T. Sumner[1], S. Fillo[1], S.B. Shears[1], S. Collins[2] and W.C. Wetsel[1,*], [1]Lab. of Cell. and Mol. Pharm., NIEHS, Research Triangle Park, NC 27709, and [2]Dept. of Psychiatry, Duke Univ. Med. Ctr., Durham, NC 27710.

Activation of α_1-adrenoceptors (α_1ARs) stimulates LHRH secretion *in vitro* and LH secretion *in vivo*. The purpose of the present study was to determine whether immortalized hypothalamic LHRH neurons (GT1 cells) possess α_1ARs, which second messenger pathways are stimulated by these receptors, and whether activation of these α_1ARs affects LHRH secretion. Ligand binding studies with [^{125}I]-HEAT and WB4101 or (±)-niguldipine revealed that the GT1 cells contain two populations of receptors which correspond to the α_{1A}- (23%) and α_{1B}- (77%) subtypes. Northern blots confirmed that these neurons contain transcripts for the $\alpha_{1a/d}$ and α_{1b} receptors. Activation of α_1ARs with phenylephrine or with norepinephrine in the presence of a β-blocker (1-alprenolol) stimulated inositol phosphate production, and prostaglandin E_2 and LHRH secretion in a dose-dependent manner. These data suggest that LHRH neurons *in situ* may contain several different α_1ARs subtypes, that α_1AR activation is coupled to two different intracellular signaling pathways, and that these actions may regulate LHRH secretion.

405.9

THE ROLE OF Ca^{2+} IN PULSATILE RELEASE OF LHRH FROM CULTURED LHRH NEURONS DERIVED FROM EMBRYONIC OLFACTORY PLACODE OF THE RHESUS MONKEY. K.L. Keen, P.Claude*, L.L.Luchansky, and E. Terasawa. Wisconsin Regional Primate Research Center & Neuroscience Training Program, University Wisconsin, Madison, WI 53715

In a previous study we have reported that cultured LHRH neurons derived from the olfactory placode of monkey embryos release LHRH into media in a pulsatile manner at approx. 50 min intervals (Neuroscience Abst. 272.4. 1994). To further study the mechanism of LHRH pulse generation, in this experiment we have examined the role of calcium and the LHRH agonist, Des-Gly^{10}. (D-Ala^6)-LHRH Ethylamide, in LHRH release. The olfactory placode and the migratory pathway of LHRH neurons from monkey embryos at E35-37 were dissected out and cultured on collagen-coated coverslips in a growth medium. Two to four weeks later coverslips with cultured cells were mounted in Sykes-Moore chambers and perifused with a Krebs-Ringer phosphate buffer solution containing BSA and glucose (pH=7.4). Perifusates were collected in 10-min fractions for 5-6 h and LHRH was measured by RIA. After the perifusion experiment, cells were fixed and immunostained for LHRH and neuron-specific enolase. LHRH cells released the decapeptide hormone in a pulsatile manner at the interpulse interval 47.3 ± 3.5 min (N=18). LHRH release from cultured LHRH cells was stimulated by 1μM Bay K 8644 as well as 30 and 56 mM K^+, while it was suppressed by 1 μM nifedipine. Exposing the cells to a low Ca^{2+} (10 nM) buffer solution suppressed LHRH release after 117 ± 29 min (N=11). Finally, the LHRH agonist at 10 nM consistently stimulated LHRH release. These results suggest that 1) release of LHRH is influenced by the state of Ca^{2+} channels, and the presence of extracellular Ca^{2+} is necessary for LHRH pulsatility, although intracellular mobilization of Ca^{2+} may be able to maintain the pulsatile release of LHRH for a short time, and that 2) LHRH itself may modify its pulsatility in these neurons, as shown in GT-1 cells. We are currently examining how intracellular Ca^{2+} oscillations correlate with the pulsatility of LHRH release.(Supported by NIH grants, HD15433, HD11355 & RR00167).

405.10

DOPAMINE INCREASES TRANSFORMING GROWTH FACTOR-ß1 SYNTHESIS AND SECRETION IN THE ANTERIOR PITUITARY GLAND. D.K. Sarkar*, M. Pastorcic, A. De and N. Boyadjieva. Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA 99164-6520.

We have previously shown that transforming growth factor ß1 (TGFß1) is produced and secreted from the lactotropes of the anterior pituitary gland, and controls prolactin secretion and lactotropic growth. Since dopamine is known to be a physiological regulator of lactotropic function, we determined the action of dopamine and its agonist bromocriptine on TGFß1 synthesis and secretion. Determination of dopamine action on PRL and TGFß1 secretion from anterior pituitary cells in primary cultures revealed that dopamine concentration-dependently inhibited PRL secretion but stimulated TGFß1 secretion from these cells. When these cultured cells were treated with bromocriptine in the presence of estrogen, this dopamine agonist also stimulated TGFß1 secretion. In addition, bromocriptine increased TGFß1 mRNA levels in cultures in the presence of estrogen. Determination of bromocriptine action on TGFß1 protein and mRNA levels in the pituitary of estrogen-primed ovariectomized rats indicated that the agonist increased anterior pituitary content of TGFß1 protein and mRNA. These data suggest that dopamine and TGFß1 may interact to control lactotropic function. Supported by the National Institutes of Health Grant CA 56056.

405.11

AMPLIFIED LH SECRETORY BURST FREQUENCY WITH SELECTIVE ATTENUATION OF PULSATILE (BUT NOT BASAL) TESTOSTERONE SECRETION IN HEALTHY OLDER MEN: POSSIBLE LEYDIG CELL DESENSITIZATION TO ENDOGENOUS LH SIGNALING. T. Mulligan,[1] A. Iranmanesh,[2] S. Gheorghiu,[1] M. Godschalk,[1] S. Pincus,[3] J.D. Veldhuis.[4] [1]Hunter Holmes McGuire VA MC, Richmond, VA 23249, [2]Salem VA MC, Salem, VA 24153, [3]990 Moose Hill RD, Guilford, CT 06437, and [4]Div Endocrinology, Univ of Virginia, Charlottesville, VA 22908.

The specific mechanisms underlying the relative hypogonadism of aging remain to be elucidated fully. We used frequent venous sampling (every 2.5 min), sensitive and specific LH and testosterone assays, and deconvolution analysis (*Proc.Natl.Acad.Sci.* 84:7686, 1987) of the LH time series to assess overnight LH and testosterone (T) secretion in healthy young (N = 10, ages 21-34 years) and older (N = 8, ages 62-74 years) men. Elderly men had more frequent bursts of LH secretion (1.4 vs 0.9 per hr, P = 0.003), less T secreted per burst (265 vs 490 ng/dL, P = 0.021), and per hour (308 vs 721 ng/dL/hr, P = 0.05). Basal T secretion was similar. The frequency of LH and T secretory bursts correlated inversely (R = -0.746, P = 0.034). An approximate entropy (ApEn) statistic (*Proc.Nal.Acad.Sci.* 88:2297, 1991) was used as a scale-invariant measure of the orderliness of LH and T release. ApEn values for LH in older men (1.50 ± 0.12) were significantly higher (P = 0.0008, rank-sum) than those in young (1.17 ± 0.21), which indicates greater process randomness in LH secretion. We conclude that healthy aging in men is associated with: (i) diminished T production due to a decrement in the mass of T released per burst; (ii) an attenuated ability of the gonad to augment T production despite an accelerated LH secretory pulse frequency, suggesting partial desensitization of Leydig cells to available LH; and (iii) increased process irregularity in the time-structure of LH release.

AXON GUIDANCE MECHANISMS AND PATHWAYS V

406.1

SPROUTING OF ENDPLATES IN TRANSGENIC ANIMALS CONSTITUTIVELY EXPRESSING NCAM IN SKELETAL MUSCLE M.S.Fazeli*, C. Hobbs, D.J.Wells and F.S.Walsh. Exp. Path., UMDS, Guy's Hosp. & Dept. Basic Vet Sci., Royal Vet. Coll., London, U.K.

The neuromuscular junction is a versatile model system for the analysis of the role of cell adhesion molecules in synapse formation and plasticity. Neural cell adhesion molecule (NCAM) has been postulated to play an important role during the development and regeneration of the neuromuscular system. We have generated animals expressing the lipid-anchored isoform of human NCAM (125kD) under the control of skeletal muscle specific α-actin promoter. Examination of the EDL of transgenic mice revealed a significant number of endplates with fine terminal and nodal sprouts (21% compared with 1.17% in the non-transgenic littermates; 16.23μm± 1.35). Sprouting in the soleus muscle was also noted, however this was at a lower rate than in the EDL. A significant number of nerve terminals in the EDL displayed an altered morphology, with most terminal branches ending in conspicuous enlargements. Nerve terminals in both the soleus and EDL of transgenic mice displayed an increased arborization, as indicated by an increase in the number of branch points from 2.82± 0.21 to 4.25 ± 0.13 in the soleus. Endplate size was also increased in the Soleus and EDL by 30.5% and 50.7% respectively, compared with littermates. At the ultrastructural level, junctions in transgenic animals displayed an increase in the number of postsynaptic folds (8.7 ± 0.52 compared with 6.85 ± 0.57). The number of terminals with greater than 7 folds increased from 35% in the non-transgenic animals to 76% in the transgenics. The cross sectional area of the nerve terminal in these junctions was not significantly altered. Botulinum toxin induced paralysis of the EDL of transgenic animals resulted in terminal sprouts that were 67% longer than those in non-transgenic animals. Our observations indicate a role for NCAM in the development of neuromuscular junctions.

406.2

SELECTIVE INTERACTION BETWEEN SRC-FAMILY TYROSINE KINASES AND NEURAL CELL ADHESION MOLECULES. P.F. Maness*, S.G. Klinz, H.E. Beggs and W. R. Morse. Dept. of Biochemistry, UNC Chapel Hill School of Medicine, Chapel Hill, NC 27599-7260.

Members of the src-family of tyrosine kinases participate in neural cell adhesion molecule (CAM) mediated neurite outgrowth. Specifically, L1-dependent neurite outgrowth is mediated by the *c-src* gene product ($pp60^{c\text{-}src}$), whereas NCAM-dependent neurite outgrowth is mediated by the *fyn*-protein ($p59^{fyn}$). To elucidate the molecular basis for this selectivity we investi-gated interactions among neural CAMs and src-family kinases.

Physical association between $pp60^{c\text{-}src}$ and L1 in growth cone-enriched membranes from fetal (E18) rat brain was demonstrated by coimmunoprecipitation from Brij96-solubilized extracts. This interaction was specific as $pp60^{c\text{-}src}$ was not observed in immunoprecipitates with NCAM antibodies. However, overexpression of $pp60^{c\text{-}src}$ and L1 in transiently transfected COS cells did not result in detectable levels of association, indicating that their interaction in growth cones may involve an additional neuronal molecule. In contrast, overexpression of $p59^{fyn}$ and NCAM in transfected COS cells did result in an association demonstrated by coimmunoprecipitation The physiological relevance of these findings was underlined by *in vivo* immunostaining that revealed L1 and $pp60^{c\text{-}src}$ and NCAM and $p59^{fyn}$ coexpression on primary olfactory axons during axonogenesis in the mouse embryo (E11).

A principal substrate for $pp60^{c\text{-}src}$ in growth cones is tubulin. We also demonstrate that tyrosine phosphorylation of tubulin in a fetal rat brain cytosolic extract influences the dynamics of microtubule assembly, suggesting that CAM-*src* kinase interactions regulate cytoskeletal properties affecting axonal outgrowth.

406.3

FOCAL ADHESION KINASE ($pp125^{FAK}$) IS TYROSINE PHOSPHORYLATED DURING THE INITIAL FORMATION OF NEURITES ON LAMININ. P.S. Leventhal* and E.L. Feldman, Dept. of Neurology, University of Michigan, Ann Arbor, MI 48109-0588

In SH-SY5Y human neuroblastoma cells, an *in vitro* model of neuronal growth and differentiation, adhesion to laminin (LN) induces rapid neurite outgrowth (NTO). This has previously been shown to correspond to tyrosine phosphorylation of $pp125^{FAK}$, although the role of this protein is not clear. We found that adhesion of SH-SY5Y cells to LN-coated tissue culture plates resulted in tyrosine phosphorylation of $pp125^{FAK}$ before there was measurable NTO. Tyrosine phosphorylation of $pp125^{FAK}$ was maximal as the rate of neurite formation peaked, but phosphorylation of $pp125^{FAK}$ decreased thereafter, even as the neurites continued to lengthen. Colchicine, which eliminates elongation but not formation of neurites on LN, had no effect on the stimulation of $pp125^{FAK}$ phosphorylation. Finally, cytochalasin D inhibited LN-stimulated tyrosine phosphorylation of $pp125^{FAK}$ at levels that prevent initial neurite formation. These results suggest that the tyrosine phosphorylation of $pp125^{FAK}$ is related to changes in the actin cytoskeleton that occur during initial neurite formation and that its phosphorylation is not directly associated with the elongation of neurites.

406.4

POSTTRANSCRIPTIONAL REGULATION OF LAMININ RECEPTORS IN SENSORY NEURONS IS DETERMINED BY LIGAND CONCENTRATION. M. L. Condic*, and P. C. Letourneau, Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, MN 55455.

Neurons are able to extend axons on Laminin (LM) absorbed to the substratum over a wide range of concentrations. This is somewhat surprising, in that LM is known to be an adhesive extracellular matrix molecule, and most cells need to maintain a precise balance between cell-substratum adhesion and deadhesion in order to remain motile. The most likely explanation for the ability of neurons to extend processes on a wide range of LM concentrations is that the levels of extracellular LM a growth cone encounters can induce a compensatory regulation of LM receptors (LM-Rs), such that high levels of LM cause a decrease in the amount of LM-R expressed (to prevent excessive adhesion to the substratum), and low levels of LM increase the expression of LM-R (to maintain cell motility).

We have examined the RNA and protein levels for two LM-Rs, α6 and α3 integrin. Dorsal root ganglia neurons from E12 chick embryos were dissociated and cultured on glass coverslips coated with either Hi- or Lo-LM (20 or 1 μg/ml). Quantitation of the adsorbance of LM using 3H-labeled LM indicated that there was a ten-fold difference in the concentration of bound LM between the two conditions. Neurons extend axons under both conditions. Overnight cultures were either processed *in situ* for RNA, or total cellular proteins were metabolically labeled and cell surface proteins were biotinylated prior to extraction for immunoprecipitation. Surprisingly, the levels of LM-R RNA were consistently lower in cells cultured on Lo-LM relative to those grown on Hi-LM (~60%). However, the amount of LM-R expressed at the cell surface was on average 3.4 fold greater in cells grown on Lo-LM. Total cellular integrin α6 protein was approximately equal in the two conditions. Controls cultured on Fibronectin showed RNA and protein levels similar to those seen on Hi-LM. These results suggest that LM regulates its own receptor, both at the level of translation and posttranslationally through an effect on protein stability or transport to the surface. (Supported by NIH grant HD19950 and NS28807 to PCL).

406.5

GROWTH CONES AND FIBROBLASTS EXHIBIT SUBSTRATE PREFERENCE IN THE ABSENCE OF DIFFERENTIAL ADHESION

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The guidance of cells to distant locations is an important process during development of organisms as well as throughout adulthood. Growth cones, for example, must find and form synapses with distant neurons for proper functioning of the nervous system. Following injury, the normally stationary fibroblasts migrate to the wound site to aid in the healing process. The mechanism by which these cells are guided, however, is still poorly understood. An influential model suggests that cells and growth cones are guided by "differential adhesion" whereby guidance is mediated by either the most or least adhesive surface. The role of differential adhesion in substrate preference was examined using a combination of patterned substrates and interference reflection microscopy (IRM). N1E-115 neuroblastoma growth cones and 3T3 fibroblasts exhibited preferences for laminin and fibronectin, respectively, over amine, and amine over alkane. A quantitative analysis of cell-substrate adhesion was developed using digital image processing techniques and correlative immunofluorescence microscopy. The results revealed that substrate preference was not correlated with substrate adhesivity. Further, the adhesion profiles on the preferred and non-preferred substrates were indistinguishable, showing that differential adhesion did not mediate preference on patterned substrates. Moreover, the adhesion profiles as well as the general cell morphology and cytoskeletal organization were determined by the context in which the chemicals were presented, i.e., singly or alongside another. These results are, therefore, consistent with the notion that substrate preference and guidance may be mediated by some signal transduction mechanism.

This research was supported by a grant from The Whitaker Foundation.

406.6

MECHANISMS OF INTRARETINAL AXON GUIDANCE. B. Schlosshauer* and H. Stier. *Naturwissenschaftliches und Medizinisches Institut an der Universität Tübingen (NMI), 72762 Reutlingen, FRG.*

During development ganglion cell axons are guided into the inner optic fiber layer (OFL) by a dual mechanism. Using tissue explants on retinal cryosections, we have shown that in the OFL endfeet of radial glia/neuroepithelial cells provide a permissive substratum, whereas outer retina layers are inhibitory (Development, 121 (1995). Similarly, isolated endfeet used as substratum in vitro, are permissive in contrast to cell somata of radial glia purified by complement mediated cell lysis. Axonal outgrowth on glial somata is smaller by 2 orders of magnitude compared with glial endfeet. In addition, scanning electron microscopy shows axons to grow in a highly fasciculated manner avoiding contact with glial cell somata. When outgrowing axons approach outer layers of retinal cryosections, growth cone collapses are induced. Exposure of axon tips to purified retinal membrane fractions yields similar results as shown by time lapse video recording. In both cases the collapse inducing activity can be inactivated by heat denaturation. Because radial glia appears to be a likely candidate, cytoplasmic membranes are currently enriched from purified glial cells for collapse assays. In control experiments, cytoplasmic membranes from the synaptic target region (tectum opticum) of retinal ganglion cell axons do not significantly induce growth cone collapses. The biochemical characterization of the inhibitory activity is under way.

The data suggest that radial glia/neuroepithelial cells are instructive in guiding ganglion cell axons. Radial cells appear to be polarized having permissive endfeet and inhibitory somata which affect axon growth differentially. Supported by DFG.

406.7

DISTINCT TENASCIN-C DOMAINS INFLUENCE NEURON ADHESION, NEURON REPULSION AND PROMOTION OF NEURITE OUTGROWTH. A. Faissner*, A. Scholze, A. Clement, A. Joester, K. Schütte, F. Wigger, R. Frank[2], P. Ekblom[3] and B. Götz, Department of Neurobiology, INF 364, [2]Center of Molecular Biology (ZMBH), INF 282, University of Heidelberg, D-69120 Heidelberg, Germany, [3]Department of Animal Physiology, Uppsala University Biomedical Center, S-75124 Uppsala, Sweden.

The glia-derived extracellular matrix protein tenascin (TN-C) exhibits modular structure with aminoterminal EGF type- followed by fibronectin type III (FN III)-repeats and homologies to fibrinogen β and γ at the carboxyterminus. Isoforms are distinguished by alternatively spliced FN III motives between FN III repeats 5 and 6 of the basic structure. Functionally, TN-C promotes neuron-astrocyte adhesion, neuron migration and neurite extension. On the other hand, the glycoprotein displays repulsive properties for some neurons and their growth cones. Antibody perturbation studies suggest that these functions are encoded by distinct sites on TN-C. In order to characterize these further, bacterially expressed fusion proteins were generated to map domains adhesive, counteradhesive and neurite outgrowth promoting for neuronal cells. In short term (1 hour) assays, several recombinant proteins proved adhesive for postnatal day 6 (P6) cerebellar and embryonic day 18 (E18) hippocampal neurons, but only TNfn1-3 supported the development of a monolayer of cerebellar neurons within 24 hours. In contrast, TNegf and the alternatively spliced segment TNfnA1,A2,A4 exposed on patterned substrates displayed repulsive properties for both types of neurons and for growth cones leaving P6 cerebellar explants. Finally, neurite outgrowth promoting properties for E18 hippocampal neurons and P1 DRG explants could be attributed to TNfnB,D, TNfnD,6 and TNfn6, a region centered around the distal splice site of TN-C. The functional properties of isoform-specific FNIII variants indicate functional relevance of the alternative splicing of TN-C. Supported by DFG (SFB 317/A2 and Graduiertenkolleg).

406.8

SOLUBLE CHONDROITIN SULFATE PROTEOGLYCAN REDUCES DORSAL ROOT GANGLION NEURITE LENGTH AND RATE OF ELONGATION ON FIBRONECTIN, BUT NOT ON LAMININ, BY BINDING DIRECTLY TO GROWTH CONES. D.M. Snow*, E.M. Brown, and P.C. Letourneau, The University of Minnesota, Cell Biology and Neuroanatomy, Minneapolis, MN 55455.

Chondroitin sulfate proteoglycan (CSPG) is a complex macromolecule having a wide variety of effects on neurons *in vivo* and *in vitro*. Studies on CSPG function have involved the use of both soluble and substratum-bound forms. Therefore, this study examined whether soluble CSPG influenced neurite outgrowth differently than substratum-bound CSPG. Previous reports (Snow et al., 1990, Exp. Neurol. **109**:111; Snow et al., 1994, Dev. Biol., **106**:87), showed that bound, but not soluble, CSPG inhibited neurite outgrowth, and induced the elevation of intracellular calcium in neurons grown on laminin (LN). A further report (Snow, D.M., 1994, ASCB Abst. **364a**) showed that the addition of soluble CSPG at physiological concentrations had little effect on the attachment of dorsal root ganglion neurons (DRGs), or on their filopodial lifespan or length, when DRGs were grown on fibronectin (FN) or laminin. Interestingly, however, soluble CSPG reduced neurite length of DRG neurons grown on FN, but not on LN.

In the present study, we grew DRGs on either of two FN peptides, CS-1 (Humphries et al., 1987, J. Biol. Chem. **262**:6886), or recombinant 9.5GST (Maejne, A. et al., *in prep.*), both of which permit DRG attachment and outgrowth, but do not bind CSPG, and compared neuronal behavior to DRGs grown on intact FN. Our results suggest that: 1) soluble CSPG reduces neurite outgrowth on FN or FN peptides up to 80%, and reduces elongation rate on FN up to 50%, and 2) soluble CSPG regulates neuronal behavior by binding directly to growth cones elongating on FN. Therefore, CSPG may affect growth cone behavior by either binding to the substratum, i.e. LN, or by binding directly to growth cones and not the substratum ligand, i.e. FN. *Supported by NIH grant EY10545 to DMS, and HD19950 to PCL.*

406.9

ENDOGENOUS CHONDROITIN SULFATE MODULATES LAMINAR-DEPENDENT INTERACTIONS OF THALAMIC NEURONS WITH CULTURED SLICES OF DEVELOPING CEREBRAL NEOCORTEX.

D. E. Emerling* & A. D. Lander. Dept. of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139.

In the embryonic mouse brain, thalamic axons extend within the intermediate zone (IZ) and subplate (SP) layers of the cerebral cortex but do not enter the adjacent cortical plate layer (CP) until the latter begins to differentiate, eventually becoming layers 2-6 in the adult. Previously, we found that dissociated, embryonic mouse thalamic neurons attach well to the IZ and SP of living, vibratome-cut slices of embryonic cerebral cortex, but attach poorly to the CP. On postnatal slices, attachment to the CP improves markedly. (Development **120**, 2811-2822, 1994). We now find that treatment of embryonic cortical slices with chondroitinase ABC (which degrades chondroitin sulfate [CS]), causes the CP to support thalamic neuron attachment at levels comparable with the other layers. Interestingly, such treatment also causes the IZ and SP to become less permissive for attachment. Both effects are dose-dependent and correlate with the disappearance of CS-immunoreactivity. Treatment of slices with another glycosaminoglycan lyase, hyaluronidase, fails to produce any of the effects seen with chondroitinase. These results suggest that CS, within different cortical laminae, may contribute *both* to local inhibition and local promotion of neuronal adhesion and may thereby differentially regulate axon growth during development. Whether these actions result from direct effects of CS on neurons, or indirectly, via interactions of CS with other molecules in the neural environment is under investigation.

406.10

CHARACTERIZATION OF *C. ELEGANS* SEMAPHORIN I AND II. P.J.Roy, H. Zheng, A. Roach*, and J. Culotti. Samuel Lunenfeld Research Institute, 600 University Ave., Toronto, On, M5G 1X5, Canada.

Semaphorins are a family of proteins implicated in nerve guidance, and are found in both invertebrates and vertebrates, as described by Kolodkin *et al.*, (1993 Cell **75**, 1389-1399). Using *C. elegans*, we wish to understand how the semaphorins help guide axons to their proper targets. To this end, we have identified a *C.elegans* semaphorin cDNA fragment through degenerate PCR. By splicing a partial clone we isolated from a cDNA library together with one obtained from a *C. elegans* cDNA sequencing project (Waterston *et al.*, 1992 Nature Genet. 1, 114-123), we constructed a full length cDNA (2.7kB) which corresponds to the size on the mRNA on Northern blots. Ce-sema cDNA sequence analysis elucidated a signal sequence and a transmembrane domain, similar to the Semal class of semaphorins, hence the designation Ce-Semal. Ce-Semal was mapped to cosmid F14B11 on chromosome I. A 12.8 KB subclone of F14B11 contains most of the *Ce-semal* gene; it has sufficient promoter elements to drive the expression of Lac-Z in a defined set of cells, and has the entire Ce-Semal coding sequence and 3' UTR. *Ce-semal* maps within 80 KB to the left of *unc-54*. We are currently using the 12.8 KB subclone to try to rescue prospective mutants which lie to the left of *unc-54*.

3.8 KB of *Ce-semal* predicted promoter sequence was fused to both nuclear and cytoplasmic lac-Z reporter constructs. Preliminary staining analysis of several stable lines of animals with the reporter constructs shows lac-Z expression early in embryogenesis, and strong posterior hypodermal staining in early larval stages, which is decreasingly maintained into adulthood. Weak staining is also seen in the head, among other cells.

Y. Kohara's cDNA sequencing project (Kohara *et al.*, 1994, p.comm.) yielded a second *C. elegans* semaphorin cDNA fragment. In combination with Kohara's clone, we have generated and used a 5'RACE product to construct a full length cDNA (2.4KB), which matches the predicted size indicated by Northern analysis. Our sequence analysis revealed a signal sequence, and an IG domain, placing this protein within the second class of semaphorins, hence the designation Ce-SemaII.

406.11

THE MURINE SEMAPHORINS: A DIVERSE GENE FAMILY ENCODING GROWTH CONE GUIDANCE CUES. A. W. Püschel, R. H. Adams and H. Betz*. Abteilung Neurochemie, Max-Planck-Institut für Hirnforschung, Deutschordenstr. 46, D-60528 Frankfurt/Main, FRG.

During development vertebrate spinal sensory and motor axons navigate with remarkable specificity to their peripheral targets. Members of the semaphorin gene family have been proposed to act as growth cone guidance signals in vertebrates and invertebrates. To identify candidate molecules involved in axonal pathfinding during mouse embryogenesis we isolated cDNAs encoding seven new members of the semaphorin family (semA - G). Four subgroups can be distinguished by comparison of the murine semaphorins to known vertebrate and invertebrate sequences. In situ hybridization analysis shows that the murine semaphorin genes are differentially expressed in mesoderm and neuroectoderm before and during the time when axons select their pathways in the embryo (E9 - E15).

In explant cultures recombinant Sem D/collapsin converts a matrix permissive for axonal growth into one that is inhibitory for neurites of peripheral ganglia. Our data demonstrate that semaphorins may provide local signals to specify territories inaccessible for growing axons.

406.12

DECORIN IN THE DEVELOPING MOUSE NERVOUS SYSTEM: EXPRESSION IN THE FLOORPLATE, AND BINDING TO NETRIN-1. Litwack, E.D.*[1], M.J. Galko[2], K. Danielson[3], M. Tessier-Lavigne[2], R.V. Iozzo[3], and A.D. Lander[1]. [1]Dept. of Biology & Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139. [2]Dept. of Anatomy, UCSF, San Francisco, CA 94143. [3]Dept. of Pathology and Cell Biology, Thomas Jefferson University, Philadelphia, PA 19107.

Decorin, a small extracellular matrix proteoglycan with a single chondroitin sulfate (CS) or dermatan sulfate (DS) chain, is a major component of several connective tissues, and binds via its core protein to collagen fibrils. Both *in situ* hybridization and immunohistochemistry revealed that decorin is also expressed in the developing central nervous system: Decorin mRNA and protein occur uniquely in and are present throughout the floorplate of E11 mouse neural tube, a midline structure important for the guidance of commissural axons. Since the floorplate has little obvious intercellular matrix, it seemed likely that decorin's function there might involve interactions with ligands other than collagens. The recently described protein netrin-1 is specifically expressed by the developing floorplate, and is a secreted chemoattractant for commissural axons. To test whether netrin-1 and decorin might interact, salt extracts of cells expressing epitope-tagged netrin-1 were passed over a column prepared from bovine skin decorin (gift of L. Rosenberg). Netrin-1 was retained and could be eluted with 1M NaCl. In contrast, no netrin-1 bound a BSA column. Affinity coelectrophoresis experiments indicate that netrin-1 binds decorin with an apparent K_d of 0.035 μM, near the apparent K_d of netrin-1 for heparin (0.030 μM). Neither CS nor DS bound netrin-1 as tightly (apparent K_ds of 2.2 μM for bovine tracheal CS and 1.1 μM for porcine skin DS). Consistent with this view, DS released from decorin by β-elimination showed no binding of netrin-1 (up to 0.5 μM DS). The data suggest that decorin's core protein may bind netrin-1 directly, a hypothesis now being tested. The idea that netrin-1 binds to decorin in the developing nervous system raises the possibility that decorin may regulate the diffusibility of netrin-1, or influence its chemotropic effects on axons. (Supported by R01-NS26862 to A.D.L.)

406.13

AXONAL GUIDANCE OF MOTOR NEURONS INVESTIGATED IN VIVO AND IN VITRO. S. Guthrie*, A. Tucker and A.Pini+. Division of Anatomy and Cell Biology, UMDS, Guy's Hospital, London SE1 9RT
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In the developing nervous system, motor axons grow away from the ventral midline floor plate, suggesting that the latter may be a source of repulsive axon guidance cues. Transplantation experiments in the chick hindbrain showed that motor axons diverted from their normal pathways to avoid grafted floor plates, often traversing abnormally long circuitous trajectories to reach exit points. When ventral explants of rat or chick hindbrain or rat spinal cord were co-cultured at a distance from floor plate explants within collagen gels, the outgrowth of motor axons was dramatically reduced from explant regions that faced the floor plate. Thus, the floor plate secretes diffusible repulsive cues, which may exclude motor axons from the midline during development. Chick hindbrain motor neurons showed increased axon outgrowth when cultured with cranial sensory ganglia, which are an intermediate target for some cranial motor neurons. This suggests that ganglia may provide a trophic influence for growing motor axons.

407.1

HOMOSYNAPTIC LONG-TERM DEPRESSION OF *APLYSIA* SENSORIMOTOR SYNAPSES IN CELL CULTURE. X. Y. Lin* and D. L. Glanzman. Dept. of Physiological Science, UCLA, Los Angeles, CA 90024.

Although synapses between sensory and motor neurons of *Aplysia* exhibit short-term depression in response to low-frequency presynaptic stimulation, long-term depression (LTD) of sensorimotor synapses in cell culture has not been demonstrated. Indeed, it has been reported (Montarolo et al., 1988) that in vitro *Aplysia* sensorimotor synapses lack the capacity for LTD due solely to homosynaptic activation. Based on this result, it was suggested that the long-lasting depression of sensorimotor synapses in the abdominal ganglion of *Aplysia* induced by repetitive stimulation of the siphon or branchial nerve—which is thought to play a role in long-term habituation of the defensive withdrawal reflex (Carew and Kandel, 1973)—is due to heterosynaptic inhibitory pathways recruited by nerve shock.

We have previously shown that sensorimotor synapses in cell culture can exhibit homosynaptic long-term potentiation (LTP) (Lin and Glanzman, 1993, 1994a, 1994b). Moreover, induction of this LTP appears to be mechanistically similar to induction of LTP of synapses in the CA1 region of the mammalian hippocampus. We therefore examined whether in vitro *Aplysia* sensorimotor synapses, like CA1 synapses, can exhibit homosynaptic LTD. To induce LTD of sensorimotor synapses in cell culture, we stimulated the sensory neuron at 2 Hz. Compared to control stimulation, the 2-Hz presynaptic stimulation produced significant LTD of the sensorimotor EPSP. The EPSPs in synapses which received 2-Hz stimulation were significantly smaller than the EPSPs in control synapses 90 min after the end of the experimental stimulation period ($p < 0.02$, Mann-Whitney U test). Because there were no interneurons in our cell cultures, these results demonstrate that *Aplysia* sensorimotor synapses can undergo LTD that is independent of heterosynaptic input. Future experiments will investigate whether the induction of homosynaptic LTD of sensorimotor synapses requires a postsynaptic rise in Ca^{2+} and activation of NMDA-related receptors.

407.2

THE NMDA RECEPTOR ANTAGONIST APV DISRUPTS THE CELLULAR ANALOGUE OF CLASSICAL CONDITIONING IN *APLYSIA*. G.G. Murphy* and D. L. Glanzman. Interdepartmental Program in Neurosci., Sch. of Med., and Physiological Sci., UCLA Los Angeles, CA 90024.

Classical conditioning of the *Aplysia* siphon-withdrawal reflex (Carew et al. 1981,1983) is generally thought to be due to a presynaptic mechanism—activity-dependent presynaptic facilitation of sensorimotor connections (Hawkins et al. 1983; Walters and Byrne, 1983). Recently, however, it has been found that, contrary to an earlier report (Carew et al., 1984), *Aplysia* sensorimotor synapses possess the capacity for Hebbian LTP (Lin and Glanzman, 1994). Furthermore, the induction of Hebbian LTP of sensorimotor synapses requires a rise in postsynaptic Ca^{2+} and activation of NMDA-related receptors.

To determine whether classical conditioning in *Aplysia*—like LTP of sensorimotor synapses—might involve activation of NMDA-related receptors, we utilized the cellular analogue of classical conditioning of siphon withdrawal (Hawkins et al., 1983). The preparation we used consisted of the CNS of *Aplysia* together with two posterior pedal nerves (P9) which connect the animal's tail to the CNS. During training brief activation of a siphon sensory neuron (the CS) was paired with strong shocks delivered to the pedal nerves (the US). Paired stimulation carried out in normal artificial seawater (ASW) produced significant enhancement of the monosynaptic sensorimotor EPSP measured 60 min after the end of training (mean EPSP = 194 ± 29% of the pretest EPSP, $n = 4$). By contrast, paired stimulation carried out in ASW which contained the specific NMDA receptor antagonist APV (100 μM) produced significantly less enhancement of the monosynaptic EPSP (mean EPSP = 111 ± 18% of the pretest EPSP, $n = 5$, $p < 0.05$, t-test). These results—together with our previous finding that postsynaptic infusion of the Ca^{2+} chelator BAPTA completely blocks the cellular analogue of classical conditioning of siphon withdrawal (Murphy and Glanzman, *Soc. Neurosci. Abstr.* 20:1072, 1994)—suggest that classical conditioning in *Aplysia* is mediated, at least in part, by LTP-related modulation of sensorimotor connections.

407.3

ROLE OF ADENYLYL CYCLASE AND cAMP IN ASSOCIATIVE SYNAPTIC FACILITATION AT SENSORY NEURON SYNAPSES IN *APLYSIA* . T. W. Abrams* and J. E. Galun. Department of Neuroscience, University of Pennsylvania, Phila., PA 19104-6018.

Activity-dependent facilitation has been proposed to result from enhancement by paired spike activity of the modulatory effects produced by facilitatory transmitter in the presynaptic SNs. Several cAMP-mediated effects of the facilitatory transmitter 5HT have been found to be enhanced when paired SN activity and Ca^{2+} influx immediately precede the arrival of 5HT. Ca^{2+}/CaM-sensitive cyclase is a candidate site of stimulus convergence since its properties would enable it to integrate two temporally paired stimuli, Ca^{2+} and 5HT, with optimal cyclase activation occurring when Ca^{2+} precedes 5HT. Murphy and Glanzman (1994) found Ca^{2+} elevation in the postsynaptic MN may be critical in triggering activity-dependent facilitation. To investigate the role of presynaptic cAMP in activity-dependent facilitation, we injected the competitive antagonist Rp-cAMPS into a SN soma. 30 min were allowed for diffusion of the inhibitor throughout the presynaptic processes. Associative synaptic facilitation was produced by pairing a 0.5 sec 10 Hz SN spike train with a 1 sec train of mantle nerve stimuli. 15 min after the end of 5 pairing trials, EPSPs from Rp-cAMPS-injected SNs were <35% of their pre-pairing amplitude. In contrast, at the 15 min posttest, EPSPs from control paired SNs were significantly facilitated. These results suggest that if an increase in postsynaptic Ca^{2+} is a necessary signal in the initiation of associative synaptic facilitation at these SN synapses, then there must be a trans-synaptic interaction between this postsynaptic signal and the presynaptic cAMP cascade.

407.4

HEBBIAN SYNAPSE-SPECIFIC FACILITATION AT *APLYSIA* SENSORIMOTOR CONNECTIONS: A POSSIBLE MECHANISM FOR CONDITIONED RESPONSE SPECIFICITY. G. A. Clark*, P. M. Balaban, and X. Fang. Program in Neuroscience, Psychology Dept., Princeton Univ., Princeton, NJ 08544.

The *Aplysia* siphon-withdrawal response exhibits conditioned response (CR) specificity: following training with different unconditioned stimuli (USs) (e.g., tail or mantle shock), animals exhibit different forms of CRs which resemble the unconditioned response to the particular US used. One possible neural mechanism is synapse-specific facilitation, which could preferentially enhance connections onto one motor neuron relative to others. To explore this possibility, here we examined whether Hebbian facilitation at sensorimotor connections (Lin and Glanzman, 1994) in the abdominal ganglion could be synapse-specific. In each preparation, we recorded intracellularly from an individual LE siphon sensory cell which connected to two different LF_S siphon motor neurons. We then paired spike train conditioned stimuli in the sensory neuron (12 spikes, 25 Hz) with simultaneous depolarization (3 nA) of one siphon motor neuron, and hyperpolarization of the other motor neuron. This manipulation of postsynaptic voltage was meant to mimic the effects with behavioral training with tail and mantle USs, which excite specific motor neurons but inhibit others. Following cellular training, EPSPs elicited by intracellular stimulation of the siphon sensory cell were preferentially enhanced at connections onto the depolarized motor neuron, relative to connections of the same sensory cell onto the hyperpolarized motor neuron ($P < 0.01$). These results indicate that Hebbian facilitation at sensorimotor connections can be synapse-specific, and thus might serve as one associative mechanism for conditioned response specificity.

407.5

PRELIMINARY EVIDENCE FOR SYNAPSE-SPECIFIC LONG-TERM FACILITATION AT CONNECTIONS OF *APLYSIA* SIPHON SENSORY NEURONS. C. M. Gooch* and G. A. Clark. Program in Neuroscience, Department of Psychology, Princeton Univ., Princeton, NJ 08544.

Synapse-specific plasticity provides a potentially precise means of modifying neural pathways. Although short-term facilitation in *Aplysia* LE siphon sensory cells can be synapse-specific (Clark & Kandel, PNAS 81: 2577-2581, 1984), long-term facilitation (lasting ≥ 24 hr) requires protein synthesis and probably the modification of gene expression. To investigate whether long-term facilitation can also be synapse-specific, we recorded the intracellular monosynaptic excitatory synaptic potentials (EPSPs) evoked simultaneously in a central and peripheral motor neuron by stimulation of individual sensory cells. We then examined whether 1-hr, 40-min applications of 10-30 μM serotonin (5-HT), restricted to either the central or peripheral synapses, could produce differential increases in synaptic strength at these two sites when tested 24 hours later. For each sensory cell in a preparation, difference scores were calculated by subtracting the amount of facilitation at peripheral synapses from the amount of facilitation at central synapses. We found that 5-HT application to the central synapses and soma of the sensory cell produced a greater increase in synaptic strength at central synapses than at peripheral ones, relative to effects of application of 5-HT restricted to peripheral synapses (all n's = 5, $P < 0.05$, one-tailed). In contrast, control applications of artificial seawater produced a difference score that was intermediate between the effects of central and peripheral 5-HT applications. These results provide preliminary evidence that long-term heterosynaptic facilitation may be in part synapse-specific.

407.6

APL-EF1α: A CANDIDATE MODULATORY FACTOR FOR UBIQUITIN-MEDIATED DEGRADATION OF PROTEINS DURING DEVELOPMENT OF LONG-TERM FACILITATION IN *APLYSIA* SENSORY NEURONS. K. Inokuchi, W. Pei, E. R. Kandel, J.H. Schwartz* and A. N. Hegde. Center for Neurobiology & Behavior and Howard Hughes Medical Institute, Columbia University College of Physicians & Surgeons, New York, NY, 10032.

The cAMP-dependent protein kinase becomes persistently active during the maintenance of long-term presynaptic facilitation (LTF) of sensory-to-motor neuron synapses underlying sensitization of defensive reflexes in the marine mollusk *Aplysia*. Kinase activity persists for at least 24 h, requires new protein synthesis, and results from proteolysis of regulatory (R) subunits. Hegde et al. (*PNAS* **90:** 7436, 1993) presented evidence that this proteolysis is mediated by the ATP-ubiquitin-proteasome pathway. Based on these and earlier data, we postulated that among the new proteins synthesized would be specific regulatory components for the ubiquitin pathway. Earlier, we found that a ubiquitin carboxyl-terminal hydrolase (Hegde et al., *in preparation*) is induced in sensory neurons by the facilitatory neurotransmitter 5-HT. Now, using LTF-producing protocols, we show that a protein similar to vertebrate elongation factor 1 α (EF1α) is also induced in sensory neurons by 5-HT. Conventionally, the elongation factor, a GTP-binding protein, participates in the formation of the peptide bond between nascent proteins and amino acyl tRNAs on ribosomes. *Aplysia* EF1α (Apl-EF1α) may be needed for the increased synthesis of new protein required for LTF (Winicov et al., *Cold Spring Harbor Symp. on Learning and Memory*, 1993). In addition, Gonen et al. (*PNAS* **91:** 7648, 1994) have demonstrated a role for an EF1α in ubiquitin-mediated degradation of proteins with blocked N-termini in rabbit reticulocyte lysates. We obtained full-length cDNA clones for Apl-EF1α and raised anti-peptide antibodies based on its inferred amino acid sequence. These antibodies recognize an M_r 50,000 protein in nervous tissue. Our working hypothesis is that this factor facilitates regulated proteolysis of R subunits and other protein substrates with blocked N-termini that are necessary for the maintenance of LTF.

407.7

EXPRESSION OF THE IMMEDIATE-EARLY GENE ApC/EBP IS REQUIRED FOR THE STRUCTURAL CHANGES THAT ACCOMPANY LONG-TERM FACILITATION IN *APLYSIA*. C. H. Bailey*, M. Chen, M. Ghirardi, C. M. Alberini, and E. R. Kandel. Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, NY, NY 10032.

Activation of the C/EBP immediate-early gene is part of the molecular switch required for consolidation of long-term facilitation in *Aplysia* (Alberini et al., 1994). To explore the role of ApC/EBP in the induction of learning-related synaptic growth, we have examined in parallel the effects of blocking the expression of this gene on the 5-HT-induced long-term increases in both the functional strength of the sensory-to-motor neuron connection and on the number of fluorescently-labeled sensory neuron varicosities in dissociated cell culture. To compete for the binding activity of ApC/EBP to its target sequence we injected into sensory neurons an oligonucleotide encoding the enhancer response element (ERE). Control cells were injected with an ERE oligonucleotide mutated at two bases (ERE mutant) and unable to bind to ApC/EBP. Sensory neurons injected with the ERE mutant showed a significant long-term increase 24 hrs following the application of 5-HT in both the amplitude of the evoked EPSP in the motor neuron L7 (79% ± 14, n = 7) and in the number of sensory neuron varicosities contacting the major axons of L7 (54% ± 12, n = 7). By contrast, injection of the ERE oligonucleotide completely blocked both the 5-HT-induced long-term increase in EPSP amplitude (2% ± 6, n = 9, $p < 0.001$) and in the number of sensory varicosities (0.1% ± 3, n = 9, $p < 0.001$). These findings suggest that the induction of ApC/EBP (and perhaps other immediate-early genes) may constitute a necessary and intermediate step for the structural changes, which might then carry the memory stably into the long-term stage.

407.8

LONG-TERM FACILITATION IN *APLYSIA* REQUIRES COORDINATE TRANSCRIPTIONAL ACTIVATION BY AF-1 AND CONCOMITANT DEREPRESSION OF CREB-2. D. Bartsch, M. Ghirardi, P. Skehel, C. H. Bailey, M. Chen*, S. Herder, K. Karl, and E.R. Kandel. HHMI, Ctr. Neurobiol. & Behav., Columbia Univ., NY, NY 10032.

The learning-related synaptic plasticity in the monosynaptic sensory-motor connection of the *Aplysia* gill-withdrawal reflex requires new protein and mRNA synthesis during the consolidation phase in which the short-term synaptic facilitation is transformed into a long-term form. During this period the transcription factor ApC/EBP is rapidly induced as an immediate-early gene by serotonin and cAMP. Perturbation of ApC/EBP function selectively blocks long-term facilitation (Alberini et al., Cell 76:109, 1994). Using the yeast two-hybrid cloning system, we have identified two constitutively expressed b-Zip domain transcription factors (AF-1 and CREB-2) that specifically interact with ApC/EBP. Both AF-1 and CREB-2 interact with ApC/EBP and each other via C-terminal leucine-zipper domains and can be coprecipitated from *Aplysia* neuron extracts. Injection of AF-1-specific antisera into the presynaptic neuron of *Aplysia* sensory-motor neuron coculture selectively inhibits long-term facilitation induced by serotonin but has no effect on short-term facilitation. Injection of CREB-2-specific antisera has no effect on long-term facilitation induced by repeated 5-HT stimulation, but potentiates the effect of 5-HT, such that in contrast to control a single stimulus is now sufficient to induce long-term facilitation. Therefore CREB-2 appears to act as a repressor of the transcription required for long-term facilitation. These data indicate that the transition from short- to long-term facilitation requires the coordinated positive regulation of the activator AF-1 and concomitant inactivation of the repressor CREB-2.

407.9

CONTRIBUTION OF LE SIPHON SENSORY NEURONS TO HABITUATION, DISHABITUATION AND SENSITIZATION OF THE GILL-WITHDRAWAL REFLEX IN *APLYSIA*. R. D. Hawkins* and L. Frost Ctr. Neurobiol. & Behav., Columbia Univ., NY, NY 10032.

We have developed a simplified preparation, consisting of the isolated mantle organs of Aplysia, that undergoes habituation, dishabituation, sensitization, and classical conditioning of the gill-withdrawal reflex. We previously found that the LE siphon sensory neurons contribute to the reflex elicited by controlled force stimulation of the siphon. However, a weak water movement stimulus that can produce PSPs in motor neurons and withdrawal of the gill does not cause the LE cells to fire, suggesting that other, unidentified sensory neurons can also contribute to the reflex (Cohen et al., 1991). Using a more sensitive mechanical stimulator (Byrne, 1975), we have replicated these results, and in addition have found that the unidentified neurons have lower thresholds than the LE cells. We previously recorded evoked spiking, the hyperpolarized complex PSP, and the PSP in high divalents in the gill motor neuron LDG1 in response to controlled force stimulation of the siphon during habituation, dishabituation, and sensitization of the reflex (Hawkins et al., 1992; Kaplan et al., 1993, 1994). Computer modeling of these results suggests that: (1) habituation can be accounted for by depression of sensory neurons PSPs; (2) dishabituation and sensitization by mantle shock involve heterosynaptic facilitation of the sensory neurons, and both facilitation and inhibition of interneurons; (3) 2.5 min after shock, inhibition of both excitatory and inhibitory interneurons produces net inhibition of the complex PSP, but contributes less to changes in evoked spiking; (4) 12.5 min after shock, facilitation of excitatory interneurons and inhibition of inhibitory interneurons contribute importantly to facilitation of evoked spiking.

407.10

A POSTSYNAPTIC CONTRIBUTION TO POSTTETANIC POTENTIATION (PTP) AND PAIRING-SPECIFIC FACILITATION AT APLYSIA SENSORY-MOTOR SYNAPSES IN CELL CULTURE. J.-X. Bao* and R. D. Hawkins. Ctr. Neurobiol. & Behav., Columbia Univ., NY, NY 10032.

Infusion of the Ca^{2+} chelator BAPTA into the postsynaptic neuron or postsynaptic hyperpolarization during tetanic stimulation reduces long-term potentiation (LTP) at *Aplysia* sensory-motor synapses (Lin and Glanzman, 1994). We have examined whether these treatments also affect PTP and pairing-specific facilitation at these synapses in isolated cell culture. The excitatory postsynaptic potential (EPSP) between a pleural sensory neuron and a gill or siphon motor neuron (L7 or LFS) was tested at 5 min intervals for 40 min. In test-alone controls, the evoked EPSPs declined over time (homosynaptic depression). When the sensory neuron was stimulated with a tetanus (20 Hz for 2 s), EPSPs were potentiated for about 15 min. Infusion of BAPTA (200 mM) into the postsynaptic neuron or strong postsynaptic hyperpolarization (2-4 nA) during the tetanus reduced the amplitude and duration of PTP. Pairing tetanus with a single puff of 5-HT (50 μM), which by itself produced short-lasting facilitation, induced greater facilitation than unpaired tetanus and 5-HT. Postsynaptic BAPTA reduced this pairing-specific facilitation, but did not affect facilitation by 5-HT alone. BAPTA also did not affect the amplitude of EPSPs after BAPTA infusion, homosynaptic depression, or paired-pulse facilitation, suggesting that BAPTA does not diffuse from the motor neuron to the sensory neuron. Since both PTP and pairing-specific facilitation are expressed presynaptically (Eliot et al., 1994), these results suggest that a postsynaptic increase in intracellular Ca^{2+} may lead to formation of some retrograde messenger, which participates in presynaptic facilitation of transmitter release.

407.11

ANATOMICAL EVIDENCE THAT SENSITIZATION OF MULTIPLE REFLEXES MAY BE MEDIATED BY AN IDENTIFIED CELL. W. G. Wright*, K. Jones, P Sharp, & B. Maynard. Department of Biology, Colorado State University, Ft. Collins, CO 80523.

Although sensitization-related changes in the neural circuitry of withdrawal reflexes in *Aplysia* are well studied, relatively few studies address the organization of the modulatory components of sensitization. In particular, it is not known whether individual modulatory loci can simultaneously effect change in multiple reflex circuits. There is, however, evidence that a single modulatory transmitter, serotonin, plays a pivotal role in facilitating several different reflex circuits during sensitization. Furthermore, it is known that activation of a pair of serotonergic neurons, the CB1s, produces heterosynaptic facilitation of the sensorimotor connections of one of these reflex circuits. These data together raise the possibility that the CB1s may produce changes in the neural elements of other reflex circuits, thereby effecting sensitization of multiple reflex systems simultaneously. In the present study, we utilized immunocytochemistry and intracellular labeling to obtain anatomical evidence of CB1's possible role in modulating other reflex circuits. We found 1) profuse fine processes given off by CB1 in the posterior neuropile of the cerebral ganglion, 2) extensive branching and fine processes in the pleural ganglion, and 3) a branch of CB1 that projects into the pedal ganglion. These three observations are consistent with, but do not prove, the hypothesis that CB1 modulates synaptic connections between 1) the sensory and motor neurons of the tentacle withdrawal reflex, 2) the sensory and interneurons of the tail and tail/siphon withdrawal reflex, and 3) the sensory and motor neurons of the tail withdrawal reflex.

408.1

NEUROSTEROIDS IN BRAIN MICRODIALYSATES.

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Coupling high performance liquid chromatography with gas chromatography-mass fragmentography (GC-MF) using negative ion detection, it has been possible to measure simultaneously subpicogram concentrations of allopregnanolone (ALLO) and of its parent compounds [pregnenolone (PREG), progesterone (PROG) and 5α-dihydroprogesterone (5α-DHP)] in discrete brain regions.Because measurements of neurosteroid steady state in brain may not reflect the dynamic state of neurosteroids at the synaptic level, we have used implanted microdialysis probes in the striatum and used the sensitive GC-MF method to establish whether neurosteroids are released and detected in the CNS extracellular fluid of awake freely moving rats. To this end, normal or adrenalectomized castrated (ADX/CX) rats were implanted stereotaxically with microdialysis probes. Continuous probe perfusion with Ringer solution (pH6, 1μl/min, 30 min fraction collection) in normal rats revealed a basal efflux of PROG of 25 to 30 pg and of ALLO of 4 to 6 pg/fraction. This efflux was constant for 240 min of dialysis. In ADX/CX rats the efflux of PROG was reduced to approximately 5 pg but that of ALLO was maintained between 3.5 and 5 pg/fraction. The intravenous infusion of PREG-SO_4 (20 mg/kg) in ADX/CX rats elicited a 7 to 8 fold increase in the content of ALLO in brain but not in plasma. In the first hour following PREG-SO_4 infusion the microdialysate content of ALLO increased from 3.5 to 15 pg/fraction. These experiments indicate that: 1. Neurosteroids are released in significant amounts from brain stores into the extracellular fluid and 2. The stable baseline release of neurosteroids can be utilized to study the mechanism of action of agents which modify synthesis and release of neurosteroids.(Supported by Grant MH49489-03).

408.2

FLUOXETINE TREATMENT SELECTIVELY CAUSES BRAIN ACCUMULATION OF ALLOPREGNANOLONE (ALLO) IN RATS.

D. P. Uzunov, D. L. Cheney*, E. Costa and A. Guidotti; Center for Neuropharmacology, Nathan S. Kline Institute for Psychiatric Research, Orangeburg, and Department of Psychiatry, NYU.

Fluoxetine (20 mg/kg, i.p., 1 hr.) administered to normal and adrenalectomized/castrated (ADX/CX) male rats increased by 2-3 fold the brain content of the neurosteroid ALLO measured by gas chromatography-mass fragmentography in the selected negative ion monitoring mode (J. Neuro. 1995). The extent of fluoxetine-induced ALLO increase was identical in normal (from 3 to 9 pmol/g) and ADX/CX rats (from 2 to 8 pmol/g). Incubation of brain slices of normal and ADX/CX male rats with fluoxetine (10 μM; 15 min) followed by addition of 10 μM 5α-dihydroprogesterone (5α-DHP), the immediate precursor of ALLO, resulted in a significant time-dependent accumulation of ALLO as compared to vehicle treated brain slices. Interestingly, conversion of ALLO to 5α-DHP by brain slices or by cultured glial cells was inhibited by 10 μM fluoxetine. Since the content of progesterone and pregnenolone in brain slices or glial cells was not altered by fluoxetine, the data support the hypothesis that fluoxetine induces an accumulation of brain ALLO by inhibiting the 3α-hydroxysteroid oxidoreductase which is the brain enzyme responsible for the conversion of ALLO to 5α-DHP. The observation that in fluoxetine treated ADC/CX rats the extent of brain ALLO accumulation is comparable to that occurring in normal rats suggests that the effect of fluoxetine is brain specific and that it is independent from peripheral hormone mobilization sources. The high potency of ALLO to decrease neuronal excitability via an action on $GABA_A$ receptors supports the working hypothesis that $GABA_A$ receptors may participate in the antidepressant, antidysphoric action of fluoxetine.
Supported by Grant MH49486-03.

408.3

BIOCHEMICAL SENSITIZATION TO THE NEUROSTEROID, 3α-HYDROXY-5α-PREGNAN-20-ONE (3α5α-THP) DURING ETHANOL WITHDRAWAL IN RATS.

A. Leslie Morrow* and Leslie L. Devaud. UNC School of Medicine, Chapel Hill, NC 27599.

Neurosteroids are potent modulators of $GABA_A$ receptors in the CNS. Behavioral and neurochemical cross-tolerance to most positive modulators of $GABA_A$ receptors have been observed in ethanol dependent rats. We recently demonstrated sensitization to the anticonvulsant effects of 3α5α-THP during ethanol withdrawal (Alc. Clin. Exp. Res. 19: 350, 1995). We now report enhancement of 3α5α-THP potentiation of $GABA_A$ receptor-mediated Cl^- uptake in cerebral cortical microsacs of ethanol withdrawn rats. Rats were administered ethanol by liquid diet for 2 wks using a pair-fed design and tested 6 hrs following the removal of diet. $GABA_A$ receptor-mediated Cl^- uptake was measured 5 sec after the addition of GABA (20μM) and various concentrations of 3α-THP (5-600 nM). GABA mediated Cl^- flux was reduced by 28%. 3α5α-THP potentiation of the GABA response was enhanced by 44.8±7.2% in ethanol withdrawn rats. Neurosteroid sensitivity in recombinant $GABA_A$ receptors is enhanced by the expression of γ1 vs. γ2 subunits. The level of γ1 subunit mRNAs (measured by competitive RT/PCR) was increased 70% in cerebral cortex (3.5 ± 0.6 vs. 6.0 ± 1.3 pg/μg total RNA). These results suggest that neurosteroid sensitization may be mediated by alterations in $GABA_A$ receptor subunit assembly in ethanol dependent rats. Interactions between ethanol and 3α5α-THP may influence the development of ethanol dependence. (Supported by AA09013, AA00191 and N.C. Governor's Institute).

408.4

INTRACEREBROVENTRICULAR INJECTION OF ALLOPREGNANOLONE ANTAGONIZES THE NEGATIVE EFFECTS OF ISONIAZID AND PTZ ON $GABA_A$ RECEPTOR FUNCTION.

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The effect of intracerebroventricular (i.c.v.) administration of 3α-hydroxy-5α-pregnan-20one (allopregnanolone; AP), a neurosteroid endowed with anxiolytic and anticonvulsant properties, on the function of $GABA_A$ receptors has been investigated in the rat brain. AP dissolved in 45% 2-hydroxypropyl-ß-cyclodextrin induced a dose-dependent (5-15 μg/5 μl i.c.v.) decrease (maximal effect -55% at 15 μg) of [^{35}S]TBPS binding measured "ex vivo" in unwashed membrane preparations from various rat brain regions (cerebral cortex, striatum, cerebellum, thalamus, hypothalamus, hippocampus). The effect of AP on [^{35}S]TBPS binding was evident within 2 min. after injection, lasted for at least 30 min. and returned to control values by 60 min. AP antagonized dose-dependently (2.5-15 μg/5 μl i.c.v.) both the increase of [^{35}S]TBPS binding and the convulsions induced by isoniazid (375 mg/kg s.c.). The same treatment antagonized convulsions induced by pentylentetrazole (PTZ) (75 mg/kg i.p.): 10 μg of AP produced a marked delay (8 folds) in the latency of convulsions induced by pentylentetrazole while at a dose of 15 μg this drug completely prevented the appearance of seizures. These effects were evident 2 min. after AP administration and lasted for at least 30 min. The time course of these effects well correlated with the time-related changes in brain AP concentrations which were maximal within 5 min. after i.c.v. infusion and returned to control values by 60 min. These results provide evidence that pharmacological doses of AP enhance the function of the $GABA_A$ receptors. The correlation between physiological concentrations of AP and GABAergic transmission will be discussed.

408.5

ALLOPREGNANOLONE REDUCES BASAL AND STRESS-INDUCED RELEASE OF ACETYLCHOLINE AND DOPAMINE IN DIFFERENT BRAIN AREAS OF FREELY MOVING RATS.

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Pharmacological, biochemical and electrophysiological evidences suggest that neurosteroids possess anxiolytic activity, possibly via a direct interaction with the GABA-gated chloride ion channel ($GABA_A$ receptor). As agonists and antagonists of $GABA_A$ receptor modulate in opposite manner the basal release of acetylcholine (ACh) and dopamine (DA) in the rat brain, we investigated using the microdialysis technique the effect of 3α-hydroxy-5α-pregnan-20-one (allopregnanolone)(AP) on basal and stress-induced release of these neurotransmitters in the prefrontal cortex and other brain areas of freely moving rats. AP (5, 10, 15 μg/5 μl, i.c.v.) dose dependently decreased the basal output of both cortical ACh and DA. This effect was maximal (-50%) 40 min after injection, lasted for about 60 min and returned to basal values within 120 min. AP also significantly decreased the basal output of ACh and DA in the hippocampus and nucleus accumbens, respectively. Footshock (0.2 mA/500 ms per s) is a stressful stimulus known to induce a rapid and marked increase in ACh (~90%) and DA (~100%) release in the above brain areas, an effect that lasts for at least 30-40 min. AP, at a dose (5 μg/5μl) per se ineffective on basal ACh and DA release, prevented the effect induced by footshock stress in the output of these neurotransmitters, an effect mimiked by anxiolytic drugs like diazepam. Our results show that AP shares with anxiolytic drugs the capability to modulate ACh and DA release and to prevent the stress-induced enhancement in the release of these neurotransmitters. This pharmacological finding supports the idea that endogenous neurosteroids partecipate in the modulation of central cholinergic and dopaminergic function during and following stressful conditions.

408.6

MODULATORY EFFECTS OF THE NEUROACTIVE STEROID THDOC ON GABA-INDUCED CHLORIDE CURRENTS IN CULTURED RAT HYPOTHALAMIC NEURONS.

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Steroid hormones control a variety of physiological functions, utilising both, genomic and non-genomic mechanisms in the central nervous system. The neuroactive steroid 5α-pregnane-3α,21-diol-20-one (THDOC) exerts its action either via activation of the progesterone receptor, following oxidation of THDOC to 5α-pregnane-21-ol-3,20-dione (Rupprecht et al., Neuron, 11:523-530, 1993) or through direct membrane actions via modulation of the GABA(A) receptor-mediated chloride currents. We investigated the modulatory potency of THDOC on the GABA(A)-induced chloride currents in serum-free cultured rat hypothalamic neurons, employing the whole-cell voltage-clamp technique. GABA (1 - 10 μM) and/or THDOC (10 nM - 1 μM) were locally applied via a multibarrel/single tip superfusion device. The amplitudes of the GABA-induced chloride currents were measured at a holding potential of -70 mV. The effect of various concentrations of THDOC (10 nM - 1 μM) on different concentrations of exogenously applied GABA (1 μM and 10 μM) was tested. Currents induced by 10 μM GABA were increased to 128% and 125% of control, by simultaneous application of 100 nM and 1μM THDOC, respectively. Ten nM THDOC had no consistent effect on the GABA-induced chloride currents. However, responses to 1 μM GABA were dose-dependently modulated: 10 nM THDOC reduced the GABA-induced current to 80% of control, whereas 100 nM and 1 μM THDOC enhanced the GABA response to 115% and 180% of control, respectively. In summary, the naturally occuring metabolite THDOC exhibited marked effects on the GABA-induced chloride currents in cultured rat hypothalamic neurons. The bidirectional modulatory action of THDOC depended on the concentration of exogenously applied GABA and THDOC. Through this mechanism, THDOC may modulate the signal to noise ratio of the GABAergic inhibitory synaptic transmission.

408.7

CO 2-1970: A PARTIAL AGONIST FOR THE NEUROACTIVE STEROID BINDING SITE OF THE $GABA_A$ RECEPTOR. J.A. Drewe*, E.R. Whittemore, C.L. Kimbrough, J.-S. Chen, D. Hogenkamp, R.M. Woodward and J.E. Hawkinson. CoCensys Inc., Irvine, CA 92718

Neuroactive steroids bind to a unique site on the $GABA_A$ receptor complex and allosterically modulate the binding of [^{35}S]TBPS, GABA ([^{3}H]muscimol) and benzodiazepine ([^{3}H]FLU) site ligands. 3a,5α-P is a full agonist at the neuroactive steroid site inhibiting 100% of [^{35}S]TBPS binding, enhancing [^{3}H]FLU binding to 180% and ≈225% of control in rat cortical and recombinant α1β1γ2L membrane preparations, respectively, as well as enhancing [^{3}H]muscimol binding to 160% in rat cortical membrane. Numerous synthetic neuroactive steroids have been examined, one of which denoted CO 2-1970 having limited efficacy for allosteric modulation [^{35}S]TBPS binding, displacing maximally 45% and ≈55%, enhancing [^{3}H]FLU binding, 140% and ≈160% in rat and α1β1γ2L, respectively, and enhancing [^{3}H]muscimol binding to <110%. To experimentally verify that CO 2-1970 is a partial agonist, competition studies were performed between 3α,5α-P and CO 2-1970. CO 2-1970 reduced the apparent potency of 3α,5α-P for modulating the binding of all three radioligands in rat cortex and for [^{3}H]FLU and [^{35}S]TBPS in recombinant α1β1γ2L membranes. In *Xenopus* oocytes expressing the α1β1γ2L subunit combination, Cl^- currents constituting 5% of the maximum GABA response were potentiated to 90% by 3α,5α-P, but to only 25% by CO 2-1970. Levels of potentiation induced by 3α,5α-P were reduced by coapplication of CO 2-1970 with a decrease in apparent affinity for 3α,5α-P. Collectively, these data provide compelling *in vitro* evidence that CO 2-1970 is a partial agonist for the neuroactive steroid binding site of the $GABA_A$ receptor.

408.8

STEROID RECEPTOR MEDIATED EFFECTS OF NEUROACTIVE STEROIDS: CHARACTERIZATION OF STRUCTURE ACTIVITY RELATIONSHIP. R. Rupprecht*, J.M.H.M. Reul, B. Berning, C.A.E. Hauser and F. Holsboer. Max Planck Institute of Psychiatry, Clinical Institute, Kraepelinstr. 10, 80804 Munich, Germany.

Neuroactive steroids rapidly alter neuronal excitability through binding sites on neurotransmitter-gated ion channels. The 3α-hydroxy ring A-reduced pregnane steroids enhance γ-aminobutyric acid (GABA)-mediated chloride currents while pregnenolone sulfate and dehydroepiandrosterone display functional antagonistic properties. Based on our previous findings that the 3α-hydroxy ring A-reduced pregnane steroids allotetrahydroprogesterone and allotetrahydrodeoxycorticosterone may regulate also gene expression via the progesterone receptor after intracellular oxidation, we have characterized the effects of a series of natural and synthetic neuroactive steroids at the genomic level using a cotransfection system. Pregnanolone and pregnenolone were able to activate both the chicken and the human progesterone receptor while the synthetic 3α-hydroxylated derivative alphaxolone and dehydroepiandrosterone were only active via the chicken progesterone receptor. Moreover, the antigluocorticoid activity of dehydroepiandrosterone reported at the systemic level could not be reconstituted in the cellular cotransfection system. This study provides evidence for differential genomic effects of neuractive steroids in a structure specific manner which may have impact on the development of these steroids for therapeutic application in neurology and psychiatry.

408.9

DIFFERENTIAL MODULATION OF $GABA_A$ RECEPTOR BY LORECLEZOLE, PROPOFOL AND DIAZEPAM M.Serra*, C.A. Ghiani, G.Tuligi, E. Maciocco and G. Biggio Dept. Exp. Biology, University of Cagliari, Cagliari Italy.

Recently, molecular biology and functional studies have shown that, differently to benzodiazepines and more similar to barbiturate, the broad spectrum anticonvulsant loreclezole (LRC) acts via a specific modulatory site on the ß subunit of the $GABA_A$ receptor complex. A similar specificity for the ß subunit has also been shown for the action of the general anesthetic propofol (PPF). On the basis of these data, the effect of LRC on the function of $GABA_A$ receptor complex in the rat cerebral cortical membrane preparation was compared with those of PPF and the benzodiazepine receptor full agonist diazepam (DZ). LRC and PPF modulated [^{3}H]muscimol and [^{35}S]TBPS binding with potencies and efficacies greater than those of DZ. The combination of LRC and PPF or DZ resulted in addictive effect on [^{3}H]muscimol binding, suggesting different sites of action for these drugs. Both LRZ and PPF showed biphasic effects on [^{35}S]TBPS binding to well washed membranes (in the absence of endogenous GABA): at low concentrations (5 or 10 μM) both drugs enhanced [^{35}]TBPS binding, whereas at higher concentrations (30 to 100 μM) they inhibited this biochemical parameter. In contrast, DZ enhanced [^{35}S]TBPS binding to washed membranes at all concentrations tested. The combination of LRC with GABA, at concentration (0.3 μM) that alone only slightly increased [^{35}S]TBPS binding to washed membranes, not only abolished but reversed the increase in binding elicited by low concentrations (5 to 10 μM) of LRC and significantly potentiated the inhibitory effect exerted by higher concentrations (30 to 100 μM) of this drug. Similar effects were observed with the combination of GABA with PPF. However, GABA had no effect on the enhancement of [^{35}S]TBPS binding induced by DZ. Since LRC modulates $GABA_A$ function receptor with higher efficacy than DZ its lack of sedative and hypnotic effects may depend from the capability to interact with selective $GABA_A$ receptor subtypes. Moreover, the lack of anesthetic action of LRC may reflect its much weaker capability to directly activate the chloride channel compared to PPF.

408.10

DUAL MODULATION OF THE $GABA_A$ RECEPTOR/IONOPHORE BY α-SUBSTITUTED γ-BUTYROLACTONES. K.D. Holland, G.C. Mathews, A.M. Bolos-Sy, J.B. Tucker, P.A. Reddy, D.F. Covey*, J.A. Ferrendelli, and S.M. Rothman. Department of Molecular Biology & Pharmacology and Department of Neurology & Neurosurgery, Washington University School of Medicine, St. Louis, MO 63110.

Alkyl-substituted γ-butyrolactones (GBLs) and γ-thiobutyrolactones exhibit convulsant or anticonvulsant activity depending on the alkyl-substituents. Alpha-substituted lactones with small alkyl substituents are anticonvulsant and potentiate GABA-mediated chloride currents, while β-substituted compounds are usually convulsant and block $GABA_A$ currents. We have now found that this distinction is not so clearcut, in that some compounds can both block and augment $GABA_A$ currents, but with different time courses. For example, α,α-diisopropyl-GBL (α-DIGBL) potentiates exogenous GABA currents in cultured rat hippocampal neurons, but diminishes GABA-mediated inhibitory postsynaptic currents. A more detailed analysis demonstrates a triphasic effect of α-DIGBL on GABA currents, with a rapid inhibitory phase, a slower potentiating phase, and then an "off-response" when the GABA/α-DIGBL perfusion is stopped. Thus, α-DIGBL can inhibit and potentiate GABA currents with kinetically different time courses. Inhibition is more rapid, but at steady-state potentiation dominates. Using a simplified model of the $GABA_A$ receptor/ionophore, we have simulated our experimental observations with α-DIGBL. Another lactone, β-ethyl-β-methyl-γ-thiobutyrolactone, also has dual actions, with inhibition predominating at low concentrations and potentiation predominating at high concentrations. We propose two distinct γ-butyrolactone modulatory sites on the $GABA_A$ receptor: an inhibitory "picrotoxin" site and an enhancing "lactone site". New information on the structure of the $GABA_A$ receptor/ionophore may allow the molecular dissection of these two sites.

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408.11

EVIDENCE FOR A POSITIVE MODULATORY SITE OF THE γ-BUTYROLACTONES ON THE $GABA_A$ RECEPTOR DISTINCT FROM THE PICROTOXIN SITE. K.L. Williams [1], D.A. Gurley [2], D.S. Weiss [3], G. White [2], D.F. Covey [1], J.A. Ferrendelli [1] and S.M. Rothman [1]*. [1]Washington Univ. Sch. of Med., St. Louis, MO 63110; [2]Neurogen Corp., Branford, CT 06405; [3]Univ. of South Florida College of Medicine, Tampa, FL 33612.

γ-Butyrolactones and related compounds target the $GABA_A$ receptor and modulate GABA-induced chloride flux. α-Substituted compounds generally potentiate, while β-substituted compounds potently inhibit GABA-induced currents. Both modulatory effects were believed to be mediated through opposite effects on the picrotoxin site. To test this hypothesis, a picrotoxin-insensitive $GABA_A$ receptor was created by introducing two point mutations in the second transmembrane domain of the α1, β2 , and γ2 subunits of the rat $GABA_A$ receptor, to make them identical to the glycine β subunit. Only the presence of a single mutated subunit (α, β, or γ) was required for picrotoxin insensitivity.

We examined the responses to α- and β-ethyl, methyl-thiobutyrolactone (α-EMTBL and β-EMTBL) of *Xenopus* oocytes injected with rat α1, β2, and γ2 RNA. Concentration responses were determined for each compound at the GABA EC_{10}. α-EMTBL (3-5,000 μM) potentiated GABA-induced currents in both wild-type α1β2γ2 - injected cells and in cells containing the mutated β_2 subunit (i.e., α1β2 ^{mut}γ2). In contrast, β-EMTBL, which inhibited GABA-induced currents at concentrations below 1 mM and potentiated GABA currents above 1 mM, in the α1β2γ2 - injected cells only potentiated over the entire concentration range in the oocytes injected with the α1β2 ^{mut}γ2 subunits. Additionally, β-EMTBL potentiated GABA-induced currents at 100 and 1000 μM in cells injected with the mutated γ_2 subunit (i.e., α1β2γ2^{mut}). These results indicate that the lactones act through two distinct effector sites: a negative modulatory (picrotoxin) and positive modulatory (undefined) site.

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409.1

NEURONS AND ASTROCYTES CONTAIN THE MITOCHONDRIAL PERMEABILITY TRANSITION PORE. Janet M. Dubinsky*, Dept. of Physiology, University of Minnesota, Minneapolis, MN 55455.

The permeability transition pore (PTP) is a mitochondrial channel associated with hypoxia in liver, that opens in response to calcium and oxidation, resulting in swelling of mitochondria in isolated preparations (Thomas et al '94 Am J Physiol 267:C313). Since both calcium and oxidation are required for delayed glutamate neurotoxicity (Kristal et al., Nsci. Abstr. 20:649) and mitochondrial swelling follows both excitotoxic and ischemic injury, I have hypothesized that the PTP exists in neural mitochondria and may participate in the pathogenesis of excitotoxic injury. To determine if nervous tissue contains the PTP, mitochondria in cultured astroglia and hippocampal neurons were visualized using conventional and confocal microscopy with nonyl acridine orange, a dye that partitions into mitochondrial membranes irrespective of mitochondrial membrane potential. Plasma membranes were permeabilized by a 5' exposure to 0.0005-0.001% digitonin in a high potassium or predominantly sucrose containing medium. Under these conditions, mitochondria were observed to change from rod shaped to rounded in response to calcium. Addition of an excess of EGTA could restore a rod-like shape. Phenylarsine oxide, a PTP activator, also produced shape changes in healthy mitochondria. Thus neuronal and astroglial mitochondria behave similarly to liver mitochondria under conditions that stimulate the PTP. Supported by the Mn. Medical Foundation and the Mn. American Heart Association.

409.2

CGP 37157: A NEW TOOL FOR STUDYING MITOCHONDRIAL BUFFERING OF GLUTAMATE- INDUCED CALCIUM INFLUX IN CULTURED FOREBRAIN NEURONS. R. J. White* and I. J. Reynolds. Center for Neuroscience and Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Mitochondria are a potentially relevant target for Ca^{2+}-mediated damage following excitotoxic stimuli, and we have previously documented the importance of mitochondria in buffering modest calcium loads induced by 15 seconds of 3 μM glutamate (glu). Using indo-1 microfluorimetry to measure $[Ca^{2+}]_i$ in single cells, we now report the effect of CGP-37157 (CGP), a reversible, specific inhibitor of the mitochondrial Na^+/Ca^{2+}-exchange. Following application of a control (15s, 3μM) glu pulse, we stimulated primary cultures of rat forebrain neurons with an identical dose of glu and then washed the glu-containing buffer out with 2 minutes of 25 μM CGP. We consistently observed that $[Ca^{2+}]_i$ returned to baseline more rapidly than in controls. Rarely, we observed a small $[Ca^{2+}]_i$ "hump" while washing the CGP out with Ca^{2+}-free buffer, presumably reflecting reactivation of the mitochondrial Na^+/Ca^{2+}-exchange and subsequent Ca^{2+} release from the mitochondria.

To explore mitochondrial Ca^{2+} buffering processes in paradigms relevant to excitotoxicity, we exposed neurons to 100 μM glu for 5 minutes. Under control conditions, recovery of $[Ca^{2+}]_i$ to baseline took up to an hour. In contrast, when glu stimulation was terminated with a Ca^{2+}-free solution containing CGP, some cells were able to buffer $[Ca^{2+}]_i$ to baseline within minutes. Thus, by preventing Ca^{2+} movement from the mitochondria to the cytoplasm, CGP revealed a large mitochondrial contribution to the prolonged elevation of $[Ca^{2+}]_i$ following this intense glu stimulus. In each experiment with the 100μM glu treatment, we observed a $[Ca^{2+}]_i$ "hump" after we washed the CGP away with Ca^{2+}-free buffer; the "hump" was long-lasting and presumably reflected the substantial store of Ca^{2+} which the mitochondria had taken up during the glu stimulus. Our data reveal a significant, novel cellular transport process and establish the importance of mitochondria in buffering a glutamate-induced Ca^{2+} influx. CGP will prove to be an invaluable tool for further defining the role of mitochondria in excitotoxic neuronal injury.

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409.3

GLUTAMATE EXCITOTOXICITY AND MITOCHONDRIAL MEMBRANE POTENTIAL. L. Kiedrowski*. The Nathan S. Kline Institute for Psychiatric Research, New York University, Orangeburg, NY 10962.

Young cultures of cerebellar granule cells are less susceptible to glutamate excitotoxicity than older ones in spite of the fact that application of glutamate in a Mg^{2+} free medium leads to an influx of similar amounts of $^{45}Ca^{2+}$ (Eimerl & Schramm, J. Neurochem. 62 (1994) 1223-26). The Ca^{2+} influx that is diverted to mitochondria during glutamate pulse may compromise mitochondrial function. Therefore, the mitochondrial ability to retain the inner mitochondrial membrane potential ($\Delta\psi$) in younger and older cultures was studied. Cerebellar granule cells were simultaneously loaded with Fura-2 and tetramethylrhodamine (TMR) to study $[Ca^{2+}]_i$ and $\Delta\psi$, respectively. In the 7 day old cultures an exposure to 1 μM antimycin A1 for 30 min failed to dissipate $\Delta\psi$. In contrast, in the 10 day old cultures an incubation with antimycin A1 for only 7 min was sufficient to dissipate $\Delta\psi$ and release TMR from mitochondria to cytoplasm. Hence, the inability to retain $\Delta\psi$ may account for the increased susceptibility to excitotoxicity in older cultures. In fact, with the increasing age of cultures a progressively larger percentage of cerebellar granule cells failed to buffer $[Ca^{2+}]_i$ during 15 min exposure to 100 μM glutamate and 10 μM glycine in a Mg^{2+} free medium; after glutamate withdrawal, the neurons that failed to buffer $[Ca^{2+}]_i$ also failed to reaccumulate TMR in mitochondria. This indicates that in cerebellar granule cells that are more susceptible to excitotoxicity, the $\Delta\psi$ collapses during the exposure to glutamate. A possible cause of the $\Delta\psi$ collapse in these neurons may be an excessive Ca^{2+} uptake by mitochondria. Supported by NIH grant NS 28130-05.

409.4

PHOSPHOCREATINE-DEPENDENT GLUTAMATE UPTAKE BY SYNAPTIC VESICLES C.J.Xu[a*], W.E.Klunk[a], Q. Xiong[a], J.N. Kanfer[b], J.W.Pettegrew[a] a: Laboratory of Neurophysics, University of Pittsburgh Medical Center, Pittsburgh, PA 15213. b: Department of Biochemistry and Molecular Biology, University of Manitoba, Winnipeg, Canada.

Glutamate is a major excitatory neurotransmitter in the vertebrate central nervous system. Glutamate is taken up by synaptic vesicles in an ATP-dependent manner, which is markedly stimulated by low concentrations of chloride and magnesium. Phosphocreatine (PCr) is an important high-energy phosphate that is a substrate for the creatine kinase catalyzed conversion of ADP to ATP. PCr was found to strongly stimulate glutamate uptake by bovine synaptic vesicles (about 180%) in the presence of 50 μM glutamate. Furthermore, we found that: (1) PCr could stimulate glutamate uptake by synaptic vesicles even in the absence of exogenous ATP; (2) ATP alone at maximal concentration (2 mM) could only achieves 60% of the maximal PCr-stimulated uptake; (3) 0.5 mM of iodoacetoamide completely inhibits creatine kinase activity, but does not inhibit glutamate uptake activity, suggesting that the effect of PCr on the glutamate uptake activity is not due to the conversion of ADP to ATP by creatine kinase; (4) PCr-dependent glutamate uptake, in contrast to ATP-dependent glutamate uptake, is not magnesium or chloride-dependent; (5) 0.5 mM of N-ethylmaleimide (NEM), a selective H^+-ATPase inhibitor, completely inhibits ATP-dependent glutamate uptake, but only slightly inhibits PCr-dependent glutamate uptake activity. We conclude that in addition to an ATP-dependent glutamate uptake system, there is a previously unrecognized PCr-dependent glutamate uptake system in the synaptic vesicles that appears to be able to stimulate greater uptake than the ATP-dependent system. (This work is supported by NIA grant AG08974)

409.5

MK-801 REDUCES UPTAKE AND STIMULATES EFFLUX OF EXCITATORY AMINO ACIDS VIA MEMBRANE DEPOLARIZATION. M.C. Longuemare*, E.C. Keung, F.R. Sharp, P.H. Chan, R.A. Swanson. Unversity of California and Veterans Affairs Medical Center, San Francisco, CA 94121.

MK-801 blocks excitotoxic neuronal injury by preventing Ca^{2+} flux through NMDA receptor (NMDA-R) gated ion channels. MK-801 and related compounds also have other effects, such as neuronal vacuolization and blockade of glutamate-induced astrocyte swelling, that may be unrelated their action at the NMDA-R. One mechanism by which these agents may act on neurons and glia is through impairment of glutamate uptake. In the present study, MK-801 inhibited glutamate uptake in both astrocyte and neuronal cultures in a dose-dependent manner. MK-801 (500μM) reduced astrocyte glutamate uptake from 24.0 ± 0.6 to 16.8 ± 0.5 nmol/mg protein/minute for [glutamate] = 100μM. Both the V_{max} and K_M were reduced by 45%, suggesting uncompetitive inhibition. In addition, astrocyte GABA uptake was inhibited by more than 50%. The related NMDA-R channel blockers, PCP and ketamine, also inhibited glutamate uptake, while NMDA-R antagonists which block the glutamate-binding site or the glycine site showed no effect. Since glutamate and GABA uptake are electrogenic, blockade of ion channels leading to membrane depolarization could indirectly inhibit uptake. Whole-cell patch clamping demonstrated a 35 ± 3 mV depolarization by MK-801 (1mM), suggesting that MK-801 inhibits uptake by reducing membrane potential. As predicted by models of glutamate transport, MK-801-induced depolarization caused excitatory amino acid efflux from astrocytes. It is possible that depolarization and subsequent impairment of glutamate transport may contribute to neurotoxic effects of NMDA-R channel blockers.

409.6

LUBELUZOLE REDUCES EXTRACELLULAR GLUTAMATE IN THE PERI-INFARCT ZONE OF A THROMBOTIC INFARCT IN RATS. D. Scheller, M. De Ryck, A. Lesage,* G. Clincke and F. Tegtmeier. Janssen Research Foundation, D-41470 Neuss, Germany and B-2340 Beerse, Belgium.

Lubeluzole, the S-isomer of a novel benzothiazole, is a compound aimed at the treatment of acute stroke. It protected neurologic function and reduced infarct size in thrombotic infarction. The R-isomer was inactive. As excess extracellular glutamate (glu) is known to exert cytotoxic phenomena, we determined extracellular glu changes in the peri-infarct zone and measured the possible effect of both compounds on that parameter. A microdialysis probe (2x0.5 mm, perfusate: aCSF; flow rate: 2 μl/min) was implanted into the histologically verified peri-infarct area of male Wistar rats (250 g, urethane 1.55-1.95 g/kg). After 90 min, Rose Bengal was given i.v. (70 mg/kg) and illuminated through the skull for 5 min. Either lubeluzole (1.25 mg/kg), its R-isomer (1.25 mg/kg) or vehicle solution (10% cyclodextrin, pH 5) were infused 10 min afterwards. Amino acids were determined using HPLC with fluorescence detection. Extracellular glutamate rose from 0.844±0.409 (μmol/l, mean±SD, n=8) to 4.578±1.772 (6 h after infarction) in controls and increased from 0.869±0.385 to 1.119±0.685 (n=9) in the lubeluzole treated group (p=0.007, ANOVA for repeated measures). In the R-isomer series, glutamate rose from 0.603±0.362 to 4.782±2.002 (n=6) in the controls and increased from 0.668±0.354 to 2.016±1.265 (n=9) in the treated group (p=0.19). In conclusion, lubeluzole prevented the slow rise of extracellular glutamate in the peri-infarct zone of a thrombotic infarct. Thus, lubeluzole may, by preventing the rise of extracellular glu, contribute to an improved neurological outcome after infarction.

409.7

N-METHYL-D-ASPARTATE- AND OXIDANT-INDUCED LUNG INJURY: CRITICAL ROLE OF POLY(ADP-RIBOSE) POLYMERASE. S. I. Said*, H. Pakbaz, and H. I. Berisha.

Poly(ADP-ribose)polymerase (PARP), a nuclear enzyme that is activated by DNA damage and catalyzes the polymerization of poly (ADP-ribose), is involved in cell differentiation, proliferation, and DNA repair. Excessive activation of PARP may lead to depletion of cellular NAD and ATP, and eventual cell death. We have investigated the importance of PARP activation in the production of acute lung injury due to oxidant stress caused by: 1) *N*-methyl-D-aspartate (NMDA, 1 mM) in the presence of L-arginine (10 mM), in rat lungs; 2) Infusion of paraquat (100mg/kg)in guinea pig lungs; and 3) Combination of xanthine (1mM) and xanthine oxidase (0.15 U/ml) in rat lungs. The lungs were mechanically ventilated with air or O_2 + 5% CO_2, and perfused at constant flow with Krebs solution containing 4% BSA. The importance of PARP activation was assessed by the use of either of 2 selective inhibitors of this enzyme, benzamide or 6(*5H*)-phenanthridinone. In all 3 models, injury occurred within 1 h, as evidenced by marked increases in airway and perfusion pressures, wet/dry lung weight ratio, and protein leakage into bronchoalveolar lavage fluid; lung tissue NAD levels decreased. Infusion of benzamide (1 mM) or 6(*5H*)-phenanthridinone (1 mM) greatly attenuated or totally prevented all signs of lung injury in each model (n=4-6). All 3 forms of injury were also prevented by the neuropeptide VIP, infused at 10 μg/kg. min.$^{-1}$. The results suggest that: 1) poly(ADP)-ribosylation, through PARP activation, is an essential step on a common pathway leading to tissue injury from NMDA and oxidants; and 2) VIP may act in part as a PARP inhibitor.

409.8

IDENTIFICATION OF A NOVEL NMDA AGONIST C.L. Willis*, J. Humphrey*, A.R. Chamberlin*, R.J. Bridges, Dept. of Pharm. Sci., Univ. of Montana, Missoula, MT 59812, Dept. of Chem., Univ. of Calif.*, Irvine, CA 92717

During the course of studies aimed at the design and synthesis of conformationally constrained EAA analogues, we prepared the novel compound L-*trans*-2,3-pyrrolidine dicarboxylate (L-*trans*-2,3-PDC). Consistent with its action as an NMDA agonist, earlier studies with L-*trans*-2,3-PDC demonstrated that it blocked ^{3}H-L-glutamate binding to NMDA receptors and produced an excitotoxic response in rat cortical cultures that was blocked by MK-801. In the current study we examined the *in vivo* neurotoxicity of L-*trans*-2,3-PDC, as well as that of its newly synthesized 5-methyl derivative, by focal injection into the dorsal hippocampus of male Sprague-Dawley rats. Cellular damage in the pyramidal (CA1-CA4) and dentate granule (DG) neurons induced by L-*trans*-2,3-PDC or 5-Me-L-*trans*-2,3-PDC was assessed histologically and compared to pathology produced by NMDA, AMPA, and kainate. Although the two novel analogues exhibited similar potencies in blocking of ^{3}H-L-glutamate binding to NMDA receptors (IC_{50} values of 1-3 μM), a significant difference was observed in their ability to induce excitotoxicity *in vivo*. Thus, while an injection of 20 nmols of L-*trans*-2,3-PDC produced little or no damage in the hippocampus, 0.25 nmols of 5-Me-L-*trans*-2,3-PDC induced a significant level of neuronal injury. CA1, CA3 and CA4 subfields exhibited between 70-90% neuronal injury, whereas only 10-30% CA2 and DG were damaged. The pattern and extent of pathology was comparable to that produced by 25 nmols of NMDA. The neuronal damage induced by 5-Me-L-*trans*-2,3-PDC was prevented by MK-801 (3mg/kg i.p.), but not by NBQX (25 nmols: a dose that protects against 1 nmol of AMPA). These results identify 5-Me-L-*trans*-2,3-PDC as a very potent NMDA agonist and highlight its utility in studying NMDA receptor-mediated neurodegeneration. This work was supported in part by NIH/NINDS NS30570 and NS27600.

409.9

HIGH-POTASSIUM-INDUCED NEURONAL DAMAGE *IN VIVO*: EFFECTS OF NMDA-RECEPTOR BLOCKADE. D. G. Fujikawa* and T.B. Sohn. Exp. Neurol. Lab., VA Med. Ctr., Sepulveda, CA and Dept. of Neurology and Brain Res. Inst., UCLA Sch. of Med., Los Angeles, CA.

We have found that microdialysis probe delivery of 12 mM K^+ to the rat amygdala for 60-70 min produces edema, neuronal necrosis and elevations of extracellular glutamate to 262-322% and aspartate to 186-198% of baseline, without inducing local seizure discharges . In this study we investigated the effects of the competitive NMDA-receptor-antagonist CGP 40116. Bilateral CMA/11 guide cannulae with attached bipolar electrodes were placed in the basolateral amygdaloid nuclei of adult Wistar rats (n=6). The next day microdialysis probes were inserted and perfused with Krebs-Ringer-bicarbonate (KRB) solution for 2 h in freely-moving rats. The solution was then switched to 100 mM KCl in modified KRB (delivering 12 mM K^+ to tissue) bilaterally for 60 min; one side contained 36 μM CGP 40116. Preliminary data suggest that about 12 μM (29%) of the CGP 40116 was delivered to tissue, more than 100-fold greater than its *in vitro* K, for inhibition of NMDA-sensitive L-[^{3}H]-GLU binding. Five of 6 normally-behaving rats had amygdalar epileptiform discharges on the CGP 40116 side during high-K^+ perfusion, with a latency to onset of 40.0 ± 7.4 min (mean ± S.E.M) and a total spike-wave duration of 8.0 ± 2.9 min. After *in situ* brain perfusion-fixation and removal, 6-μm-thick coronal brain sections stained with H & E were prepared and examined. Cells were counted in 270 x 540 μm areas immediately medial and lateral to each probe. Tissue edema was graded on a 0-3 scale. On the control high-K^+ side there were 45 ± 6 acidophilic and 86 ± 14 normal neurons, compared to 39 ± 5 acidophilic and 97 ± 8 normal neurons on the side exposed to CGP 40116 (p = 0.17 and 0.50 respectively). Severe edema was also present bilaterally: 3.0 ± 0.0 on the control side and 2.9 ± 0.1 on the CGP 40116 side (p = 0.36). Delivery of CGP 40116 to the amygdala during high-K^+ perfusion induced local epileptiform activity and was not neuroprotective.

409.10

SPECIES DIFFERENCES IN QUINOLINIC ACID SYNTHESIS AND RESPONSES TO SPINAL CORD INJURY A. R. Blight[1]*, E. C. LeRoy, Jr.[1], K. Saito[2] and M. P. Heyes[2], Div. Neurosurgery,[1] Univ. North Carolina, Chapel Hill, NC 27599, and Lab. of Clinical Science,[2] NIMH, Bethesda, MD 20892.

Experimental contusion injury to the spinal cord of guinea pigs results in delayed neurologic deficits that continue to increase in severity for several days following trauma, coincident with the invasion of the lesion by mononuclear phagocytes and increased levels of the neurotoxin quinolinic acid (QUIN), which is synthesized by macrophages. Inflammatory responses and accumulations of QUIN also occur following spinal cord contusion in rats, yet there is little or no indication of delayed onset of neurologic deficits. In this study, the responses of guinea pigs and rats to closely similar injuries of the spinal cord were examined. Tissue levels of QUIN were measured using mass spectrometry and gas chromatography at various times post-injury. In guinea pigs, increases in QUIN levels at the lesion site began at 1 day post injury, rose steeply during the first week, achieved potentially neurotoxic, maximal values, well in excess of serum levels, by 12 days, and remained elevated above serum levels at 25 days post-injury. A similar profile of increases in QUIN occurred in adjacent areas of the spinal cord, with lower peak levels. In rats, increases in tissue QUIN were much smaller, remaining below serum concentrations at all times, approached a plateau within 24 hours and decreased slowly over the succeeding weeks. Correlated with these observations, peritoneal macrophages from guinea pigs were shown to convert L-tryptophan to both L-kynurenine and QUIN *in vitro*, while rat macrophages show a much smaller capacity, and only under conditions of intense activation. Behavioral measurements confirmed the striking difference in the time course of functional deficits in the two species. QUIN accumulations in spinal cord following contusion injury may be of direct pathophysiologic significance. (Supported in part by grant NS21122 from NIH, NINDS).

INGESTIVE BEHAVIOR IV

410.1

TOTAL BUT NOT SPARED-GASTRIC VAGOTOMY REVERSES OLEIC ACID-INDUCED SATIETY. D. Greenberg*, J. McCaffery, D.L. Lewis, G.P. Smith and A.J. Strohmayer. Bourne Laboratory, and Departments of Psychiatry and Neurology, Cornell Univ. Medical College, White Plains, NY 10605 USA.

Total subdiaphragmatic vagotomy attenuates the satiating effects of intestinal nutrients (Yox et al. 1992). To further investigate the neural mediation of satiation elicited by intestinally infused fats, we tested the satiating potency of intestinal infusions of the long chain fatty acid oleic acid (OL) in sham feeding rats with total abdominal vagotomy or with spared-gastric vagotomy.

Rats received total vagotomy where both the right and left vagal trunks were severed (n=8), spared gastric vagotomy where all branches of the abdominal vagus except the two gastric branches were severed (n=10), or sham vagotomy (n=8). All rats were subsequently fitted with gastric cannulas and duodenal catheters to allow duodenal infusions during sham feeding. Rats were food deprived overnight and given access to liquid food for 90 min. Sham fed intakes were measured every 5 min. Intestinal infusions (10ml) were delivered at a rate of 0.44 ml/min and began 12 min after sham feeding and were of sodium oleate (0.65 or 0.325 kcal) or 0.15M NaCl.

Intestinal infusions of both concentrations of OL rapidly suppressed sham feeding in the sham operated and spared-gastric vagotomy groups but not in the total vagotomy group [$F(2,41) = p < .005$]. We conclude that the gastric vagal nerves, presumably the afferent fibers are sufficient to mediate the satiating effect of intestinal infusions of OL.

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410.2

SYNERGISTIC INCREASE IN FOOD INTAKE AND BRAIN FOS-LIKE IMMUNOREACTIVITY (FOS-LI) AFTER TREATMENT WITH METHYL PALMOXIRATE (MP) AND 2,5-ANHYDRO-D-MANNITOL (2,5-AM). C.C. Horn* and M.I. Friedman. Monell Chemical Senses Center, Philadelphia, PA, 19104.

We investigated the neural mechanism that integrates signals from carbohydrate (CHO) and fat metabolism to control food intake. Rats maintained on a diet providing equal amounts of calories from CHO and fat were given p.o. 5 mg/kg MP, a fatty acid oxidation inhibitor, or vehicle (methyl cellulose). Three hr later rats were injected i.p. with 150 mg/kg 2,5-AM, a fructose analogue that inhibits liver CHO metabolism, or saline. By 2 hr after i.p. injection, rats treated with MP and 2,5-AM showed a synergistic increase in food intake (MP+2,5-AM=2.0±0.3 gm, Veh.+2,5-AM=0.4±0.2, MP+Sal.=0.6±0.3, and Veh.+Sal.=0; n's=8; interaction, $p<0.05$). Fos immunocytochemistry was employed to investigate the neural pathways involved in this synergistic response. Groups of rats were treated as above (n's = 4). Brains from rats given MP+2,5-AM showed a large amount of Fos-li in the solitary tract nucleus and paraventricular nucleus of the hypothalamus whereas the other groups showed little or no Fos-li in these areas. These findings suggest that signals for feeding produced by MP and 2,5-AM treatment are integrated in the brain stem or peripherally. (This research was supported by NIH grants DK36339 and DC00014, and a grant from the Howard Heinz Endowment.)

410.3

DIET-RELATED EFFECTS OF 2,5-ANHYDRO-D-MANNITOL (2,5-AM) ON FOOD INTAKE AND LIVER ATP. J.E. Koch*, G. Graczyk-Milbrandt, M.D. Osbakken, H. Blum, M.A. Ketchum, J.L. Nuss & M.I. Friedman. Monell Chemical Senses Center, Philadelphia, PA 19104.

2,5-AM, a fructose analogue, stimulates eating by its action in liver and decreases liver ATP. The eating response is attenuated by a high-fat diet. To determine whether this diet effect was related to liver ATP, 300 or 400 mg/kg 2,5-AM was injected (i.p.) into rats fed either a chow, low fat/high carbohydrate (LF/HC), or high fat/low carbohydrate (HF/LC) diet. 2,5-AM increased food intake compared with saline, but rats fed the LF/HC diet increased food intake more than rats fed the HF/LC diet or chow. One week later rats were re-injected, livers were freeze-clamped in liquid N_2 30 min later, and assayed for ATP. Relative to saline, 2,5-AM decreased liver ATP in all diet groups; however, the drop was greater in rats fed LF/HC (82%) than in rats fed chow (43%) or HF/LC diets (46%). In vivo ^{31}P NMR spectroscopy also revealed diet-related changes in liver phosphate metabolism during i.v. infusion of 2,5-AM. Results are consistent with the hypothesis that the signal for eating after 2,5-AM is related to hepatic ATP levels. (Supported by NIH grant MH-50363)

410.4

METABOLIC ACTIVITY IN THE BRAINSTEM ASSOCIATED WITH INTESTINAL INFUSIONS OF GLUCOSE VERSUS SALINE IN RAT PUPS. **C.B. Phifer* and C.A. Crady.** Louisiana Scholars' College, Northwestern State University, Natchitoches LA 71457.

Responsiveness to ingested nutrients develops in rats during the second week of life. In a recent study involving independent ingestion by 15-day-old rat pups, direct duodenal infusions of 0.6M glucose decreased intake more than 50% relative to saline infusions. In the present study, [^{14}C]2-deoxyglucose autoradiography was used to study brainstem regions mediating inhibition of ingestion by intestinal glucose.

Polyethylene cannulas were implanted in the duodenum 4mm from the pylorus. Following [^{14}C]2-DG injections, pups were allowed to ingest milk for 30min. During the first 8min of the test, pups received duodenal infusions of 0.6M glucose or isotonic saline at 0.25% BW/min. Average autoradiograms were made from brainstem sections of eight pups in each treatment group. Images showing differences in activity were obtained by subtracting one average image from the other average image.

Pups that received glucose infusions showed lower relative metabolic activity in the lateral lemniscus, lateral parabrachial nu, principal sensory nu of the trigeminal n, oral portion of the spinal trigeminal nu, nu of the solitary tract, and medullary reticular fields. Glucose-infused pups showed higher activity than saline-infused pups in inferior colliculus, paraolivary regions, prepositus hypoglossal nu, medial vestibular nu, cuneate nu, and interpolar regions of the spinal trigeminal nu. These results will be discussed in relation to earlier studies on gastric distension and to nutrient-induced satiety.

410.5

CELIAC VAGAL AFFERENTS DETECT INTESTINAL MACRONUTRIENT INFUSIONS THAT SUPPRESS MEAL SIZE. E.K. Walls*, R.J. Phillips, F.B. Wang, M.C. Holst and T.L. Powley. Psychological Sciences, Purdue Univ. West Lafayette,IN 47907.

Examination of the chemosensory functions of gastrointestinal vagal afferents has been facilitated by the recent development of a surgery that achieves highly specific deafferentation of vagal projection fields in the abdomen. Concurrent use of a WGA-HRP nodose ganglion injection and i.p. Fluorogold provide histological verification of the surgeries (Walls, et.al. 1993, Soc. Neur. Abst. 19, 816). To specify more clearly the locations and pathways of chemosensory functions, the suppression of first meal size following acute intraduodenal infusion of 11 different macronutients was compared in sham surgical control rats (SH, n=15) and rats that had received unilateral intracranial vagal afferent rhizotomy combined with contralateral celiac vagal branch cautery (CV, n=22). Only data from rats with verified surgeries were used for statistical analysis. Duodenal nutrient infusions (1.2 or 2.4 kcal/10ml/ 10min) immediately prior to the first (30 min) evening meal produced three distinct profiles of response. 1. Infusions of isotonic saline, 3% fructose, 3% D-phenylalanine or 5.6% glycerol failed to suppress first meal intake in either group. 2. Equicaloric 1.2 kcal infusions of 3% glucose, 3% maltose, 3% L-phenylalanine, 12% Isocal, and 1.4% oleic acid significantly reduced intake in SH but not in CV rats. 3. Intestinal infusion of 3% casein hydrolysate or 24% Isocal significantly reduced meal size in both SH and CV groups but the elimination of celiac afferents in CV rats blunted this response. Measures of baseline intake on noninfusion days also revealed that celiac deafferentation reduces the size of the first meal without affecting overall daily food intake or body weight. The finding that some nutrients failed to reduce intake shows that detection is nutrient-specific. The loss of suppression of meal size in CV rats suggests that afferents of the celiac vagus are critical for intestinal preabsorptive detection of macronutrients. The blunted nutrient-induced reduction in meal size reveals that when celiac vagal afferents have been eliminated some macronutrients are still detectable. NIH DK27627 and NIMH MH01023.

410.6

RAPID STIMULATION OF SALT APPETITE BY INTRAVENOUS ANGIOTENSIN II IN RATS. R.L. Thunhorst*, J.R. Lane, and D.A. Fitts. Dept. of Psychology, Univ. Iowa, Iowa City, IA 52242 and Dept. Psychology, Univ. Washington, Seattle, WA 98195.

A role for the renal renin-angiotensin system in the direct stimulation of salt appetite in the rat remains controversial because attempts to stimulate salt appetite by intravenous (iv) administration of angiotensin II (ANG II) have been unconvincing. Our recent demonstration that circulating ANG II is critical for the expression of sodium depletion-induced salt appetite calls for a re-examination of the role of renally-derived ANG II. The present experiment replicates our finding that depletion-induced salt appetite is prevented by selective blockade of ANG II synthesis with an iv dose of converting-enzyme inhibitor (captopril, CAP) that does not cross the blood-brain barrier. We now show that iv ANG II rapidly re-establishes salt appetite in CAP-blocked rats. Rats maintained on regular food, water and 0.3 M NaCl were depleted in the afternoon by furosemide (10 mg/kg sc) and were placed into metabolism cages with only water available. CAP infusions (2.5 mg/h iv) began just before the furosemide injections and continued overnight. The next morning, all rats received access to saline and water in graduated burettes. For the first 90 min of saline access, iv CAP continued in all rats. For the next 90 min, Group 1 received iv ANG II (30 ng/min, n = 4), and Group 2 continued on iv CAP (n = 6). For the last 90 min, all rats received iv ANG II.

	Group 1			Group 2		
	CAP	ANG	ANG	CAP	CAP	ANG
water	0.1±0.1	4.0±1.6*	3.5±2.5	0.2±0.2	0.0±0.0	3.4±1.1*
saline	0.4±0.4	4.3±1.3*	1.0±0.4	0.5±0.4	0.7±0.5	2.6±0.8*

*p<.05, ml/90 min, M and SE. The results suggest that ANG acts on the blood side of the blood-brain barrier to stimulate salt appetite in the sodium-depleted rat.

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410.7

EFFECT OF HINDBRAIN LESIONS ON WATER AND SALINE INTAKE AFTER SHORT TERM SODIUM DEPLETION. G.L. Edwards* and J.D. Power. Dept. of Physiol. & Pharmacol., Coll. of Vet. Med., Univ. of Georgia, Athens, GA 30602.

Treatment with a single dose of furosemide (10 mg/kg) and a low dose of captopril (5 mg/kg) results in an increase in sodium appetite in rats (Am. J. Physiol. 267: R171, 1994). We have found that overnight sodium depletion causes an increase in saline intake in rats with lesions of the area postrema/immediately adjacent nucleus of the solitary tract (AP/mNTS-lesions) (Am. J. Physiol. 264: R1242, 1993). In these studies, we examined the effect of short term sodium depletion with furosemide and captopril on water and 2% saline intake in rats with AP/mNTS-lesioned rats and SHAM-lesioned rats. We have found that this protocol stimulates increased water and saline intake in SHAM-lesioned rats (baseline: water 0.8±0.5 ml, saline 0.7±0.4 ml vs. depletion: water 6.4±0.7 ml, saline 4.1±0.9 ml) as previously reported. Interestingly, saline intake was not increased in AP/mNTS-lesioned rats (baseline: 26.4±3.8 ml vs depletion: 28.5±2.2 ml). However, water intake was significantly elevated in the AP/mNTS-lesioned rats after furosemide/captopril treatment (baseline: 4.8±1.0 ml vs depletion: 12.1±1.7 ml). These data provide further evidence for a role of the AP/mNTS in the control of fluid and electrolyte balance. (Supported by NIH DK 42533)

410.8

EVIDENCE THAT GLYCINE COTRANSMISSION MAY BE ESSENTIAL TO FEEDING STIMULATION BY LATERAL HYPOTHALAMIC (LH) NMDA RECEPTORS. B.G. Stanley* and B.S. Butterfield. Departments of Neuroscience & Psychology, University of California, Riverside, CA 92521.

We have recently shown that intense eating is elicited by LH injection of N-methyl-D-aspartic acid (NMDA), and that LH pretreatment with NMDA receptor antagonists suppresses both NMDA-elicited and natural eating. Cell physiology studies have suggested that NMDA receptor activation requires costimulation by glycine and glutamate. To examine whether glycine co-transmission might participate in NMDA-elicited eating, LH injection of 7-CK (7-chlorokynurenic acid), an NMDA-receptor glycine site antagonist, was tested in cannulated adult male Sprague-Dawley rats. First, we examined whether LH injection of 7-CK suppressed eating elicited by LH injection of NMDA (10 nmol), and found that the 6-10 gm elicited eating response was abolished by doses of 7-CK above 10 nmol. To examine behavioral specificity, 7-CK pretreatment was tested on eating elicited by LH injection of kainic acid (1 nmol). In contrast to its block of NMDA-elicited eating, 7-CK actually potentiated eating elicited by kainic acid, demonstrating the behavioral specificity of its actions. To confirm that 7-CK's effects were actually due to its actions at the glycine site, we tested whether pretreatment with LH injection of glycine would reverse 7-CK's block of NMDA-elicited eating. Glycine at doses above 20 nmol reversed 7-CK's effects. Finally, bilateral LH injection of 7-CK suppressed eating in 24 hr fasted rats. These findings support a role for NMDA receptors in LH feeding control mechanisms, and argue that glycine may act as a cotransmitter at these receptors.

410.9

REMNANTS OF A CTA PERSIST IN RAT NTS AFTER FULL BEHAVIORAL EXTINCTION. S. A. McCaughey[1]*, B. K. Giza[1], L. J. Nolan[2], J. C. Smith[3], J. A. Rhinehart-Doty[3], and T. R. Scott[1] ([1]Dept. of Psych., Univ. of Delaware, Newark, DE 19716; [2]St. Luke's/Roosevelt Hospital and Columbia University, NY, NY 10025; [3]Dept. of Psych., Florida State Univ., Tallahassee, FL 32306)

The development of a conditioned taste aversion (CTA) to saccharin in rats is accompanied by changes in taste-evoked activity in nucleus tractus solitarius (NTS). Responses to the CS are increased in a burst of activity that peaks 950 msec after stimulus application and that is restricted to a sugar-sensitive subgroup of cells. While a CTA can prevent ingestion of toxins, it also risks eliminating the ingestion of nutrients that were only coincidentally paired with illness. Thus, the ingestion of the CS without subsequent illness should extinguish the CTA. The aim of this study was to extinguish a CTA thoroughly and observe whether NTS responses return to normal. Subjects were female Wistar rats. One group (experimental) was conditioned to avoid 2.5 mM saccharin (the CS) by pairing its taste three times with intraperitoneal LiCl. The other group (control) received isotonic saline injections after the CS. Experimental rats were then given 10 min per day access to the CS after 23 hours of fluid deprivation until they accepted the CS at pre-CTA levels. Control rats were given access to the CS for the same length of time. All subjects were then tested in a two-bottle preference situation until the experimental rats preferred the CS over water to the same extent as the control rats. All behavioral vestiges of the aversion were extinguished after 13 daily sessions. Recording then took place from single neurons in NTS in response to 13 stimuli, including the CS. Responses to the saccharin CS in the experimental group still included a burst of activity, limited to the sugar-sensitive subgroup of cells, that peaked 1200 msec after stimulus onset. Thus, even after total behavioral extinction of a CTA, a neural vestige of the experience remains in the hindbrain. This may support the rapid relearning of an aversion that occurs upon a subsequent pairing of the CS with illness. (Supported by grant DK30964 from the NIDDKD.)

410.10

COLOCALIZATION OF bZIP PROTEINS AND THEIR REGULATION BY CONDITIONED AND UNCONDITIONED STIMULI IN RAT BRAINSTEM SUGGEST A ROLE IN TASTE AVERSION LEARNING. C.A. Wilson and M.W. Swank*. Department of Psychology, Furman University, Greenville, SC 29613.

Expression of the bZIP transcription factor c-Fos in the intermediate region of the nucleus tractus solitarius (intNTS) is a reliable correlate of both unconditioned stimulus (US) and post-training conditioned stimulus (CS) presentation during taste aversion learning. Because cFos must dimerize with other bZIP proteins to regulate putative genes involved in plasticity, examination of other bZIP proteins is a crucial step in understanding the role of cFos in this learning paradigm.

Although previous studies have shown expression of FosB, JunB, and JunD throughout the brainstem, the present study examined whether FosB is regulated by CS saccharin or US LiCl. Relative to control animals which received saline injections or ingested water, LiCl ($p < .001$) and novel saccharin ($p < .05$) caused an increase in FosB expression within intermediate NTS. In contrast to the effects of these two salient stimuli, familiar saccharin did not produce an increase in FosB above control, suggesting that regulation of FosB by novel saccharin is a crucial component of bZIP-mediated plasticity.

Colocalization of bZIP proteins is an obvious prerequisite for dimerization and interaction. To examine this, fluorochrome-avidin was used to sequentially label cells for c-Fos followed by FosB, JunB, or JunD. Within the intermediate NTS almost complete colocalization of c-Fos with FosB, JunB and JunD was found. We propose a model in which cFos and FosB compete for dimerization with JunB and/or JunD as part of a computational network of transcription factors which mediate plastic events associated with taste aversion learning.

410.11

PREFERENCE OR AVERSION FOR ETHANOL OR SACCHARIN AFTER BILE DUCT LIGATION IN RATS. J. R. Lanc*, E. M. Starbuck, & D. A. Fitts. Dept. of Psychology, University of Washington, Seattle, WA 98195.

A bile duct ligation (BDL) in rats produces cholestasis, cirrhosis, portal hypertension, hyperkinetic circulation, and eventual hepatorenal failure. This syndrome is superficially similar to alcoholic cirrhosis, and thus yields the opportunity to observe the effects of cirrhosis on ethanol intake in relatively alcohol-naive subjects. Rats that were allowed to drink 6% (v/v) ethanol for 3 wk persistently reduced their daily intake of ethanol by about 50% after BDL. The ethanol intakes of sham ligated rats did not change. To determine whether BDL-treated rats associate the taste of a completely novel, non-drug solution with the ill-effects of the BDL, dehydrated rats were given access to a 0.06% saccharin solution for 2 hr either immediately (paired) or 2 days (unpaired) before BDL or sham ligation surgery. Sham ligated rats all drank copious amounts of saccharin regardless of the pairing. All BDL rats avoided saccharin for about the first week, and after that the unpaired BDL rats developed a high preference for saccharin. The aversion in paired BDL rats persisted until the termination of the experiment at 27 days. When the association between ethanol and the BDL surgery was broken by providing either ethanol alone or ethanol with saccharin 15 days after BDL, the BDL groups drank as much as the sham ligated groups. Thus, BDL appears to induce a chronic aversion for either saccharin or ethanol when these flavors are paired with the onset of the illness. The reduction in ethanol intake does not seem to result from reduced ethanol metabolism, because chronic BDL rats drank large amounts of saccharin-flavored ethanol when it was unpaired with the BDL surgery. The results support the view that chronic illnesses can produce persistent aversions for flavors. Supported by NS22274, AA05390, and the U.W. Alcohol and Drug Abuse Institute.

410.12

ADRENALECTOMIZED RATS DEFEND A NEW, LOWERED BODY WEIGHT FOR AT LEAST 10 WEEKS. A.M. Strack*, C. Horsley, M.F. Dallman. Dept. Physiology, UCSF, San Francisco, CA 94143-0444.

After adrenalectomy (ADX), the rates of body weight gain and food intake decrease over the first few weeks. To determine whether these decreases continue or whether they represent an altered set point for body weight (Green et al. Endocrinology 130:269, 1992), we studied ADX and sham-operated (SHAM) male rats through 10 wks after surgery on wk 4 of life. Body weight, food intake, ACTH, corticosterone (B) and insulin were measured at 1-2 wk intervals. Groups of 6-8 rats were killed 1,3,5 and 10 wks after surgery and tissues were weighed; DNA and protein were measured in brown adipose tissue (BAT).

Throughout the 10 wk period, plasma B levels at the PM peak or after 30 min restraint stress were < 1 μg/dl in all ADX rats used in these studies, demonstrating complete surgery. Body weight of ADX decreased to ~ 70% SHAM by 3 wks and remained at this level through 10 wks. Food intake in ADX was decreased by 2 d and remained at 75-80% of SHAM through wk 5 then it increased to ~ 90% SHAM in wks 7-10. BAT DNA increased by 40% between 1 and 3 wks and remained at this level until 10 wks; BAT protein (μg/mg wet weight) increased ~ 50% at 5 wks and 123% of SHAM at 10 wks. These increases in BAT indicate persistently increasing activity of the sympathetic nervous system after ADX. By contrast, white fat weight decreased by ~ 50-70% over 10 wks and plasma insulin levels were reduced by ~ 50-70% compared to SHAM at all times.

The increase in food intake, which coincided with the further increase in sympathetic activity, resulted in maintaining the reduced body weight constant in ADX rats. These results strongly suggest that ADX does reduce the reference, or set-point for body weight. (Supported in part by DK28172)

ISCHEMIA: NEUROPROTECTION AND TROPHIC FACTORS

411.1

EFFECTS OF MILD AND MODERATE HYPOTHERMIA ON NEUROLOGIC OUTCOME, INFARCT SIZE AND ADENYLATE LEVELS FOLLOWING TRANSIENT MCA OCCLUSION IN RATS C. M. Maier*, K. VB. Ahern, R. R. Gonzalez, and G. K. Steinberg. Dept. of Neurosurgery, Stanford University Medical Center, Stanford, CA 94305.

We studied 24 male Wistar rats who underwent a 2 hour occlusion of the ICA, MCA, and ACA by an intraluminal suture placed 18-22 mm into the ICA. During occlusion the animal's rectal and brain temperature were kept either normothermic (36.5-37.5°C; n=8), mildly hypothermic (32.5-33.5°C; n=8), or moderately hypothermic (29.5-30.5°C; n=8). After suture removal the animals were returned to normothermia and allowed to reperfuse for 24 hours. The animals were closely monitored throughout the recovery period and evaluated for neurological findings at 24 h post-ischemia. Average post-anesthesia recovery time showed that animals in the 33°C group recovered at a significantly faster rate (21±11 min; $p<0.05$) compared with the normothermic animals (37±12 min), but no significant improvement was found in the 30°C group. Neurological outcome showed that hypothermic animals in the 33°C group scored consistently higher (40±3.9) than either the normothermic (34±8.5) or the 30°C group (36±7.4). Histopathology revealed an 83% and 68% reduction in the area of cortical ischemic neuronal damage for the 33°C group (6.5±11.5%; $p<0.01$) and the 30°C group (11.9±17%; $p<0.025$), respectively, compared with the normothermic animals (37±20.5%). Using the intraluminal suture model, a separate group of animals was studied to determine brain adenylate levels. After the animals were anesthetized, the brains were frozen *in situ* with liquid nitrogen, removed, sectioned into 3 coronal slices and the homogenates processed for extraction of adenylates. Initial results of HPLC elution profiles in control animals show that this method assays adenylates with a high degree of accuracy and thus may be useful in studying the mechanisms of neuroprotection by mild hypothermia.

411.2

BODY TEMPERATURE IN ACUTE HUMAN STROKE: RELATION TO STROKE SEVERITY, MORTALITY AND OUTCOME. J. Reith*, H.S. Jørgensen, P.M. Pedersen, L.L. Jeppesen, H. Nakayama, H.O. Raaschou and T.S. Olsen. Dep. of Neurology, Bispebjerg Hospital, Copenhagen, Denmark, 2400.

Despite substantial experimental evidence of the benefit of peri- and postischemic hypothermia and the dangers of hypothermia, the validity of these claims in man is unproven. In the Copenhagen Stroke Study, which is a community-based, consecutive and prospective study of acute stroke, we measured body temperature on admission in 1197 patients. Stroke severity was measured using the Scandinavian Stroke Scale (SSS), on admission, weekly and at discharge. The CAT-scan rate was 82%. Multiple logistic and linear regression analyses were used. Among confounding factors recorded and taken into account were: Age, gender, stroke severity (SSS), Prior stroke, diabetes, hypertension, atrial fibrillation, ischemic heart disease, comorbidity, smoking, infections and leukocyte counts of blood. For patients admitted within 6 (n= 390), 12 (n=556), and 24 (n=788) hours, body temperature was significantly and independently related to stroke severity, mortality and outcome. Although our investigation does not provide evidence of a causal relationship between early body temperature and outcome in acute human stroke, such a relationship explain our findings.

411.3

NEURONAL N-TYPE CALCIUM CHANNEL ANTAGONIST, SNX-111, PREVENTS APOPTOSIS IN A FOCAL MODEL OF ISCHEMIA. K. Gohil*, T. Singh, D. Xue, G. Miljanich, R.R. Luther, C.M. Bitler, and S.S. Bowersox. NEUREX Corporation, Menlo Park, CA 94025.

The mechanisms of delayed neuronal death after a reversible ischemic insult are poorly understood. Recent studies in rodents have shown that apoptosis may play an important role in this process. A commonly used indicator of apoptosis is the presence of fragmented DNA (f-DNA), formed by the action of calcium-activated endonucleases. We have, therefore, examined the effects of SNX-111 (synthetic ω-conopeptide MVIIA), a selective N-type neuronal calcium channel blocker, on ischemia-induced DNA fragmentation in spontaneously hypertensive rats. Temporary focal cerebral ischemia was produced by transient occlusion of the middle cerebral artery and permanent occlusion of the right common carotid artery. Rats were given either saline or a single dose of 5 mg/kg SNX-111 (infused intravenously over 30 minutes) immediately following 90 minutes of focal cerebral ischemia. Animals were sacrificed 24 hours after reperfusion, and their brains were rapidly removed and frozen. Coronal sections were analyzed for f-DNA using digoxigenin-dUTP and terminal transferase. Neurons with f-DNA were present only on the ipsilateral side of the injury. The highest densities of apoptotic nuclei (1800-3000) occurred in penumbral regions of parietal and insular cortices. Although total cell counts were comparable in saline and SNX-111 treated animals, the number of apoptotic neurons in brain sections from rats infused with SNX-111 was decreased by 63%. These findings suggest that the previously demonstrated neuroprotective actions of N-type calcium channel blockers (Buchan et al. *J. Cereb Metab Blood Flow* 14:903, 1994) may be due, in part, to suppression of apoptosis.

411.4

NPS 102, A NOVEL NMDA RECEPTOR ANTAGONIST, REDUCES ISCHEMIC DAMAGE IN RATS WITH INTRALUMINAL MIDDLE CEREBRAL ARTERY OCCLUSION. Y. Chien, L.D. Artman, N. Alasti, J.L. Raszkiewicz*, and A.L. Mueller. NPS Pharmaceuticals, Inc., Salt Lake City, UT 84108.

NPS 102 is a synthetic polyamine spider toxin analog that blocks NMDA/glycine-induced Ca^{2+} influx noncompetitively in cultured rat cerebellar granule cells (IC_{50} =93 nM). NPS 102 produces marked neuroprotection (90%) of hippocampal CA1 pyramidal cells in a gerbil model of global ischemia. In order to study the potential neuroprotective effects of NPS 102 in focal cerebral ischemia, we administered NPS 102 (10 mg/Kg, *i.p.* given immediately after the onset of ischemia) in male adult Wistar rats subjected to either permanent or temporary (2-hour) occlusion of the middle cerebral artery with an intraluminal suture. Cerebral infarct volumes were measured with triphenyltetrazolium staining 24 hours after permanent, or 72 hours after temporary occlusions. Compared with the control group which received saline, NPS 102 reduced the total infarcted volume by 20% in the permanent occlusion group (mean ± SEM): 286 ± 18 (control, n = 9) vs 229 ± 16 (NPS 102, n = 11) mm^3, $p < 0.03$; and 37% in the temporary occlusion group: 150 ± 20 (control, n = 9) vs 94 ± 14 (NPS 102, n = 9) mm^3, $p < 0.05$. The reduction in infarct volume involved both cortical and subcortical areas, and was most apparent in the striatum; this profile distinguishes NPS 102 from most other NMDA receptor antagonists. Moderate reduction in arterial pressure (25-30 mm Hg lasting 2 hours) was noted, while no PCP-like or other behavioral effects were observed. The results indicate that NPS 102, administered immediately after ischemic insult, reduces the infarct size in rats with focal cerebral ischemia, and that the neuroprotection is more effective in the setting of temporary ischemia followed by reperfusion.

411.5

PROTECTION FROM DELAYED HYPOXIC NEURONAL INJURY WITH THE NOS INHIBITOR, METHYLARGININE. R.A. Wallis* and K.L. Panizzon, Dept. of Neuro., UCLA and Sepulveda VAMC, Sepulveda, CA 91343.

During stroke, neurons appear to be injured by mechanisms of both acute and delayed injury. Nitric oxide has been demonstrated to mediate both hypoxic-, and NMDA-induced acute neuronal injury. Since NMDA-receptor activation has been shown to induce delayed neuronal injury, we evaluated the effectiveness of the nitric oxide synthase (NOS)inhibitor, methylarginine against delayed neuronal injury from hypoxia in the hippocampal slice. Subjecting hippocampal slices to hypoxic exposure which was acutely sub-lethal, produced later evidence of CA1 delayed neuronal injury. To induce hypoxia, slice perfusion was changed to artificial CSF saturated with 95% N_2/5% CO_2. Hypoxia was discontinued at the peak appearance of the Hypoxic Injury Potential, which is an electrophysiological marker of acute irreversible hypoxic injury in this preparation. Slices given hypoxia recovered 100 ± 0% of initial CA1 PS amplitude after 1 hour. Thereafter, however, they lost all CA1 PS activity at a mean 7.3 ± 1.2 hours following hypoxia. In contrast, paired slices not receiving hypoxia, maintained PS activity for 19.8 ± 0.5 hours. The appearance of delayed neuronal injury from hypoxia was prevented with L-methylarginine (170 μM) treatment, a concentration also protecting against acute neuronal injury in this preparation. When L-methylarginine was given for 35 mins. after hypoxia, CA1 PS response could be elicited thereafter for 21 ± 1.4 hours after, compared to paired slices exposed to hypoxia alone in which CA1 PS response could be elicited for only 7.7 ± 0.1 hours. The protection seen in these studies with L-methylarginine, a stereospecific inhibitor of NOS, indicates the involvement of nitric oxide mechanisms in CA1 delayed neuronal injury induced by hypoxia. Supported by the VA Research Service.

411.6

INDUCIBLE NITRIC OXIDE SYNTHASE GENE EXPRESSION IN TRANSIENT FOCAL CEREBRAL ISCHEMIA: PROTECTION BY AMINOGUANIDINE C. Iadecola*, X. Xu, F. Zhang, R. Casey, H. B. Clark and M. E. Ross. Dept. of Neurology, Univ. of MN, Minneapolis, MN 55455.

We have demonstrated that in permanent focal cerebral ischemia there is expression of inducible nitric oxide (NO) synthase (iNOS) and that the resulting NO production contributes to the secondary phase of tissue damage (AJP 268, R286, 1995; JCBF&M 15, 378, 1995). In the present study we investigated whether iNOS is expressed also after transient focal ischemia and, if so, we sought to define its role in the mechanism of brain injury. The middle cerebral artery was occluded for 2 hrs by an intraluminal filament in Sprague-Dawley rats and iNOS message was determined by the reverse-transcription polymerase chain reaction (RT-PCR) in the ischemic neocortex 6 hrs to 7 days after reperfusion. After transient ischemia iNOS mRNA was maximally expressed at 12 hrs after reperfusion and then began to decline. No expression was observed in non-ischemic cortex. iNOS immunoreactivity in the infarct was found in cells associated with the wall of small blood vessels at 12-48 hrs after reperfusion and in inflammatory cells as well at 48-96 hrs. To determine whether iNOS contributes to ischemic damage rats were treated for 4 days with the iNOS inhibitor aminoguanidine (AG) starting 6 hours after reperfusion. AG treatment (n=10) reduced infarct size in neocortex by 32±7% ($p<0.05$). Thus, transient focal ischemia leads to iNOS expression in selected cells. The observation that AG treatment reduces ischemic damage suggests that NO produced by iNOS contribute to the tissue damage. Interestingly, in transient ischemia maximal iNOS mRNA expression occurs 36 hrs earlier than in permanent ischemia (JCBF&M above). Perhaps, reperfusion of previously ischemic tissue triggers the release inflammatory cytokines which, in turn, induce iNOS in susceptible cells. We conclude that iNOS expression is an important determinant of ischemic brain damage and that AG may be a useful agent for the treatment of ischemic stroke. (Supported by AHA and NIH)

411.7

LUBELUZOLE IMPROVES ISCHEMIC PENUMBRA MEASURED BY CORTICAL SPREADING DEPRESSIONS IN RATS. M. De Ryck*, E. De Prins, C. Nolten, R. Marrannes and G. Clincke. Janssen Res. Found., Dept. Neuropsychopharmacol., Beerse, Belgium.

A photochemical thrombotic infarct was induced in rat parietal cortex (n=30). Two glass microelectrodes recorded extracellular [K^+] and/or DC. A platinum electrode was placed frontally for electrical stimulation. In vehicle-treated rats (n=14), in sites up to 900 μm proximal (P) to the infarct edge, infarct-related spreading depressions (SDs) progressively increased in duration, decreased in amplitude, and developed slower de- and/or repolarization slopes. By contrast, in sites 1-3 mm distal (D), SDs maintained a sharp profile, while somewhat increasing in duration. Following a one-hour SD-free period, an elicited SD, propagating towards the infarct, revealed further penumbral worsening, i.e., further changes in SD profile. The novel neuroprotective benzothiazole lubeluzole (R087926; 0.63 or 1.25 mg/kg iv; 5 min after irradiation; n=8/dose) did not reduce the number of infarct-related SDs (it is not an NMDA blocker), but following the one-hour SD-free period, lubeluzole protected against penumbral worsening. Lubeluzole (1.25 mg/kg iv) increased the penumbral SD amplitude by 24 % ($p < 0.05$) and accelerated the de- and repolarization rates by 89 % ($p < 0.05$) and 100 % ($p < 0.01$), resp., towards normal values. Lubeluzole prevents the increase of extracellular glutamate and normalizes neuronal excitability in the penumbra. It inhibits the glutamate-activated nitric oxide pathway.

411.8

NEUROTROPHINS INDUCE NMDA RECEPTOR EXPRESSION IN CULTURED RAT NEOCORTICAL NEURONS. H.S. Ying*, B.J. Gwag, M.M. Behrens, J. Koh, D. Lobner, and D.W. Choi. Dept. of Neurology and Center for the Study of Nervous System Injury, Washington Univ. School of Medicine, St. Louis, MO 63110.

Neurotrophin pretreatment increases the vulnerability of cultured mouse cortical neurons to oxygen-glucose deprivation-induced neuronal death, via an NMDA receptor-mediated mechanism (Koh *et al.*, *Science* 268:573-575, 1995). Here, we used RT-PCR (30-35 cycles) to examine the effect of neurotrophin pretreatment on NMDA receptor expression in rat cortical cell cultures. 24 hr pretreatment with 100 ng/ml BDNF, NT-3, or NT-4/5 (but not NGF) increased mRNA levels of NMDAR-2A relative to cyclophilin, neurofilament light chain, or GFAP, whereas NMDAR-1 mRNA levels were unchanged. Consistent with this neurotrophin agonist profile, high levels of trk B and trk C mRNA, but not of trk A or p75 mRNA, were present in the cultures. Immunoblots revealed that the same neurotrophins selectively upregulated NMDAR-2A/B protein over NMDAR-1 protein. ^{35}S-methionine incorporation during neurotrophin application confirmed that the increased immunoreactivity corresponded to newly synthesized protein. These experiments suggest that the ability of neurotrophins to potentiate oxygen-glucose deprivation-induced neuronal death may be explained in part by an upregulation of NMDA receptor gene expression.

Supported by NIH NINDS grants NS 30337 and NS 322626 (DWC); Spanish Ministry of Science and Education (MMB). Neurotrophins were a generous gift from Genentech.

411.9

BDNF POTENTIATION OF OXYGEN-GLUCOSE DEPRIVATION-INDUCED NEURONAL DEATH: INVOLVEMENT OF GENE EXPRESSION AND NITRIC OXIDE SYNTHASE. D. Lobner*, B.J. Gwag, J. Koh, H.S. Ying, M.M. Behrens, and D.W. Choi. Dept. of Neurology and the Center for the Study of Nervous System Injury, Washington Univ. Sch. of Med., St. Louis, MO 63110.

We have shown previously that the neurotrophins BDNF, NT-3, and NT-4/5, but not NGF, potentiate neuronal necrosis induced by oxygen-glucose deprivation or addition of NMDA in murine cortical cell culture (Koh et al., *Science*, 268: 573-575, 1995). Murine cortical cell cultures briefly (30-40 min) deprived of oxygen and glucose normally developed little neuronal degeneration by the next day, but more neuronal death occurred in cultures treated with BDNF (50-100 ng/ml). This BDNF injury potentiation was associated with increased neuronal cell body swelling and was not blocked by late addition of cycloheximide, consistent with increased necrosis. BDNF injury potentiation was largely dependent on multi-hour pretreatment exposure; a small potentiation was induced by acute addition of BDNF during oxygen-glucose deprivation alone, and postreatment had no effect. The large potentiating effect of 24 hr BDNF pretreatment was blocked by concomitant addition of 100 ng/ml actinomycin-D. Acute addition of inhibitors of nitric oxide synthase (NOS), 1 mM N^{ω}-Nitro-L-arginine (N-Arg) or 1 mM N^{G}-Nitro-L-arginine methyl ester (L-NAME), during oxygen-glucose deprivation did not itself reduce neuronal death caused by longer oxygen-glucose deprivation (50-55 min), but attenuated BDNF-potentiated death by about 50%. These data suggest that BDNF potentiation of oxygen-glucose deprivation-induced neuronal cell death is dependent on gene expression and at least in part on NOS activity.

Supported by NIH NINDS NS 30337 (DWC), Spanish Ministry of Science and Education (MMB). BDNF was a generous gift from Genentech.

411.10

Suppression of Specific Nerve Growth Factor Genes by Antisense DNA to *c-fos*. PK Liu*, JK Cui, YY He, CY Hsu. Div. Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, TX 77030 and Dept. of Neurology, Washington University St. Louis, MO 63110

In a rat stroke model, expression of immediate early genes (c-fos, c-jun and junB), late effector genes (neurotrophin genes: nerve growth factor [NGF] and brain-derived neurotrophic factor [BDNF]) and transcription factors (AP-1, and cyclic AMP response element binding [CREB]) increase within 2 hr after focal cerebral ischemia (FCI). Transcription factor activator protein-1 (AP-1) is a DNA-binding complex that recognizes the enhancer site of several late effector genes, including NGF. AP-1 dimeric complex contains proteins of Fos and Jun families. Cerebral ischemia increases the levels of AP-1 DNA binding activity. To investigate the effect of AP-1 activation on the regulation of late effector genes, we synthesized a 26-mer DNA (*rncfosr*$_{115}$) in both antisense and sense orientation to the c-*fos* mRNA. *rncfosr*$_{115}$ was infused into the left lateral ventricle of adult male Long-Evans rats (n=20). Brain uptake and distribution of oligo DNA were studied using digoxigenin- or biotin- labeled *rncfosr*$_{115}$. Neuronal uptake in the hippocampus was noted as early as 6 hr, and lasted for at least 41 hr. A 30-min ischemia with a 90-min reperfusion were induced 16 hr post *rncfos*$_{115}$ infusion. We determined the effect of *rncfosr*$_{115}$ on the expression of transcription factor activities, and of neurotrophin mRNA after FCI. We observed inhibition of Fos/AP-1 activities and suppression of NGF mRNA by antisense *rncfosr*$_{115}$, but not CREB activities or the expression of γ-actin and neurotrophin-3 genes in the hippocampus. We concluded that post ischemic NGF expression *in vivo* is dependent upon Fos/AP-1 activities, and this model is suitable for studying the regulation of effector genes controlled by AP-1 binding activities.

411.11

INDUCTION OF NGFI-B EXPRESSION AFTER FOCAL CEREBRAL ISCHEMIA. T.N. Lin*, J.J. Chen, S.J. Wang, J.T. Cheng, S.I. Chi. Institute of Biomedical Sciences, Academia Sinica, Taipei and Tzu-Chi Medical College, Hualien, Taiwan, ROC

NGFI-B (also known as nur77) is a nuclear receptor, membrane of the thyroid/steroid receptor family, which is rapidly induced in cells stimulated with nerve growth factor (NGF). Altered expression of NGF mRNA has been reported after ischemia challenge. In the present study, the temporal and spatial expression of NGFI-B was investigated, using a three-vessel occlusion focal cerebral ischemia model. Northern blot analysis showed that, in the ischemic cortex, transient increased expression of NGFI-B immediately following ischemia-reperfusion and peaked (15-fold increase) at 30 min after reperfusion. Ipsilateral hippocampus also showed a similar induction of NGFI-B. Interestingly, increased expression of NGFI-B was also noted, with less extent, in the contralateral cortex which was sustained only very mild ischemia. On the other hand, there was no obvious increase in the contralateral hippocampus region. These results were in lined with the results from in situ hybridization studies. Which further revealed that enhanced expression of NGFI-B were also observed in the ipsilateral subcortical region but not the contralateral side. These results suggest that NGFI-B may play a crucial role in ischemia induced neuronal injury and/or recovery.

411.12

THE NEUROPROTECTIVE EFFECT OF CLENBUTEROL IS MEDIATED BY NGF. J. Krieglstein*, J. Semkova and M. Schilling. Department of Pharmacology, Philipps-University, Ketzerbach 63, D-35032 Marburg, Germany

Adrenergic stimulation is known to induce NGF synthesis in the brain. Therefore, we attempted to find out whether the β2-mimetic clenbuterol could protect neurons against damage by increasing NGF synthesis. In primary cultures of hippocampal neurons obtained from newborn rats we tested NGF induction and neuroprotection by clenbuterol. In the mouse model of focal cerebral ischemia the capacity of clenbuterol was examined to reduce the infarct size. Exposure of the hippocampal cells to 100 µM clenbuterol for 4 h enhanced the level of NGF in the culture medium five-fold as measured by two-site ELISA. Furthermore, 1-100 µM clenbuterol was capable of protecting concentration-dependently the hippocampal neurons against damage caused by 1 mM glutamate added to the medium for 1 h. When NGF was bound in the medium of the hippocampal cells by an antibody the neuroprotective effect of clenbuterol was no longer demonstrable. Doses of 0.3 or 1 mg/kg clenbuterol injected intraperitoneally 5 h prior to occlusion of the middle cerebral artery reduced significantly the infarct area on the mouse brain surface. The results offer strong evidence that the neuroprotective effect of clenbuterol demonstrated *in vitro* and *in vivo* is mediated by NGF. The induction of NGF in the brain by lipophilic drugs could become an elegant approach to the treatment of neurodegenerative diseases.

POTASSIUM CHANNEL STRUCTURE, FUNCTION AND EXPRESSION II

412.1

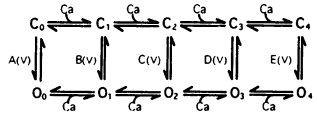
MACROSCOPIC PROPERTIES OF MSLO CA-ACTIVATED K^+ CURRENTS J. Cui *, D. H. Cox, and R. W. Aldrich. Dept. of Mol. and Cell. Physiol., HHMI, Stanford University School of Medicine, Stanford CA 94305

Previous studies of the gating of Ca-activated K^+ channels have focused primarily on the calcium dependence of steady-state single-channel recordings. To address the relationship between Ca-dependent and voltage-dependent gating, we have studied the kinetic and steady state properties of macroscopic currents from mSlo Ca-activated K^+ channels expressed in excised macropatches from *Xenopus* oocyte membranes. In response to voltage steps activation and deactivation kinetics were sensitive to both internal Ca^{2+} ([Ca]i) and membrane voltage over a wide range of [Ca]i (1 to 1000 µM) and voltages (-180 to +200 mV). Activation rates increased, and deactivation rates decreased with increasing [Ca]i or voltage. Both activation and deactivation could be approximated by a single exponential function. G-V plots could be fitted with a simple Boltzman function which maintained a similar shape as [Ca]i was varied. The position of the G-V curve shifted in the hyperpolarizing direction along the voltage axis as [Ca]i was increased. The magnitude of the G-V curve shift was not constant as [Ca]i was increased in 10 fold increments, but rather was larger at low [Ca]i. These results suggest that mSlo gating involves a voltage dependent process that is intrinsic to the channel protein and is cooperative among the subunits. Further supporting the existence of an intrinsic voltage dependent gating transition, the rate of activation at each test potential increased with [Ca]i reaching a plateau at high [Ca]i whose level was voltage dependent. Also, at voltages greater than +90 mV activation of the mSlo channel could be observed in solutions whose free Ca^{2+} was buffered to as low as ~ 2 nM. The activation rate under these conditions is much faster than the diffusion limit for Ca^{2+} binding, indicating that the channel can open without binding to Ca^{2+}. From measurements of the voltage-dependence of channel activity at Popen between 10^{-4} to 10^{-2} we have estimated the amount of charge associated with mSlo gating to be between 1 to 1.5 charges per channel, an amount approximately equal to the sum of the charges associated with the rate limiting steps in activation and deactivation. These data lead us to favor a model in which each channel must undergo a single, intrinsic voltage dependent conformational change separate from Ca^{2+} binding before opening.

412.2

A KINETIC MODEL FOR THE MSLO CA-ACTIVATED K^+ CHANNEL D. H. Cox, J. Cui, and R. W. Aldrich *. Dept. of Mol. and Cell. Physiol., HHMI, Stanford University School of Medicine, Stanford CA 94305

We have studied macroscopic currents from mSlo Ca-activated K^+ channels expressed in *Xenopus* oocytes. These studies have revealed several constraints on any system designed to model the behavior of this channel (see accompanying abstract). Among these constraints are the following: 1) In response to voltage steps, activation and deactivation kinetics are sensitive to both [Ca]i and voltage over a wide range of these stimuli, and yet maintain approximately single exponential kinetics. 2) Conductance vs [Ca]i curves are best fit to the hill equation using coefficients between 1 and 3 (depending on membrane voltage) suggesting that at least 3 Ca^{2+} ions bind to the channel and affect activation. 3) There appears to be a single, intrinsic voltage dependent conformational change which the channel must undergo in order to open. And 4), even at very low [Ca]i (~ 2 nM), activation is observed at voltages greater than +90 mV. We have used the general kinetic scheme below to model the behavior of this channel.



Horizontal transitions represent Ca^{2+} binding steps. No voltage dependence has been assigned to these steps. Vertical transitions represent a single voltage dependent conformational change necessary for channel opening. Ca^{2+} can bind to both *closed* (C) and *open* (O) conformations, but binds with higher affinity to O thus favoring channel opening. Models of this type can account for the major properties of our data. In one form of this scheme the affinities of all Ca^{2+} binding sites in any one conformation are the same. In this case the model amounts to a voltage dependent version of the MWC model of allosteric proteins replacing the T and R nomenclature with C and O respectively.

412.3

TISSUE-SPECIFIC TRANSCRIPTIONAL REGULATION OF THE SLOWPOKE CA^{2+}-ACTIVATED K^+ CHANNEL GENE. N. S. Atkinson* and R. Brenner. Dept. of Zoology., The University of Texas at Austin, Austin TX 78712-1064.

The stereotypical properties of an electrically excitable cell results from the coordinated activity of different transmembrane ion channels. Little is known about the gene-regulation of the ion channel genes which underlie a cell's electrical profile. We are studying transcriptional control of the slowpoke Ca^{2+}-activated K^+ channel gene. We have shown that slowpoke has both neuronal and muscle/gut/tracheal cell specific promoters. Each promoter causes the production of mRNAs that differ slightly in the 5' end and therefore encode slightly different polypeptides. For each of these tissue types we have also identified promoter proximal and/or enhancer elements that direct expression of the promoters. Furthermore, developmental-stage specific and eye specific enhancer elements have been discovered.

412.4

N-terminal domain of GIRK1 muscarinic potassium channel facilitates activation by muscarinic receptors. P.A. Slesinger*, E. Reuveny, Y.N. Jan and L.Y. Jan. Howard Hughes Med. Instit. and Dept. of Physiology. Univ. of California, San Francisco. San Francisco, CA 94143.

Stimulation of G-protein coupled receptors leads to the dissociation of the G-protein trimer into activated Gα and Gβγ subunits which can each interact with various effectors. Although the specificity of this interaction for Gα effectors can be determined by the kind of Gα subunit, it is unclear what determines the specificity for effectors which respond to Gβγ subunits. For example, the muscarinic potassium channel (I_{KACh}) in the heart can be activated by a variety of different recombinant Gβγ subunits, yet stimulation of muscarinic receptors but not beta-adrenergic receptors activates I_{KACh}. Expression of GIRK1, a cloned subunit of I_{KACh}, in *Xenopus* oocytes generates Gβγ-sensitive inwardly rectifying potassium channels which couple to muscarinic receptors and have many properties similar to those of I_{KACh}. To identify structures involved in the activation of GIRK1 by muscarinic receptor stimulation, we have constructed chimeras of GIRK1 and IRK1, a G-protein-insensitive inward rectifier. In oocytes expressing GIRK1 and the m2 receptor, the m2 agonist carbachol rapidly induces an inwardly rectifying potassium current (τ = ~7 s.). Replacing the hydrophobic core (M1-H5-M2) and part of the C-terminal domain of IRK1 with the corresponding region of GIRK1 produces a chimeric channel which is Gβγ-sensitive but activates slowly (τ = 44 s.) after m2 stimulation and has a high agonist-independent (basal) current. Adding the N-terminal domain of GIRK1 to this chimeric channel restores fast activation by m2 stimulation (τ = ~7 s.) and reduces the basal current. Our results demonstrate that the N-terminal domain of GIRK1 serves two roles: facilitating rapid activation by m2 stimulation and reducing the agonist-independent current. The interaction of the N-terminal domain with G-proteins provides one mechanism for ensuring specific activation of I_{KACh} in the heart.

412.5

EVIDENCE THAT NEURONAL G PROTEIN-GATED INWARDLY RECTIFYING K^+ CHANNELS ARE ACTIVATED BY Gβγ SUBUNITS AND FUNCTION AS HETEROMULTIMERS. Paulo Kofuji, Craig Doupnik*, Norman Davidson and Henry A. Lester. Division of Biology 156-29, Caltech, Pasadena, CA 91125

Guanine nucleotide-binding proteins (G proteins) activate K^+ conductances in cardiac atrial cells to slow heart rate and in neurons to decrease excitability. Complementary DNAs encoding three isoforms of a G protein-coupled inwardly rectifying K^+ channel have recently been cloned from cardiac (GIRK1) and brain cDNA libraries (GIRK2 and GIRK3). Here we report that GIRK2 but not GIRK3 can be activated by G protein subunits Gβ1 and Gγ2 in *Xenopus* oocytes. Furthermore when either GIRK3 or GIRK2 was coexpressed with GIRK1 and activated either by muscarinic receptors or by Gβγ subunits, G protein-mediated inward currents were increased by 5 to 40 fold. The single-channel conductance for GIRK1 plus GIRK2 coexpression was intermediate between those for GIRK1 alone and for GIRK2 alone, and voltage-jump kinetics for the coexpressed channels displayed new kinetic properties. On the other hand, coexpression of GIRK3 with GIRK2 suppressed the GIRK2 alone response. These studies suggest that formation of heteromultimers involving the several GIRKs is an important mechanism for generating diversity in expression level and function of neurotransmitter-coupled inward rectifier K^+ channels. Supported by NIH, AHA and ICAgen Inc.

412.6

DETERMINATION OF THE SUBUNIT STOICHIOMETRY OF AN INWARD RECTIFIER POTASSIUM CHANNEL. J. Yang, Y.N. Jan and L.Y. Jan*. HHMI, Dept. of Physiol., Univ. of California, San Francisco, CA 94143

Inward rectifier K^+ channels have a distinct architecture that is different from voltage-gated K^+ channels: they contain only two putative transmembrane segments instead of six. It has been shown that voltage-gated K^+ channels are composed of four subunits. We examined the stoichiometry of an inward rectifier K^+ channel, IRK1, by linking together the coding sequences of three or four identical subunits and tagging the expression of an individual subunit with a mutation (D172N-E224K) that weakens rectification and reduces single-channel conductance. Tandem trimers or tetramers with 0-3 tagged subunits at various positions were examined alone or in coexpression with the mutant monomer in Xenopus oocytes. We found that the four subunits in a tandem tetramer are equivalent and are all incorporated into the functional channel. More compellingly, whereas the tandem tetramer made up of four wild-type (wt) subunits behaved the same with or without coexpression of the mutant monomer, the macroscopic and single-channel properties of the wt tandem trimer coexpressed with the mutant monomer differ from those of the wt tandem tetramer, but are similar to those of the tandem tetramers with one mutant subunit at any of the four positions. These results indicate that IRK1 channel expressed in oocyte is composed of four identical subunits. Surprisingly, the tandem tetramers made up of three mutant (in this case, D172N-E224G) subunits and one wt subunit retain nearly identical sensitivity to Mg^{2+} and ~1/6 of the sensitivity to spermidine as compared to the wt channel, suggesting that Mg^{2+} and spermidine can interact with only one subunit in a tetrameric channel. The tetrameric structure of both inwardly rectifying and voltage-gated K^+ channels is consistent with an evolutionary linkage between these two families of channels.

412.7

STRUCTURE OF THE PORE REGION OF CYCLIC NUCLEOTIDE-GATED CHANNELS AND ITS FUNCTION IN GATING. Z.-P. Sun*, M.H. Akabas, E.H. Goulding, A. Karlin and S.A. Siegelbaum. Depts. Pharmacol., Physiol., Biochem., Ctr. Neuro. & Behav. & Ctr. Molec. Recog., HHMI, Columbia University, N.Y., NY 10032

The P-region (or H5) linking the extracellular ends of the S5 and S6 transmembrane segments of cyclic nucleotide-gated (CNG) ion channels is the major determinant of their ion-permeation properties and forms the channel pore. The P-region of the voltage-gated ion channels, which is homologous to the P-region of the CNG channels, has been proposed to fold into and out of the membrane as a β hairpin. We have applied the substituted-cysteine accessibility method (SCAM) to investigate the structure of the P-region. Each P-region residue (positions R345-S371, numbered R1-S27 here) of the cloned bovine retinal channel (BRET) was substituted with cysteine individually. The accessibility of each cysteine substitute to charged, sulfhydryl-specific reagents applied to either side of the membrane, in both the open and closed states, was studied in inside-out and outside-out patch-clamp recordings of the mutant channels expressed in *Xenopus* oocytes. V4C, T20C and P22C were accessible to reagents applied to either side of the membrane, V4C only in the closed state, and T20 and P22C in both the open and closed states. L7C and V24C were accessible to reagents in the closed state only from the cytoplasmic side. The two sided accessibility of V4C, T20C, P22C indicates that the P-region is a loop which extends towards the central axis of the channel. Together, the P-regions of the subunits of the oligomeric CNG channel form an iris that is the permeability barrier or gate in the closed state and the cation-selectivity filter in the open state.

412.8

A ROLE FOR THE "PRE-PORE" REGION OF VOLTAGE-DEPENDENT K^+ CHANNELS IN GATING AND ZINC MODULATION. G.Talukder[1], M.M. Tamkun[2], and N.L. Harrison*[1]
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Zn^{2+} modulates the gating of certain cloned voltage-gated K^+ channels, such as Kv1.5 (Harrison et al. 1993 Mol. Pharm. 43 482-486). Two cloned potassium channels, Kv1.2 and Kv2.1, show decreased sensitivity to zinc. The amino acid sequence in the pre-pore domain between S5 and S6 is quite variable between these channels and Kv1.5. We have studied mutations of Kv1.5 in the pre-pore domain which affect gating, activation kinetics and zinc modulation. Whole cell recordings were made at 25°C, using intracellular solutions based on K gluconate and extracellular perfusion with saline containing 3mM K^+. All channels were transiently expressed in HEK 293 cells. Measurements of $t_{1/2}$ (activation) were conducted at or as near as possible to $V_{1/2}$ (midpoint of activation). We have studied several mutations of Kv1.5, including A455K, H461K, and E454Q. The gating of E454Q, A455K, and H461K is shifted in the depolarized direction relative to the wild type (w.t.) channel ($V_{1/2}$: w.t. = 4.5 ± 1.8mV; E454Q = 19.5 ± 3.1; A455K = 19.6 ± 1.9; H461K = 18.9 ± 2.3). There is approximately a two-fold slowing of the activation kinetics of E454Q relative to the wild type channel ($t_{1/2}$: w.t. = 5.33 ± 0.7ms; E454Q = 11.75 ± 2.6ms [$p<0.01$]). The three mutations also show a decreased sensitivity to zinc, especially E454Q, which shows a ten-fold lower affinity for zinc. Based on these data, we suggest that the region of the channel protein between S5 and the pore may interact with extracellular ions and the "voltage sensor" in the S4 region to modulate channel gating.

412.9

IN SITU OXIDATION OF AN ENGINEERED CYSTEINE IN THE K⁺ CHANNEL Kv2.1 (DRK1) SUGGESTS CONTACT BETWEEN THE PORE AND S6. R.H. Joho*, Y. Liu, R.D. Zuhlke, and H.-J. Zhang. Department of Cell Biology and Neuroscience. The University of Texas Southwestern Medical Center, Dallas, Texas 75235.

The segment between S5 and S6 of voltage-gated K⁺ channels has been implicated to form the major part of the ion conduction pathway. Little is known about the conformation of this region although various models have been proposed. To gain insight into the secondary structure, we used cysteine-substitution mutagenesis to probe side-chain accessibilities of mutated amino acids in the pore region of Kv2.1 and to monitor possible disulfide formation between cysteines by *in situ* oxidation. Of 28 cysteine substitutions, we identified one mutant, I379C, that showed >90% current reduction after superfusion of channel-expressing *Xenopus* oocytes with 0.1% H_2O_2. The effect could be reversed by 1 mM DTT. This finding suggested that the new cysteine at position 379 might form a disulfide either with the same cysteine of another subunit or with one of the three cysteines (C232, C393, and C394) present in the hydrophobic core (S1 - S6) of the channel, rendering the channel nonfunctional after *in situ* oxidation. To show if any of the three cysteines might interact with position 379, we mutated either singly or in combination C232, C393, and C394 to serine. The "cysteineless" mutant C232S/C393S/C394S/I379C was H_2O_2 resistant. The double mutants C232S/I379C and C393S/I379C were still H_2O_2 sensitive, in contrast, C394S/I379C was H_2O_2 resistant, suggesting that positions 379 and 394 are close enough to support formation of a disulfide bridge after *in situ* oxidation. (Supported by grants from the NIH and MDA [RHJ], and the American Heart Assoc./Texas Affiliate [HJZ].)

412.10

ACIDIC AMINO ACID E283 IN S2 SEGMENT INTERACTS ELECTROSTATICALLY WITH S4 BASIC RESIDUES IN SHAKER K⁺ CHANNEL S. K. Tiwari-Woodruff and D. M. Papazian*, Department of Physiology, UCLA School of Medicine, Los Angeles, CA 90024-1751.

Electrostatic interactions between conserved acidic residues in putative transmembrane segments and basic amino acids of the voltage-sensing S4 segment stabilize the structure of voltage-dependent Shaker K⁺ channels (Papazian et. al., *Neuron*: in press, 1995). Using charge reversal mutations, we have now identified likely short range electrostatic interactions of E283 in S2 with R368 and R371 in the S4 segment.

Single and double mutations (in an inactivation-removed [IR] background) were expressed in Xenopus oocytes for analysis of protein maturation and channel function. The mutation E283R-IR eliminated functional expression and significantly reduced maturation of the protein, suggesting that E283R affects proper folding. The double mutant combinations E283R+R368E-IR and E283R+R371E-IR restored efficient maturation. In contrast, charge reversal mutations at other S4 mutations did not rescue E283R. Only the double mutant combination E283R-R371E-IR restored functional expression; the activation curve was shifted to the right. These results suggest that E283 interacts electrostatically with R368 and R371. However, these two S4 positions are not equivalent because R371E restores channel activity to E283R whereas R368E does not. Perhaps the interactions are not of equal strength or E283 interacts with R368 and R371 in different channel conformations. The latter possibility would be consistent with the need for charged amino acids to undergo conformational changes during voltage-dependent activation.

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RETINA II

413.1

A METABOTROPIC GLUTAMATE RECEPTOR ON GANGLION CELLS IN CAT AND PRIMATE RETINAL SLICES E.D. Cohen* Dept. of Ophthalmology and Visual Science, Yale Univ. Med. School, New Haven, CT 06520-8061

Bipolar cells transduce the visual signal through release of glutamate onto the dendrites of ganglion cells. I have examined the effect of glutamate agonists upon voltage-gated calcium currents of cat and primate ganglion cells in a slice preparation. Calcium currents were enhanced by 10mM barium, Na^+ channels were blocked by the addition of 200nM TTX, and NBQX 5μM blocked AMPA/kainate receptors. Ganglion cells were recorded using whole-cell and perforated patch clamp techniques. When held at -80mV and stepped to -10mV, application of 10μM glutamate depressed voltage gated calcium currents on cat and primate ganglion cells up to 50%, while evoking little inward current at rest. Quisqualate (10-3μM) and t-ACPD (10-100μM) produced similar depressions. An agonist potency series for this effect revealed glutamate≈quisqualate >ACPD. Depression of calcium currents were observed on both on- and off-center midget/β ganglion cells and on α/parasol cells. This suggests that regulation of calcium influx by metabotropic receptors could play an important role in modulating the light-evoked responses of retinal ganglion cells. Supported by EY10617.

413.2

NICOTINIC ACETYLCHOLINE RECEPTORS ARE EXPRESSED BY AMACRINE AND GANGLION CELLS IN THE RABBIT RETINA. Kent T. Keyser[1]*, Agnieszka Brzozowska-Prechtl[1], Jon M. Lindstrom[2] and Richard H. Masland[3] Department of Neurosciences, University of California, San Diego CA[1]; The Institute of Neurological Sciences, University of Pennsylvania, Philadelphia PA[2]; Howard Hughes Medical Institute, Mass. General Hospital, Boston MA[3].

Acetylcholine (ACh) is a transmitter in the retina that affects the response properties of many ganglion cells, including those that display directional selectivity. Three structural (β) and eight ligand binding (α) subunits of neuronal nicotinic acetylcholine receptors have been purified and antibodies have been raised against some of them. As part of a continuing effort to understand the cholinergic circuitry in the retina, we used a monoclonal antibody that recognizes native α3 or α5 subunits to determine the pattern of expression of these subunits in the rabbit retina. Immunoreactive cells in the Inner Nuclear Layer were restricted to the first one or two tiers of cells. They ranged from 7 to 14 μm in diameter in dehydrated and cleared sections, and comprised at least three distinct populations based upon somata size and intensity of immunoreactivity. Several populations of cells in the Ganglion Cell Layer were distinguishable on these bases as well. The labeled cells in this layer ranged from 8 to 16 μm. The dendrites of some of the immunoreactive cells could be traced until they entered one of two dense bands of immunoreactive processes in the Inner Plexiform Layer. These bands divided the IPL roughly into thirds. They overlap the levels of stratification of the starburst (cholinergic) amacrine cells, but were wider and less sharply defined than the strata defined by starburst processes. Viewed in horizontal sections, the nAChR-positive plexi had a honeycomb appearance resembling the mosaic of starburst dendrites. These results are consistent with the known location of nicotinic synapses upon ganglion cells. The identify of the nAChR-containing amacrine cells remains to be learned.

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413.3

VOLTAGE-GATED NON-SELECTIVE ION CURRENT IN RETINAL GANGLION CELLS. T. Tabata & A.T. Ishida*. Section of Neurobiology Physiology & Behavior, University of California, Davis CA 95616.

Using whole-cell patch-clamp methods, we have found a voltage-gated non-selective ion current (I_{non}) in retinal ganglion cell (RGC) somata dissociated from adult goldfish. Both rapidly and gradually rising components of I_{non} were more activated at more depolarized voltages; the activation of these components was apparent at command potentials between -50 mV and +50 mV. The current-voltage curve of I_{non} did not shift upon replacement of monovalent cations or anions in the superfusate saline by other small inorganic ions or by large organic ions (e.g., N-methyl-D-glucamine and D-gluconate). These measurements suggest that I_{non} can be carried by small as well as large ions and that the corresponding conductance has little ion selectivity. I_{non} was resistant to 3 μM tetrodotoxin (TTX), 30 mM tetraethylammonium (TEA) plus 10 mM 4-aminopyridine (4AP), and 2.4 mM Co^{2+}, which are effective blockers in goldfish RGCs against Na, K, and Ca currents, respectively. However, I_{non} could be reduced in amplitude by roughly 50% with 1 mM halothane. When depolarized by exposure to high-K^+ saline containing Lucifer yellow (10 mM), TTX, TEA, 4AP, and Co^{2+}, dissociated RGCs could be loaded with Lucifer yellow within several minutes. Some, if not all, of I_{non} may therefore pass through channel-like hemi-gap junctions. Whether these form gap junctions *in situ* or function otherwise (e.g., spike shaping) remains to be established.

Supported by NIH grant EY 08120.

413.4

CAN WE DETERMINE THE CONTRIBUTION OF NMDA AND KA/AMPA RECEPTORS TO EPSPs IN GANGLION CELLS? T.J. Velte[1]*, W. Yu[2], and R.F. Miller[1]. [1]Dept. of Physiol., Grad. Program in Neuroscience, Univ. of Minnesota, Mpls, MN 55455; [2]Johns Hopkins Univ., Sch. of Med., Baltimore, MD 21205.

Both NMDA and KA/AMPA receptors contribute to light evoked synaptic inputs of most retinal ganglion cells. However, the role and magnitude of each receptor subtype to EPSP generation has not been determined. We used NBQX and D-AP7 to segregate NMDA and KA/AMPA receptors, and study the relative contribution of each class, examined under voltage-clamp conditions in the slice preparation of the amphibian retina. Synaptically evoked currents were measured alternately in NBQX vs. D-AP7. Algebraic summation of the two currents were compared to the control response in standard Ringer. Most often the KA/AMPA + NMDA sum was less than the control, although occasionally the sum exceeded the control.

Computer simulations were carried out to understand the confounding summation experiments. We used an equivalent cylinder or a realistic compartmental model of a ganglion cell, with a passive membrane (R_m=70,000 Ω cm²), a linear KA/AMPA current, and a non-linear NMDA current. We could duplicate the physiological summation data under the following conditions: when the stimulus was placed on or near the soma, the synaptic response was well clamped and the currents summed to produce the same current magnitude seen in the control. When the stimulus was located on a proximal dendrite, there was significant local escape voltage which caused the membrane potential to approach the peak potential for maximum NMDA current, which augmented the response. When the stimulus was placed distally, the escape voltage moved beyond the NMDA peak potential and closer to the reversal potential for both channel types (0 mV) which attenuated the overall current. Thus both clamp failures and non-linear summation may account for our observations.

Work supported by PHS Grant: EY-07133 to TJV and EY-03014 to RFM)

413.5

MODULATION OF VOLTAGE-GATED Na' CURRENTS IN RETINAL GANGLION CELLS BY SEROTONIN RECEPTOR ACTIVATION.

S. Hidaka, M. Sutter*, and A.T. Ishida. Section of Neurobiology Physiology & Behavior, University of California, Davis, CA 95616.

It is unknown whether serotonin (5HT) directly affects retinal ganglion cells, although it is known that serotonergic amacrine cells densely ramify in the inner plexiform layer, and that exogenous 5HT can modulate retinal ganglion cell light responses. We have tested for effects of 5HT on whole-cell currents in retinal ganglion cell somata dissociated from adult goldfish (*Carassius auratus*), using amphotericin-B-perforated patch-clamp methods. No pronounced change in holding current was produced by 5HT agonists at E_{hold} between -65 mV and -90 mV. We therefore tested for effects of these ligands on voltage-activated currents, beginning with regenerative Na' currents. Under control conditions, maximal Na' current (-4 nA to -10 nA) was elicited by depolarizations from -90 mV to -10 mV. The decay of these currents could be fit by double exponential functions, with fast and slow time constants of 0.6 msec and 2-5 msec, respectively. Application of $5HT_1$ receptor agonist [1-(2-methoxyphenyl) piperazine; 3μM] and $5HT_{1A}$ receptor agonist [8-hydroxydipropylaminotetralin; 3μM] decreased maximal Na' current amplitude by 35% and slowed its decay, without markedly altering voltage-dependence of current activation. Current decay could then be fit by a time constant of roughly 1.5 msec. These effects developed over 1-10 min, could be reversed by washing with control saline, and resemble effects we recently reported of cAMP- (but not cGMP-) analogs on Na' currents in these cells. These results show that activation of $5HT_{1A}$ receptors in retinal ganglion cells can modulate Na' currents in these cells. We are now studying mechanisms coupling 5HT receptors to Na' channels, and functional consequences of their activation.

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413.6

DIFFERENTIAL DISTRIBUTION OF $GABA_A$ RECEPTORS IN IDENTIFIED AMACRINE CELL TYPES IN THE RABBIT RETINA. Berndt Ehinger and Charles L. Zucker* Department of Ophthalmology, University of Lund, Sweden, and Schepens Eye Research Institute, Harvard Medical School, Boston, MA.

$GABA_A$ receptor synapses are known to participate in movement detection in the retina, for which acetylcholine (ACh) containing starburst amacrine cells also play a role. However, the circuitry involved is not well understood. We have studied the distribution of $GABA_A$ receptor subunit proteins in rabbit retina, taking advantage of the improved resolution attainable by confocal microscopy and the availability of monoclonal $GABA_A$ receptor subunit antibodies. Further, starburst amacrine cells and other cells in the inner nuclear and ganglion cell layers of the retina stained by intraocular injection of DAPI were classified by intracellular injections of Lucifer yellow in living tissue. Immunohistochemical staining for choline acetyltransferase was also used to identify starburst amacrine cells in rabbit retina.

Starburst amacrine cells were found to contain no or only very little $GABA_A$ receptors of the α_1 and β_1/β_2 subtypes. On the other hand, such receptors occur in high concentration in a cell type with morphologically characteristic features, called the DAPI-3 cell. This cell can be stained by an intraocular injection of DAPI, which is an aid for finding it for intracellular dye injection. Perikarya of the DAPI-3 cells are in the proximal cell rows of the inner nuclear layer and send processes into two sublayers in the inner plexiform layer, where they abut the processes of the two mirror symmetric populations of starburst amacrine cells. The high $GABA_A$ receptor content of the DAPI-3 cells can be used to identifying them immunohistochemically. They occur with roughly the same density as OFF starburst amacrine cells. $GABA_A$ receptor proteins appear also in other cell types in the retina, most notably in indoleamine accumulating S2 cells. The observations suggest that movement detection is accomplished by complex circuits formed by several different cells, and presumably comprising at least starburst amacrine cells as well as a second $GABA_A$ receptive amacrine cell type, perhaps the DAPI-3 cells. Supported by NIH grant EY07552 to CLZ.

413.7

ZINC ENHANCES $GABA_A$ CURRENTS OF SKATE MULLER CELLS.

R.L. Chappell*[1], H. Qian[2], R.P. Malchow[3], and H. Ripps[3]. Hunter College and Graduate School, CUNY[1]; Harvard University[2]; Department of Ophthalmology and Visual Sciences, University of Illinois Chicago[3]; Marine Biological Laboratory, Woods Hole, MA 02543[1,2,3]

Perforated (amphotericin B) patch recordings were used to examine the modulatory effects of zinc on the GABA-induced currents of Muller cells (radial glia) isolated from the skate retina. Earlier studies indicated that the GABA currents of these elements are mediated solely by $GABA_A$ receptors.

We report here pharmacological data suggesting that the modulatory sites on the $GABA_A$ receptors of skate Muller cells exhibit several unique features. First, the benzodiazepine inverse agonists (10μM DMCM or 10μM CCB) potentiated the GABA-mediated responses. Second, 10μM zinc greatly enhanced the current elicited by 1μM GABA. Perhaps even more surprising was the fact that zinc alone induced a current comparable to that elicited by GABA; the reversal potential was the same as for the GABA current, the current was blocked by bicuculline, and the I-V relation was virtually indistinguishable from that produced by GABA. Moreover, cell-attached patch recordings showed that spontaneous channel activity was almost completely abolished by 100μM bicuculline, whereas the frequency of channel openings was significantly enhanced by 10μM zinc. These findings suggest that zinc acts directly on the $GABA_A$ receptors of Muller cells, and raise the possibility that the subunit composition of the $GABA_A$ receptor of the Müller cell differs from that of neuronal $GABA_A$ receptors studied previously in the CNS. It is noteworthy that zinc could be detected in skate photoreceptors using the Neo-Timm sulfide-silver method. These results support the notion that zinc may play a role in modulating GABAergic activity in the vertebrate retina.

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413.8

MOLECULAR CLONING AND NITRIC OXIDE MODULATION OF KAINATE/AMPA-TYPE GLUTAMATE RECEPTOR IN THE HYBRID BASS RETINA. L.V.Ponomareva and D.G.McMahon* Department of Physiology, University of Kentucky, Lexington KY 40536-0084

Emerging evidence suggests that kainate/AMPA-type glutamate receptors (KA GluR) are widespread in the retina. We have used 0.5kbp PCR-generated fragment from the coding region of a rat KA GluR (RGluR1) as a probe to investigate whether the bass retina expresses a KA GluR gene. In parallel,we studied the effects of the nitric oxide/cGMP pathway on the KA GluR of dissociated bass horizontal cells(HC). A clone BGluR1 was isolated by screening a bass retinal cDNA library. It was sequenced and compared to previously sequenced mammalian GluR genes. BGluR1 represents 3 kbp and shows 68% identity to rat GluR1 and GluRA at the nucleotide and 40% identity at the amino acid level. BGluR1 contains the same regions of high homology as are found in all previously sequenced cDNA of ionotropic GluRs from mammals, frog and chicken. It includes 4 homologous transmembrane domains and the "flop" region, which also exists in RGluRA. Retinal HC were dissociated and maintained in culture using standard techniques. Whole-cell patch-clamp recordings were made from HC while kainate (10 or 50 μM) was applied by pressure ejection and test agents were applied by adding to the bath. The current traces elicited by application of L-glutamate, AMPA and kainate on HC support our conclusion that bass HC have KA/AMPA GluRs. Application of 1mM sodium nitroprusside (SNP) or 100 μM S-nitroso-N-acetylpenicillamine (SNAP) reduced the overall responsiveness of HC GluR to KA, reducing the peak amplitude of the response to a standard puff of kainate by approximately 50% (N=8 and 7). The effects of SNP were mimicked by application of 2mM 8-Br-cGMP (-63%,N=5) and were blocked by addition of cGMP cyclase inhibitor LY 83,583 (N=3). Future experiments will focus on cell localization of BGluR1, its expression and defining the mechanisms of nitric oxide modulation.

413.9

GABA ρ SUBUNITS IN RAT RETINA: STRUCTURE AND FUNCTION. Dongxian Zhang*, Zhuo-Hua Pan, Amy D. Brideau, and Stuart A. Lipton. Dept. of Neurology, Children's Hospital, and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

Unlike the human GABA ρ receptors (Cutting et al, *PNAS* 1991) and $GABA_C$ receptors found in other species (Qian and Dowling, *Nature* 1993), the rat $GABA_C$ receptor is uniquely picrotoxinin-insensitive (Feigenspan et al., *Nature* 1993). We previously isolated from a rat retinal cDNA library two clones, homologous to the human ρ1 and ρ2 subunits. A unique single amino acid substitution (methionine instead of threonine) was identified in the second membrane region (M2) of the ρ2 clone compared to the ρ1 clone. Here, we undertook a systematic site-directed mutagenesis study involving 6 amino acid residues in the M2 region and found that the native met/thr site on ρ2 is critical for picrotoxinin-insensitivity. We characterized the expression levels of ρ1 and ρ2 transcripts in the rat retina. Northern blot analysis of either total RNA or poly (A)-selected RNA with a ρ1-specific probe labeled a band of 4.2 kb. A ρ2-specific probe labeled a predominant band of 2.2 kb in total RNA and an additional weak band of 3.4 kb in poly (A)-selected RNA. In addition, using a probe derived from a conserved region of ρ1 and ρ2 subunits, we found that ρ2 subunit mRNA appeared to be more abundant than that of the ρ1 subunit. This difference in expression levels was confirmed by combining primer extension in the presence of dideoxynucleotide, leading to early chain termination; in this method, the two ρ subunits could be distinguished by differences in the lengths of their products. If protein levels approximately mirror mRNA expression, our results suggest that an adequate amount of the ρ2 subunit is present in the rat retina to form native ρ1/ρ2 heteromeric receptors that are insensitive to picrotoxinin.

413.10

THE ACTIN-BASED CYTOSKELETON REGULATES VOLTAGE-GATED POTASSIUM CHANNEL ACTIVITY IN RETINAL BIPOLAR NEURONS. G. Maguire*#, V. Connaughton#, A. Pratt@, R. Jackson@, and H. Cantiello@, # Department of Neurobiology & Anatomy, University of Texas Medical School- Houston, TX 77030, and @The Renal Unit, Massachusetts General Hospital, Charlestown, MA 02129 and Department of Medicine, Harvard Medical School.

The actin-based cytoskeleton is a rich intracellular network linked either directly or indirectly to membrane ion channels. Actin filaments exhibit ionic condensation and support the propagation of ionic charge waves along their longitudinal axis. Although actin has been well documented to play a role in regulating ion channel activity in epithelial cells, its role in regulating ion channels in neurons is less well understood.

We used whole-cell and single channel recording techniques in the retinal slice and in isolated retinal neurons to examine the effects of manipulating the actin-based cytoskeleton on voltage gated K+ channel activity in retinal bipolar neurons.

Under whole cell recording conditions, the addition of cytochalasin D to the bath, an agent that disrupts the actin-based cytoskeleton, breaking actin into shorter filaments, led to an increase in the amplitude of the macroscopic voltage gated K+ channel currents as compared to control. The onset of this effect was about 5 min. following cytochalasin D application and the maximal effect was at about 15 min. Single K+ channel activity was similarly effected under cell-attached conditions. i.e. the frequency of K+ channel opening greatly increased about 5 min. following cytochalsin D application to the bath. Inside-out patches demonstrated that the addition of medium length actin filaments to the bath also increased the frequency of single K+ channel openings. These findings are consistent with actin filaments existing in a dynamic equilibrium, with long actin filaments in a cytoskeleton network leading to decreased channel opening, and shorter actin filaments leading to an increased frequency of opening.

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413.11

MULTIPLE ROLES OF CALMODULIN IN MELATONIN BIOSYNTHESIS BY PHOTORECEPTOR CELLS: REGULATION OF CALCIUM INFLUX AND ADENYLATE CYCLASE ACTIVITY. P.M. Iuvone* and A.L. Alonso-Gómez, Dept. of Pharmacology, Emory Univ. Sch. of Med., Atlanta, GA 303022

Serotonin *N*-acetyltransferase (NAT), a key regulatory enzyme in melatonin biosynthesis, is induced in depolarized photoreceptors. Induction of NAT activity by depolarization involves a signaling cascade that requires calcium influx through dihydropyridine-sensitive voltage-gated channels, stimulation of adenylate cyclase activity, cAMP-dependent protein phosphorylation, and *de novo* RNA and protein synthesis. Calcium influx is required for all subsequent steps in the cascade. The objective of this study was to examine the possible role of calmodulin in this intracellular signaling cascade. In photoreceptor-enriched chick retinal cell cultures, CGS 9343B and KN-62, selective inhibitors of calmodulin and CaM kinase II respectively, inhibit K^+-evoked $^{45}Ca^{2+}$ influx through dihydropyridine-sensitive channels, cAMP accumulation, and induction of NAT activity. In contrast, they have no effect on induction of NAT activity by cAMP analogues, indicating a site of action prior to activation of protein kinase A. The inhibition of K^+-evoked $^{45}Ca^{2+}$ influx by CGS 9343B, KN-62, and KT5970, a structurally dissimilar CaM kinase II inhibitor, was blocked by Bay K 8644, a dihydropyridine channel agonist. CGS 9343B, but not KN-62, inhibited induction of NAT activity elicited by the calcium ionophore A23187, suggesting a site of action distal to the channel. Membranes prepared from photoreceptor-enriched cell cultures contain calmodulin-sensitive adenylate cyclase that is inhibited by CGS 9343B. These results suggest that Ca^{2+}/calmodulin modulates two steps in the cascade regulating induction of NAT activity, enhancing Ca^{2+} channel activity via a CaM kinase II-dependent mechanism and directly stimulating adenylate cyclase.

413.12

THE RETINAL NIGHT-DAY SWITCH: A NEURONAL FLIP-FLOP DEVICE. M.K. Boelen[1], P.L. Megaw[1], I.G. Morgan[2] and D.W. Marshak[3]*. [1]La Trobe University, VIC 3550, Australia, [2]Australian National University, ACT 2601, Australia, [3]University of Texas Medical School, Houston, Texas 77225.

Reciprocal inhibitory circuits act as flip-flop devices with only one of the two elements active at any time. In chicken retina, photoreceptors (PRs) are involved in reciprocal inhibitory interactions like these with dopaminergic amacrine cells (DACs). Melatonin, synthesized and released by PRs in the dark, inhibits the DACs. Dopamine, released by the DACs in the light, inhibits the synthesis and release of melatonin by the PRs (1). The DACs are involved in a second set of reciprocal inhibitory interactions with the enkephalin-, neurotensin- and somatostatin-like immunoreactive amacrine cells (ENSLIACs). Here, dopamine, released by the DACs in the light, inhibits the ENSLIACs, and the enkephalins, released by the ENSLIACs in the dark, inhibit the DACs. A similar circuit may operate in primate retinas although the peptidergic element is different. In macaques, there are associational amacrine cells immunoreactive for both somatostatin and corticotropin releasing factor but not for the enkephalins. These cells co-stratify with DACs, and the majority of their contacts are with amacrine cells. In addition, somatostatin's effects resemble those of dopaminergic antagonists in rabbit retina (2,3). This PR:DAC:peptide triad exhibits two states. In the dark, the PRs and peptidergic amacrine cells are active, releasing melatonin and a combination of peptides while the DACs are inactive. In the light, the DACs release dopamine, while the PRs and peptidergic amacrine cells are inactive. The switch is flipped from the dark state to the light state by withdrawal of melatonin inhibition of the DACs plus light-induced drive to the DACs through an ON-bipolar cell pathway.

1. Besharse, J.C., et al. (1988) Progr. Ret. Res. 7, 21-61.
2. Jensen, R.J. (1992) Vis. Neurosci. 8:463-467.
3. Zalutsky, R. and Miller, R. (1990) J. Neurosci. 10:383-393.

CELL DIFFERENTIATION AND MIGRATION IV

414.1

CEREBELLAR GRANULE CELL DIFFERENTIATION IN MUTANT AND X-IRRADIATED RODENTS AS REVEALED BY THE NEURAL ADHESION MOLECULE TAG-1 D. Karagogeos*[1], K. Kyriakopoulou[1], N. Delhaye-Bouchaud[2], J. Mariani[2] and Y. Bailly[2]
1 Dept of Basic Sciences, P.O. Box 1393, University of Crete Medical School, Heraklion 711 10, Crete, Greece
2 Laboratoire de Neurobiologie du Developpement, CNRS URA 1488, Universite P. & M. Curie, Paris 75005, France

In the external granular layer (EGL) of the cerebellum, the granule cell (GC) precursors express the axonal glycoprotein TAG-1, a molecule involved in adhesion and neurite outgrowth. GC express TAG-1 transiently just as they extend neurites prior to migrating over the radial glia. We studied *in vivo* models in which Purkinje and/or GC defects occur during postnatal development to test whether such defects influence the premigratory phase of TAG-1 expression. These models include the cerebellar mutant mice *staggerer* and *lurcher* as well as rats irradiated during early postnatal development.

Neither alterations in Purkinje cell differentiation nor the related GC loss in the mouse mutants impair the ability of the surviving GC precursors to express TAG-1. Also, early GC loss in the X-irradiated rats does not alter the normal pattern of TAG-1 expression in the surviving precursors. In the adult cerebellum of X-irradiated rats, ectopic GC remain located superficially in the molecular layer and stop expressing TAG-1 beyond the first 3 weeks of postnatal development.

Thus, the transient expression of the molecule is maintained as long as the GC precursors are in a postmitotic, premigratory state, even if this state is prolonged as is the case in sg/sg. We suggest that TAG-1 expression is regulated primarily by factors intrinsic to the GC and is unaffected by epigenetic events that kill the GC without altering their gene expression program.

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414.2

GENE EXPRESSION WITHIN THE DEVELOPING RAT CEREBELLUM. **V. Narayanan*, B. Ripepi, S. Olinsky, and W. Weng. Department of Pediatrics, Neurology, and Neurobiology, University of Pittsburgh, Pittsburgh, PA.**

The most numerous of the cell types in the cerebellum, the granule cells, are derived from the external germinal layer, a structure that persists until P21 in the rat. The goal of our research is to isolate and characterize molecules that are expressed at specific stages of granule cell development, and eventually study the genetic control of neuronal differentiation. We ablated the cerebellar granule cells and the germinal cells by low dose X-irradiation of newborn rat pups. We next prepared a subtracted cDNA library (CONTROL - IRRADIATED), the expectation being that this would be enriched in genes expressed in the granule cells. We have identified 20 genes that are differentially expressed (by Northern blot analysis), about half of these being novel genes and the rest corresponding to cloned cDNAs. We present here our in situ hybridization data on the cellular distribution of mRNAs corresponding to two of these genes, that may have important functions in the cytoskeletal rearrangements that accompany granule cell differentiation. The first (clone GCE.B5) is the rat MARCKS cDNA, which encodes a protein kinase C substrate. MARCKS is expressed in a variety of cells within the cerebellum and cerebrum, including the granule cells, cells within the Purkinje layer, as well as in the hippocampus. MARCKS is known to interact with calmodulin and the cytoskeleton, and this interaction is modified by phosphorylation. The second gene we have studied (clone GCA.H2) corresponds to elongation factor-1α. This gene is strongly expressed in the external germinal layer, and is down-regulated during cell differentiation and migration. It has recently been determined that this protein is involved in the dissolution of microtubules.

414.3

THE EXPRESSION OF MEF2 GENES IS IMPLICATED IN NEURONAL DIFFERENTIATION X. Lin* and R.F. Bulleit Dept. of Pharmacology, Univ. of Maryland Sch. of Med., Baltimore, MD21202

We cloned a cDNA encoding the mouse homologue of the transcription factor MEF2A from a postnatal mouse cerebellar cDNA library. In the adult mouse, MEF2A RNA expression occurs throughout the brain with high levels of expression in olfactory bulb, hippocampus and cerebellum. A similar pattern of protein expression was observed in immunostaining of brain sections using a polyclonal antibody generated against 21 amino acids at the C-terminal of MEF2A. Because the polyclonal antibody may crossreact with MEF2C and MEF2D isoforms, we also examined the expression of RNA for these two isoforms. By using PCR we obtained mouse cDNA clones of MEF2C and MEF2D. The pattern of MEF2D RNA expression resembles MEF2A in adult mouse brain; MEF2C differs with a high level of RNA expression in the cortex and a low level of expression in the cerebellum. In developing cerebellum, MEF2A and MEF2D RNA levels increase after birth reaching a peak at postnatal day 12 and are maintained in adults. More interestingly, this time course of MEF2A and MEF2D RNA expression coincides with that of GABAα6, a marker of the mature cerebellar granule cell phenotype. We further observed by using the polyclonal antibody, in the developing cerebellum, that MEF2 expression only occurs after granule cells have migrated into the internal granule layer(IGL). Thus, like in skeletal muscle, the products of MEF2 genes may be involved in determining the mature phenotype of cerebellar granule neurons. MEF2A and MEF2D may be particularly important in turning on a set of neuronal specific genes in these cells.

414.4

GRANULE NEURONS OF THE MIGRATION DEFICIENT WEAVER MOUSE MUTANT LACK A FUNCTIONAL NMDA-RECEPTOR. **Päivi Liesi* and Jerry M. Wright. LMCN, NIAAA, NIH, 12501 Washington Ave, Rockville, MD 20852.**

The cerebellar granule neurons of the homozygous weaver mouse mutant fail to migrate and die within the first two weeks of postnatal life. The weaver gene is mapped in the chromosome 16 in the mouse, but the defective gene(s) has not yet been identified.

P7-P9 normal and homozygous weaver granule neurons were cultured on a laminin substratum for 24-48 hrs. Within this time period, the normal granule neurons extended long neurites whereas the weaver granule neurons showed impaired neurite outgrowth. Immunocytochemistry for NMDAR1 subunit (mouse Σ1) of the receptor showed expression of the R1-subunit in both the weaver and the normal granule neurons. The mRNA levels for the NMDAR1 subunit were comparable in the normal and homozygous weaver mutant brain tissues. *Patch clamp studies showed, however, that the homozygous weaver* granule neurons differed from their normal counterparts with respect of the expression of a functional NMDA receptor. The normal neurons expressed a functional NMDA receptor whereas the homozygous weaver neurons failed to express the functional receptor.

These results indicate that the migration deficiency of the weaver granule neurons may be related to their lack of expression of the functional NMDA receptor. The mechanisms of neuronal dying and subunit composition of the NMDA receptor of the normal and homozygous weaver mouse cerebellum are further discussed.

414.5

MORPHOLOGICAL AND IMMUNOCYTOCHEMICAL STUDIES OF CEREBELLAR GRANULE CELLS ASSOCIATED WITH INHIBITION OF MIGRATION INDUCED BY K252a IN VITRO. S. Tanaka, S. Kobayashi and T. Shirao* Department of Neurobiology and Behavior, Gunma University School of Medicine, Maebashi, 371, Japan.

We examined the migration of cerebellar granule cells on laminin in primary reaggregation cultures. we found that K252a, a potent protein kinase inhibitor, specifically inhibited the migration through signal pathway other than protein kinase A, protein kinase C and CaM kinase II. In the absence of K252a, neurites extended from aggregations of granule cells and the cells migrated on the neurites. Most of the migrating cells were small spindle shaped. Immunocytochemical study demonstrated that both of actin filament and actin-binding protein drebrin were co-localized in submembranous regions of migrating cells. While in the presence of K252a, migration was significantly inhibited. Within five minutes after the addition of K252a, the shapes of most migrating cells started to change their forms. Within thirty minutes migrating cells slipped off the neurites and their shapes changed into large polygonal shapes with many filopodia-like process. Immunocytochemical study demonstrated that drebrin distributed diffusely in the cytoplasm, while actin filament formed stress fiber-like structures. These results suggested that the signal transductions in the cells for the dissociation of drebrin from actin filament were associated with protein kinase. Then actin filament formed into stress fiber-like structures, and thereby the migration was inhibited.

414.6

LAMININ-1 IS NON-PREFERRED AND ANTI-ADHESIVE FOR DEVELOPING CEREBELLAR CELLS. B. Lom* and P. E. Hockberger. Northwestern Univ., Chicago, IL 60611. Previous studies have indicated that the extracellular matrix molecule laminin-1 (EHS laminin) may be involved in guiding granule cell migration along radial glial fibers in the postnatal rodent cerebellum. This conclusion was based primarily upon three observations: *in situ* immunolocalization of laminin-1 along Bergmann glial fibers, *in vitro* studies showing that cerebellar neurite outgrowth, cell adhesion, and cell migration were enhanced on laminin-1 coated surfaces when polyamino acids were present, and inhibition of neurite outgrowth and cell migration in culture by laminin-1 antibodies. Other immunostaining and antibody blocking observations have produced contrary results. We have reinvestigated these observations and found that laminin-1 was not associated with radial glia, but rather, immunolocalized to blood vessels and meninges in postnatal cerebella by three different laminin antibodies. In culture, it was an anti-adhesive and non-preferred substrate for developing cerebellar cells, and laminin-1 antibodies did not influence cell adhesion or preference. Specifically, dissociated cerebellar cells adhered poorly to culture surfaces coated with laminin-1, compared with collagen IV, fibronectin, simple amines, or glass. Avoidance of laminin-1 regions on chemically patterned culture surfaces was not affected by the addition of laminin-1 antibodies. In comparison, neuroblastoma cells preferred laminin regions over glass and this preference could be eliminated by the addition of laminin-1 antibodies. Cerebellar cells preferred and adhered to laminin-1 regions only when it was combined with polylysine. These results suggest that laminin-1 acts as an anti-adhesive, non-preferred molecule for developing cerebellar cells.

414.7

DYNAMICS OF CALCIUM OSCILLATIONS IN NEURONAL CELL MIGRATION. H. Komuro* and P. Rakic, Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510

During neuronal cell migration, extension of the leading process and translocation of cell soma are regulated by a cooperated activity of voltage- and ligand-gated channels (Komuro & Rakic, Science **257**: 806, 1992; **260**: 95, 1993). Moreover, the movement of migrating neurons is characterized by alternations of short stationary phases with saltatory forward or backward movement (Komuro & Rakic, J. Neurosci. **15**: 1110, 1995). Here, we examined the role of intracellular calcium ions as candidate intracellular second messengers that may control saltatory movement of migrating neurons. Rectangular pieces of cerebella from 2-6 day-old mice were placed on poly-L-lysine and laminin-coated Petri dishes containing serum-supplemented culture medium. After a 2-3 day incubation, cells were bathed in serum-free, defined culture medium with 1-3 μM Fluo-3/AM, Ca^{2+} indicator dye, for 30 min at 37 °C. After rinsing with defined culture medium, changes in the rate of cell movement and the levels of intracellular Ca^{2+} of migrating granule cells were examined using confocal laser scanning microscopy. During saltatory movement, itracellular Ca^{2+} levels of cell somas spontaneously increased an average of 14.2 ± 3.2 % of baseline intensity, for an average duration of 1.3 ± 0.4 min, with average frequencies of 13.2 ± 3.6/hr. This frequency is comparable to the cycle of saltatory movements of cell soma. Fast-moving cells exhibited a larger amplitude and higher frequency of Ca^{2+} oscillations than slow-moving cells. Blockade of Ca^{2+} influx by antagonists to voltage- and ligand-gated channels depressed these oscillatory responses, and resulted in a retardation of cell movement. Blockade of Ca^{2+} release from intracellular Ca^{2+} stores slightly decreased the amplitude of Ca^{2+} elevations and slowed cell movement. Moreover, Ca^{2+} levels within leading process of migrating cells fluctuated spontaneously; although the amplitude and frequency of Ca^{2+} oscillations of the leading process differed from that of the cell somas. Therefore, our results suggest that the amplitude and frequency of Ca^{2+} oscillations of the somas are positively correlated with the saltatory movement of migrating cell. (Supported by NS22807)

414.8

NMDA-MEDIATED DEPOLARIZATION CAN ACT AS A STOP SIGNAL DURING NEURONAL MIGRATION *IN VITRO* TAURINE MODULATES. E. Trenkner*, C. Harris, W. Hou, S. Gartenberg. NYS Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

Early postnatal mouse cerebellar granule cells (GC) migrate *in vitro* as they would *in vivo* under 'non-depolarizing' conditions. Here, we have studied granule cell migration after 2 DIV in the presence of various concentrations of depolarizing reagents NMDA, glutamate and their antagonists APV, MK801, CCP as well as K^+. We found that NMDA in concentrations higher than 10^{-4}M arrested ~85% of migrating granule cells in less than 3 min. Similarly, K^+ inhibited migration in osmolarity corrected depolarizing conditions (> 7 mM) in our medium (BME, HS, 2 μM Ca^{2+}). While the effect of K^+ could not be prevented, migration continued with NMDA-antagonists for more than 16 h. Furthermore, after pre-treatment of cultures with Ca^{2+} ionophore A23187 as well as with taurine, GC continued to migrate. We also demonstrate that physiological concentrations of taurine regulate intracellular Ca^{2+} concentrations to approximately 3-5 μM during NMDA or glutamate induced depolarization, suggesting that intracellular Ca^{2+} concentrations determine the degree of GC migration. K^+-depolarization was uneffected. We propose that both the expression of NMDA type glutamate receptors on migrating GC and the event of depolarization through synapse formation with incoming mossy fibers determine the spatial distribution of individual granule cells after migration. Taurine seems to play a crucial role in modulating or even controlling intracellular Ca^{2+} concentrations and thus the extent of migration.
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414.9

A ROLE FOR THE CELL ADHESION MOLECULE FASCICLIN II DURING NEURONAL MIGRATION IN THE INSECT ENTERIC NERVOUS SYSTEM. P.F. Copenhaver*, M. Snyder, and A. Horgan, Cell Biology L215, Oregon Health Sciences U., Portland, OR 97201.

The directed migration of embryonic neurons in the developing nervous system requires the specification of their migratory pathways by extrinsic guidance cues. In the enteric nervous system (ENS) of the moth, *Manduca sexta*, this process guides a population of ~300 neurons (the EP cells) along a well-defined set of visceral muscle bands that form on the gut surface during embryogenesis. We have now found that both the EP cells and these muscle bands express a protein related to the cell adhesion molecule fasciclin II (named MFas II), an immunoglobulin-related cell adhesion receptor. Antisera against this protein stained both the EP cells and the muscle bands during the migratory period. An affinity-purified fraction of this antiserum caused a dose-dependent inhibition of migration in embryo culture. Microsequence data obtained from the purified protein revealed strong homology with forms of fasciclin II that have been described in other species. Immunoblot analyses of proteins extracted from the developing ENS revealed several putative isoforms of MFas II, including a ~91 kd form that (by analogy to fasciclins in other species) may be a glycosyl phosphoinositol-linked form of MFas II. Brief exposure of the developing ENS to PI-specific phospholipase C (but not other phospholipases) caused a loss of MFas II immunostaining in the ENS, a defasciculation of the visceral muscle bands, and a dose-dependent inhibition of EP cell migration. We are currently cloning the moth form of MFas II to clarify the role of this cell adhesion molecule in the guidance of EP cell migration both *in vitro* and *in vivo*. Supported by NIH # MH51833-01 and a grant from the MRF of Oregon.

414.10

PARTICIPATION OF THE HETEROTRIMERIC G PROTEIN Go AND POTENTIAL DOWNSTREAM EFFECTORS IN REGULATING NEURONAL MIGRATION. A.M. Horgan* and P.F. Copenhaver, Cell Biol. & Anat. L215, Oregon Health Sciences U. Portland, OR 97201.

The intracellular signaling molecules responsible for regulating neuronal migration in an embryo are poorly understood. In the enteric nervous system of *Manduca sexta*, ~300 neurons (the EP cells) migrate along defined pathways to form the enteric plexus. One member of the heterotrimeric G protein family, Go_α, is expressed in the EP cells coincident with their migration. Manipulations of G protein activity in a semi-intact embryonic preparation suggests an inhibitory role for this signaling molecule during migration. Intracellular injections of mastoparan caused a marked decrease in migration, whereas injections of pertussis toxin (PTX) had no effect. However, when the toxins were co-injected, the inhibitory effect of mastoparan was eliminated. Two likely candidate downstream effector systems that may be affected by G_O are intracellular calcium and components of the cytoskeleton. When intracellular calcium was elevated in the EP cells, we saw an inhibition of migration, but neuronal motility was not obviously affected by injections of the calcium buffer BAPTA. We are now examining whether G_O stimulation requires intracellular changes in calcium to down-regulate migration. Increased polymerization of both actin and tubulin have also been shown to coincide with EP cell migration, while transient exposure to cytochalasin D inhibited migration. We are now investigating whether Go stimulation in the EP cells acts via the regulation of specific classes of actin binding proteins in the course of neuronal migration. Supported by NIH # MH51833-01 and a grant from the MRF of Oregon.

414.11

MIGRATION OF OLIGODENDROCYTE PRECURSORS ON ASTROCYTES AND MENINGEAL CELLS

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During development oligodendrocyte precursors migrate extensively through a CNS environment consisting mainly of astrocytes and neurones. However in some demyelinating conditions such as MS, remyelination fails in a predominantly astrocytic environment. In order to determine whether failure of oligodendrocyte precursor migration might be responsible we have examined *in vitro* the effects of astrocytes and meningeal cells on migration.

Migration rates were measured by time lapse microscopy, and by attaching precursors to shards of coverslip, inverting them on the test surface, and observing migration distances. Oligodendrocyte precursors and the CG4 and Oli-*neu* precursor cell lines migrate rapidly on laminin, very slowly on mature astrocytes and meningeal cells, but rapidly on embryonic astrocytes and EHS sarcoma cells. Astrocyte extracellular matrix, whether derived from mature cells, astrocyte cell lines whose matrix is inhibitory to axon growth or embryonic astrocytes, promotes migration as well as laminin, and migration on laminin is not affected by astrocyte conditioned medium. No correlation was found between adhesiveness and rate of migration. Time lapse microscopy showed that oligodendrocyte precursor contacts with meningeal cells were followed by rapid collapse and withdrawal of the process, while contacts with astrocytes were long-lasting, with flattening of the contacting process

414.12

GLIAL PROGENITORS FROM THE POSTNATAL SUBVENTRICULAR ZONE MIGRATE ALONG RADIAL GLIA *IN VITRO*. S.R. Newman, M. Zerlin, and J.E. Goldman*. Dept. Of Pathology, Columbia Univ., College of Physicians & Surgeons, NY, NY 10032.

Glial cells of the mammalian forebrain arise from the subventricular zone (SVZ), a germinal matrix that lies adjacent to the lateral ventricles. Migration of glial progenitors from the SVZ into gray and white matter may be guided by radial glia. *In vivo*, the collapse of radial glia coincides temporally with the inability of SVZ cells to migrate out into the cortex, and columns of SVZ-derived cells oriented perpendicularly to the pial surface have been observed in postnatal cortex. We report here that postnatal SVZ-derived cells migrate along radial glial cables *in vitro* using a model system of postnatal SVZ cell development, combined with high resolution time-lapse video microscopy and immunocytochemistry. We establish cultures from early postnatal rat forebrain in which aggregates of SVZ cells are connected by cables made up of processes that resemble radial glia, morphologically and antigenically. Immature cells migrate along cables, and even translocate between neighboring tracks. The cells that are apposed to the cables are unipolar and bipolar, and many express the immature neuroepithelial cell markers GD3 and A2B5. In addition, cells that express the oligodendrocyte (OL) markers O4 and O1 also align along the cables. The O4+ and O1+ that align along the cables display a unipolar or bipolar morphology, in contrast to the complex, branched O4+ and O1+ cells that lie directly on the culture dish. This difference in morphology between OL progenitors associated with cables and those on the culture substrate suggests that contact with the cable inhibits the morphological transformations of the progenitors. We suggest a model in which postnatal SVZ cells migrate along radial glia into gray and white matter. Radial glia may provide a permissive, preferential track that allows intrinsically migratory cells to disperse throughout the CNS.

414.13

EXPRESSION OF 9-O-ACETYLATED GANGLIOSIDES IS CORRELATED WITH TANGENTIAL CELL MIGRATION. R. Mendez-Otero and L. A. Cavalcante*. Depart. Neurobiology, Instituto de Biofisica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ 21941-590, Brazil.

9-O-acetylated gangliosides (9-O-aG) are expressed in regions of the developing brain during radial neuronal migration such as occurring during histogenesis of the cerebral cortex and the cerebellar granular cell layer (Mendez-Otero et al., J. Neurosci., 8: 564, 1988). In addition, we have demonstrated that an antibody against 9-O-aG (Jones mAb) interferes with axonal growth (Mendez-Otero et al., Soc. Neurosc. Abs., 18:36,1992) . To determine the generality of 9-O-aGs expression in directional movements, we have examined 9-O-aG immunoreactivity in the cell stream from the telencephalic subventricular zone (SVZ) to the olfactory bulb in developing and adult rats. Our results demonstrate that Jones antigens are expressed in the subventricular zone of the lateral ventricle of developing (postnatal) and adult rats and along the pathway purported to be followed by newly generated cells to their final destination in the olfactory bulb. Our present observation thus support the hypothesis that 9-O-acetylated gangliosides are expressed by neurons capable of dynamic changes and may contribute to the molecular mechanisms permiting cell migration not only during development but also in the adult.

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414.14

A SEX COMPARISON OF NEURONAL MIGRATION INTO THE FERRET SEXUALLY DIMORPHIC PREOPTIC AREA/ANTERIOR HYPOTHALAMUS. J-J. Park*, R.G. Paredes, M.J. Baum and S.A. Tobet. Dept. of Biology, Boston University, Boston, MA 02215 and The Shriver Center, Waltham, MA 02254

The sexually dimorphic male nucleus of the dorsal preoptic area/anterior hypothalamus (Mn-POA/AH) is formed in male ferrets due to the action of estradiol during the last quarter of the 41-day gestation. We (Tobet et al., J. Neurobiol. 26:75, 1995) previously suggested that neurons migrate along radial glial guides into the POA/AH from proliferative zones lining the lateral as well as the third ventricles. We attempted to compare the routes and rate of this migration by a cohort of cells born in male and female fetuses on embryonic(E) day 24. Fetuses were injected with the thymidine analog, bromodeoxyuridine (BrdU) on E24 and subsequently killed on E30, E34, or E38. Brains were sectioned and processed immunocytochemically for BrdU as well as glial fibrillary acidic protein (GFAP), which is found in radial glial fibers. The distribution of BrdU immunoreactivity throughout the POA/AH was analyzed by quantitative image analysis in equivalent brain sections of ferrets of each sex killed at these fetal ages, which bracket the period when the Mn-POA/AH is formed in males. The results support our previous suggestion that neurons migrate into the dorsal POA/AH from proliferative zones lining the lateral as well as the third ventricles. There was no indication, however, that the migration of cells born on day E24 differs in males and females. Thus the action of estradiol in promoting the formation of the male's Mn-POA/AH may depend on other processes, such as cell specification or differential cell death. (supported by HD21094 & MH00392 to MJB and IBN94-21697 to SAT)

414.15

MULTIPLE CLASSES OF GABA RECEPTORS MEDIATE EMBRYONIC RAT CORTICAL CELL MOTILITY *IN VITRO*. T.N. Behar*, C.A. Scott, and J.L. Barker. Lab. of Neurophysiology, NINDS, NIH, Bethesda, Md. 20892

Embryonic rat cortical cell migration to μM GABA was analyzed *in vitro* using a chemotaxis chamber. GABA stimulated chemokinesis that was partially inhibited by saclofen and picrotoxin, but was unaffected by bicuculline. Agonists of $GABA_B$ (R-baclofen) and $GABA_C$ (CACA and TACA) receptors also stimulated chemokinesis. Baclofen-induced motility was only blocked by saclofen. Motility induced by CACA or TACA was blocked by picrotoxin, but not by bicuculline or saclofen. Bicuculline potentiated migration to CACA or TACA, suggesting that $GABA_A$ receptor activation may arrest chemokinesis in a subpopulation of cells. Whereas picrotoxin and saclofen each partially inhibited chemokinesis to GABA, significant levels of motility were completely attenuated in the combined presence of the two antagonists. Taken together, these results suggest that GABA's chemokinetic effects involve both $GABA_B$ and $GABA_C$ receptor proteins.

414.16

CELL DISPERSION PATTERNS IN THE MOUSE RETINA

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How retinal neurons acquire their phenotype is not yet determined. The cell's birth date, lineage, migration path and interactions with surrounding cells and the microenvironment may all influence its fate. The relative role of each of these factors remains controversial. We are interested in the relationships between cell lineage, their dispersion patterns and cell-cell interactions, and how these may determine cell fate.

We have generated a transgenic mouse carrying the *lacZ* gene on the X chromosome. In hemizygous female transgenic mice, clones of *lacZ* +ve or -ve cells are formed as a consequence of early X inactivation. In retina, each clonal cluster is composed of a radially-aligned column of cells spanning the retinal layers and invariably contains rod photoreceptors, bipolar and Muller glial cells. The remaining 15% of retinal neuroblasts, forming the ganglion, amacrine, horizontal and cone photoreceptor cells are tangentially displaced from the radial columns. As all seven cell classes share a common lineage those dispersion patterns must be linked to fate rather than lineage.

We are interested in identifying those forces which maintain the radial columns as a clonal cluster. Likely candidates are the cell-surface-expressed cell-cell adhesion molecules. We have focused on the cadherin superfamily using reverse-transcriptase PCR analysis of eye RNA and oligonucleotide primers directed towards conserved consensus sequences in the cytoplasmic domain. Of the currently known cadherins. E, K, OB-1, P, N, R and VE-cadherins are expressed at the mRNA level in the developing mouse eye. In addition, a novel cadherin most closely related to human cadherin 8 has been isolated from post-natal eyes. Differential expression of cell-cell adhesion molecules may determine the dispersion patterns of retinal neuroblasts.

This work was supported by the NH & MRC, the ARC and the NIH.

414.17

MIGRATION OF FACIAL MOTONEURONS IN THE DEVELOPING BRAZILIAN OPOSSUM BRAIN.

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The Brazilian opossum, *Monodelphis domestica*, is a small marsupial whose young are born in an extremely immature state with a protracted postnatal period of neurogenesis. We have previously shown that the facial motor nucleus (FMN) demonstrates a transient expression of cholecystokinin (CCK)-binding sites during development (Kuehl-Kovarik et al., Soc. Neurosci. Abstr. 19: 729, 1993). In addition we have also shown, using markers of synapse-associated proteins, that the FMN does not receive afferent innervation until postnatal day 15 (15 PN) and mature synapses are formed between 15 and 25 PN (Swanson et al., Soc. Neurosci. Abstr. 19: 644, 1993). In this study we have used postnatal injection of the retrograde tracer cholera toxin subunit B (CtB) and immunohistochemical detection of CtB to describe the migration of facial motoneurons in the developing brainstem. Injections of CtB were made into the cheek or lip region of opossum pups. Facial motoneurons in newborn opossum pups (1 PN) exhibited CtB labeling and their cell bodies were localized near the developing genu. At 3 and 5 PN, CtB labeled facial motoneurons were observed in and migrating to the region of the adult FMN. Between 7 and 10 PN, facial motoneurons had all migrated to their final destination. During postnatal days 1 through 5 the CtB labeled facial motoneurons appeared to migrate from the genu ventrally and laterally to where the motor nucleus will be located. These results indicate that facial motoneurons innervate their targets by 1 PN and migrate to their destination in the brainstem thereafter. Studies are underway examining the relationship of the location of facial motoneurons to the distribution of the markers of synaptic development.

414.18

DORSAL MIGRATION OF NEURONS OF THE "U-SHAPED" GROUP CAN BE PERTURBED IN SPINAL CORD SLICE CULTURES.

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Developing rat spinal cord contains a "U-shaped" group of neurons that surrounds the ventral 50% of the ventricular zone on E16 and that expresses both choline acetyltransferase and NADPH-diaphorase. Over a 2-3 day period, some of these cells appear to migrate into the dorsal horn *in vivo*. A relatively histotypic dorsal migration of the "U-shaped" group has been replicated in *in vitro* slice cultures of E16 cervical spinal cord. To attempt to perturb this migration, E16 slice cultures were *microsurgically divided into dorsal and ventral parts. After removing the* ventral cord, the isolated dorsal cord was cultured for 3 days. Both parts were processed for diaphorase histochemistry to determine the amount of ventral cord removed, as well as the presence and location of the "U-shaped" group. All dorsal cultures contained a late-forming medial cluster of diaphorase cells (Wetts *et al.*, Dev. Dyn. 202:215), but the number and position of cells derived from the "U-shaped" group varied with the amount of spinal cord excised. When the ventral 40% of cord was removed, numerous cells from the "U-shaped" group were present in dorsal cultures, but had disoriented processes and uncharacteristic locations. If over 55% of the spinal cord was removed ventrally, none of the diaphorase cells derived from the "U-shaped" group were present in dorsal cultures. Intermediate excisions produced a mixture of the above results. These findings demonstrate that the "U-shaped" group originates in ventral spinal cord, whereas the late-forming medial diaphorase cells originate dorsally. The disoriented processes and lack of migration of the "U-shaped" group may have resulted from the surgically induced loss of commissural axons, supporting our hypothesis that these axons may guide dorsal migration in spinal cord. *Supported by NIH grant NS 18858.*

CELL DIFFERENTIATION AND MIGRATION V

415.1

MIGRATION AND MATURATION OF CELLS OF THE EARLY NEOCORTEX IN RODENT CENTRAL NERVOUS SYSTEM MAINTAINED IN CULTURE.

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The entire central nervous system from the fetal rat (E15) has been shown to survive in culture for periods of up to 10 days. The details of the dissection and culture conditions have been published previously (Saunders et al, 1992. Proc. Roy. Soc. (B) 250, 171). The level of protein in the medium was adjusted to one which was similar to what would normally be present in the CSF of the fetal rat *in vivo*. After periods of up to 24 hours in culture preparations were fixed for light microscopy using Bouin's fixative and processed using histological and immunocytochemical techniques (Saunders et al, 1992. Anat. Embryol. 186, 477). During the first 24 hours ventricular zone cells of the neocortex, in the presence of 10% fetal calf serum (FCS), continued to divide and proliferate as they would *in vivo*. These newly formed cells were then able to migrate to more superficial regions of the neocortex. Although the neocortical wall had increased in thickness the cells accumulated outside the ventricular zone and there was no clearly defined cortical plate. However, one known marker for cells in the early cortical plate, fetuin, was identified in cells in the outer region of the cultured neocortex. The position of fetuin positive cells corresponded to that of the early cortical plate, *in situ*. Additionally, specific staining for fetuin, a protein derived from FCS, confirmed the integrity of the CSF/brain barrier even after 24 hours in culture. This was demonstrated by the total lack of fetuin and albumin (medium-derived) staining in the extracellular spaces of the brain. Although the overall morphological appearance of the neocortical wall was not preserved *in vitro* (E15 + 24 hours), the characteristic ability of early cortical plate cells to specifically take up fetuin was maintained in this model.

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415.2

GENETIC ENGINEERING OF HUMAN EMBRYONIC BRAIN CELLS BY RETROVIRAL VECTOR-MEDIATED GENE TRANSFER.

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In contrast to chemical and physical cellular transfection methods, replication deficient murine retroviruses have proven to be extremely efficient vectors to transfer genes into proliferating mammalian cells. Embryonic cells are highly mitotically active and can be transfected *in vitro* with retroviral DNA that integrates into the host genome and is subsequently expressed. We have established a procedure for the *in vitro* transduction of first trimester embryonic brain cells of different gestational ages in primary culture. An 80-100% transduction efficiency was detected when *E.coli LacZ* reporter gene expression was driven by the 5'LTR viral promoter, and the cell proliferation was stimulated by the addition of bFGF and/or TGF-alpha to the culture medium. Recombinant gene expression in cultured cells was qualitatively studied by use of a modified retroviral vector encoding nuclear expression of the bacterial ß-galactosidase enzyme, with subsequent X-gal staining. In long-term dissociated cell culture, X-gal co-staining with cytoplasmic cell type-specific markers revealed the expression of either neuronal or glial phenotypic markers in recombinant brain cells The temporal pattern of recombinant gene expression was studied by CPRG spectrophotometric analysis of cytoplasmic ß-galactosidase expression. Preliminary results from the current study indicate possible future clinical utility of *in vitro* genetically modified embryonic human cells for replacement cell therapy in neurodegenerative diseases including Parkinson's disease.

415.3

NEURONAL DEVELOPMENT AND MIGRATION IN EXPLANT CULTURES OF CEREBRAL CORTEX OF THE MOUSE.

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Explant cultures may be used to investigate mechanisms of neuronal migration. Fragments (250-350 µm thick) taken from the cerebral mantle of 16 day embryonic mouse (E16) were grown *in vitro* on a membrane with large pores. The tissue fragment was embedded in a collagen 'sandwich', with medium placed both above and below the membrane. The plates were rocked slowly so that the surface of the explants was intermittently exposed to the ambient environment (5% CO2 and 20% oxygen). At 7 days in vitro, culture thickness was on average 172 µm; other cultures were maintained for up to 14 days. Immunohistochemical staining for GluR1, a glutamate receptor subunit, and A-60, (NeuN, neuron-specific nucleoprotein), indicated a high density of viable neurons throughout the explant at day 7 and at day 14. Glial cells were plentiful as seen with GFAP immunohistochemistry. Explants also stained intensely for GAD, glutamic acid decarboxylase, a GABA precursor, and GABA at days 7 and 14 in culture. Neurons also stained positively for microtubule-associated protein (MAP-2) at day 7. Time-pregnant mice were injected the day prior to explanting with 5-bromo-deoxy-uridine (BrdU) which becomes incorporated into the thymidine of dividing cells. Using a histochemical reaction with an antibody to BrdU, labeled cells were found at E16 in the ventricular zone. At 7-14 days in culture, labeled cells were found throughout the cortical plate, indicating that the neuronal cells had migrated *in vitro*, although it was not possible to differentiate cortical layers. In summary, this culture system permits embryonic cortical tissue to survive and differentiate for up to two weeks *in vitro*, while neuronal migration is occuring. (Supported by the Bickell Foundation and by the Children's Hospital of Eastern Ontario Research Foundation.)

415.4

CELLULAR PROPERTIES OF LEECH RETZIUS NEURONS CO-CULTURED WITH VARIOUS TARGET TISSUES.

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Retzius (Rz) neurons of *Hirudo medicinalis* differentiate into one of two possible phenotypes--which differ with respect to peripheral target, central morphology, synaptic inputs, and response to ACh applied to the soma--depending upon the environment in which they develop. Normally the peripheral processes of Rz neurons in the 5th and 6th midbody segment [Rz(5,6)] contact embryonic reproductive ducts, whereas all other Rz neurons [Rz(X)] contact body wall muscles; ablating the reproductive ducts at 30-35% of development causes Rz(5,6) neurons to adopt the properties of Rz(X).

To explore the interaction between Rz neurons and their targets, we cultured adult Rz neurons with several target tissues and then evaluated their morphology and physiological responses. Rz neurons were dissected from standard or reproductive ganglia and placed into culture, either in isolation on concanavallin A or directly on top of a target: body wall muscle, juvenile male reproductive duct, juvenile female reproductive duct, or germinal plate from an embryo at 30-35% of development. Cultures were maintained 3-17 days; then each neuron was impaled, its electrical properties and response to ACh were recorded, and it was filled with Lucifer Yellow. Filled cells were treated with anti-Lucifer antibody and HRP-conjugated second antibody, drawn with *camera lucida*, and digitized; the length of the processes was then measured. As a control, pressure-sensitive mechano-receptor neurons (P cells) were cultured under the same conditions.

Rz(X) and Rz(5,6) neurons responded to the targets similarly. Neurons grew more processes on some targets than on others, in the order: germinal plate > female duct > male duct > body wall. Surviving neurons responded to ACh, even after 17 days in culture, and no neuron changed the sign of its response to ACh, whether it was grown on its usual target or on the "wrong" target and regardless of its process length. We conclude that the ACh response of Rz neurons is a very robust cellular property that is maintained even when the cells produce large amounts of new cell membrane. Supported by NIH Grant NS25916.

415.5

CYTOSKELETAL DYNAMICS IN GROWTH CONE RETRACTION INDUCED BY THE mAb JONES H.M. Araujo, and R. Mendez-Otero*, Instituto de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro,RJ 21941-590 Brasil.

During development axons must find their targets navigating through environments with both atractive and repulsive cues. The ganglioside 9-O-acetyl GD3 may be one of these cues (Mendez-Otero et al., J.Neurosci. 8:564-579, 1988). Through blockage of this ganglioside with the JONES mAb, axonal migration is inhibited and growth cone collapse can be seen in primary culture. We have treated dorsal root ganglion (DRG) neurons *in vitro* with JONES, as a model to investigate intracellular events mediated by the ganglioside. We have utilized cytoskeleton-interfering drugs to characterize cytoskeletal filaments target or driving growth cone retraction. Adding taxol or nocodazole to the culture medium we show that microtubule rearrangements play an important role in JONES-induced retraction. On the other hand, cytochalasin D, which depolymerizes F-actin, induces axonal retraction with a pattern similar to that seen with JONES. We were able to establish two domains of JONES action: the proximal portion of the axon where rearrangements are dependent of microtubule dynamics, and the distal portion where microfilaments have an important role. We conclude that JONES-induced retraction involves changes in both tubulin and actin-based filaments.

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415.6

INCOMPLETE TARGET SEGREGATION OF FACIAL, VESTIBULAR AND COCHLEAR EFFERENTS IN RODENTS. D.H. Nichols*, J.D. Kingsley, L.L. Bruce and B. Fritzsch, Creighton Univ., Dept. Biomed. Sci., Omaha, NE 68104.

Otic efferents of many non-mammalian vertebrates share both fiber pathways and perikaryal position with facial motoneurons. Moreover, developmental studies in chicken and mice showed that postmitotic efferents and facial motorneurons first colocalize then segregate by differential migration (Fritzsch et al., 1993, J. Neurobiol. 24: 1481). Here we report that subpopulations of rodent facial motor, vestibular and cochlear efferent neurons possess collaterals to each other's target areas. Specifically, DiI applied to the facial nerve distal to the geniculate ganglion labeled collaterals to the horizontal canal and occasionally the utricle. A few contralateral facial motoneurons were labeled as well. Applications of DiI to the: 1)utricle labeled collaterals to the facial nerve, saccule and posterior vertical canal and 2)saccule labeled collaterals to the first turn of the cochlea. These saccular collateral branches had terminal swellings in the vicinity of spiral ganglion cells. Thus, subpopulations of otic efferent and facial branchiomotor neurons project to the same peripheral targets during development until at least postnatal day 9. This branching pattern varied quantitatively between individuals.

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415.7

SHORT EXPOSURE TO METHYLAZOXYMETHANOL (MAM) CAUSES A LONG-TERM INHIBITION OF AXONAL OUTGROWTH FROM CULTURED EMBRYONIC RAT HIPPOCAMPAL NEURONS. J.R. Hoffman*, L.J. Boyne, P.R. Levitt, I. Fischer. Beaver College, Glenside PA, 19038; Med. Coll. of Penn. & Hahnemann Univ., Phila. PA, 19129; UMDNJ-Robert Wood Johnson Med. Sch., Piscataway NJ, 08854.

MAM is an antimitotic, alkylating agent that is used to induce microencephaly, but its direct effects on developing neurons are not known. We examined the outcome of short exposure to MAM on postmitotic embryonic hippocampal cultures during the early stages of establishment of axonal polarity. At 0, 1, or 2 days in vitro (DIV), neurons were treated with 0.1nM-1uM MAM for 3 hr and then transferred to glial conditioned media. At 3 DIV, the cells were fixed and analyzed by immunofluorescent staining for viability and neurite differentiation. Control cells initiate several minor processes; one process elongates rapidly at about 1 DIV eventually becoming an axon, while extensive dendritic growth occurs after 3-4 DIV. Neurons treated with 1uM MAM at 0 or 1 DIV showed a withdrawal of axons and marked inhibition of neurite growth without affecting cell viability. Cells achieved only minimal recovery of neurite length at 7 DIV, even when transferred to a glial co-culture. In contrast, cells treated initially with MAM at 2 DIV showed no effect on axonal growth. To determine the effects of MAM on the neuronal cytoskeleton, we performed *in vitro* microtubule assembly assays using brain extracts. There were no changes in the total amount of microtubules assembled in the presence of MAM. However, MAM caused a decrease in the coassembly of MAP2, but not MAP1b, with microtubules. These results demonstrate that MAM can directly affect developing neurons, indicating that an early disruption of axonal outgrowth can have long-term effects.

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415.8

GRANULE NEURON REGULATION OF PURKINJE CELL DEVELOPMENT: ROLE OF NEUROTROPHINS AND C-AMP. M.E. Morrison, R.C. Marcus*, and C.A. Mason. Dept. of Path., Coll. P & S. Columbia Univ., NY, NY 10032.

Previous work in our laboratory established a method for purification of cerebellar Purkinje cells (Baptista et al., 1994, Neuron 12:243), and demonstrated that granule neurons, presynaptic afferents of Purkinje cells, are potent regulators of Purkinje cell differentiation. Purified Purkinje cells cultured alone survive poorly, never developing dendrites, while Purkinje cells cocultured with granule neurons undergo full dendritic development. This model system allows us to identify factors supplied by granule neurons that induce Purkinje cell differentiation in vitro. Purkinje cell survival and development were assessed in three culture combinations: purified Purkinje cells cultured alone or with purified granule cells, or unpurified Purkinje cells in cultures of whole dissociated cerebellum. Neurotrophins were included in the culture medium at all times. Cultures were fixed at various times up to 3 weeks in vitro, and Purkinje cells were visualized with an antibody against calbindin. Results to date indicate that in cultures from perinatal animals, NT-3 and CNTF decrease Purkinje cell survival, while BDNF improves Purkinje cell survival and may accelerate dendritic spine development when Purkinje cells are cocultured with granule neurons. Preliminary results from embryonic and perinatal mixed cultures also reveal a developmental switch in Purkinje cell neurotrophin responsiveness.

Addition of a chlorophenylthio derivative of cAMP to the culture medium increased the survival of purified Purkinje cells, and enhanced dendritic development of purified Purkinje cells cultured with granule cells. Experiments are under way to test the specificity of these effects, and to examine the hypothesis that cAMP is one of the second messengers involved in granule cell-triggered Purkinje cell dendritic development.

(Growth factors generously provided by Dr. G. Yancopoulos, Regeneron. Supported by NIH grant NS16951 (CAM) and NRSA Award NS09864 (MEM).)

415.9

ROLE OF TARGET CELLS IN THE SURVIVAL AND DIFFERENTIATION OF PURIFIED PURKINJE CELLS IN VITRO. C. Carroll, M.E. Morrison, and C.A. Mason*, Dept. Path., Coll. P&S, Columbia Univ., NY, NY 10032.

Cell-cell interactions between afferents and their target neurons comprise a general class of mechanisms controlling cell survival and differentiation. Previous results from our lab (Baptista et al., 1994, Neuron 12:243) show that in isolation, Purkinje cell survival is poor, but is improved by neuronal contacts with either purified granule neurons or with high densities of other Purkinje cells. Moreover, proper Purkinje cell differentiation is driven only by co-culture with granule cells, the presynaptic afferents of Purkinje cells, producing dendrites with spines receiving synapses.

To address the role of postsynaptic or target cell influences on Purkinje cell survival and differentiation, we cocultured purified rodent Purkinje cells with their target cells, the deep cerebellar nuclei. Results to date show a twofold increase in the survival of Purkinje cells cocultured with enriched cell fractions of deep nuclei neurons, compared to survival of Purkinje cells cocultured with granule neurons. Furthermore, Purkinje cell axonal outgrowth and arborization is enhanced by coculture with deep nuclei cells, even though dendritic differentiation does not progress beyond immature forms. These results suggest that targets of the Purkinje cells, the deep cerebellar nuclei, regulate Purkinje cell survival and axonal, but not dendritic, outgrowth. Experiments are in progress to further characterize the relative contributions of pre- and postsynaptic cells at different developmental stages to the survival and differentiation of the Purkinje cell, and to assess the potential contribution of growth factors from the deep nuclei targets.

Supported by NIH grant NS16951 to CAM.

415.10

NEUROTROPHIN-3 IS ESSENTIAL FOR THE DEVELOPMENT OF THE CHICK RETINA. A. Rodríguez-Tébar*, J.M. Frade, E. Martí, M.A. Rodríguez-Peña and P. Bovolenta. Instituto Cajal, CSIC. Madrid, Spain, 28002.

Chicken embryos treated with a monoclonal antibody specifically blocking the activity of neurotrophin-3 (NT-3) showed severe alterations in the development of the retina, presenting abnormalities in all layers. Early in retinogenesis, fewer differentiated neurons were identified in NT-3 deprived embryos. The impairment of differentiation affected all types of neurons, and the undifferentiated precursors proliferated for longer. Accordingly, optic nerves from treated embryos contained 30-50% of axons with respect to controls. At earlier developmental ages, NT-3 is mostly located in the pigment epithelium, as assesed by both immunohistochemistry and northern analysis. As development proceeds, NT-3 expression shifts towards the neural retina. The NT-3 receptor trkC is expressed early in development and was found in most undifferentiated cells of the neural retina.

Together this data indicates that NT-3 is a decisive factor for differentiation and the survival of the different phenotypes of retinal neurons, and for the control of the final cell number in the vertebrate retina.

415.11

ROLE OF CYTOKINES IN NEURODEVELOPMENT: RELEVANCE TO DEVELOPMENTAL MODEL OF SCHIZOPHRENIA. B.Garimella, A. Jazayeri, C.M.Spangler, K.Essani and K.M.Merchant[1]*, Dept of Biological Sciences, Western Michigan Univ., and [1]CNS Diseases Research, The Upjohn Co., Kalamazoo, MI 49001

The role of a cytokine in neurodifferentiation is evident in earlier studies demonstrating that IL-6 exposure of PC12 cells produces morphological and biochemical markers of differentiation (neurite outgrowth, c-*fos* gene expression, and expression of Na^+ channels) similar to that seen after NGF treatment. The present study characterized further the neurodifferentiation effects of IL-6 and other cytokines. Exposure of PC12 cells to IL-6 (300 to 1000 ng) in the absence of serum did not appear to have a significant mitogenic effect as detected by uptake of ^{3}H-thymidine. However, uptake of ^{3}H-methionine was increased 8- to 10-fold after IL-6 treatment in the absence of serum. Interestingly, in the presence of IL-6, PC12 cells also remained viable for 8-10 d in the absence of serum. These data support the role of IL-6 in differentiation. Neurodevelopmental effects of immune system components were also examined *in vivo* by endotoxin treatment (S. enteritidis, 0.5 µg/rat, i.p.) of rat pups on postnatal days 1, 3 and 5. We compared the expression of genes thought to be involved in apoptotic processes or differentiation (Bcl-2, Bax, c-*fos*) in distinct brain regions in rats pretreated with endotoxin or vehicle at various times (2, 4, 6, 14 or 21 days) after the treatment. Finally, the relationship between developmental abnormalities produced by immune system modulation and psychotic disorders was evaluated by examining the responses of endotoxin-treated rats to amphetamine and clozapine. (Supported by Scottish Rite Research Foundation and Stanley Research Foundation).

415.12

IN VIVO INJECTION OF FGF2 INCREASES BRAIN WEIGHT AND CORTICAL VOLUME IN RAT EMBRYOS. F. M. Vaccarino*, D. L. Stull and M. L. Schwartz. Child Study Center and Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06520.

Growth factors may regulate both the number and the commitment of progenitor cells to different lineages. We have previously reported that basic fibroblast growth factor (FGF2) increases the number of neurons containing glutamate, but not of those containing GABA, in primary cultures of embryonic telencephalon (Vaccarino et al, 95). This action is due in part to an increase in the proliferation of progenitors which is selective for the glutamate lineage. To test whether FGF2 exerts a similar effect *in vivo*, FGF2 was microinjected into the cerebral ventricles of E15.5 rat embryos. FGF2-injected fetuses (N=10), sacrificed at E21, had a mean increase in cerebral weight of 6% compared to littermate controls (N=13) ($p<0.05$). In addition, the volume of the cerebral cortex of FGF2-injected fetuses was increased by 5-13% over that of control littermates due to an increased thickness of both the intermediate zone and cortical plate. To identify the cells responding to FGF2, *in situ* hybridization was used to determine the distribution of two FGF receptor types, FGFR-2(bek) and FGFR-3. At E15.5, these receptors were found primarily in cells of the germinative and marginal zones of cortex. At E21, FGFR-2 and FGFR-3 were restricted primarily to cells of the cortical plate. FGF2 injections greatly increased the number of FGFR-2 and FGFR-3 expressing cells, which were mainly found in the intermediate zone and morphologically resembled migrating neurons. In both control and FGF2-injected brains, FGFR-2 and FGFR-3 were only occasionally present in the subventricular zone, suggesting that these receptors may not be involved in gliogenesis. These data are consistent with the *in vitro* results suggesting a role for FGF2 in the regulation of neuronal number and cortical size during fetal development.

415.13

A NOVEL HEPARAN SULFATE PROTEOGLYCAN INVOLVED IN THE REGULATION OF FGFs IN MOUSE EMBRYONIC BRAIN IS A PUTATIVE PERLECAN HOMOLOGUE. S.J. Joseph*, M.D. Ford and V. Nurcombe. Department of Anatomy, Univ. of Melbourne, Vic, 3052.

A novel heparan sulfate proteoglycan (HSPG); called HSPG-BRM, for Brain Regulated Molecule, is expressed in embryonic mouse neuroepithelial tissue. This proteoglycan has been shown to differentially bind and activate FGF-1 and FGF-2 during the respective stages of neural proliferation and differentiation (1). The HSPG-BRM core protein has been purified from embryonic mouse brain, and partial peptide sequence indicates unique regions but also amino acid homology to Perlecan(2), a large HSPG that has been recently shown to bind to bFGF (3). Oligonucleotide primers have been designed from the amino acid data for the use in Polymerase Chain Reaction (PCR). The PCR derived products have been subcloned and sequenced. A 96bp PCR product, homologous to both HSPG-BRM and Perlecan was used as a probe in Northern analysis of poly(A^+) RNA from the embryonic day 10 neuroepithelium-derived cell line, 2.3D. The expected 12.6kb mRNA species of Perlecan was detected, as well as two other variants: a 6kb and 3.5kb mRNA species. This latter species we believe to be the putative HSPG-BRM mRNA as it is expressed in relatively high abundance and is the expected size for a 45kD protein. Results from the PCR studies indicate the existence of alternatively spliced variants of Perlecan in the embryonic mouse brain, and that HSPG-BRM is a putative homologue of Perlecan.

1. Nurcombe, V *etal.* (1993) Science 260, 103-106.
2. Noonan, D.M. *etal* (1991) J.Biol Chem. 266, 22939-2294
3. Aviezer, D. *et al* (1994) Cell,. 79, 1005-1013.

415.14

INVOLVEMENT OF JANUS KINASES IN SIGNAL TRANSDUCTION OF AN IMMORTALIZED DIENCEPHALIC CELL LINE. *K. Shimoda*, T. Oshima, K. Inoue, D. Kaneto, T. Kitagawa, H. Takahashi# and J.W. Commissiong##.* Div. of Neurology, National NishiTottori Hospital, Tottori 68902, JAPAN. #Lab. Histochem., Mitsubishi-kasei, Inst. of Life Sci. Machida 163, JAPAN. ##NTU, LMCN, NINDS, NIH, Bethesda, MD 20892, USA.

Many cytokines have recently been identified in the CNS. However, for many, their physiological functions have not been elucidated. We have prepared an immortalized cell line from E13 diencephalic region of rat, tested the hypothesis that IL-3 and bFGF may be involved in signal transduction mechanisms. E13 diencephalic cells were immortalized by transfection with the SV40 LT antigen temperature sensitive mutant, carried by a retrovirous vector. Degenerate oligoprimers were amplified by PCR methods, and used to clone PTKs . The clones from one cell line designated S1 were sequenced, and used to identified the following PTKs: Jak1, Jak2, bFGFR, hck/bmk and Try10. Using the Northern blotting technique, we identified the following Janus kinases: Jak1, Jak2, Jak3 and Tyk2. When the S1 cells were cultured and treated with bFGF and Il-3, both of which were coupled to Jak2, they extended processes and differentiated into cells with a neuron like morphology. The result strongly suggested that PTks are involved in signal transduction mechanisms in the S1 cell lines. They might also have a similar function during development in the CNS. The S1 cell line that we have described may therefore become a model for studies of the molecular mechanisms involved in neuronal differentiation and signal transduction in the CNS.

PATTERN FORMATION, COMPARTMENTS AND BOUNDARIES III

416.1

IDENTIFICATION OF CHICK PAX GENES LOCALIED IN THE SPECIFIC NEURON CIRCUITS.
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In the development of nervous system, several distinct cellular and molecular mechanisms cooperate to ensure correct neuronal network formation. Identification of the relevant molecules and their functions is our main goal. Pax molecules are the vertebrate homologues of *Drosophila* paired box containing genes and expressed regionally in the vertebrate brains. As those contain the paired box or both the paired and homeo boxes, it can be postulated that they are the transcription regulators involved in the development of specific neuronal cells.

To investigate the roles of pax molecules in the neuronal network formation, we screened for pax molecules expressed during middle-late stages of neuronal development in the chick brain, and identified two molecules, pax 6 and 7. Using in situ hybridization and immunohistochemical staining, we clarified that in the late stage of chick neuronal development pax 6 and 7 were expressed extensively in the retina and optic tectum, respectively, and scarcely in other part of the embryo. Expression was observed in the differentiated neuronal cells in these tissues. From these expression patterns, it was suggested that pax 6 and 7 molecules might regulate genes involved in the neuronal circuit formation.

416.2

SEGMENTAL DIFFERENCES IN SMALL CARDIOACTIVE PEPTIDE-CONTAINING NEURONS CORRELATE WITH EXPRESSION OF Lox2, A LEECH Hox GENE. V. Berezovskii * and M. Shankland. Department of Neurobiology, Harvard Medical School, Boston, MA 02115

Expression of Hox genes at the level of single neurons may play a significant role in establishing their segmental identity, and we therefore studied cellular colocalization of LOX2 protein and Small Cardioactive Peptide (SCP)-like immunoreactivity in the leech *Helobdella triserialis*. LOX2 is expressed at embryonic stage 10, and its expression remains stable until adulthood. The onset of SCP expression was later, and varied greatly for different neurons. Immunostaining revealed that the Medial Paired SCP (MPS) neurons located in the midbody segments 7-15 of the adult express LOX2 protein. In the 11th-15th ganglia large MPS neurons begin to accumulate SCP shortly after the end of embryonic development, while in the 7th-10th ganglia the MPS neurons are smaller and begin to express SCP at a later stage. In the 16th and 17th ganglia we saw large MPS neurons which express lower levels of SCP starting from mid-juvenile stage, but these cells did not show detectable LOX2 expression. Lineage tracer injections showed that the MPS neurons are descended from the O-teloblast stem cell. Two medial O-derived neurons in ganglia 11-15 contained LOX2 protein on each side of the nerve cord, while in ganglia 16 and 17 we found only one such neuron. In ganglia 1-6 and 18-21 these neurons did not express LOX2 protein or SCP. The results suggest that some segmental differences in the MPS neuron correlate with the segmental pattern of LOX2 expression.

416.3

THE RXRα GENE IS EXPRESSED IN A MEDIOLATERAL GRADIENT IN THE VENTRICULAR ZONE OF A DISCRETE STRETCH OF THE CHICKEN EMBRYO NEURAL TUBE. F. Hoover*, E.A.P. Seleiro, P. Brickell & J. C. Glover. Dept. of Anatomy, Univ. of Oslo, 0317 Norway.

Characterization of the expression patterns of the various retinoid receptors (RARα, β, γ and RXRα, β, γ) is a prerequisite to an understanding of the regulation of pattern formation by retinoids. We have used non-radioactive in situ hybridization to localize RXRα transcripts in wholemounts of chicken embryos. The highest levels of expression are found in the neural tube. At the earliest stage yet examined (stage 10 = 11 somites), expression is localized to a short stretch of the neural tube adjacent to somites 4 and 5. Between stages 10 and 15 the domain of expression expands into the hindbrain to a sharp rostral border just caudal to the otic vesicle, remaining high to about the level of somite 8, and then gradually decreasing along the spinal cord. This pattern is maintained at least until stage 22, the latest stage yet examined. As the hindbrain develops, expression as seen in wholemounts is clearly predominant in the rhombic lip. Transverse sections show expression throughout the ventricular zone, in a mediolateral gradient peaking at the rhombic lip. The level of expression outside the ventricular zone is markedly lower if not absent. These results suggest that the RXRα gene is selectively expressed in progenitor cells along a specific stretch of the neural tube. The mediolateral gradient of expression in this progenitor cell population may be related to the mediolateral gradient of proliferative activity previously described in the hindbrain neural tube (Hemond and Glover (1993) J. Neurosci. 13:1387-1402).

416.4

DISRUPTION OF BF-2 WINGED HELIX GENE BY HOMOLOGOUS RECOMBINATION IN ES CELLS Hatini V.*, Huh S. O., Balas G., Soares V., and Lai E. Cornell University Graduate School of Medical Sciences, Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021

Several members of transcription factor families are expressed in restricted domains within the developing forebrain and are believed to control their fate, growth and differentiation. We have previously shown that BF-1 and BF-2, winged helix (WH) transcription factors, are expressed in the telencephalon and rostral diencephalon, respectively. Their expression forms a boundary within the rostral forebrain and divides the optic stalk and retina into adjacent domains. The expression of BF-2 is the highest in the ventricular zone suggesting a role in the control of cell fate and cell proliferation within this region of the neuroepithelium. To examine the role of BF-2 during development we deleted the BF-2 gene by homologous recombination in the mouse. A targeting vector in which most of the BF-2 coding region was replaced by a lacZ neo cassette was constructed. Both J1 and CJ7 ES cell lines were electroporated with the BF-2 targeting vector and 4 correctly targeted clones were isolated. These clones were injected into blastocytes and one of the CJ7 clones contributed the mouse germ line. Analysis of heterozygote animals reveals that the β-galactosidase activity is identical to the *in situ* expression and may be useful in the analysis of the BF-2 mutant phenotype. Heterozygote animals were bred to generate BF-2 homozygote animals. Preliminary analysis of E11 litters of heterozygote intercrosses reveals that the homozygote animals are viable until this stage. Homozygote animals will be analyzed to determine the molecular basis of the putative developmental abnormalities and the possible mode of action of BF-2 within the developing neuroepithelium.

416.5

CEREBELLAR COMPARTMENTS IN TRANSGENIC MICE EXPRESSING AN OLFACTORY MARKER PROTEIN-LacZ FUSION GENE. M.G.Nunzi[1]*, M.Grillo[2] F.L.Margolis[2] and E.Mugnaini[1]. [1]Neuromorphology Lab., Univ. of Conn., Storrs, CT 06269 and [2] Roche Inst. Molec. Biol., Nutley, NJ 07110.

The organization of the cerebellar cortex into compartments defined by distinct Purkinje cell (PC) phenotypes is an hallmark of the mammalian cerebellum. possibly linked to functional modules within the cerebellar circuitry. The molecular basis underlying PC diversity has been previously explored in transgenic mouse lines bearing different constructs of the PC specific gene L7/Pcp-2 (Vandaele, et al.,1991; Oberdick et al.,1993). We report that a murine line (HpY-1) with a *lacZ* transgene containing 0.3kb of upstream and 0.35kb of downstream sequence from the olfactory marker protein (OMP) gene ectopically expresses the *lacZ* reporter within restricted cerebellar PC domains. The spatial pattern of expression was analyzed from embryonic to adult life in whole mount cerebella and sections with X-gal or anti-beta-galactosidase staining. At E13, a cluster of *lacZ*-positive cells outlined the PC plate in the cerebellar primordium. By E17, PCs expressing *lacZ* showed a banded pattern more evident in the caudal and lateral cerebellum than in the anterior lobe. Between P5 and P20, on and off parasagittal bands of *lacZ* expression became more prominent in the posterior vermis and hemispheres, and a wide expression region marked the rostral paraflocculus, whilst *lacZ* expression in the anterior vermis and crus I subsided. By P30, *lacZ* expression was detectable within parasagittal bands in the posterior cerebellum with a boundary slightly rostral to the primary fissure. These data suggest that the transgene contains regulatory elements recognized by transacting factors present in specific PC subsets and involved in the formation of cerebellar parasagittal and anterior-posterior compartments, or that the site of integration has placed the transgene under the control of such regulatory elements (Supported in part by NIH Grant NS 09904).

416.6

EXPRESSION OF AN OLFACTORY MARKER PROTEIN-LacZ CONSTRUCT MARKS GRANULE CELLS IN CEREBELLAR CORTEX AND COCHLEAR NUCLEAR COMPLEX. [1]E. Mugnaini*, [2]C. Stein-Isak [2]F.L. Margolis and [1]M.G. Nunzi [1]Lab.of Neuromorphology, Univ. of Connecticut, Storrs, CT 06269 and [2] Roche Inst. Molec. Biol.,Nutley, NJ 07110.

Embryological investigations have shown that cerebellum and dorsal cochlear nucleus (DCoN) originate from nearby regions of the embryonic metencephalon and may share in common precursors of different cell types. Accordingly, the DCoN contains cerebellar-like granule, stellate, Golgi and unipolar brush cells, as well as cells analogous to the Purkinje cells, the cartwheel neurons. In the cerebellum, granule cells originate postnatally from the external granular layer, and then migrate inward through the molecular and Purkinje cell layers to form the granular layer. In the DCoN the granule cells show a similar developmental pathway, while in the ventral cochlear nucleus they mantain, for the most part, a superficial position. Histochemical analysis of the spatial and temporal distribution of an olfactory marker protein-*lacZ* fusion gene in a line of transgenic mice (B-OMP-1; Walters et al., submitted) has revealed ectopic *lacZ* expression within the granule cell populations of both cerebellum and cochlear nuclei. In the cerebellum, the transgene expression appears developmentally regulated. At P4, X-gal marks differentiating granule cells in all lobules. By P7, granule cells show stronger *lacZ* expression in vestibulocerebellar folia than in the rest of the cerebellum. In the adult, *lacZ* expression appears exclusively in the vestibulocerebellum. In the cochlear nuclear complex, *lacZ* is expressed at high levels at all stages of development and appears substantial also in the adult. Taken together, these data corroborate the notion that the granule cells systems of the vestibulocerebellum and cochlear nuclear complex form analogous systems that share in common not only morphological phenotypes, but also aspects of gene regulation. (Supported by NIH grant NIDCD 01805 and NS 09904).

416.7

BrdU BIRTHDATING AND THE PURKINJE CELL COMPARTMENTATION OF THE MOUSE CEREBELLUM

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Zebrin II is a 35 kDa polypeptide antigen in rat cerebellum, identified by using a monoclonal antibody. Zebrin II immunoreactivity is confined to a subset of Purkinje cells that form 14 rostrocaudal parasagittal bands interposed by non-immunoreactive Purkinje cells. To explore the origins of compartmentation in the adult mouse cerebellum, pregnant dams were injected once with 0.05 mg/g BrdU at different gestation periods between E10 - E14. From each litter, pups were taken at three stages: at 4 h after injection, at P6 and as adults (>P30). The animals were ethanol-fixed, paraffin-embedded, sectioned, and immunostained for zebrin II + BrdU (adult), calbindin + BrdU (P6), or for BrdU alone (embryos). Statistical comparisons revealed a strong correlation between zebrin $II^{+/-}$ compartments and Purkinje cell birthdates. By comparing the BrdU labeling in animals from the same litter as embryos, neonates and adults, it is possible to correlate specific zebrin compartments with early Purkinje cell clusters and with the organization of the cerebellar anlage. The data support the hypothesis that birthdate-related Purkinje cells share a common zebrin II phenotype.

416.8

THE EFFECTS OF THE MURINE MUTATION, meander tail, ON CEREBELLAR PURKINJE AND GOLGI EPITHELIAL CELLS ARE SECONDARY TO THE LOSS OF GRANULE CELLS. K.M. Hamre* and D. Goldowitz. Dept. of Anat. & Neurobiol., Univ. of Tenn. Coll. of Med., Memphis, TN 38163.

The murine mutation meander tail (symbol: *mea*) results in numerous anterior lobe cerebellar perturbations including misorientation of Purkinje cell dendrites and somas, distorted and shortened radial glial fibers, foliation abnormalities, and the loss of virtually all granule cells. Previously, we presented evidence that the loss of granule cells in *mea* mice may be intrinsic to the granule cells or their precursors. In the present study, we further examine this issue, and additionally examine whether the glial and Purkinje cell abnormalities are also intrinsic defects to these cells or whether they are secondary to another target of the mutant gene such as the loss of granule cells. To address this issue, experimental murine chimeras were made between homozygous *mea* embryos and wild-type embryos. All chimeric mice were allowed to survive until 3-4 weeks of age. Cell genotype and phenotype were labeled in the same section. Cell genotype was identified with in situ hybridization. Subsequently, cell phenotype was immunocytochemically identified with antibodies to calcium-binding protein to label Purkinje cells or GFAP to label radial glial processes. In the cerebellums of chimeric mice, no obvious evidence of abnormal Purkinje cell alignment or dendritic arborization was found. Serial reconstructions of 22 genotypically *mea* Purkinje cells demonstrated that each of these cells was normally positioned with normally projecting dendritic arbors. Likewise, evaluation of Golgi epithelial cells showed no evidence of abnormal glial processes within the cerebellum of chimeric mice and genotypically *mea* Golgi epithelial cells had phenotypically normal radial glial processes. In contrast to the normal appearance of genotypically mutant Purkinje cells and radial glial processes, mutant granule cells were selectively deleted in the chimeric cerebellum. Thus, *mea* gene action is extrinsic to cerebellar Purkinje cells and Golgi epithelial cells but intrinsic to cerebellar granule cells. Support: NS23475.

416.9

SAGITTAL COMPARTMENTS OF THE CEREBELLUM ARE DISRUPTED IN *EN-2* MUTANT MICE

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The mouse *Engrailed-2* gene (*En-2*) is a homologue of the Drosophila *en* gene that functions in establishing the posterior identity of compartments. Null alleles of mouse *En-2* have distinct effects on cerebellar foliation, which may reflect defects in anterior/posterior (A/P) patterning. We wished to know whether *En-2* might also play a role in mediolateral organization. Three markers of sagittal compartmentation were used: Zebrin II and ppath, antibodies that reveal the adult banding pattern, and a transgene, L7:ßgal, whose expression reveals a transient perinatal pattern of sagittal stripes. In the adult, the thin-anterior/thick-posterior pattern of the sagittal bands is preserved, but many individual bands are fragmented or attenuated. In the neonate, the basic temporal pattern of the marker is preserved (staining begins medially and spreads laterally), but distinct differences are also found. *En-2* mutant mice are always more lightly stained than age matched controls; there is an unusual fusion of the two most medial bands; and there is a precocious appearance of a band in the Crus I folium. The observed disruption of M/L banding in *En-2* mutants is consistent with our finding that *En-2* is expressed in the embryonic cerebellum in a pattern of sagittal bands. Our data suggest that *En-2*, which has previously been associated with A/P organization, also has important functions in specifying the M/L pattern of the mouse cerebellum.

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416.10

***Tlx-1 (Hox11)* EXPRESSION IS ASSOCIATED WITH SENSORY AXON INNERVATION OF THE DEVELOPING CHICK HINDBRAIN.**

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We have isolated and characterised the chick homologue of *Tlx-1* (*Hox11*), a divergent homeobox-containing gene. *Tlx-1* was originally identified in human due to chromosomal translocations which resulted in its deregulated expression. The corresponding mouse homologue has a very complex pattern of expression within the developing embryo and plays a critical role in normal development of the spleen. The predicted protein sequence (297 aa) of the chick gene shares 73% and 71% identity overall with the human and mouse homologues respectively. Transcripts are first detected at HH stage 7 within the developing branchial region and later within several components of first the peripheral (HH stage 16) and then central nervous system (HH stage 20) including the trigeminal and facial ganglia and subsequently within the developing hindbrain.

We have begun to investigate the association between *Tlx-1* expression within the hindbrain and the ingrowth of sensory axons. The central pathways of sensory axons from the trigeminal and facial nerves at various stages (E5-E9) were mapped by injection of DiI into the respective ganglia of fixed hindbrains and axon morphologies reconstructed using confocal microscopy. Each respective sensory tract was found to occupy a successively more lateral position parallel to the marginal expression of *Tlx-1*. Axonal sidebranches of the afferent trigeminal axons extend medially into the *Tlx-1* expressing region. Deafferentation of the developing hindbrain at HH stages 14-16, either by ablation of the fifth ganglion, or by insertion of tantalum foil between the ganglion and hindbrain, will allow us to test the functional relationship between *Tlx-1* expression and distribution of trigeminal sensory axons.

416.11

SPECIFIC ADHESION DIVIDES ODD- AND EVEN-NUMBERED RHOMOBOMERES

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Although neuroepithelial cells can move freely within a rhombomere, cell mingling between rhombomeres is almost neglegible. To address the question of what cellular mechanisms are responsible for restricting cell movements between rhombomeres, we have established two *in vitro* culture systems. The first system was designed to assess if cells from different rhombomeres would adhere to membranes prepared from individual rhombomeres. The results showed that cells from any given rhombomere exhibited a preferential adhesion to their own membranes and, to a lesser degree, to those of alternate rhombomeres. The second system was based on the aggregation of dissociated rhombomere cells. Cells were allowed to aggregate with cells of the same rhombomere or with cells of neighbouring rhombomeres. Screening the aggregates revealed that mixtures of cells from both even- and odd-numbered rhombomeres either formed separate aggregates or became segregated within mixed aggregates. Selective removal of classes of adhesion molecules by different enzymatic treatments (Urushima et al., 1979) was used to uncover the adhesion systems involved. The general adhesion between hindbrain cells relies on Ca- dependent (CDS) and Ca-independent (CIDS) mechanisms. In contrast, the selective adhesion was only seen when CDS were left on the surface and not when only CIDS were present. The introduction of an RGD peptide, which interferes with integrin mediated adhesion, to aggregation cultures did not effect this selective adhesion. Our results suggest that cells from even- and odd-numbered rhombomeres differ in their adhesive properties as to a lesser extent do cells from different even or odd-numbered rhombomeres. Our experiments also suggest that the specific adhesion between cells of the some rhombomeric origin is due to a Ca-dependent mechanism, probably involving Cadherins. These functional adhesion assay systems provide a useful tool to dissect the molecular basis of these interactions.

416.12

CHANGES IN DENDRITIC BRANCH PATTERN OF THE MEDIUM SPINY NEURONS IN THE STRIATUM OF THE WEAVER MOUSE: A GOLGI STUDY.

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In order to determine if there were changes in the dendritic arbor of the medium spiny neurons of the striatum as a result of the loss of dopaminergic input which is seen in the weaver neurological mutant mouse, a series of animals were prepared for Golgi analysis. Weavers and homozygous littermate controls were perfused with 2% glutaraldehyde - 2% paraformaldehyde in a 0.1M phosphate buffer and processed following the method of Shimono and Tsuji (JCN 259:122-130, 1987). The centrifugal method of numbering the first, second, etc., order dendritic branches was used in the count. There were no statistical differences in cell size or total dendritic area, but there were significantly fewer third and fifth order dendrites in weaver. These findings suggest that the loss of dopamine input alters the formation of the characteristic branch pattern of the dendritic arbor in the medium spiny neurons.

416.13

DEVELOPMENT OF THE NEOSTRIATAL MOSAIC IN THE MOUSE.

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The patch/matrix organization of the striatum has been demonstrated in primates and rodents. Many studies have characterized these compartments in terms of connectivity and expression of transmitters, receptors, enzymes, and glycoconjugates. Elegant studies in the rat have addressed the development of the two compartments. We have undertaken the investigation of the development of this compartmentation in the mouse. We are studying the distribution and time course of expression of several markers of the patch/matrix compartments in perinatal mice. Markers that we are currently investigating include glycoconjugates recognized by a lectin, peanut agglutinin (PNA); tyrosine hydroxylase (TH)-containing nigrostriatal fibers; and birth dates of striatal cells, established by incorporation of the thymidine analog, 5-bromo-2'-deoxyuridine. In the embryonic day 18 (E18) mouse, the patch compartment can be identified as focal concentrations of TH fibers. As in previous studies in other species, there is a caudal-to-rostral gradient of TH fiber patch formation. This can be seen around the time of birth, when anterior regions of the striatum have a homogeneous distribution of TH fibers while more posterior regions show emerging TH fiber patches. Patches of PNA staining correspond to patches of TH fibers, and PNA staining is absent in striatal areas where TH fibers are homogeneously distributed. Interestingly, in areas where TH patches are just beginning to appear, patching of the PNA label is already clearly visible. We are also investigating how striatal cell birth date correlates with the appearance of PNA-positive patches. We have established that on P3, cells which become postmitotic on E12 are found in patches, while cells born on E14 are found in the matrix of the same striatal region. In addition, the caudal-to-rostral gradient of birth dates of patch neurons reported for the rat is evident in the mouse.

416.14

TEMPORAL AND SPATIAL REGULATION OF TRANSCRIPTION FACTOR EXPRESSION IN THE DEVELOPING STRIATUM.

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We have found that in the prenatal rodent striatum, NGFI-A, FRA and FOS expression follow temporal and spatial patterns typical of developing striosomes. We now have tested the precision of this match pre- and post-natally. There was, at E19, a strict match of the TF cell clusters with DARPP-32 and TH-positive patches, and double staining for FRA and NGFI-A showed that NGFI-A was expressed by a large majority of the FRA-positive cells both before and after the cells formed clusters. In contrast to the tight linkage of the TFs prenatally, their expression patterns diverged postnatally. FOS expression rapidly declined, FRA expression remained predominantly striosomal until P7 and then decreased, and NGFI-A expression increased and then spread to the matrix. Thus, although the three TFs are early markers of the striosomal compartment, they lose compartmental constraints as the striatum matures postnatally.

To test whether the early striosome-association of the TFs could be related to the early differentiation of this compartment relative to that of the matrix, we tested whether MAP-2 expression, which marks the differentiation of neurons as they acquire dendrites, would be linked to that of the TFs. MAP-2 immunostaining of striatal processes first appeared diffuse, but, by E17, MAP-2 clusters began to form and they then predominated until after P7. Throughout this period, virtually every MAP-2 cluster matched a TF-positive cluster. We suggest that early, selective TF expression in striosomes may reflect the early, time-shifted differentiation of this compartment relative to that of the matrix. We thank Drs. J. Milbrandt, M. Iadorola, H. Hemmings Jr. and P. Greengard for antisera, and acknowledge NIH 1 R01 HD28341.

416.15

EXPRESSION OF THE NMDA RECEPTOR NR1 SPLICE VARIANTS AND NR2 mRNAS IN THE STRIATUM DURING PRENATAL DEVELOPMENT
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Excitatory amino acids are thought to play an important role in the development of the mammalian central nervous system. Of the variety of glutamate receptors, NMDA is the best known. The subtype NR1 as well as NR2B and NR2D are expressed in the rat prenatal period. We found that this was true in the developing striatum in particular, but in addition found that the alternatively spliced variants, cassette insertion I (exon 5) and the deletion I (exon 21) (Sugihara et al., 1992) were expressed differentially. Using in situ hybridization technique, with oligonucleotide probes for the NMDA receptor subtypes NR1 InsI, NR1 DelI, NR2A, NR2B, NR2C, and NR2D, we studied the expression of the receptor mRNA in the prenatal period from E14 to E20 at two day intervals of the prenatal rat striatum. InsI mRNA was seen as early as E14 particularly in the periventricular area. By E18, the differentiating field in the striatum exhibited the densest signal. DelI expression was not observed in the prenatal period. NR2A expression also was not seen during this period. NR2B expression was seen in the striatum as early as E16. Density was uniform in all axes and across the neuroepithelium, subventricular zone and the differentiating field of the striatum up to E20. NR2C expression was low, but at E18 was noted in the striatum to be slightly denser than hippocampus, thalamus, and pallidum. NR2D expression showed a medial to lateral gradient with the periventricular zones being the densest in mRNA expression. This was seen as early as E16. The specialization of the NMDA receptor subtype and the variable expression in the prenatal period, particularly of the alternative spliced variants of NR1 may suggest a role in the early genesis of the rat striatum.

416.16

A NOVEL TRANSGENIC MOUSE LINE IDENTIFIES CELLS IN THE DEVELOPING AND MATURE CEREBRAL CORTEX.
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Molecular approaches have been used recently to investigate the mechanisms underlying pattern formation in the vertebrate brain. This issue is of particular interest in the telencephalon, which is comprised of the cerebral cortex and subcortical areas such as the basal ganglia. We report further characterization of a transgenic mouse line in which a reporter gene is expressed exclusively in cells of the cerebral cortex. This line was established (Lee et al., Mol. Repro. Develop.35:382) in studies investigating the expression of IGF-2, in which a construct containing 30kb upstream from the IGF-2 initiation site was fused to the lacZ reporter. Expression patterns of lacZ in this line were mapped from e10 through birth. Cells expressing lacZ were first observed at e10.5 in the developing cerebral wall while all other regions were lacZ-negative. The positive cells were located underneath the pial surface, where the earliest neurons settle. A large increase in lacZ-positve cells was evident in the wall at e11.5. At subsequent ages, the anatomical pattern of lacZ expression suggests that differentiating, nonproliferative cells are the predominant, if not the only, lacZ-positive cells. Almost all lacZ expression was confined to cells within the borders of the cerebral cortex and absent subcortically, although a few labelled cells were present in the diencephalon at early ages. Homozygous offspring from matings of hemizygotes do not reach adulthood; preliminary evidence indicates that homozygotes die near the time of birth. In summary, ontogenetic analysis of this transgenic line indicates intergration into a site that drives unique expression in cells related by a common origin in the cerebral wall of the developing telencephalon. Supported by NS-21970 (JP) and MH-45507 (PL).

416.17

CELL DISSOCIATION OF THE CEREBRAL CORTEX AND CHEMOSENSITIVE AREAS BY THE TISSUE PRINT METHOD.
J.W. Peters[1]*, J.M.Wright[2], D.W.Frederick[3], and C.Q.Trouth[1]. Dept.of Physiology & Biophsyics[1], Dept.of Psychology[3], Howard Univ., Wash., DC 20059, Lab.of Molecular and Cellular Neurobiology[3], NIAAA, NIH, Bethesda, MD 20892.

As a prelude to ion channel studies on the cerebral cortex and chemosensitive areas of the ventrolateral medulla neurons of Post natal(P)1 to P3 rats, neural cells and their respective processes were isolated through tissue printing dissociation (Barres,et al.(1990) Neuron 5:527-544). Tissue slices were incubated in 0.2% papain (Worthington) for 5-30 minutes, and washed with a soybean trypsin inhibitor (Worthington, 50% solution). Tissues were placed on nitrocellulose paper and transferred to glass coverslips treated with a 1% collodion solution (Ladd). Time trial studies were conducted to yield intact axonal and dendritic processes. Results have yielded patch-clamp recordable cells. Studies are in progress to define their respective ion channel properties.
This work is supported by ONR Grant # N00014-94-1-0523.

416.18

RESTRICTED CEREBRAL CORTEX EXPRESSION OF A CANDIDATE G-PROTEIN COUPLED RECEPTOR ISOLATED BY PCR DIFFERENTIAL DISPLAY.
Margaret E. Levin, Anjen Chenn, and Susan K. McConnell*. Department of Biological Sciences, Stanford University, Stanford, CA 94305.

We are interested in studying the mechanisms by which neurons within the cerebral cortex attain their lamina-specific identities during development. We hypothesize that genes involved in laminar determination will be expressed in layer-specific patterns, and are thus using PCR differential display techniques (Liang and Pardee, 1992) to identify candidate genes. In order to isolate differentially-expressed mRNAs, we have compared mRNAs obtained separately from the upper layers (1-4) and the lower layers (4-6) of P6 rat cortex. Of 15 differentially-expressed PCR products, in situ hybridization revealed that the expression of one candidate (1A) is localized within layers 2/3 and 4 in sections of P6 and P15 rat cortex. This mRNA is also expressed in the striatum in P6, P15, and adult rat brains. The 1A PCR product was used to isolate a cDNA clone from an upper layer cortical cDNA library. Sequencing and examination of the data bank revealed that 1A encodes a candidate G-coupled receptor for which the ligand is unknown (Song et al., 1994). We are currently continuing our search for layer specific genes using both PCR differential display and subtractive hybridization. Supported by NEI EY06524, NIH GM07365, and McKnight Scholars.

416.19

ONTOGENETIC DISTRIBUTION OF NADPH-DIAPHORASE IN MOUSE SOMATOSENSORY CORTEX.
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We have investigated the expression of NO synthase activity (NOS) during development of the mouse somatosensory cortex using NADPH-diaphorase histochemistry. Diffuse distribution of NOS was detectable in the entire width and over the entire area of the somatosensory cortex at birth but appeared more concentrated in the lower part of the cortical plate. At postnatal day 3 (P3) soon after layer 4 has started to develop the typical barrel field pattern, NOS accumulated in the hollows of barrel fields. Maximal expression of NOS in the hollows was reached at P6. At P9, NOS expression was decreased when compared to P6 and barrel fields could not be seen P15. In addition to the diffuse distribution of NOS, NOS was accumulated at all ages studied in individual cells, possibly interneurons, which were sparse in layer 4, where they were mostly located in the septa. These strongly NOS positive cells were more abundant in the deeper layers of the cortex. After ablation of the C row of whiskers at P1, NOS expression at P6 reflected the altered barrel fields. The somatotopic distribution of NOS was also observed in barreloids and barrelettes at P6. Our experiments show that expression of NOS correlates with the establishment of connections in the somatosensory pathway and with other activity dependent changes during stabilization of synaptic contacts.

416.20

DEVELOPMENTAL CHANGES ALTER THE ABILITY OF MOSSY FIBERS TO REESTABLISH THEIR NORMAL INNERVATION PATTERN IN HIPPOCAMPAL SLICE CULTURES.
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Mossy fibers (MF) from dentate gyrus (DG) granule cells establish synapses on CA3 pyramidal neurons during the first 3 postnatal weeks in the rat (Gaarskjaer,1986). Cultured hippocampal slices from postnatal day 4 rat pups show a similar MF termination pattern in stratum lucidum (SL) when examined by Timm stain after 10-14 days *in vitro*. Thus, axon guidance cues used by MF appear to be preserved in these cultured slices. Three experimental manipulations were performed on hippocampal slice cultures to examine whether the axon guidance cues used by MF are developmentally regulated. First, MF were transected on the day of culture or day 7 *in vitro*. MF transected on either day were able to reestablish their synaptic pattern in SL of CA3. Second, DGs and hippocampi (H) of same age or different age were co-cultured. Same-age co-cultures (P4 DG to P4 H or P11 DG to P11 H) showed good MF reinnervation of SL, as did different age co-cultures from P4 DG to P11 H. However, P11 DG to P4 H co-cultures showed little MF reinnervation of SL. Third, new P4 or P11 DG were co-cultured onto hippocampal slices in which MF had been allowed to degenerate. New MF reinnervated these hippocampi, but did not reestablish their normal synaptic pattern in SL. These 3 experimental manipulations suggest that the expression of axon guidance molecules in the hippocampus and on MF growth cones are developmentally regulated, and that existing MF play a role in directing MF reinnervation of SL.
This study was supported by The PEW Memorial Trust.

417.1

INDUCTION OF NFκB MEDIATES REGULATION OF NERVE GROWTH FACTOR MRNA BY IL-1 IN RAT HIPPOCAMPAL ASTROCYTES Wilma J. Friedman*[1], Sanjay Thakur[3] and Arnold B. Rabson[2,3] Departments of [1]Neuroscience and Cell Biology, and [2]Molecular Genetics and Microbiology, UMDNJ/Robert Wood Johnson Medical School, and [3]CABM Piscataway, N.J.

Cytokines such as interleukin-1ß (Il-1) are produced in the brain during development and during inflammatory processes which result from lesions or disease. One function of Il-1 in the brain appears to be the stimulation of astrocytes to proliferate and produce a variety of cytokines and trophic factors, including nerve growth factor (NGF). The mechanisms by which Il-1 exerts its actions on astrocytes remain poorly defined. We present evidence that this cytokine elicits activation of the NFκB transcription factor in astrocytes, in particular the p50 and p65 subunits. Moreover, this transcription factor mediates effects of Il-1 on NGF mRNA expression. Inhibition of NFκB activation prevents the induction of NGF mRNA by Il-1. Elucidation of the processes by which cytokines activate astrocytes and influence trophic factor expression may provide insight into mechanisms governing inflammatory processes within the CNS. Supported by NIH grant NS 31357-02 to WJF.

417.2

INDUCTION OF bFGF GENE EXPRESSION *in vivo* IN RAT PHOTORECEPTORS BY THE α_2-ADRENERGIC AGONISTS XYLAZINE AND CLONIDINE. Rong Wen[†§], Tong Cheng[†§], Yiwen Li[¶], and Roy H. Steinberg*[†§]. Depts of †Physiology, §Ophthalmology, and ¶Neurology, University of California, San Francisco, CA 94143

We observed an induction of bFGF mRNA in the rat retina following systemic administration of the α_2-adrenergic agonists xylazine and clonidine. A single injection of xylazine (6mg/kg, i.m.) resulted in a more than 5-fold increase in bFGF mRNA in the retina by 12 hrs, which then declined to the baseline in 48 hrs. This increase in bFGF mRNA was completely inhibited by pre-injection of yohimbine, a specific α_2-adrenergic antagonist (5mg/kg, i.p., 20 min before xylazine). Injection of another α_2-adrenergic agonist, clonidine (0.5 mg/kg, i.p.), also up-regulated bFGF mRNA in the retina with a similar temporal pattern. Pre-treatment with yohimbine (15 mg/kg, i.p., 20 min before xylazine) completely abolished the effect of clonidine. A higher dosage of yohimbine (15 mg/kg, i.p.) also inhibited the normal expression of bFGF in the retina by 40%, indicating that α_2-adrenegic stimulation was of physiological importance in regulating bFGF expression in the retina. Of particular interest is the finding that the xylazine-induced bFGF expression was found almost exclusively in the inner-segment region of photoreceptors, as accessed by in situ hybridization. However, in the eight different brain regions examined: septum, striatum, thalamus, hypothalamus, hippocampus, olfactory bulb, cerebellum, and cerebral cortex, there was virtually no change in bFGF mRNA expression after either xylazine or clonidine treatment. These findings indicate that bFGF expression in photoreceptors can be regulated by α_2-adrenergic stimulation, and may provide new insights into the mechanisms that regulate bFGF expression in general.

Supported by NIH grant EY01429.

417.3

REGULATION OF NGF SYNTHESIS BY DEXAMETHASONE IN HUMAN CELL LINES OF NEURONAL ORIGIN. R. Ortmann, B. Hengerer, D. Mathé, A. Schade, J.-G.Meisburger and G. Fagg* CNS Research Department, Pharmaceutical Division, Ciba, CH-4002 Basel, Switzerland.

Dexamethasone (DEX) increases and inhibits NGF synthesis in cultured hippocampal neurons and in astrocytes, respectively. We studied the effect of DEX on NGF expression in a series of established neuroblastoma and astrocytoma cell lines in order to find a cell line with an 'neuronal-type' up-regulation of NGF synthesis by DEX.

Levels of NGF mRNA and NGF protein were measured by Northern blotting and ELISA respectively (detection limits 1pg NGF mRNA and 1pg NGF, respectively). NGFmRNA and NGF protein levels were below detection limits in most of the neuroblastoma cell lines studied (SH-SY5Y, SK-N-SH, SK-N-MC, IMR-32). In most astrocytoma cell lines (U-373MG, U-118MG, U-87MG, T-98G), basal NGF protein and NGF mRNA levels were between 10-20pg NGF/ml and 1-5pg/10^6 cells, respectively. DEX (0.01-10µM) increased NGF protein and mRNA levels only in U-373MG astrocytoma cells (2-4fold and 4-5fold, respectively). Under the same experimental conditions DEX had no effect or inhibited NGF mRNA and NGF protein in all other astrocytoma cell lines studied. In the absence of fetal calf serum (fcs), or in the presence of dialyzed fcs during the drug treatment period DEX did not increase protein levels of U-373MG cells. Cell viability was not affected under these experimental conditions.Our data suggest that fcs contains a molecule acting as a cofactor for the up-regulation of NGF synthesis by DEX in human U-373MG cells. In conclusion, in a series of astrocytoma and neuroblastoma cells tested, only U-373MG cells increased NGF synthesis in response to DEX.

417.4

EXPRESSION AND REGULATION OF FGF-2 AND FGFR-1 mRNAs IN PC12 AND CHROMAFFIN CELLS: EFFECTS OF NGF AND GLUCOCORTICOID. C. Meisinger and C. Grothe*. Institute of Anatomy, University of Freiburg, D-79104 Freiburg, Germany.

FGF-2 and FGF receptor (FGFR)-1 mRNAs are present in the adrenal cortex and the adrenal medulla of rat and bovine glands as we have shown after ribonuclease protection assay. *In situ* hybridization studies reveal that both FGF-2 and FGFR-1 mRNAs are localized to a chromaffin subpopulation in the medulla.

In order to help to elucidate the physiological function of FGF-2 for chromaffin cells we analyzed the expression of both mRNAs in PC12 cells under different culture conditions. FGF-2 and FGFR-1 transcripts are expressed in PC12 cells under serum-containing and serum-free culture conditions. Neither application of growth factors in serum-free cultures (FGF-2, NGF, FGF-2/NGF, dexamethasone), nor stimulation of cholinergic receptors with carbachol or of adenylate cyclase with forskolin does affect FGFR-1 expression. In serum-containing cultures, however, NGF stimulates a significant increase of FGFR-1 mRNA as compared to untreated cultures suggesting that NGF could increase the sensitivity of PC12 cells for FGF. Dexamethasone stimulates a significant increase of the FGF-2 transcript level in serum-free and serum-containing cultures of PC12 cells suggesting that the glucocorticoid-effect on catecholamine synthesis could be mediated via the endogenous FGF. According to our results an autocrine/paracrine role of FGF-2 as a maintenance and differentiation factor for chromaffin cells is conceivable.
Supported by DFG Gr 857/8-1

417.5

REGULATION OF FGF-1 AND FGF-2 GENE EXPRESSION IN RAT CORTICAL ASTROCYTES. M.A. Riva[a,*], R. Molteni[b], E. Lovati[b], F. Fumagalli[b] and G. Racagni[b]. [a] Di.Bi.T., San Raffaele Hospital, and [b] Center for Neuropharmacology, University of Milan, Via Balzaretti 9, 20133 Milan, Italy.

Neurotrophic factors play a major role in the maturation of specific neuronal pathways as well as in the maintenance of cell homeostasis. Moreover trophic molecules can be useful for the treatment of neurodegenerative disorders, as indicated by several observations showing that they can protect neurons against different types of damage. The present study was undertaken to investigate the regulatory mechanisms of FGF-1 and FGF-2 gene expression, as compared to other trophic molecules, in rat cortical astrocytes. We found that glucocorticoid hormones and the beta adrenergic receptor (βAR) agonist isoproterenol are potent inducers of FGF-2 gene expression in type I astrocytes. The effect of isoproterenol is mimicked by cAMP analogues or IBMX, a cAMP phosphodiesterase inhibitor, to indicate that intracellular elevation of this second messenger is indeed related to the induction of FGF-2 gene expression. On the contrary, the gene expression for FGF-1 and CNTF is markedly reduced after exposure to isoproterenol. However, different mechanisms may be involved in the modulation of trophic factor expression by these agents. The induction of FGF-2 mRNA by dexamethasone and isoproterenol does not depend upon new protein synthesis, while the reduction of CNTF expression following exposure to the βAR agonist was blocked by concomitant incubation of the protein synthesis inhibitor cycloheximide. Moreover experiments with actinomicyn D, an inhibitor of gene transcription, suggest that the effects of isoproterenol on FGF-2 expression do not depend only on transcription activation, but may be the result of mRNA stabilization. These data suggest that complex mechanisms may contribute to the regulation of neurotrophic factor biosynthesis in astroglial cells. These mechanisms can be important in the maintenance of neuronal homeostasis and may provide useful informations to develop new therapeutical agents able to modulate the expression of trophic molecules, as an alternative strategy for the treatment of acute and chronic neurodegenerative disorders.

417.6

GLUCOCORTICOIDS REGULATE THE GENE EXPRESSION OF NEUROTROPHIC FACTORS: NGFβ, S100β, AND FGF-2 IN CULTURED HIPPOCAMPAL ASTROCYTES. H. Niu, D.A. Hinkle, D.F.Speck*, P.M. Wise. Dept.of Physiology, Univ. of Kentucky College of Medicine, Lexington, KY 40536.

Glucocorticoids are major mediators of hippocampal functions in the adult as well as during development and aging. Astrocytes can synthesize a variety of neurotrophic factors which may mediate neuron-astrocyte interactions. We hypothesized that glucocorticoids may regulate the expression of neurotrophic factors in hippocampal astrocytes. In this study, three neurotrophic factors known to be important for hippocampal neuron development and survival were investigated: NGFβ, S100β, and FGF-2. Enriched type I astrocyte cultures from hippocampus were treated with 1µM dexamethasone (DEX), a synthetic glucocorticoid, for up to 120 hours. Total RNAs were isolated at 6, 12, 24, 48, 72, 96 and 120 hours after the initiation of the treatment. The neurotrophic factor mRNAs were measured by solution hybridization-RNase protection assays using highly specific cRNA probes for rat NGFβ, S100β, and FGF-2 with cyclophilin as an internal standard. The mRNA levels were quantitated with a PhosphorImager and 4 to 8 independent cultures were analyzed. We report that DEX suppressed NGFβ mRNA continuously over the 120-hour treatment, with a significant decrease detectable as early as 6 hours. In contrast, there were transient increases of approximately 1.5-fold in the S100β and FGF-2 mRNA levels which peaked at 24 hours. At 96 and 120 hours of treatment, S100β mRNA levels decreased approximately 2.5-fold. In addition, since GFAP is considered a marker of the functional status of astrocytes, GFAP mRNA levels were monitored. DEX suppressed GFAP mRNA level continuously over the 120-hour treatment, but a significant decrease was not detected until 48 hours into the treatment. Our results demonstrate that glucocorticoids can differentially regulate the gene expression of important neurotrophic factors in hippocampal astrocytes *in vitro*. This suggests that one of the mechanisms through which glucocorticoids affect hippocampal functions may be through regulation of neurotrophic factors gene expression (supported by NIH AG02224 to PMW).

417.7

TRANSCRIPTIONAL REGULATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR mRNA LEVELS IN HIPPOCAMPUS BY KAINIC ACID IS INDEPENDENT OF PROTEIN SYNTHESIS AND ACTIVATION OF CALMODULIN KINASE IIα. Eero Castrén*, Benedikt Berninger, Axel Leingärtner, Dan Lindholm and Hans Thoenen. *A.I. Virtanen Institute, University of Kuopio, Finland, Department of Neurochemistry, Max Planck Institute for Psychiatry, Martinsried, Germany.

We have investigated the pathways through which kainic acid increases brain-derived neurotrophic factor (BDNF) mRNA levels in cultured hippocampal neurons and in transgenic mice. Kainic acid induced the transcription of BDNF mRNA without influencing the mRNA stability. BDNF gene produces two transcripts with identical coding regions but with the 3' untranslated regions of different length. Interestingly, the half-life of the 4.2 kb BDNF transcript was much shorter than that of the 1.6 kb transcript (23±4 min. vs. 132±30 in), indicating that the 3' untranslated region of BDNF mRNA regulates mRNA stability. Increase in the BDNF mRNA levels by kainic acid was not blocked by the protein synthesis inhibitor cycloheximide demonstrating that BDNF is regulated as an immediate early gene in hippocampal neurons. Although calmodulin antagonists are known to abolish the effect of kainic acid on BDNF mRNA, this effect was very similar in Ca^{++}-calmodulin-dependent protein kinase IIα knock-out mice and in wild-type mice suggesting that either other CAM-kinase subtypes can compensate for the lack of the α type in these mice or that BDNF mRNA regulation is mediated by other calmodulin-dependent pathways than that involving CAM kinases.

417.8

DIFFERENTIAL REGULATION OF BDNF TRANSCRIPTS THROUGH A CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE PATHWAY. V. Y. Hayes*[1], K. D. Murray[2] and P. J. Isackson[2]. [1]Dept. of Molecular Neuroscience and [2]Dept. of Biochemistry and Molecular Biology, Mayo Clinic, Jacksonville, Fl 32224.

BDNF mRNA dramatically increases in cortical and hippocampal regions of the rat brain following recurrent seizures. The BDNF gene consist of at least four distinct 5' exons (exon I-IV) with different transcription initiation sites and a 3' exon (exon V) encoding preproBDNF (Timmusk et al., Neuron 10:475). Transcripts containing exon I and III are primarily responsible for increased BDNF mRNA expression following seizure induced by intraperitoneal injection of kainic acid, however they are distinctly regulated in different neuronal populations. Intracerebroventricular injection of KN62, an inhibitor of calcium/calmodulin-dependent kinase activity, results in a blockade of BDNF mRNA (exon V) induction in the neocortex, but not significantly in the hippocampus (Murray et al., Soc. Neurosci. Abstr. 19:111.15). In this study we have used in situ hybridization to demonstrate that seizure-induced BDNF transcripts containing exon I and II are inhibited in neocortex and to a lesser extent in hippocampus. In contrast, expression of transcripts containing exons III and IV are unaffected by KN62. These results provide further insight into mechanisms regulating CNS expression of BDNF mRNA.

417.9

EFFECTS OF ELEVATED INTRACELLULAR CALCIUM ON GDNF GENE EXPRESSION. J. F. Bishop, W. Wang, G. P. Mueller* and M. M. Mouradian. Genetic Pharmacology Unit, Experimental Therapeutics Branch, NINDS, and Department of Physiology, USUHS, Bethesda, MD 20892.

Glial cell line-derived neurotrophic factor (GDNF) has both protective and restorative effects on nigrostriatal dopaminergic neurons *in vivo* (Tomac et al., Nature 373:335-339, 1995) and is therefore an important candidate for treatment of Parkinson's disease. In the present study, the role of calcium in the regulation of GDNF gene expression in rat C6 glioma cells was investigated. Confluent C6 cells were exposed to the Ca^{2+} ionophore A23187 and GDNF mRNA levels were measured by reverse transcription (RT) followed by quantitative PCR (QPCR). Cells were incubated with A23187 (10^{-5} M) or vehicle for 1 or 4 hours, and total RNA was extracted. QPCR results indicated that GDNF transcripts were elevated between 5 and 10 fold compared with vehicle treated controls. Inclusion of the RNA polymerase inhibitor actinomycin D (10 μg/ml) in the culture medium for 4 hours abolished basal GDNF expression, suggesting that GDNF mRNA turnover is rapid in C6 cells. Actinomycin D decreased but did not abolish A23187-stimulated GDNF expression, suggesting that a calcium-dependent mechanism increases transcript stability. These results indicate that calcium is a prominent second messenger involved in the regulation of GDNF gene expression in C6 cells. The degree to which these observations can be extended to other cell types remains to be determined.

417.10

DIFFERENTIAL EFFECTS OF INTERLEUKIN-6 ON CALCIUM RESPONSE AND MEMBRANE RESPONSE TO NMDA IN DEVELOPING CNS NEURONS. Z. Qiu*, J.G. Netzeband and D.L. Gruol. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037

We previously found that the amplitude of the calcium response to NMDA in cultured rat cerebellar granule neurons was significantly enhanced by chronic treatment with interleukin-6 (IL-6) during development, and that calcium influx and calcium release from intracellular stores contributed to mechanism mediating such increases. To determine the role of the calcium influx via various pathways to this enhancement, we have examined (a) calcium influx through voltage-sensitive calcium channels in response to NMDA, domoate and K^+ depolarization, (b) the membrane response to NMDA and K^+ depolarization and (c) membrane passive properties in both control and IL-6 treated neurons at different ages. The granule neuron cultures were exposed to IL-6 (5 ng/ml) by addition to the culture medium. Blocking L-type, N-type and P-type calcium channels with nimodipine, ω-conotoxin GVIA and ω-Aga-IVA toxin respectively all significantly decreased the amplitude of the calcium response to NMDA and K^+ in control and IL-6 treated neurons. However, the effects were more pronounced for the IL-6 treated neurons compared to controls and age dependent differences in the sensitivity of control and IL-6 neurons to these manipulations were observed. The sensitivity of the calcium responses to calcium channel blockers was similar for K^+ and domoate in both control and IL-6 treated neurons but differed from that observed for NMDA. Electrophysiological recordings from the granule neurons showed that at 11 days *in vitro* (DIV) there were differences in membrane input resistance and resting membrane potential between control and IL-6 treated neurons. These differences were not observed at 4 DIV. However, the membrane depolarizations to NMDA stimulation were similar in control and IL-6 treated neurons at all ages studied. These results suggested voltage-sensitive calcium channel play a role in the IL-6 induced enhancement of calcium response to NMDA, whereas a larger membrane response to NMDA does not. Supported by MH47680.

417.11

REGULATED SECRETION AND VECTORIAL TARGETING OF NEUROTROPHINS IN NEUROENDOCRINE AND EPITHELIAL CELLS. J.V. Heymach Jr., A. Krüttgen, U. Suter, and E.M. Shooter*, Dept. of Neurobiology, Stanford Univ. School of Medicine, Stanford, CA 94305.

As a first step towards understanding how neurotrophins are targeted and secreted by neurons, we have investigated these questions in two model cell lines which mimic certain aspects of neuronal protein sorting: MDCK cells, a polarized epithelial cell line which targets proteins to either its apical or basolateral membranes; and AtT-20 cells, a neuroendocrine cell line which sorts secreted proteins to either regulated or constitutive pathways. When stably or transiently expressed in MDCK cells, NGF (both long and short splice variants) and NT-3 were secreted predominantly via the basolateral membrane,which is believed to correspond to the somatodendritic domain of neurons. In AtT-20 cells, both neurotrophins were sorted to the regulated secretory pathway as demonstrated by their release upon stimulation with 8-bromocyclic AMP. To determine if the NGF propeptide directs the sorting of mature NGF, we used site directed mutagenesis to generate mutants in which regions of the propeptide were deleted; in every case in which expression could be detected, these mutants were sorted similarly as wild-type NGF in both cell lines. Furthermore, NGF sorting was not significantly altered by mutations which specifically abolished N-linked glycosylation or proteolytic processing sites within the NGF precursor. These results indicate that NGF and NT-3 are vectorially targeted and secreted in a similar manner in the model cell lines examined, and suggest that the determinants of neurotrophin sorting are likely to be present within the mature neurotrophin moiety.

417.12

CHARACTERIZATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) SECRETION FROM HIPPOCAMPAL NEURONS. O. Griesbeck[1], A. Blöchl[1], J.F. Carnahan[2], H. Nawa[3] and H. Thoenen.[1]* Max-Planck-Institut für Psychiatrie, Abteilung Neurochemie, 82152 Martinsried, Germany[1]; Amgen Inc., Thousand Oaks, CA 91320, USA[2] and Beckman Neuroscience Center, Cold Spring Harbour Laboratory, Cold Spring Harbour, NY 11724, USA[3]

In previous experiments it has been demonstrated that the activity-dependent secretion of NGF from hippocampal neurons (slices and primary cultures) showed very unusual characteristics. In contrast to the activity-mediated secretion of neurotransmitters and neuropeptides, the high potassium-, veratridine- and glutamate-initiated release of NGF was independent of extracellular calcium. It could be blocked by tetrodotoxin and depended on extracellular sodium and intact intracellular calcium stores (Blöchl and Thoenen, 1995, Eur. J. Neurosci., in press). It was important to know whether these unusual characteristics of activity-dependent secretion of NGF are unique for this molecule or whether other members of the same gene family show the same features of secretion. Using a novel two-site enzyme immunoassay for BDNF, we also found an activity-dependent release of BDNF from hippocampal slices of adult rats induced by high potassium (50 mM) or glutamate (100 μM) which could be blocked by tetrodotoxin, but not by the high-affinity extracellular calcium chelator BAPTA. However, BAPTA/AM which penetrates the neuronal membrane abolished the activity-dependent release of BDNF, indicating that the observations made on the activity-dependent secretion of NGF are representative also for the other members of the NGF gene family.

417.13

CNTF RELEASE FROM CULTURED RAT HIPPOCAMPAL ASTROCYTES AND ITS REGULATION BY CYTOKINES. H. Kamiguchi*, K. Yoshida, M. Sagoh, H. Wakamoto, M. Inaba, H. Sasaki, M. Otani and S. Toya. Deptment of Neurosurgery, Keio University, Tokyo 160, Japan.

Ciliary neurotrophic factor (CNTF) has been shown to rescue various types of lesioned neurons, and it needs to be released from astrocytes into the extracellular space to have an effect on neurons. However, direct evidence for CNTF release has not been unequivocally demonstrated. We hypothesized that the rapid sequestration by CNTF receptor present on cultured astrocytes might be the cause of the inability to detect CNTF released from astrocytes into the medium. Therefore, by two-site ELISA, we measured CNTF immunoreactivity in medium conditioned by astrocytes treated with phosphatidylinositol-specific phospholipase C (PI-PLC) which was used to prevent released CNTF from binding to the CNTF receptor on cultured astrocytes, since PI-PLC cleaves the glycosyl-phosphatidylinositol anchor of CNTFRα, the unique component involved in CNTF binding. CNTF immunoreactivity was not detectable in untreated astrocyte-conditioned medium (ACM), but was detectable in PI-PLC-treated ACM. These results together with the evidence that PI-PLC treatment used in this experiment did not have a toxic effect on astrocytes prove the fact that CNTF can be released from astrocytes without cell lysis. Subsequently, the effects of cytokines such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and epidermal growth factor (EGF), which are likely to be present in the brain following injury, were examined on CNTF release. Those cytokines increased CNTF protein levels in PI-PLC-treated ACMs without increasing CNTF protein levels in astrocyte-extracts, indicating that those cytokines enhance CNTF release from astrocytes.

417.14

IDENTIFICATION OF A STRUCTURAL DOMAIN RESPONSIBLE FOR SECRETION OF A NEUROTROPHIC FACTOR FOR CILIARY GANGLION NEURONS C. Gary Reiness*, Sean Sweeney, and Rae Nishi#, Dept. of Biology, Lewis & Clark College, Portland, OR 97219 and #Dept. of Cell Biology & Anatomy, Oregon Health Sciences University, Portland, OR 97201

Although growth promoting activity (GPA) and ciliary neurotrophic factor (CNTF) are both potent trophic factors for chick ciliary ganglion neurons in vitro, they differ in their abilities to be released from cells. The present study was undertaken in order to determine the structural basis for this difference in release between GPA and CNTF. GPA is homologous (50% amino acid identity) to CNTF; however, when GPA and CNTF were expressed by transfected cells, biological activity due to GPA was detectable in the medium, whereas CNTF was not (Leung et al., Neuron, 8: 1045, 1992). Neither GPA nor CNTF contains an N-terminal signal sequence for secretion, but GPA contains a sequence between positions 43-63 that is significantly more hydrophobic than the corresponding sequence in CNTF. We hypothesized that this internal hydrophobic domain might serve as a signal for secretion. To test this hypothesis, we generated a chimeric sequence that coded for the N-terminal 57 amino acids of GPA and the C-terminal 143 amino acids of CNTF. COS cells transfected with this vector expressed and secreted a protein that supported the survival of embryonic chick ciliary neurons in culture and was recognized by anti-rat CNTF antisera in an immunoblot assay. When CNTF was expressed in the same vector in COS cells, neurotrophic activity was found within cell extracts and no detectable activity was found in the culture medium. Thus the first 57 amino acids of GPA contain a signal that can target the molecule into a secretory pathway. We are currently conducting experiments to characterize the pathway by which the chimeric neurotrophic factor is secreted by COS cells. *Supported by the Murdock College Science Program (CGR), the American Heart Association, Oregon Affiliate (CGR), and NINDS (RN).*

417.15

INTRAVENTRICULAR AND INTRASTRIATAL INFUSION OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) IN THE CYNOMOLGOUS MONKEY: RETROGRADE TRANSPORT AND COLOCALIZATION WITH DOPAMINE CONTAINING SUBSTANTIA NIGRA NEURONS. E. J. Mufson*[1,3], J. S. Kroin[2], Y-T. Liu[1], T. Sobreviela[1], R. D. Penn[2], J. A. Miller[3] and J. H. Kordower[1]. Depts. Neurol. Sci.[1] and Neurosurg.[2], Rush Med. Ctr, Chicago, IL 60612 and Amgen Inc.[3], CA.

The distribution and retrograde transport of BDNF was examined using MRI guided stereotaxic intracerebroventricular (ICV) and intrastriatal infusion in the *cynomolgous* monkey. Two ICV animals were infused with BDNF at a dose of 3 µg/h for 21 and 28 days and a third ICV animal received sequential infusions of 15, 30 and 60 µg/h BDNF each for 7 days using an Alzet minipump. For the multiple intrastriatal animals (n=5) a dose of 3 µg/h was infused into each site. Following the lower dose ICV infusion, BDNF immunoreactivity was confined to the ventricular ependymal layer. In the sequential higher dose ICV case the cannula was located mainly within the lateral ventricle, although there was damage to the ependymal wall and adjacent caudate nucleus. BDNF immunoreactivity revealed spread of injectate within the ipsilateral and to a lesser extent the contralateral caudate nucleus, septum, orbital cortex and ventricular ependymal wall. In this case, retrogradely labeled BDNF neurons were found within the parafascicular thalamus and substantia nigra, pars compacta, as well as within cortex and basal forebrain. BDNF intrastriatal infusion retrogradely labeled perikarya within sensory motor cortex, parafascicular thalamus and substantia nigra, pars compacta. Sections from these cases dual immunoreacted for BDNF and tyrosine hydroxylase, the synthesizing enzyme for dopamine, revealed a subpopulation of pars compacta dopaminergic neurons which contained retrogradely transported BDNF. These findings indicate that a select subgroup of nigral dopamine neurons retrogradely transport BDNF in the primate and that a subpopulation of nigral cells may be responsive to the trophic influences of BDNF in the normal and pathologic state.

417.16

RETROGRADE TRANSPORT OF rhBDNF FOLLOWING NEO AND LIMBIC CORTEX INFUSION IN RAT. T. Sobreviela[1]*, M. Pagcatipunan[2], J. S. Kroin[3], R. D. Penn[3], J. Miller[4] and E. J. Mufson[1]. Depts. of Neurol. Sci.[1], Neurosurgery[3], Rush Presbyterian Med. Ctr. Chicago, IL 60612, Univ. Illinois[2], Chicago and Amgen[4], CA.

Brain-derived neurotrophic factor (BDNF) mRNA distribution differs from that of other members of the NGF family of neurotrophins within the central nervous system. To determine those neuronal populations which transport BDNF, rats were infusioned with rhBDNF at a dose of 3 µg/h for 7 days using an Alzet 2002 minipump into the frontal, occipital, entorhinal cortex, amygdala and lateral ventricle. Immunohistochemistry was performed using a turkey anti-BDNF antibody. Frontal cortex infusion revealed retrogradely labeled neurons within frontoparietal, cingulate and perirhinal cortex, endopiriform nucleus, diagonal band, nucleus basalis, dorsal thalamus, lateral hypothalamus and locus coeruleus. Occipital infusion retrogradely labeled neurons in the temporal, ento/perirhinal, retrosplenial and occipital cortex as well as the claustrum, medial septum, diagonal band, dorsal thalamus, dorsolateral geniculate and hypothalamus. Entorhinal infusion retrogradely labeled neurons in the frontal, temporal, perirhinal cortex, CA fields, subiculum, endopiriform nucleus, septal/diagonal band complex, amygdala, dorsal thalamus, nucleus basalis, lateral hypothalamus, supramammilary region, brainstem tegmentum, raphe nuclei and locus coeruleus. Amygdaloid infusion retrogradely labeled neurons in the diagonal band/nucleus basalis, intra-amygdaloid nuclei, piriform, ento/perirhinal and occipital cortex as well as dorsal and epithalamus, hypothalamus, substantia nigra pars compacta, dorsal raphe and parabrachial regions. In contrast, after intracerebroventricular rhBDNF infusion, immunoreactivity was restricted to the ependymal wall of the ventricular system. These findings indicate that selective neuronal subgroups retrogradely transport BDNF. Moreover, these neurons may respond to this neurotrophin in the normal or pathologic condition.

417.17

NEUROLOGIC SYMPTOMS EXHIBITED BY TRANSGENIC MICE EXPRESSING ANTISENSE INTERLEUKIN 3 (IL3) RNA. Y. Sugita*, C.E. Dunbar†, S. Doren†, D.M. Cockayne¶, J.P. Schwartz. Clinical Neuroscience Branch, NINDS, †Hematology Branch, NHLBI, NIH, Bethesda, MD 20892 and ¶DNAX Res. Inst., Palo Alto, CA 94131.

IL3 is an important mediator in physiological and pathophysiological processes affecting the central nervous system (CNS). Microglia react to brain injury or inflammation with proliferation, migration and differentiation. IL3 stimulates proliferation of microglia, but also is a trophic factor for central cholinergic neurons. The major source of IL3 in the CNS is activated astrocytes and microglia. To examine the role of IL3 in the CNS, transgenic mice expressing murine antisense IL3 (AS-IL3) RNA were generated. RT-PCR confirmed expression of the AS-IL3 transgene in the CNS and hematopoietic tissues, and ELISA showed decreased IL3 levels in the periphery. AS-IL3 transgenic mice exhibit either progressive neurologic dysfunction or a lymphoproliferative syndrome: occasional mice with the neurologic syndrome show splenomegaly. Neurologic symptoms include paralysis (mono-, para- or hemiplegia) and ataxia or bradykinesia, followed by wasting and death resulting from an inability to eat or drink. Histopathological examination shows multifocal proliferation /infiltration of activated microglia in the brain stem and spinal cord, with some astrocytosis. In addition, B lymphocytes infiltrate around the brain and spinal cord, resulting in compression of the parenchyma. However, AS-IL3 transgenic mice do not exhibit abnormalities of the neuronal network or altered cell number in the hematopoietic system. Thus, IL3 may be required for appropriate expression of other cytokines/growth factors, which in turn regulate the environment of the CNS.

418.1

DIFFERENT CELLULAR MECHANISMS UNDERLIE THE POTENTIATION OF TRANSMITTER SECRETION AT DEVELOPING NEUROMUSCULAR JUNCTIONS BY BDNF AND CNTF.

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Extracellular application of brain-derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF) have been shown to cause rapid potentiation of spontaneous and evoked transmitter secretion from presynaptic nerve terminals at developing neuromuscular junctions. Three lines of evidence suggest that these two factors may potentiate the transmitter secretion by acting on different sites in the secretory machinery. First, CNTF requires signaling with the cell body for its potentiating effect, whereas BDNF can still potentiate secretion from a presynaptic nerve terminal transected from the cell body. Second, at a concentration of 100 ng/ml of BDNF or CNTF, which produces saturating effects on potentiating transmitter secretion, addition of CNTF or BDNF (100 ng/ml), respectively, further increases the potentiation significantly. The two factors may act synergistically, since addition of a low concentration (1 ng/ml) of CNTF, which by itself was ineffective, greatly elevated the potentiation effect of a low concentration (1 ng/ml) of BDNF. Third, application of BDNF (100 ng/ml) significantly reduces the paired-pulse facilitation at these synapses, while CNTF at the same concentration is without effect. Since distinct receptors and transduction mechanisms are involved in the actions of BDNF and CNTF, these neurotrophic factors may act in an additive or synergistic manner in promoting the synaptic functions at developing synapses.

418.2

MECHANISM OF POTASSIUM CHANNEL REGULATION BY NEUROTROPHINS AND CNTF.

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Neurotrophic factors strongly influence the development and maintenance of neuronal excitability through the regulation of ion channel and neurotransmitter receptor expression. In this study, using electrophysiological and molecular techniques, we have focused on potassium channel regulation in the human neuroblastoma cell line SK-N-SH. This sympathetic-like cell line is responsive to several neurotrophic factors, including the neurotrophin nerve growth factor (NGF) and the unrelated neural cytokine ciliary neurotrophic factor (CNTF). In addition, we found that retinoic acid treatment induces TrkB and TrkC expression in SK-N-SH cells, and thus confers additional responsiveness in these cells to brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and NT-4.

Treatments with NGF, NT-3, NT-4, or CNTF all increase a delayed rectifier potassium current in SK-N-SH cells. Using non-stationary fluctuation analysis on whole-cell currents we provide evidence that the predominant channel species in outside-out patches underlies the macroscopic current, supporting our hypothesis that these neurotrophic factors lead to increased expression of functional channels. To test whether transcriptional regulation mediates the regulation of potassium channels by NGF, NT-3, NT-4, and CNTF we used RT-PCR with degenerate primers to identify which potassium channels are expressed in SK-N-SH cells before and after neurotrophic treatments. With this method we have identified two human potassium channels, Kv1.1 and Kv1.3, which are expressed in these cells..

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418.3

NEUROTROPHINS MODULATE SYMPATHETIC CONNECTIONS TO CARDIAC MYOCYTES.

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The classic role for neurotrophins in the nervous system are as target-derived survival factors. Recent evidence suggest that neurotrophins also act to modulate synaptic strengths at the neuromuscular junction and between central synapses. We are interested in the role of neurotrophins in the formation and modulation of synaptic connections between sympathetic neurons and their cardiac myocyte targets. We have established co-cultures of rat neonatal superior cervical ganglia (SCG) neurons and ventricular myocytes. The myocytes form clusters that spontaneously beat in culture. Functional connections between SCG neurons and myocytes were assayed by stimulating SCG neurons intracellularly and monitoring myocyte beat frequency. At early times in culture (2-3 days) stimulation of SCG neurons accelerated myocyte beat frequency. At later times (2-3 weeks) the effect was inhibitory and was blocked by atropine, indicating that the neurons had undergone a switch to a cholinergic phenotype as previously described (Furshpan, Macleish, O'Lague, and Potter 1976, PNAS 73:4225-4229). We examined the effect of NGF on synapse strength after 2-3 days in culture. NGF strongly potentiated the acceleration of myocyte beat frequency induced by SCG stimulation. The affects were rapid (2-5 minutes in onset), and reversed within 5-20 minutes of NGF washout. These results suggest that neurotrophins act to acutely modulate sympathetic synapses in culture. This modulation may be important for the initial formation and consolidation of synapses during early development.

418.4

NERVE GROWTH FACTOR ACUTELY ENHANCES HIGH-VOLTAGE ACTIVATED CA^{2+} CURRENTS IN ADULT MOLLUSCAN NEURONS.

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Murine NGF induces sprouting in specific types of *Lymnaea* neurons, trophic actions that occur over a time course of hours to days. The present study investigated acute effects of NGF in identified motoneurons previously shown to sprout in response to NGF. Right Parietal A group (RPA) neurons were isolated from adult animals and plated on a poly-L-lysine substrate in a defined medium (DM). Using standard whole-cell voltage clamp techniques, the effect of NGF on Ca^{2+} currents in RPA neurons were studied within 1 to 5 hours after plating. RPA neurons predominantly express a high-voltage activated (HVA) sustained Ca^{2+} current. Superfusion of murine 2.5S NGF evoked a rapid and reversible increase of this HVA Ca^{2+} current (n=31). In a typical experiment, the introduction of 1 ng/ml NGF in the bath induced a maximal increase in peak inward HVA current of 67% within 2 minutes. The effect completely reversed within minutes after the withdrawal of NGF. Although similar effects were observed in the majority of RPA neurons tested (n=31), the responsivity varied considerably between neurons. Data suggest that in some cells the threshold dose may be as low as 1 pg/ml. Since both the disruption of NGF's tertiary structure by alkylation of its cysteine residues,as well as preabsorption of NGF with an anti-NGF IgG abolished NGF's activity, the effect on HVA Ca^{2+} currents is unlikely to be non-specific. These data indicate that enhancement of transmembrane Ca^{2+} currents is one of the early events in NGF signal transduction in *Lymnaea* neurons. Moreover, the low threshold dose of the effect suggests the involvement of a high-affinity receptor. Supported by NCE (Canada) and MRC (Canada).

418.5

NON-TROPHIC EFFECTS OF NERVE GROWTH FACTOR (NGF) ON NEURONS OF THE MESENCEPHALIC TRIGEMINAL NUCLEUS (MES-V).

J. Yamuy*, C. Pedroarena, J. Pose, F.R. Morales and M.H. Chase. *Departamento de Fisiología, Facultad de Medicina, Montevideo, Uruguay and Department of Physiology and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA, 90024.*

Microinjection of NGF into the rostral portion of the pontine reticular formation elicits, within a short latency, an active sleep-like state (1, 2) similar to that induced by cholinergic agonists. This finding led us to hypothesize that neurotrophins may directly affect neuronal excitability. In the present study, we examined this hypothesis with respect to neurons of the Mes-V, in which we have previously reported that fast (80-120 Hz) oscillations of the membrane potential develop upon membrane depolarization and may trigger bursts of rythmic action potentials (3).

Intracellular recordings from Mes-V neurons were obtained utilizing brainstem slices of adult rats. Microdroplets of NGF, at a concentration of 1µg/µl, were applied locally by pressure ejection. Following NGF application, the amplitude of the oscillations increased. In addition, the threshold for their occurrence was reduced. The augmented oscillations could also elicit the discharge of previously silent neurons. Rheobase was lower, which was probably due to the NGF-induced enhancement of the oscillations evoked by depolarizing pulses. No consistent changes in membrane potential were detected. The configuration of the spike and the action potential's afterhyperpolarization remained unchanged. These effects occurred 5 to 30 seconds after the application of NGF and persisted for 15 to 20 minutes.

These data suggest that NGF, in addition to its well defined role in regulating the survival and phenotype of neurons, has the potential for exerting modulatory actions on their intrinsic electrophysiological properties. This work was supported by USPHS Grants NS 23426, MH 43362 and NS 09999.

1. Yamuy et al., *Soc. Neurosci. Abstr.*, Vol. 20, Part 2, p. 1104, 1994.
2. Yamuy et al., *Neuroscience*, in press.
3. Pedroarena et al., *Soc. Neurosci. Abstr.*, Vol. 20, Part 2, p. 1736, 1994.

418.6

ACTIVATION OF SPHINGOSINE KINASE DIFFERENTIATES THE SIGNALING PATHWAYS OF EPIDERMAL AND NERVE GROWTH FACTORS IN PC12 CELLS.

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Nerve growth factor (NGF) leads to neurite outgrowth and cessation of cell growth of rat pheochromocytoma (PC12) cells, whereas epidermal growth factor (EGF) stimulates cell proliferation. However, no major differences have yet been identified in the intracellular signal transduction pathways activated by the receptors for these growth factors. We have now found that NGF transiently activates cytosolic sphingosine kinase, which catalyzes the phosphorylation of the primary hydroxyl group of sphingosine to produce sphingosine-1-phosphate (SPP). SPP is a putative second messenger which mobilizes calcium in an inositol trisphosphate-independent manner, that has been implicated in the mitogenic action of platelet derived growth factor and serum (Olivera and Spiegel, Nature, 365: 557, 1993). In contrast to NGF, EGF had only a small effect on the activity of this kinase. Furthermore, long term treatment of PC12 cells with NGF induced marked increase in sphingosine kinase activity. This increase of nearly 2 fold was found after 2 days and reached a maximum of 5 fold after 3 days and remained elevated even after 2 weeks of treatment with NGF. Similar stimulations were observed in serum or serum-free media. Kinetic characterization indicated an increased *Vmax*. In contrast to NGF, chronic treatment of PC12 cells with EGF, phorbol myristate acetate, or KCl had little or no effect on sphingosine kinase activity. However, agents that increase intracellular levels of cAMP uniformly lead to increases in sphingosine kinase activity. These results indicate that activation of sphingosine kinase and subsequent production of SPP might have an important biological role in signal transduction pathways activated by NGF. Further involvement of sphingolipid metabolites in NGF action is under investigation.

418.7

MODIFICATION OF NGF SIGNALLING BY EXTRACELLULAR MATRIX ADHESION IN PC12 CELLS
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Adhesion to extracellular matrix proteins is known to exert widespread influences over many cellular processes including differentiation, cytoskeletal organization, and cell cycle progression. Many of these processes are also regulated by growth factors such as NGF. We therefore investigated the responses to NGF treatment of adherent and nonadherent PC12 cells. For these studies, PC12 cells were grown either on collagen-coated or agarose-coated dishes. Nonadherent cells grown on agarose-coated dishes showed reduced long term viability as compared to adherent cells. Treatment of adherent cells with NGF reduced proliferation rate as measured by thymidine uptake that was associated with neuronal differentiation. In contrast, proliferation of nonadherent cells was increased by NGF treatment. NGF-associated signalling was investigated by phosphotyrosine immunoblotting of cell extracts from adherent and nonadherent cells in the presence and absence of NGF. Adherent cells showed patterns of NGF-induced tyrosine kinase activity that differed from those seen in nonadherent cells. These results suggest that signal transduction events and cellular responses to NGF are modified by extracellular matrix adhesion.

418.8

PROMOTING EFFECTS OF EXTRACELLULAR MATRIX IN NEURONAL REGENERATION. SELECTIVE ROLE OF GLYCOSAMINOGLYCANS. A.M. Di Giulio*, L. Vergani, E. Germani, B. Tenconi, G. Prino and A. Gorio. Lab for Research on Pharmacology of Neurodegenerative Disorders, Dept Medical Pharmacology, Via Vanvitelli 32, Milano 20129, Italy.

The extracellular matrix plays a significant role in nerve regeneration and neurite formation and guidance in vitro. We have tested in vitro the ability of a variety of glycosaminoglycans upon promoting neurite formation in 5YSY neuroblastoma cells after exposure to serum-free medium. Heparin sulfate, chondroitin sulfate and a mixture, named Ateroid, were the most effective glycosaminoglycans in promoting neurite formation. The effective concentrations were as low as 1pM. We have assayed the in vitro active glycosaminoglycans in vivo after permanent axotomy of the sciatic nerve in the rat. Twentyone days after nerve lesion substance P content in the lumbar cord drops from 10.1 to 6.6 ng/mg protein; treatment with glycosaminoglycans by daily intraperitoneal injection at the dose of 0.2 mg/kg counteracts the loss and promotes a huge increase to 18.5 ng/mg protein. A similar effect is observed for met-enkephalin lumbar cord content. Seven and twenty days after sciatic nerve injury the abundance of NGF mRNA increased by 6 fold in the proximal stump at the site of lesion, glycosaminoglycan treatment promoted a 15 fold increase in the lesioned nerve and 10 fold in the unlesioned contralateral. The abundance of p75 NGF receptor mRNA increased 10 fold after nerve lesioning, in animals treated with glycosaminoglycans the increase was 4 fold. Also the levels of brain derived neurotrophic factor and neurotrophin-3 mRNAs increased markedly 20 days after nerve transection, in animals treated with glycosaminoglycans a similar increase also occurred in the contralateral nerve. These data suggest that glycosaminoglycans promote neurite growth and prevent retrograde degeneration, the latter effect may be mediated by an increased production of neurotrophic factors.

418.9

DIFFERENTIAL EFFECTS OF NGF AND CALPHOSTIN C IN PC12 CELLS. D. Maysinger*, S. Panarello, A.L. Padjen, T. Hashiguchi & M. Hashiguchi. Dept. of Pharmacology & Therapeutics, McGill University, Montreal, QC H3G 1Y6, Canada & Dept. of Physiology, Tokyo Medical College, Shinjuku-ku, Tokyo 160, Japan.

Pheochromocytoma cells (PC12) differentiate in the presence of nerve growth factor (NGF). Furthermore, this trophic factor also enhances enzymatic activity of Na^+,K^+-ATPase which is responsible for active transport of Na^+ and K^+ in most animal cells. The enzyme plays a central role in mediating electrical activity in the nervous system. A selective protein kinase C (PKC) inhibitor, calphostin C, also enhances the enzymatic activity of Na^+,K^+-ATPase *in vivo*.

The aim of this study was to assess the possible interaction between NGF and calphostin C effects. We examined the changes in Na^+,K^+-ATPase activity, expression of catalytic α subunit and the extent of phosphorylation and used image analysis to study differentiation as well as electrophysiological measures of Na,K pumping and excitability. Results show that: [1] Unlike NGF which induces neurite outgrowth in both serum containing and serum-free medium calphostin C had no effects; [2] Both NGF and calphostin C moderately enhanced Na^+,K^+-ATPase activity when administered individually; in combination they exert remarkable morphologic effects in PC12 cells (increased length and decreased number of neurites per cell - appearance of spindle-like cells) and also enhance significantly Na^+,K^+-ATPase enzymatic activity. [3] The effects of calphostin C was concentration-dependent and bimodal: higher concentrations (>50 nM) caused general neurite retraction whereas lower concentrations had selective effect on distal neurites.

In conclusion these results suggest that NGF and calphostin C exert their effects on morphological changes, enzymatic activity and phosphorylation pattern in PC12 cells via different mechanisms. In addition, exposure to light seems to play a role in the phototoxic effect of calphostin C. (Supported by in part by MRC).

418.10

NGF Inducible Regulatory Elements in the Rat Cyclin D1 Promoter. G-Z. Yan* and E. B. Ziff. Howard Hughes Medical Institute, and Department of Biochemistry, New York University Medical Center, New York NY 10016. Nerve growth factor (NGF) causes growth arrest and promotes rat PC12 cells to differentiate into sympathetic neuron-like cells. Our previous studies have indicated that NGF regulates the PC12 cell cycle machinery through specific inhibition of the Cdk kinases by p21, a cdk inhibitory protein, and induction of cyclin D1. Over expression of cyclin D1 in PC12 which leads to inhibition of PCNA expression is sufficient on its own to arrest the cells in G1 phase. Our recent studies show that constitutive expression of the *c-myc* gene in PC12 cells leads to a reduced level of cyclin D1 protein, while PCNA protein remains at high levels, suggesting that c-myc protein may be involved in mediating the regulation of cyclin D1 following NGF treatment. To further understand the mechanisms by which NGF regulates cyclin D1 during NGF control of PC12 cell cycle machinery, we have cloned the rat cyclin D1 promoter and analyzed the structure and function of 3kb of its 5'-flanking region. We find the upstream sequences of rat cyclin D1 gene are highly homologous to those of the human cyclin D1 gene. The cyclin D1 promoter is inducible by NGF in PC12 cells, which is similar to the induction of cyclin D1 by NGF *in vivo*. Repression by c-myc is also observed with the cyclin D1 promoter in PC12 cells. Deletion analysis has identified several regulatory regions which are involved in both basal level and NGF-induced cyclin D1 expression. We are now performing tests to determine the functional significance of these elements and the factors which interact with these elements in regulation of cyclin D1 expression.

418.11

CHARACTERIZATION OF AN mRNA REQUIRED FOR NEURITE REGENERATION IN NGF-PRIMED PC12 CELLS. J.L. Twiss* and E.M. Shooter. Depts. of Neurobiology and Pathology, Stanford Univ. Medical School, Stanford, CA 94305.

Neurite regeneration from NGF-primed PC12 cells is independent of RNA synthesis, but requires new protein synthesis. Regeneration is initiated by translation of proteins from short-lived mRNAs ($t_{1/2} \leq$ 5-6 h) produced during the NGF-priming period. Despite a careful search for short-lived mRNAs in NGF-primed PC12 cells, we found that those encoding structural proteins thought essential for neuritogenesis are all long-lived. To identify short-lived mRNAs in PC12 cells, we produced cDNA libraries from NGF-primed PC12 cells under conditions permissive and non-permissive for neurite regeneration. The permissive library [NGF x 10 d, RNA synthesis inhibition x 5 h] was screened with a subtracted probe enriched for short-lived mRNAs. The probe was generated by hybridizing cRNAs from the non-permissive libraries [NGF x 3 d and NGF x 10 d, RNA synthesis inhibition x 12 h] to ^{32}P-labeled single-stranded cDNA inserts from the permissive library to an $R_0t > 1000$. Thus far, we have identified a single clone, NSL8, with a remarkably short mRNA half-life in NGF-primed PC12 cells ($t_{1/2} \leq 4.5$ h). Antisense NSL8 oligonucleotides diminish neurite regeneration from NGF-primed PC12 cells while sense NSL8 oligonucleotides are without effect. Sequence analysis shows that NSL8 cDNA likely represents the rat homologue of the human ribosomal associated protein, L4. It is probable that rapid translation of NSL8 mRNA following neurite injury provides translational competence to regenerate neurites from a large pool of long-lived mRNAs encoding structural proteins.

418.12

CHANGES IN ANTIOXIDANT HOMEOSTASIS AND CELL VIABILITY IN NGF-PRODUCING SY5Y. D.P. Pizzo*, and J.R. Perez-Polo. Dept of Human Biological Chemistry and Genetics. Univ. of Texas Medical Branch, Galveston, TX 77555-0652

Nerve growth factor (NGF) regulates survival of several cell types both in vivo and in vitro. Protection from cell death occurs through a variety of mechanisms, including the regulation of antioxidant homeostasis. Here we describe an autocrine NGF model system consisting of NGF responsive SK-N-SH-SY5Y cells infected with a retroviral vector containing the NGF gene. The resulting cell line ($SY5Y^{NGF+}$) secretes NGF at a lower rate than that measured in fibroblast cell lines infected with the same NGF construct. The $SY5Y^{NGF+}$ has altered growth properties, and significant changes in several aspects of glutathione metabolism. The rate limiting enzyme in glutathione synthesis, gammaglutamylcysteine synthetase was reduced by greater than fifty percent in two different $SY5Y^{NGF+}$ cell lines while the corresponding levels of glutathione were slightly but not significantly reduced. The protective enzyme, glutathione peroxidase, however, was slightly elevated in one of two $SY5Y^{NGF+}$ clonal lines. The cells also had a diminished viability in defined media and a rapid onset of cell death as compared to the parental SY5Y. Within two days in defined media there was a noticeable change in morphology that resembled that typically seen in apoptotic cells. Compounds that inhibit the cell death of PC12 cells in serum free media such as NGF or cAMP did not protect the $SY5Y^{NGF+}$ cultures. However, aurintricarboxylic acid and the phorbol ester TPA enhanced cell viability. These clonal lines should be useful in studies on cell death regulation as well as the effects of chronic neurotrophin treatment. **Supported by a grant from the NASA/Texas Space Grant Consortium to DPP and NS18708 to JRPP.**

418.13

NGF COUPLES PROTEIN SYNTHESIS AND DEGRADATION IN SYMPATHETIC NEURONS. J. L. Franklin* and E. M. Johnson, Jr. Dept. of Molecular Biology and Pharmacology , Washington Univ. Sch. of Med., St. Louis, MO 63110.

Inhibitors of macromolecular synthesis are generally assumed to exert their effects on cells solely through inhibition of protein synthesis. We report here that the translational inhibitor, cycloheximide (CHX), and the transcriptional inhibitor, actinomycin D (ACT-D), also cause profound suppression of protein turnover in sympathetic neurons maintained in cell culture in the presence, but not absence of NGF. Superior cervical ganglia were dissected from embryonic-day-21 rats. Neurons were enzymatically and mechanically dissociated from the ganglia and maintained in culture in the presence of NGF for a week. For twenty-four hours before the beginning of the experiment neurons were incubated in medium containing ^{35}S cysteine/methionine Trans label to allow ample time for incorporation of the labeled amino acids into cellular proteins. The label was then washed out and the cells were incubated in cold medium for 6 hours to remove labile proteins from the analyzed pool. They were then exposed for 72 hours to either CHX or ACT-D in the presence or absence of NGF. Neurons were then lysed and proteins precipitated with TCA. Both CHX and ACT-D caused a dose-dependent suppression of turnover in cells maintained in NGF. Total turnover in untreated cells during this time was about 40% either in the presence or absence of NGF. In cells maintained for this period in medium containing NGF and either CHX or ACT-D protein turnover was suppressed by 70-80%. The IC_{50} for for suppression by both CHX and ACT-D was about 100 ng/ml of medium. In neurons maintained in the absence of NGF the inhibitors did not prevent turnover. These data indicate that a biological effect of neurotrophic factors is to maintain coupling of protein synthesis and protein degradation in neurons. Supported by the Ronald McDonald foundation.

418.14

TROPHIC FACTORS ALTER CELLULAR METABOLISM AND EXPRESSION OF THE GLUCOSE TRANSPORTERS GLUT1 AND GLUT3 DURING NITRIC OXIDE TOXICITY. K. Maiese*, S. J. Vannucci, T. M. Davis-Hill, L. Boccone, and I. A. Simpson, Dept. of Neurology, Wayne State University School of Medicine, Detroit, MI, 48201, and NIDDK, NIH, Bethesda, MD, 20816

The trophic factors basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) are neuroprotective during anoxia and nitric oxide (NO) toxicity (Maiese, et al. *J Neurosci* 13: 3034-3040, 1993) and have been shown to alter the expression of glucose transporters. Facilitated glucose transport across cellular membranes is mediated by a family of transporter proteins that include the transporters GLUT1 and GLUT3 expressed in the cental nervous system (Maher, F., et al. *FASEB* 8: 1003-1011, 1994). Given the link between metabolism, NO toxicity, and neuroprotection by trophic factors, we examined the role of metabolism and the transporters GLUT1 and GLUT3 in the protective mechanisms of bFGF and EGF during a five minute exposure to 300μM of the NO generator sodium nitroprusside in primary hippocampal neuronal cultures. Exposure to NO reduced neuronal survival from approximately 95% to 30% and increased glucose consumption (8±3 (normoxia) to 23±3 (NO), μMx10^{-5}/cell, n=10, p<0.001) and lactate production (23±3 (normoxia) to 74±5 (NO), μMx10^{-5}/cell, n=10, p<0.001). In contrast, 24 hour pretreatment with bFGF or EGF during NO exposure protected neurons (increased survival from 28±5% (NO) to 65±5% (NO + bFGF/EGF), n=10, p<0.001) and decreased glucose consumption (23±3 (NO) to 18±4 (NO+bFGF/EGF), μMx10^{-5}/cell, n=10, p<0.001) and lactate production (74±5 (NO) to 40±4 (NO+bFGF/EGF), μMx10^{-5}/cell, n=10, p<0.001). In comparison to normoxic cultures, NO decreased the expression of GLUT1 by approximately 10% and GLUT3 by approximately 45%. Yet, a 24 hour pretreatment with bFGF or EGF during NO exposure restored the expression of GLUT1 and GLUT3 similar to that observed with normoxic cultures. Our results demonstrate that during NO exposure, bFGF and EGF reduce glucose consumption and lactate production and restore the expression of GLUT1 and GLUT3 similar to that observed in normoxic cultures. Further study of NO, cellular glucose transport, and trophic factor neuroprotection may direct future therapy for neurodegenerative disease.

NEUROTROPHIC FACTORS: BIOLOGIC EFFECTS VII

419.1

DIFFERENTIATION OF EMBRYONAL CARCINOMA CELLS BY NEUROTROPHIC FACTORS AND RETINOIC ACID. W. M. W. Cheung*, A. H. Chu, M. F. Leung and N. Y. Ip. Department of Biology, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong.

Previous studies have demonstrated that cloned human embryonal carcinoma (EC) cells (NTERA-2 cl.D1 or NT2/D1) could be induced to differentiate into several morphologically distinct cell types by treatment with retinoic acid. Such differentiating actions of retinoic acid may be mediated by the induction of neurotrophic factor receptors, as suggested by a recent study with neuroblastoma cells. We have used the NT2/D1 EC cells as a model system to examine whether neurotrophic factors alone, or in combination with retinoic acid, could effect neuronal differentiation. Neurotrophic factors were found to induce differentiation of NT2/D1 EC cells in a manner similar to that observed with retinoic acid. Following treatment with specific combinations of neurotrophic factors, NT2/D1 EC cells showed a disappearance of EC phenotype and neuronal cell morphology were observed within two weeks. Of the various neurotrophic factors examined, brain-derived neurotrophic factor (BDNF) appeared to be the most critical component involved in the differentiating process. Thus, similar to the recent findings with the sympathoadrenal progenitor (MAH) cells, collaboration of neurotrophic factors plays an important role in the process of neuronal differentiation.

419.2

DIFFERENTIATION OF THE DRG: EFFECTS OF NEUROTROPHIN-3 AND LIMB BUD ABLATION PRIOR TO POST-MITOTIC NEURONAL CELL DEATH. N.E. Fox*,A.C. Rusoff*,S. Hapner*,D.Edmo*,D.O. Clary#, L.F. Reichardt## and E. Lefcort*, Dept. of Biology and WAMI, Montana State Univ.*, Bozeman, MT; Sugen, Inc., Redwood City, CA# and HHMI, UCSF, San Francisco, CA##.

We are studying the cellular and molecular mechanisms involved in the differentiation of the avian Dorsal Root Ganglia, prior to the period of post-mitotic neuronal cell death. Our previous work has pointed to a role for TrkC in DRG differentiation (Lefcort et al., Soc. NS 20: 238). TrkC is expressed throughout the formation of the DRG, with its onset in migrating neural crest cells and by E4-5 is expressed by ca 70% of the DRG cells (Lefcort et al., manuscript in preparation). In the chick, NT-3 has been shown to be expressed in the developing limb bud during the period of DRG differentiation. In that brachial DRG are larger than more rostral DRG from the onset of their formation, we are testing the effects of limb bud ablation and of exogenously supplied NT-3 on the brachial DRG prior to the period of neuronal programmed cell death. Following unilateral wing bud ablation at St. 18 (E 2.5-3), embryos are fixed at St.25 (E 4.5). We have found that even at this early stage, brachial DRG on the transected side have 22% (n=4) fewer cells than their contralateral control-side DRG. Brachial DRG in embryos treated with NT-3 from St.17 and fixed at St.25, undergo an increase of 33% in cell number (n=4) relative to controls. Since the time course for these treatments is occurring well before post-mitotic neuronal cell death is underway, our data point to a role for NT-3 and/or other factor(s) associated with the developing limb bud in the early formation of the DRG. We are now in the process of determining whether NT-3 can rescue DRG cells from limb-bud ablation. Further, we are determining what the effects of NT-3 are on DRG development: proliferation or prevention of cell death of neuroblasts, and/or promotion of their differentiation or maturation.We thank Genentech, South San Francisco, CA for NT-3. Supported by MONTS, ACS, NIH and HHMI.

419.3

REGULATION OF EARLY PHOTORECEPTOR DEVELOPMENT BY CNTF. S. Fuhrmann, M. Kirsch and H.-D. Hofmann*. Institute of Anatomy, University of Freiburg, P.O.B. 111, D-79001 Freiburg, Germany.

Ciliary neurotrophic factor (CNTF) originally purified as a survival factor for ciliary ganglion neurons has been shown to have multiple effects on central and peripheral neurons. We are interested in a possible function of CNTF in the regulation of retinal development. In the present study we have investigated the influence of CNTF on photoreceptor development in dissociated retinal cultures from embryonic chick. Using opsin-immunoreactivity as a differentiation marker for photoreceptor cells we found a 2.3-4fold increase of the number of opsin-positive cells (rods) in the presence of CNTF. Other neurotrophic factors expressed in the retina had no effect (NGF, BDNF, and bFGF). Stimulation by CNTF was dose dependent (EC_{50} 2.6 pM). Conditioned medium of glia-enriched cultures had identical effects indicating the production of a CNTF-like molecule by cultured retinal cells. CNTF exerted no effect on proliferation but acted during early stages of photoreceptor development. However, it was not capable of preventing a decrease of opsin expression during prolonged culture periods suggesting the involvement of additional factors in photoreceptor maturation. Among the agents tested only retinol prevented the disappearance of CNTF-stimulated photoreceptor cells.

We conclude that CNTF plays an important role in the determination of the rod phenotype or during its early differentiation. Retinol may be one of the factors required for further maturation and/or survival. Our results suggest a sequential action of different diffusible factors regulating photoreceptor development.

419.4

EFFECT OF NGF ON THERMAL NOCICEPTIVE THRESHOLD AND NEUROPEPTIDE SYNTHESIS IN PRIMARY AFFERENT NEURONS OF THE ADULT RAT. R. Amann*, R. Schuligoi, G. Herzeg and J. Donnerer. Dept. Exp. & Clin. Pharmacology, Graz Univ., A-8010 Graz

The aim of this study was to investigate the functional and biochemical effects of unilateral intraplantar injections (1/d on 3 consecutive days) of NGF into the rat hind paw. On each day of treatment, NGF (4 μg) caused a unilateral decrease in thermal nociceptive threshold which was apparent 10 min after injection and lasted for less than 3 h. Capsaicin denervation, but not treatment with compound 48/80 (which reduced skin histamine by > 90%) or with indomethacin (which blocked bradykinin-induced thermal hyperalgesia) abolished NGF-induced hyperalgesia. 24 h after the 3rd NGF injection, thermal nociceptive threshold was not different from control values. However, 24 h after the 3rd NGF injection neuropeptide content of the sciatic nerve was elevated: 0.5 μg NGF (but not 0.1 μg) caused an increase of CGRP in the ipsilateral sciatic nerve. 4 μg NGF produced a bilateral increase of CGRP and SP, while the CGRP and SP content of the trigeminal ganglion and several viscera was not significantly affected. Intraplantar NGF (4 μg) caused a bilateral increase of number of L-5 DRG neurons expressing CGRP- or pre-protachykinin (PPT)-mRNA. In rats which were treated with capsaicin as neonates, the number of CGRP-mRNA expressing neurons was reduced. In these rats, NGF had no detectable effect of the number of expressing cells nor on expression density per cell.

This indicates that unilateral intraplantar NGF stimulated CGRP and tachykinin synthesis exclusively in capsaicin-sensitive neurons of ipsi- and contralateral DRGs. This effect of NGF was not related to the observed immediate hyperalgesia following intraplantar injections. We assume that NGF caused sensitization of peripheral afferent nerve endings by an mechanism which was independent from mast cells degranulation or cyclooxygenase stimulation.

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419.5

NEUROTROPHINS INDUCE THE NADPH DIAPHORASE PHENOTYPE IN CULTURED SPINAL CORD NEURONS. K. Huber, K. Krieglstein, and K. Unsicker*. Dept. of Anatomy & Cell Biology; Univ. Heidelberg, INF 307, D-69120 Heidelberg, Germany

Neurotrophins are essential in the regulation of neuron survival and differentiation. One important aspect of neuron development is the acquisition of a transmitter phenotype. We have studied the influence of neurotrophins on the expression of NADPH diaphorase (NADPHd), an enzyme of the nitric oxide synthase pathway, in spinal cord cultures of 16 day old rat embryos. At this age we found NADPHd-reactivity becoming apparent in the spinal cord and predominantly expressed in preganglionic sympathetic nuclei. Spinal cords were dissociated, plated at a density of 260,000 cells/cm^2 and cultured under serum free conditions. NGF, BDNF, NT-3, or NT-4, (10 ng/ml each) were applied 24 h, 3 days or 5 days after plating. Counts of NADPHd+ neurons were obtained at various time points. Numbers of NADPHd+ neurons were very low 24 h after plating and did not change significantly until day 4 in vitro. Application of BDNF, NT-3, and NT-4 significantly increased their numbers within 24 h. BDNF, NT-3, and NT-4 were also effective when first applied after 3 or 5 days in culture. Cotreatment with NT-4 and NT-3 had no additive effect. NGF was not effective. Our findings suggest that neurotrophins are involved in the developmental regulation of NADPHd-activity in neuron populations of the spinal cord, which may include motor and preganglionic autonomic neurons. NADPHd-activity may be induced in neurons expressing the enzyme constitutively, yet at undetectable levels, or may be induced *de novo*. Supported by DFG, SFB 317/C8

419.6

MULTIPLE NEUROTROPHIC FACTORS CONTROL THE MATURATION OF SEROTONERGIC NEURONS. M.J. Eaton[1,2] and S.R. Whittemore[1,3].
The Miami Project[1], Departments of Neurological Surgery[2] and Physiology & Biophysics[3], University of Miami School of Medicine, Miami, FL 33136.

We utilized RN46A cells, an immortalized serotonergic neuronal cell line derived from the E13 raphe (White *et al.*, J. Neurosci. 14:6744-53, 1994), as a model for the maturation of embryonic raphe neurons. Differentiated RN46A cells express the low affinity neurotrophin receptor (p75^{NTR}), the BDNF receptor (trkB), the ACTH receptor, and tryptophan hydroxylase (TPH), but little 5HT synthesis was detectable. BDNF, ACTH$_{1-10}$, and adenyl cyclase activation increase the expression of TPH but the initiation of 5HT synthesis requires initial treatment with BDNF, ACTH$_{1-10}$, or adenyl cyclase activation followed by partial membrane depolarization. The L- and N-type Ca^{2+}-channel blockers reduce depolarization-dependent RN46A cell survival and eliminate the initiation of 5HT synthesis. BDNF and ACTH$_{1-10}$ increase cell survival independently of depolarization. The vesicular monoamine transporter (VMAT) and the 5HT$_{1A}$ receptor increase with differentiation, but high-affinity 5HT re-uptake remains unchanged unless RN46A cells are treated with ACTH$_{1-10}$, forskolin, or S100β. 5HT release in differentiated RN46A cells is constitutive and is not potentiated by depolarization. Collectively, these data suggest a temporal regulation of serotonergic maturation by distinct yet partially overlapping biochemical pathways: BDNF induction of trkB autophosphorylation, adenyl cyclase activation by ACTH$_{1-10}$, and voltage-gated Ca^{2+}-channel opening by partial membrane depolarization. This work was supported by The Miami Project to Cure Paralysis, General Reinsurance, and NS26887.

419.7

CYTOKINES SUCH AS INTERLEUKIN(IL)-2, IL-4, IL-7, IL-15 AND LIF ON THE EXPRESSION OF THE CHOLINERGIC PHENOTYPE IN RAT SEPTAL NEURONAL CULTURES : A SPECIFIC EFFECT OF IL-2. F. Mennicken* and R. Quirion. Douglas Hospital Research Center, Dept Psychiatry, McGill University, Montréal, Québec, Canada, H4H 1R3.

We have recently shown that IL-2 increases choline acetyltransferase (ChAT) activity in embryonic septal rat neuronal cell cultures (Soc. Neurosci. Abst., 20, 667, 1994). The aim of the present study was to determine a) the specificity of the effect of IL-2 by evaluating other cytokines and b) if the effect of IL-2 on ChAT activity was due to an increase in the growth of cholinergic cells or to a direct modulation of ChAT activity per cholinergic neuron. Cultures were obtained by dissecting septa of 17-day old rat embryos. Following dissociation, cells were grown in a chemically defined serum-free medium for 5 days with IL-2 (10^{-14} to 10^{-8}M, Sigma) or other cytokines such as IL-4, IL-7, IL-15 and LIF (0.01 to 40 ng/ml, PeproTech) added after plating. Acetylcholinesterase (AChE) staining was used to visualize cholinergic cells which represented less than 2% of all neurons in culture. Length of the neurites of AChE-positive neurons, number of primary neurites by cell and size of perikaryon were measured using computerized image analysis. No significant changes in the morphological features and the number of AChE-positive cells were observed using various concentrations of the cytokines tested, except for ChAT activity which was significantly increased only by IL-2 (10^{-13} to 10^{-10}M). Thus, IL-2 apparently acts by directly increasing the synthetic capacity of cholinergic neurons, not their survival or growth. Moreover, the effect of IL-2 is not shared by other cytokines also expressed in the CNS such as IL-4, IL-15 and LIF. Finally, in order to increase basal ChAT activity, NGF (10 and 100 ng/ml) was added to the culture medium (Hartikka and Hefti, J. Neurosci, 8, 2967, 1988). Under such conditions of highly increased (100%) ChAT activity, IL-2 failed to stimulate further the enzymatic activity. Taken together, these results suggest that IL-2 can directly modulate cholinergic neurons in the rat brain and likely sharesP metabolic pathways with NGF to induced ChAT activity. (Supported by FRSQ and MRCC).

419.8

SELECTIVE INDUCTION OF NA CHANNEL GENE EXPRESSION IN EMBRYONIC STRIATAL NEURONS *IN VITRO* IN RESPONSE TO BDNF, NT-3, AND NT-4/5. R. A. Maue[1,2*], R. Ventimiglia[3], P. E. Mather[3], R. M. Lindsay[3], and G.R. Fangcr[1]. Departments of [1]Biochemistry and [2]Physiology, Dartmouth Medical School, Hanover, NH 03755, and [3]Regeneron, Inc., Tarrytown, NY 10591.

There is considerable interest in understanding the mechanisms by which neurotrophins affect neuronal differentiation, including electrical excitability. As part of this, we have begun to assess the actions of neurotrophins on ion channel expression in striatal neurons, a neuronal population that undergoes degeneration during Huntington's disease. Neurons isolated from the striatum of E17 rats were maintained *in vitro* in the absence or presence of neurotrophins (50 ng/ml) before probes specific for type I, II, and III Na channel α subunit mRNA were used in RNase protection assays to analyze expression of these transcripts, and whole-cell patch clamp recording used to measure Na current density. In untreated cells, both type I and type II α subunit mRNA were clearly expressed, while type III α subunit mRNA was only barely detectable. In response to BDNF, NT-3, or NT-4/5, but not NGF, there was a significant increase in the level of both type I and type II α subunit mRNA, while expression of type III α subunit mRNA did not change. Furthermore, there was a significant difference in the time course of the BDNF-mediated inductions of type I and type II α subunit mRNA. The changes in α subunit gene expression were accompanied by increased expression of functional Na channels, as there was a significant increase in Na current density in the striatal neurons in response to BDNF. The results extend previous evidence from cultured cell lines that neurotrophins can regulate Na channel expression by providing the first demonstration that this can occur in primary neurons of the central nervous system as well. In addition, we provide the first indication that neurotrophins can induce type I α subunit mRNA as well as type II, and from the difference in kinetics suggest these inductions occur by distinct mechanisms. The results provide a basis for investigating the biochemical mechanisms underlying the induction of Na channel expression in striatal neurons, as well as for analysis of the functional consequences of these changes on their electrical properties. Supported by the Hitchcock Foundation and NS28767 to RAM.

419.9

DOES BDNF REGULATE THE FUNCTIONAL EXPRESSION OF NEUROPEPTIDE Y (NPY) NEURONS IN HUMAN FETAL BRAIN CULTURES ? A. Barnea* and H.N. Aguila-Mansilla. Dept. OB/GYN, UT Southwestern, Dallas, TX 75235-9032.

BDNF can regulate the human fetal NPY neuron in two possible ways: promote neuronal survival and induce functional expression. To address the latter possibility, we have used a culture model system of aggregates, formed from dissociated human fetal (12.5-18 wks old) cortical hemispheres, maintained in serum-free medium. We varied the dose of BDNF (10-50 ng/ml), the culture age (1-3 wks) and duration of BDNF exposure (2-11 days) and assayed NPY production. Under none of these conditions did we observe an increase in NPY production. These protocols were repeated in media containing 10% FCS or conditioned media derived from glia-enriched cultures and again there was no effect of BDNF. Furthermore, neither NT-3, NT-4/5, nor NGF induced NPY production. The lack of effect of BDNF is not due to poor viability/functional state of the NPY neurons, since forskolin+phorbol ester induced a dramatic 3-5-fold increase in NPY production under each of these culture conditions. Moreover, this lack of response is not due to aberrant behavior of NPY neurons in aggregate cultures, since BDNF induced a 2-3-fold increase in NPY production by aggregate cultures derived from the rat fetal cortex. Moreover, the latter positive response was observed under each of the conditions tested for the human cultures. In addition, BDNF was much more effective than NT-3 and NGF was ineffective in inducing NPY production; consistent with TrkB mediating BDNF action in the rat aggregate cultures. In summary, neither of the neurotrophins (BDNF, NT-3, NT-4/5, NGF) induced NPY production in human fetal cortical neurons in culture. If TrkB is expressed in these human

419.10

ELEVATED GLUCOSE OXIDATION AND mRNA EXPRESSION OF GLYCOLYTIC ENZYMES BY NEUROTROPHINS IN RAT CEREBRAL CORTICAL NEURONS. H. Gao*, K. Pong, C. Peterson, T. Yoshida, F. Hefti and B. Knusel. Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089.

The mechanisms of neuroprotection by trophic factors are not known. Aim of the current study was to investigate whether neurotrophins might regulate energy metabolism and free radical detoxification in brain cells. Primary cultures of embryonic rat cortex were treated with neurotrophins and mRNA was measured for phosphoglycerate kinase (PGK), phosphofructokinase (PFK) and superoxide dismutase (SOD). Cellular metabolic activity was determined by measuring production of CO_2 from cultured neurons incubated with ^{14}C-glucose. In cultures treated with BDNF or NT-4/5, mRNA levels for PGK and PFK were increased approximately twofold compared to untreated controls. NT-3 treatment resulted in a smaller increase, NGF treatment in no change. For maximal effect, the factors had to be present for at least 1 day. Very little increase was observed after 5hr treatment. However, presence of BDNF for only 4hr during ^{14}C-glucose incubation resulted in significant stimulation of CO_2 production. Further increase was observed when cultures were pretreated with BDNF for 1 to 3 days before the assay. In contrast to the stimulation of PGK and PFK expression and of cellular metabolic activity, neurotrophin treatment did not result in changes of mRNA for SOD. Our results suggest that neurotrophins might become useful in treating brain metabolic hypofunction which is observed in some neurodegenerative diseases, e.g. Alzheimer's disease.

419.11

DIFFERENTIAL EFFECTS OF LONG-TERM AND SHORT-TERM NGF TREATMENT ON NEUROPEPTIDE EXPRESSION IN AXOTOMIZED SUPERIOR CERVICAL GANGLIA (SCG) *IN VIVO*. Y. Sun* and R. E. Zigmond. Dept. of Neurosci., Case Western Reserve Univ., Cleveland, OH 44106.

Transection of postganglionic nerve trunks deprives sympathetic neurons of target-derived NGF and causes changes in their neuropeptide (NP) expression. NGF deprivation could contribute to triggering the changes in NP expression. As one test of this hypothesis, we examined whether local application of NGF to the axotomized SCG would prevent, at least partially, the changes in NP expression. At the time of axotomy, slow release pellets containing NGF or control pellets were placed in contact with the SCG. Two days after the lesion, the substance P (SP), galanin (Gal) and vasoactive intestinal peptide (VIP) immunoreactivities in creased in the placebo group. NGF treatment further increased SP and VIP, but had no effect on Gal. At 7 d, however, NGF decreased Gal, increased SP, but had no effect on VIP, and by 14 d NGF decreased both Gal and VIP by more than 50%, while still increasing SP. Corresponding effects of NGF treatment on peptide mRNA levels were also observed. The longer term effect of NGF, that is, inhibition of the axotomy-induced increases in VIP and Gal, raises the possibility that in uninjured SCG, the constant retrograde transport of NGF is involved in the suppression of VIP and Gal expression, but not SP. Since the initial effects of NGF, the increase in VIP and no effect on Gal at day 2, could be due to the interactions with acute injury responses, we examined the effect of 2 d NGF treatment on ganglia that had been axotomized for 12 d, and found NGF increased VIP but did not affect Gal. These data suggest that the duration of NGF treatment, but not when it is applied, determines the nature of its effects on NP. NGF treatment (for 10 d but not 2 d) also inhibited the small increases in VIP and Gal induced by sialectomy (which causes axotomy near the nerve endings). In summary, we found NGF had different effects on NP expression depending on the duration of treatment. Whether different signal transducing pathways of NGF or different sites of NGF action generate the differences in long- and short-term NGF effects remains to be determined.

419.12

ANTISERUM TO NERVE GROWTH FACTOR (NGF) ALTERS NEUROPEPTIDE EXPRESSION IN THE SUPERIOR CERVICAL GANGLION (SCG) AND DORSAL ROOT GANGLION (DRG) *IN VIVO*. R. E. Zigmond*, A. M. Shadiack, and Y. Sun. Dept of Neurosciences, Sch of Med, Case Western Reserve University, Cleveland, OH 44106-4975.

Axotomy of sympathetic and sensory neurons leads to a decrease in their content of the target-derived factor NGF, an increase in immunoreactivity (IR) and mRNA of certain neuropeptides including galanin (GAL), vasoactive intestinal peptide (VIP), and substance P (SP), and a decrease in mRNA for neuropeptide Y (NPY) and tyrosine hydroxylase (TH). Axotomy of the DRG leads to an increase in GAL and a decrease in SP. Administration of exogenous NGF has been shown to reverse some of the effects of axotomy for example it decreases chromatolysis (West & Bunge, 1976) and synaptic stripping (Purves & Nja, 1976) in the SCG. Also, NGF partially inhibits the increase in GAL in the SCG in culture (Sun et al., 1993) and in the SCG (Sun et al., this volume) and DRG (Verge et al., 1995) *in vivo* after axotomy. We tested whether the effects of axotomy, in adult rats, could be mimicked by decreasing endogenous NGF by daily systemic treatment with an antiserum against NGF (a gift from Dr. J. Diamond). These animals show an increase in GAL and VIP mRNA in the SCG as compared to the normal serum-treated controls at 3 d after treatment. Although, TH and NPY mRNA is unchanged at 3 d, both mRNAs show a decrease at 14 d after treatment. In the DRG, there is a doubling in GAL, VIP, and a small increase in SP mRNA by 14 d. At 14 d by immunohistochemical analysis, GAL-IR is detected in some principal neurons in the SCG and some small neurons of the DRG. No apparent change in VIP- and SP-IR was observed in the SCG or DRG at this time. A decrease in NPY-IR was observed in the SCG. The apparent lack of a decrease in TH-IR in the SCG may reflect the non-linearity of the histochemical method. These data suggest that the loss of endogenous NGF is partly, but not wholly, responsible for some of the axotomy-induced neurotransmitter changes in the SCG and DRG.

419.13

THE ROLE OF AFFERENT INPUT TO THE SUPERIOR CERVICAL GANGLION IN NGF-INDUCED PLASTICITY OF CEREBROVASCULAR INNERVATION. S.C. Billieu[1], K.L. Schwenk[1], K.A. Crutcher[2], and L.G. Isaacson[1*] [1]Center for Neurosci Res, Dept Zoology, Miami Univ, Oxford, OH 45056; [2]Dept Neurosurg, Univ of Cinci College of Med, Cinci, OH 45267.

Following a 2 week intracranial infusion of nerve growth factor (NGF; 15 µg) into the adult rat, we observe a sprouting response by sympathetic perivascular axons associated with intradural internal carotid artery (ICA), most of which originate in the superior cervical ganglion (SCG). An increase (33% and 31% respectively) in total catecholamines (µg/g protein) and the amount of norepinephrine (NE)/total catecholamines accompanies the response. Our goal was to use EM and HPLC-ECD to determine whether removal of afferent input affects the NGF-induced sympathetic response. Compared with controls, deafferentation of the SCG (dVEH) did not affect mean number of axons/µm vascular wall or total catecholamine content of the ICA, but resulted in an increase (400%) in DOPAC, a dopamine metabolite. The number of axons/µm wall following deafferentation and subsequent NGF infusion (dNGF; 15 µg) was similar to NGF cases, indicating a sprouting response in the dNGF treatment. In contrast, dNGF rats showed no corresponding increase in total catecholamines or in the proportion of NE/total but remained similar to dVEH cases. Thus, deafferentation of the SCG does not affect the morphological response elicited by exogenous NGF but apparently blocks the increase in catecholamines observed following NGF infusion.(Supported by NS17131 and NS32876)

419.14

DIFFERENTIAL REGULATION OF SUBSTANCE P BY NEUROTROPHINS IN AVIAN DORSAL ROOT GANGLIA THROUGHOUT DEVELOPMENT. P. Bernd[1*], S.C. Apfel[2], J.A. Kessler[2], and L. Yao[3]. Departments of Anatomy and Cell Biology[1,3] and Otolaryngology[1], State University of New York, Health Science Center, Brooklyn, NY 11203, and Departments of Neurology and Neuroscience[2], Albert Einstein College of Medicine, Bronx, NY 10461.

This study examined the effects of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5) on substance P (SP) levels in dorsal root ganglia (DRG) shortly after their formation (stage 26, E4.5), during mid development (stage 33, E7.5) and during late development (stage 44, E14). The neurotrophin receptors, *trk*A, *trk*B and *trk*C, have been shown to be present at all these stages. NGF has already been shown to increase levels of SP during mid and late development. DRG were isolated, rinsed with defined medium in order to dilute endogenous neurotrophins, exposed to neurotrophins for 4 hrs (100 ng/ml; 37°C), and subjected to either RIA or EIA for measurement of SP. The results showed that the levels of SP increased throughout development (2 ng/mg protein at E4.5, 3 ng/mg protein at E7.5 and 11 ng/mg protein at E14). At E4.5, none of the neurotrophins had a significant effect on levels of SP. The same was true even if DRG were incubated for 20 hrs with neurotrophins. At E7.5, both NGF and NT-3 increased SP levels by nearly 50%, while NT-4/5 did not appear to have an effect (no results were obtained for BDNF). The effects of NGF and NT-3 were blocked by their respective antibodies. At E14, all neurotrophins had significant effects on SP levels, with NT-3's effect being more pronounced (140% above control) than that of NGF (48% above control), NT-4/5 (30% above control) and BDNF (15% above control). The increases in SP following neurotrophin treatment probably reflect increases on a per cell basis, since total cell number should not be altered after a 4 hr incubation. Supported by grants from the Dysautonomia Foundation and the National Science Foundation (IBN 92-22027).

419.15

EFFECT OF NERVE GROWTH FACTOR ON THE EXPRESSION OF CHOLINE ACETYLTRANSFERASE-mRNA IN PC12 CELLS. J.L. Pongrac* and R.J. Rylett. Departments of Pharmacology and Physiology, University of Western Ontario, London, ON, CANADA, N6A 5C1.

Nerve growth factor (NGF), a neurotrophic agent, enhances cholinergic phenotypic expression. NGF increases choline acetyltransferase (ChAT) activity following *in vivo* administration, and *in vitro* in pheochromocytoma (PC12) cells and primary cultures of basal forebrain; these increases in ChAT activity are accompanied by apparent increases in steady-state levels of ChAT-mRNA. As there are multiple mRNA transcripts of the ChAT gene produced by alternative splicing, the purpose of the present study was to investigate whether NGF alters expression of all splice variants. These transcripts are variable in the promoter region as opposed to the coding region, thus translation produces a single protein product. The expression of various transcripts with different promoter regions may impact on the ability of different growth factors to elicit responses through specific transcription factors. Selective reverse transcription with polymerase chain reaction (RT-PCR) was used to amplify different upstream regions of ChAT-mRNA followed by Southern blot analysis and hybridization with a ^{32}P-labelled oligomer specific to the coding region of ChAT transcripts. We show that ChAT-mRNA transcripts associated with the M- and N-exons are expressed in PC12 cells. We also show that there is an increase in the expression of the ChAT transcript associated with the M-exon following NGF treatment. This suggests that NGF promotes the transcription of the ChAT gene in part through this variant. (Supported by MRC Canada and Ontario Mental Health Foundation)

419.16

PERIOSTEAL CELLS INDUCE CHOLINE ACETYLTRANSFERASE IN CULTURED SYMPATHETIC NEURONS. S.E. Asmus* and S.C. Landis. Dept. of Neuroscience, Case Western Reserve Univ., Cleveland, OH 44106.

Periosteum, the connective tissue covering of bone, contains osteoblasts and their progenitor cells. The transmitter phenotype of its sympathetic innervation is altered developmentally. Although initially noradrenergic, sympathetic fibers in adult rat periosteum contain vasoactive intestinal peptide (VIP) immunoreactivity and acetylcholinesterase (AChE) activity. Heterotopically-transplanted periosteum suppresses catecholamines and induces VIP immunoreactivity in fibers that would otherwise express noradrenergic traits. Assays of choline acetyltransferase (ChAT) activity have been confounded in this tissue by attached skeletal muscle. Therefore, to test whether periosteum induces cholinergic function, we cocultured rat periosteal cells and sympathetic neurons. Periosteal cells dissected from postnatal day (P) 3 rats were either grown alone or added to sympathetic neurons. Periosteal cells attained a fibroblast-like morphology and reached confluence approximately 1 week after plating. After several weeks, alkaline phosphatase, an enzyme expressed in osteoblasts, was histochemically detected in some cells. Neurons cocultured for 1 week with periosteal cells or with explants of periosteum displayed a 5-6-fold induction in ChAT acitivity over neurons cultured alone. Because transgenic mice with a disruption in the gene for leukemia inhibitory factor (LIF), a cholinergic differentiation factor, contained identical distrubutions of VIP and AChE periosteal fibers, LIF is not responsible for the switch *in vivo*. Whether LIF expression by periosteal fibroblasts *in vitro* accounts for some of the ChAT induction seen here will be addressed by coculturing neurons and periosteal cells from LIF deficient mice. Taken together with previous findings, these results suggest that periosteum, in addition to sweat glands, can regulate the neurochemical phenotype of sympathetic neurons. Supported by NS0970901 (SEA) and NS2367811 (SCL).

419.17

INDUCTIVE INFLUENCES OF CNTF ON NEURONAL GENE AND PHENOTYPE EXPRESSION: BIOCHEMICAL AND FUNCTIONAL CORRELATES. L.H. Zinman[1*], G. Lawrance[2], V.M.K. Verge[3], K.E. Dow[2], P.M. Richardson[4], D.H. Maurice[1], R.J. Riopelle[2]. [1]Dept. of Pharm. and [2]Med., Queen's Univ., Kingston, Ontario; [3]Dept. of Anat., Univ. of Saskatchewan, Saskatoon;[4]Dept. of Neurology, McGill Univ., Montreal, Que.

CNTF induces neuronal differentiation in the human LA-N-2 neuroblastoma cell line by activating its tripartite receptor complex (Lawrance et al., 1995, J. Neurochem. **64**, 1483). We have also observed that CNTF-induced increases in ChAT specific activity were time-dependent, and that changes in ChAT activity correlate with changes in ChAT protein levels. Characterization of CNTF effects on ChAT mRNA were examined by *in situ* hybridization (ISH) using a 48 bp cDNA probe for rat ChAT and Northern analysis using a 340 bp human cDNA fragment generated by RT-PCR amplification techniques. CNTF-induced increases in ChAT specific activity and protein also correlate with changes in ChAT gene expression.

In naive LA-N-2 cells, low levels of trkA and p75 mRNA are detectable, p75 protein can be chemically cross-linked to ^{125}I-NGF, and NGF-mediated trkA phosphorylation can be detected. NGF-mediated neurite growth is observed only following priming of the LA-N-2 cells by CNTF. In this setting, trkA mRNA levels and NGF-mediated trkA phosphorylation are increased, while chemical cross-linking of the trkA receptor to ^{125}I-NGF is now detectable. The observation that CNTF and NGF have additive effects on neurite growth by CNTF-primed LA-N-2 cells suggests activation of non-convergant signalling pathways subserving neurite growth. Such additive effects are not observed for ChAT induction, and NGF does not induce an adrenergic phenotype in CNTF-primed LA-N-2 cells.

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419.18

EFFECTS OF FK-506, RAPAMYCIN AND CYCLOSPORIN A ON THE IN VITRO DIFFERENTIATION OF DORSAL ROOT GANGLIA EXPLANTS AND SEPTAL CHOLINERGIC NEURONS. A. CARREAU, J. CUEUGNON, J. BENAVIDES AND X. VIGÉ. Synthélabo Recherche, CNS Research Department, BP 110, 92225 Bagneux Cedex, France.

There is increasing evidence that immunophilins (targets of some immunosuppressant drugs) play a role in neural development and differentiation. We have studied the effects of FK-506, rapamycin and cyclosporin A on dorsal root ganglia (DRG) from different segmental levels and on septal cholinergic neurons in culture from rat embryo. Low concentrations of FK-506 (1 nM) significantly increased (+83%) the number of neurites of thoracic DRG explants. Higher concentration (100 nM) also enhanced the neuritogenesis of thoracic (+100 %) and lumbar/sacral (+57%) DRG, but no significant effect was observed on cervical explants. Rapamycin displayed an opposite effect, reducing the development of DRG explants from cervical and thoracic segments (-78% at 1 nM in thoracic DRG). Cyclosporin A (from 1 to 100 nM) was without effect on DRG neuritogenesis. Both, FK-506 and rapamycin failed to affect neurite length under our experimental conditions. In cultured septal cells, FK-506 enhanced the differentiation of cholinergic neurons, as assessed by the measurement of ChAT activity, with a bell-shaped concentration-effect relationship. The maximal increase in ChAT activity (+40%) was obtained with 10 nM and 100 nM. The effect of FK-506 was additive to that of NGF. Rapamycin had no effect *per se*, but prevented the NGF-induced increase in ChAT activity.

These results demonstrate that immunophilin ligands affect neuronal differentiation. The differential sensitivity of DRG taken at various segmental levels to FK-506 and rapamycin is in agreement with the view that these drugs activate distinct transduction mechanisms. The lack of effect of cyclosporin A suggests that its receptors (cyclophilins) are not expressed by DRG.

419.19

SYNERGY BETWEEN GROWTH FACTORS AND CATECHOLAMINES (CA) REQUIRED FOR NOVEL EXPRESSION OF TYROSINE HYDROXYLASE IN NON-CA BRAIN NEURONS. **X. Du* and L. Iacovitti** Dept. of Anatomy and Neurobiology, Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA. 19102

We have previously demonstrated that induction of the catecholamine (CA) biosynthetic enzyme tyrosine hydroxylase (TH) in cultured non-CA neurons from the mouse striatum requires the cooperative interaction of two partner substances; neither of which is active alone. One agent is the growth factor, acidic fibroblast growth factor (aFGF), the other an unknown substance(s) present in several tissue extracts, including that of adult mouse midbrain. In this study, we examined 1) whether CA neurotransmitters present in the midbrain fraction were serving as aFGF partners; and 2) if so, whether other growth factors could be similarly activated by CAs to induce TH. The phenotypically plastic neurons of the embryonic mouse striatum (E13) were treated in culture with aFGF, bFGF, BDNF, CNTF, GDNF, TGFß, IGF, NGF, PDGF or IL1 in the prescence or absence of potential partners. Growth factors alone were powerless to induce TH. However, in the presence of an appropriate partner, aFGF, and to a lesser extent bFGF and BDNF, induced the novel expression of TH. Of the many potential activating substances tested, only those which contained an amine group separated from a catechol nucleus by two carbons, such as dopa, dopamine, the D1 receptor agonist SKF38393, norepinephrine and the beta adrenoceptor agonist isoproterenol were effective partners to the growth factors. However, since no receptor antagonists (SCH-23390; spiperone; halperidol, yohimbine, prazosin, propranolol) or transport blockers (mazindol; nomifensine; indatraline; nipecoic acid) could inhibit the CA effect, the mechanism of action remains obscure. We conclude that TH expression can be novelly induced by the synergistic interaction of aFGF, and to a lesser extent bFGF and BDNF, and a variety of CA-containing partner molecules. We speculate that a similar association between growth factor and transmitter may be required during development for the differentiation of a CA phenotype in brain neurons. (Supported by NIH NS 24204).

419.20

ACIDIC FIBROBLAST GROWTH FACTOR AND CATECHOLAMINES (CA) TOGETHER UP-REGULATE TYROSINE HYDROXYLASE ACTIVITY IN DEVELOPING AND/OR DAMAGED DOPAMINE NEURONS. **N.D. Stull* and L. Iacovitti** Dept. of Anatomy and Neurobiology, Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA. 19102

Our previous studies indicate that, in certain non-CA neurons, expression of the gene for the CA biosynthetic enzyme tyrosine hydroxylase (TH) can be initiated by the obligatory interaction of acidic fibroblast growth factor (aFGF) and a CA activator (See adjacent abstract). In this study, we sought to determine whether these same differentiation factors also play a role in maintaining and regulating existing TH expression in CA neurons. To do so, we studied the effects of exogenous application of aFGF and CA on TH in developing or damaged (MPP^+-treated) dopamine (DA) neurons where endogeous levels of these agents were likely to be at physiologically low levels. DA neurons from the E15 rat ventral midbrain were grown in culture on defined media $\pm$ 2.5 μM MPP^+ (24hrs); rinsed; treated for 4hrs with aFGF (10ng/ml) and DA (10μM), harvested and TH activity measured. In developing DA neurons (- MPP^+), treatment with aFGF + DA resulted in a 40-62% increase in TH activity over controls (media only). In damaged DA neurons (+MPP^+), aFGF and DA reversed the 30% loss in TH activity caused by toxin, returning TH to control levels. Since these effects were observed within 4 hrs of treatment, they were most likely due to the biochemical rather than trophic properties of these reagents. Several other CAs, such as dopa and norepinephrine, when combined with aFGF, also up-regulated TH activity levels; while the non-CA transmitter GABA did not. We conclude that the DA differentiation factors (aFGF and CAs) which are capable of first initiating expression of a quiescent TH gene may also be involved in the regulation of the enzyme during development or after damage. Although CAs have long been considered feedback inhibitors of TH, our results suggest, that in combination with aFGF, they may also serve as feedback inducers of TH transcription and/or translation. (Supported by NIH NS 24204-07).

419.21

CO-EXPRESSION OF TYROSINE HYDROXYLASE (TH) and GLUTAMIC ACID DECARBOXYLASE (GAD) IN DOPAMINE DIFFERENTIATION FACTOR (DDF)-TREATED STRIATAL NEURONS IN CULTURE. S.R. Max*, A. Bossio, and L. Iacovitti, Depts., of Neurology and Biochemistry, Medical College of PA and Hahnemann Univ., Philadelphia, PA 19102

During differentiation, neurons acquire cell-specific traits that constitute their phenotype. One critical "decision" developing neurons must make is the neurotransmitter(s) they will produce. We have shown that DDF (i.e., aFGF+Dopamine; aFGF+mazindol; aFGF+Dopamine+mazindol) can stimulate expression of TH in striatal neurons, which normally do not express TH, since they are thought to be GABAergic neurons. In the present study, immunocytochemistry showed GAD and TH to be co-expressed in striatal neurons; this result was confirmed by assay of TH and GAD activities in extracts of DDF-treated neurons. Treatment of striatal neurons with DDF resulted in a striking increase in TH expression, as revealed by immunocytochemistry and by direct enzyme assay. Assay of GAD in these neuronal extracts did not show a significant change in activity with any of the treatments. Our results indicate that those striatal neurons that can be induced by DDF to express TH are GAD-positive GABAergic neurons. The co-expression of TH and GAD in the same neurons further suggests that induction of a novel neurotransmitter phenotype does not occur at the expense of existing traits. (Supported by NIH-NS24204 awarded to LI).

420.1

NEUROTROPHIN-4/5 PROMOTES SURVIVAL OF CULTURED PRIMARY AUDITORY AND VESTIBULAR NEURONS AND PROTECTS THEM FROM OTOTOXINS. J.L. Zheng[1*], R.R. Stewart[2] and W.-Q. Gao[1]. [1]Dept. of Neurosci., Genentech, Inc., South San Francisco, CA 94080 and [2]Dept. of Anatomy, George Washington Univ. Med. Ctr., Washington, DC 20037.

Destruction of spiral and vestibular ganglion neurons (SGNs and VGNs) induced by injury and toxins is one of the major causes for hearing and balance impairments. Here we report that neurotrophin-4/5 (NT-4/5), a member of the nerve growth factor (NGF) family, promoted survival of postnatal rat SGNs and VGNs in dissociated cell cultures. The survival-promoting potency of NT-4/5 was equivalent to that of brain-derived neurotrophic factor (BDNF) and stronger than that of neurotrophin-3 (NT-3). In contrast, NGF showed no detectable effects. Immunohistochemistry with TrkB and TrkA antisera revealed that these neurons produced TrkB protein, the functional receptor for NT-4/5 and BDNF, but not TrkA protein, the high affinity receptor for NGF. The survival-promoting activity of NT-4/5 was completely inhibited by TrkB-IgG (a fusion protein of extracellular domain of TrkB and Fc fragment of immunoglobulin). In addition, NT-4/5 protected the two types of neurons from neurotoxicity of ototoxins including cisplatin, a widely used chemotherapeutic drug, and gentamicin, a commonly used aminoglycoside. Thus, NT-4/5 is a specific survival factor for SGNs and VGNs and has therapeutic value in preventing hearing and balance impairments caused by injury or ototoxins on primary auditory and vestibular afferent neurons.

420.2

DIFFERENTIAL OTOTOXICITY OF SALICYLATE, GENTAMICIN AND CISPLATIN AND PROTECTIVE EFFECTS OF SPECIFIC NEUROTROPHINS IN COCHLEAR EXPLANT CULTURES. W.-Q. Gao* and J.L. Zheng. Dept. of Neuroscience, Genentech, Inc., South San Francisco, CA 94080.

In organotypic cultures of postnatal cochlear explants in which the afferent innervation of hair cells by primary auditory neurons are intact, we have examined ototoxicity of three different classes of clinical drugs. These include sodium salicylate, an analog of aspirin, gentamicin, a commonly used aminoglycoside, and cisplatin, a widely used chemotherapeutic agent. Differential damages on auditory neurons and hair cells by the three types of drugs were observed by double labeling with a monoclonal antibody against neurofilament protein (200 kd) and a phalloidin-FITC conjugate. Sodium salicylate (3-10 mM) induced specifically degeneration of auditory neurons, but not hair cell loss. Gentamicin (0.01-0.5 mM) preferentially caused hair cell death, though loss of auditory neurons was also seen at higher concentration (1-5 mM). In contrast, addition of cisplatin to the culture resulted in degeneration of both auditory neurons and hair cells at a wide range of doses tested (1-8 µg), with a more profound damage on auditory neurons than on hair cells. When neurotrophins, members of nerve growth factor (NGF) family, were added together with sodium salicylate, gentamicin or cisplatin to the culture, neuronal degeneration was protected by neurotrophin-4/5, brain-derived neurotrophic factor and neurotrophin-3, but not by NGF or other growth factors. On the other hand, hair cell loss caused by gentamicin and cisplatin was not prevented by the presence of neurotrophins. These results suggest that ototoxic mechanisms by salicylates, aminoglycosides and chemotherapeutic agents are different and specific neurotrophins may be useful for prevention of auditory neuronal loss induced by ototoxic therapeutic drugs.

420.3

OVEREXPRESSION OF BDNF IN EPIDERMIS OF TRANSGENIC MICE CAUSES CHANGES IN TRK RECEPTOR EXPRESSION IN SENSORY GANGLIA AND ALTERATIONS IN SKIN INNERVATION. A.M. LeMaster*, B.M. Davis, and K.M. Albers. Depts of Anatomy and Neurobiology and Pathology, Univ. of Kentucky Sch. of Med., Lexington, KY 40536.

Brain-derived neurotrophic factor (BDNF) is expressed in mouse skin at approximately embryonic day 10 (E10), expression peaks at E12, and then falls to low levels which are maintained throughout adulthood. BDNF expression in skin may promote neuronal survival during a period of programmed apoptotic cell death. To examine the effect of BDNF on neuronal survival and development, two lines of transgenic mice that overexpress BDNF in the epidermis have been examined. Transgene expression is driven by the promoter and enhancer elements of the human keratin 14 (K14) gene. To characterize the K14-BDNF transgenic mice, *in situ* hybridization was used to examine trk receptor expression in trigeminal and dorsal root ganglia. Transgenic line 733 had an approximate 1.7-fold increase in trk B expressing neurons of the trigeminal ganglion. Line 632 exhibited an approximate 3-fold and 2-fold increase in trk B expressing neurons of the trigeminal and dorsal root ganglia, respectively. A 1.5-fold increase in trk A expressing neurons was also found in trigeminal ganglion of line 632. Changes in trk receptor expression correlate with changes found in whisker pad skin innervation. Immunocytochemical analysis using an antibody against PGP 9.5, a ubiquitin carboxyl terminal hydroxylase which detects both myelinated and unmyelinated nerve fibers, revealed a dense plexus of small nerve fibers in areas of dermis subjacent to the epidermis. The increased number of fibers may be derived from the superficial vibrissa nerve since it is primarily composed of small-caliber fibers. Immunolabeling of skin using an anti-neurofilament 150 antibody which detects primarily myelinated innervation, also showed an increase in fiber density. These results indicate that BDNF influences the density and character of neuronal innervation to the skin. Supported by AR40873 (KMA) and NS31826 (BMD).

420.4

TRANSGENIC MICE THAT OVEREXPRESS NT-3 EXHIBIT ENHANCED INNERVATION OF TOUCH DOMES AND INCREASED NUMBERS OF TRK C EXPRESSING NEURONS. K.M. Albers*, T.N. Perrone and B.M. Davis. Depts. of Pathology and Anatomy and Neurobiology, Univ. of Kentucky Sch. of Med., Lexington, KY 40536.

The family of neurotrophins is comprised of NT-3, NGF, BDNF, and NT4/5. Each of these compounds is expressed in target tissues during the development of the mammalian nervous system and in the adult. To examine the influence of neurotrophic compounds on the development and differentiation of neurons in the peripheral nervous system, we isolated transgenic mice that overexpress these compounds in the skin target tissue. NT-3 overexpressing mice have a phenotype unique from NGF and BDNF overexpressing mice. Flank skin of NT-3 mice stained with hematoxylin and eosin exhibited larger touch dome units associated with hair follicles. Immunolabeling using an NF-150 antibody showed innervation to touch domes was substantially increased in the number of neuronal processes and complexity. NF-150 labeling also showed an increase in the density of fibers comprising the circular component of the piloneural innervation. Numerous lanceolate endings were also observed in the transgenic skin. Immunolabeling using an anti-PGP 9.5 antibody showed a similar pattern of innervation and also revealed an increase in the density of fine caliber processes in the NT-3 expressing skin. *In situ* hybridization analysis of high affinity neurotrophin receptor expression in sensory ganglia indicated an approximate doubling in the number of cells expressing the trk C receptor mRNA. Measures of neuronal size showed no significant difference between trk C expressing neurons of transgenics and aged-matched controls. Supported by AR40873 (KMA) and NS 31826 (BMD).

420.5

COMPARISON OF BDNF-, NT4-, BDNF/NT4- AND TRKB-DEFICIENT MICE: GENE DOSING EFFECTS AND COMPENSATION. J.T. Erickson*[1], J.C. Conover[2], R.J. Smeyne[3], T.M. DeChiara[2], M. Barbacid[3], G.D. Yancopoulos[2] and D.M. Katz[1]. [1]Department of Neurosciences, Case Western Reserve University School of Medicine, Cleveland, OH 44106, [2]Regeneron Pharmaceuticals, Inc., Tarrytown, NY, 10591 and [3]Department of Molecular Biology, Bristol-Myers Squibb, Princeton, NJ 08543.

We recently reported (Conover et al., *Nature*, In Press) that mice lacking the trkB ligands NT4 or BDNF exhibit distinct sensory neuron deficits. In particular, the nodose/petrosal ganglion complex (NPG), which relays sensory information critical for regulation of autonomic functions, requires *both* BDNF and NT4 for survival of the full complement of neurons, although total cell counts from P14 BDNF, NT4 and BDNF/NT4 knockout mice revealed no obvious compensation by either factor for loss of the other in supporting survival of NPG neurons overall. This is not the case, however, for specific subsets of neurons. Analysis of tyrosine hydroxylase-containing (TH^+) dopaminergic neuronal profiles in the NPG of P1 wild type, BDNF-, NT4-, BDNF/NT4- and trkB-deficient mice revealed TH^+ cell losses that were dependent on the number of functional BDNF, but not NT4, alleles. Furthermore, the sum of the TH^+ profile losses observed following disruption of either BDNF or NT4 individually (64%) was substantially less than the loss observed following disruption of both neurotrophins simultaneously (83%). These results provide the first evidence for compensatory actions on neuron survival by trkB ligands *in vivo*. Moreover, these data indicate that different subsets of trkB-expressing neurons in the NPG may be supported by either BDNF, NT4, or both. Finally, the fact that TH^+ neuron survival was related to the number of BDNF alleles provides direct confirmation of a central tenet of the neurotrophic hypothesis, i.e., that growth factor concentration is limiting for neuronal survival *in vivo*. Supported by HL-42131 (DMK) and in part by HL-25830 (Proj. 4, DMK).

420.6

ELECTROPHYSIOLOGICAL ANALYSIS OF CUTANEOUS SENSORY NEURONS IN NEONATAL WILDTYPE MICE AND TRANSGENIC ANIMALS LACKING BDNF Martin Koltzenburg*, Gary R.Lewin[§], Klaus V.Toyka, Hans Thoenen[#], Patrick Carroll[#] *Dept. Neurology, University of Würzburg, D-97080 Würzburg, Depts. [§]Neurobiochemistry & [#]Neurochemistry, Max-Planck Institute for Psychiatry, D-82152 Martinsried, Germany.*

Brain derived neurotrophic factor (BDNF) is a member of the neurotrophin family which affects survival and differentiation of sensory neurons. Transgenic mice lacking BDNF die within the first month of life and show a 30% depletion of dorsal root ganglion cells compared to wildtypes mice (WT). Here we used electrophysiological techniques to analyze the functional properties of primary afferents. The saphenous nerve was dissected out together with the skin and single myelinated units (n= 71) were studied *in vitro* using standard teased fiber techniques from the second postnatal week until adulthood in WT mice and during the 2nd and 3rd week of life in BDNF -/- animals. In both groups conduction velocity was considerably slower during the first 3 weeks than in adults. In WT mice there was a gradual increase of conduction velocity until the 6th postnatal week when values resembled those found at 8-10 months. Thin myelinated (Aδ) afferents were classified as either nociceptive high threshold mechanoreceptors or sensitive D-hair receptors from the 2nd postnatal week onward in both BDNF -/- and WT animals. Adaptation characteristics of large myelinated (Aß) fibres appeared not to be fully mature within the first two postnatal weeks. While normal rapidly adapting afferents are found at this developmental stage, there are afferents in both BDNF -/- and WT mice which exhibit rapidly adapting responses at threshold intensities, but slowly adapting discharges at higher intensities. Since such units are not found in WT animals at 6 weeks and as slowly adapting afferents are functionally impaired in adult +/- animals (Carroll et al. Soc. Neurosci Abstr. 20, 1994, 451.5), BDNF may be involved in the functional maturation of these afferents. (Supported by the DFG, SFB 353)

420.7

NEUROTROPHIN-3 IS REQUIRED FOR THE DEVELOPMENT OF SUB-POPULATIONS OF CUTANEOUS MECHANORECEPTORS G. R. Lewin*, M. Koltzenburg§, M. S. Airaksinen, K. V. Toyka§, H. Thoenen and M. Meyer
Dept. Neurochemistry, Max-Planck Institute for Psychiatry D-82152, Martinsried. §Dept. Neurology, University of Würzburg, D-97080 Würzburg, Germany.

Elimination of the neurotrophin-3 (NT-3) gene in mice leads to a substantial loss of sensory neurons from the dorsal root ganglia. Using anatomical techniques it has been shown that at least some of the neurons lost are proprioceptive afferents. Here we have used an *in vitro* preparation to record from single neurons in the cutaneous saphenous nerve in normal and transgenic animals to ask whether specific sub-populations of skin afferents require NT-3 (Carroll et al. 1994 Neurosci. Abst 451.5). The number of myelinated axons in this nerve was reduced by 38% in NT-3 homozygous (-/-) and 24% in heterozygote mice compared to wild type littermates, indicating a gene dosing effect. Recordings were made from a total of 103 and 93 single afferents in wild type and NT3 (+/-) animals, respectively. In control and transgenic mice these afferents could be classified as high threshold mechanoreceptors, D-hair receptors, rapidly adapting mechanoreceptors, and slowly adapting mechanoreceptors. The physiological properties of all these afferent types appeared normal in NT-3 (+/-) animals. However, there was a dramatic and significant decrease in the incidence of slowly adapting mechanoreceptors (>70%) and also D-hair receptors (50%), indicating that these two sensory neuron types require NT-3 during development. These losses were very well accounted for by the loss of myelinated axons. We examined specialized Merkel cells in the touch dome complex of the skin which are the end-organs of slowly adapting mechanoreceptors and are dependent on an intact innervation. These cells were completely absent in (-/-) mice and substantially reduced in (+/-) mice. Thus NT-3 appears to be required for the development of a numerically large number of specialized sensory neurons innervating the skin.

420.8

DIFFERENTIAL EFFECT OF NEUROTROPHINS ON INJURY-INDUCED PLASTICITY OF GABA$_A$ RECEPTORS AND SODIUM CURRENTS IN CUTANEOUS AFFERENT DRG NEURONS. A.A. Oyelese*, M.A. Rizzo, S.G. Waxman, and J.D. Kocsis. Dept. of Neurology, Yale Med. Sch., New Haven, CT 06510, and Neuroscience Res. Ctr., VAMC, West Haven, CT 06516.

Axotomy enhances GABA$_A$ receptor-mediated conductances, alters action potential waveform in medium-sized DRG neurons and induces a phenotypic shift of sodium currents in cutaneous afferent DRG neurons. The present study was undertaken to determine whether the observed injury-induced changes in GABA$_A$ receptor-mediated conductance, action potential waveform and sodium currents represent changes within a specific functional class of DRG neurons (i.e. cutaneous vs muscle afferents) and to determine whether neurotrophic factors play a role in the regulation of these injury-induced changes. Whole cell recordings were obtained from cutaneous and muscle afferent DRG neurons in culture, identified by retrograde labelling with Fluoro-gold. Muscle afferent neurons had larger GABA-induced conductances and fewer action potentials with inflections on the falling phase as compared to cutaneous afferent neurons. Cutaneous, but not muscle afferent neurons, increased their GABA-induced conductances and altered their action potential waveform in response to sciatic nerve injury. *In vivo* application of NGF to the nerve stump via an osmotic pump reversed the effects of axotomy on action potential waveform and also led to an increase in the expression of kinetically slow sodium currents in cutaneous afferent neurons without affecting GABA-induced conductances. *In vivo* application of BDNF reduced the increase in GABA-induced conductance without affecting action potential waveform. These results demonstrate a differential regulation of GABA$_A$ receptor and sodium channel expression in response to axotomy in cutaneous afferent neurons by different neurotrophic factors. Supported in part by the NIH and VA.

420.9

ASSESSMENT OF PAIN THRESHOLDS IN BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) AND NEUROTROPHIN-4 (NT-4) KNOCKOUT MICE. Judith A. Siuciak*, Dacie Lewis, Joanne Conover and G. Yancopoulos, Ronald M. Lindsay, Regeneron Pharmaceuticals, Tarrytown, NY. 10591

BDNF and NT-4 are members of the nerve growth factor (NGF) family of neurotrophic factors and both signal via the TrkB receptor. Mice heterozygous for a mutation in their endogenous BDNF loci (*bdnf +/-*) or homozygous for a NT-4 mutation (*nt4 -/-*) (Conover et al., Nature, 1995) were assessed for alterations in nociceptive responses using standard tests. The tail-flick and the hot-plate tests were used to measure responsiveness to acute, noxious thermal stimuli. The formalin test examined prolonged nociceptive response to a noxious chemical stimulus. In the tail-flick test, responses in *bdnf +/-* mice and those lacking NT-4 (*nt4 -/-*) were similar to wild-type control mice. However, in the hot-plate test, *bdnf +/-* mice showed a significant increase in the nociceptive threshold compared to their wild-type controls, i.e., they were hypoalgesic. Latencies from *bdnf +/-* mice were increased by 60% ($p < 0.0001$). In contrast, the *nt4 -/-* mice showed similar hot plate latencies to wild-type controls. In the formalin test, *bdnf+/-* mice showed a decreased response to an injection of 2.5% formalin into the dorsal surface of the hindpaw. The number of flinches/min was decreased in both the early and late phases of the test. Again, *nt4 -/-* mice showed no changes in response. Although the mechanism is unclear at the present time, these data suggest that endogenous BDNF, but not NT-4, may play a role in the development of nociceptive systems. Furthermore, the lack of change in tail-flick latencies suggests the involvement of central pathways.

420.10

Comparison of rhNGF and rhNT4/5 for Modulating Capsaicin-Evoked Release of iCGRP From Peripheral Sensory Neurons WR Bowles*[1], CA Harding-Rose[1], BC Rogers[2], KM Hargreaves[1] ([1]Dept Rest Sci, Univ of MN, Mpls, MN 55455; and [2]Genentech, S SF, CA)

Recent studies suggest that nerve growth factor (NGF) may modulate neurogenic inflammation through alteration of peripheral neuropeptide secretion. Evidence implicating NGF in hyperalgesia includes behavioral studies in rats (Lewin et al., J Neurosci 13:2136, 1993, Woolf et al., Neurosci 62(2):327, 1994), and increased levels of iNGF during inflammation (Byers et al., Proc Finn Dent Soc 88(Supp 1):73: 1992). Accordingly, we compared NGF (whose preferred receptor is *trk* A), to NT4/5 (a preferred ligand for *trk* B) for altering capsaicin-evoked release of iCGRP from peripheral terminals of sensory neurons. Bovine dental pulp was chopped into 200 µm cubes, placed into 1 cc superfusion chambers, and perfused with oxygenated Krebs buffer (0.32 ml/min). After a recovery period, rhNGF or rhNT4/5 (Genentech) in concentrations of 10pM - 3µM (n=8-10/group) was added to the buffer 21 minutes before and during stimulation with 30µM capsaicin (which stimulates certain nociceptive fibers). The superfusate was collected over 7 minute periods, and iCGRP was measured using RIA. Data were analyzed using ANOVA. rhNGF pre-treatment signficantly suppressed the capsaicin-evoked release of iCGRP (80.7 ± 20.6% suppression at 7.4 pM rhNGF vs control, $p<0.05$). In contrast, rhNT4/5 suppressed the capsaicin-evoked release of iCGRP, but only at concentrations of 1µM (by 59.3 ± 30.2% vs control). The results indicate that both rhNGF and rhNT4/5 pre-treatment inhibit capsaicin-sensitive fibers. Further, the high potency of NGF and low potency of NT4/5 are consistent with a primary mechanism of action mediated by activation of the *trk* A receptor. This research was supported in part by Genentech, DE07014 and DE09860.

420.11

NEUROTROPHIN-3 PROMOTES THE SURVIVAL AND DIFFERENTIATION OF MUSCLE SPINDLE AFFERENTS IN THE ABSENCE OF PERIPHERAL TARGETS R. A. Oakley*, E. B. Lefcort, D. O. Clary, L. F. Reichardt and E. Frank.
Dept. of Neurobiology, Univ. of Pittsburgh, Pittsburgh, PA 15261; Dept. of Biology, Montana State Univ., Bozeman, MT 59717; Sugen Inc., Redwood City, CA. and Neurocsi. Unit, HHMI, UCSF, San Francisco CA 94143.

Our previous work has shown that NT3 from limb tissues is necessary for the survival of the sensory neurons that supply muscle spindles (Oakley et al., *Development* in press). To determine if NT3 is sufficient for the development of these neurons in the absence of limb targets, we studied the effect of chronic NT3 treatments following early limb bud deletion. Limb buds were unilaterally removed from E2.5 chick embryos, prior to sensory outgrowth. These embryos were then treated with either cytochrome-c (control) or NT3 from E5 to E10, during the period of target interaction and cell death. The survival and differentiation of muscle spindle afferents was assessed using antibodies to trkC and DiI injections of the dorsal roots.

In control embryos, limb bud deletion resulted in a loss of nearly 90% of the trkC-positive neurons in the lumbar dorsal root ganglia (DRG), with a concomitant loss of the Ia fiber projection to motoneurons in the spinal cord. Treatment of limb-deleted embryos with NT3 restored the normal complement of trkC-positive neurons in lumbar DRG on the deleted side and nearly doubled the number of trkC positive neurons on the contralateral side. This treatment also quadrupled the number of trkC positive neurons in thoracic DRG of unoperated segments. NT3 treatment resulted in an apparently normal Ia projection on the deleted side, despite the loss of limb motoneurons ipsilateral to the deletion. Furthermore, NT3 treatment caused an obvious increase in Ia collaterals contralateral to the deletion and in thoracic segments. These results indicate that NT3 is necessary and sufficient for the survival and differentiation of muscle spindle afferents, implicating NT3 as a factor responsible for the specification of this sensory phenotype. Supported by the NIH and the MDA.

420.12

NEUROTROPIN-3 (NT-3) TREATMENT MAINTAINS MYELINATED NERVE FIBER PROFILES AND PREVENTS A-FIBER FUNCTION LOSS IN DIABETIC RATS. K. A. Elias, N. Shinsky, G. Ingle, N. Desjardin, A. Shaheen, J. W. Winslow, M. J. Cronin* and W.-Q. Gao.
Genentech Inc., S. San Francisco, CA 94080

We have previously shown that NT-3 reverses cisplatin-induced peripheral sensory neuropathy. We now report that NT-3 treatment prevents pathological changes in sensory fibers observed in diabetic neuropathy. Female Wistar rats were made diabetic by streptozotocin (STZ; 75 mg/kg, ip). One week after STZ injection, rats were divided into 3 groups and injected 3x week s.c. for 15 weeks: non-diabetic + vehicle (n= 18), diabetic + vehicle (n=16) and diabetic + NT-3 (1 mg/kg; n=12). Sensory conduction velocities (CV) were determined via the H-reflex at monthly intervals. At 15 weeks, peroneal nerves were removed for direct recordings of the A fibers and both peroneal and sural nerves removed for histologic analysis. At 3 months, the compound sensory CV, as defined by the H-reflex, was significantly decreased in diabetic rats regardless of treatment (vehicle, 46 ± 3 ; NT-3, 46 ± 2 m/s) compared to non-diabetic controls (57 ± 2 m/s; $P<0.05$). Diabetic nerve A-fiber CVs, determined directly from peroneal nerves, were significantly decreased ($P<0.05$; 54.7 ± 1.9 m/s; n=13) compared to non-diabetic controls (67.9 ± 3.5 m/s; n=12), while NT-3 treatmnet prevented this decrease in diabetics (65.6 ± 2.4 m/s; n=9). A-fiber amplitude and integral tended to be decreased in diabetics but not significantly. Morphometric measurements of sural nerves (n=3) demonstrated the characteristic shift in fiber size frequency to smaller fibers in diabetic (81.5 ± 3.7% of myelinated nerves ≤ 35 µ) vs non-diabetics (68.8 ± 3.7%, $P<0.05$). This shift was prevented by NT-3 treatment (61.5 ± 2.2%, $P<0.05$). These results demonstrate that NT-3 can protect A-fiber CV in diabetic animals, maintain myelinated nerve profiles, and suggest NT-3 as a therapeutic in diabetic neuropathy.

420.13

FURTHER STUDIES ON THE ROLE OF NT-3 IN INTACT & INJURED PRIMARY SENSORY NEURONS K.A. Gratto*[1] B. Friedman[2] L. Liu[2] R.M. Lindsay[2] & V.M.K.Verge[1] [1]Dept of Anatomy, University of Saskatchewan, Canada S7N 5E5 [2]Regeneron Pharmaceuticals Inc., Tarrytown NY 10591

The hypothesis that NT-3 plays a role in maintaining the differentiated state of responsive trkC-expressing neurons and may be responsible for reversing changes observed after injury, *in vivo* was tested further. Adult rat right sciatic nerves were proximally cut. 14d after injury NT-3 was intrathecally infused for an additional 7d in half of the rats. Cryostat sections of intact and injured DRG with and without NT-3 infusion were processed for *in situ* hybridization to detect mRNAs encoding its receptors trkC and p75; neurofilament-medium (NFM); the immediate-early gene (IEG) cjun; and peptides galanin, SP, SOM and VIP. Results indicate that in intact neurons trkC, p75, NFM, SP and SOM are abundantly and heterogenously expressed, whereas few if any neurons express detectable galanin, VIP or abundant cjun mRNA. Two weeks after injury levels of trkC, p75, NFM, SP and SOM mRNA are dramatically reduced, while many neurons now express high levels of cjun, galanin and VIP mRNA. Infusion of NT-3 partially counteracts injury-induced decreases in trkC, p75, and NFM mRNA in primarily medium- to large-sized neurons and is also effective in downregulating the injury-induced expression of cjun and galanin in a similar neuronal subpopulation. NT-3 infusion has no apparent qualitative effect on the injury-induced changes in hybridization signal for SP, SOM and VIP. The lack of detectable influence of NT-3 on these peptides is consistent with their localization in a size range not normally associated with trkC expression, although this has not been rigorously tested. In conclusion, the ability of exogenous NT-3 to modulate expression of the receptors trkC and p75, the cytoskeletal protein NFM, the IEG cjun, and the peptide galanin, in addition to expression of the peptides NPY and α-CGRP reported previously, strongly supports a role for NT-3 in maintaining aspects of the differentiated state of responsive adult primary sensory neurons. *Canadian MRC and Saskatchewan HSURC supported.*

420.14

LOW-AFFINITY NEUROTROPHIN RECEPTOR p75 IS REQUIRED FOR THE DEVELOPMENT OF SOME MECHANORECEPTORS. K.-F. Lee[1]*, H. Dickinson-Anson[2], F.H. Gage[2], and M. Byers[3]. [1]The Salk Institute, La Jolla, CA 92037, [2]Dept. Neurosciences, UCSD, La Jolla, CA 92037, [3]Dept. Anesthesiology, Univ. Washington, Seattle, WA 98195

Because p75 binds all neurotrophins and is capable of interacting with each of the trk receptors in potentiating the activity of neurotrophins in several culture systems, mutation of the p75 gene may affect the development of both NGF-dependent and NGF-independent neurons. In the present study p75-deficient mice were tested in a battery of sensorimotor tests. Results indicated that the mutant mice were impaired on several measures compared with controls. In particular, when placed on a raised small wooden dowel bridge midway between two escape platforms, the mutant mice were unable to traverse the bridge and escape without falling off. In contrast, 100% of control mice rapidly traversed the bridge to escape. These results suggest a deficit in sensory (or sensorimotor) function. Because the skin mechanoreceptor end organs, the Meissner's corpuscles, contains p75, we examined the possibility that the phenotype is in part due to a deficit in innervation of Meissner's corpuscles in glabrous skin. Sections of glabrous skin were immunostained with an antibody to protein gene product 9.5 (PGP9.5). Meissner's corpuscle innervation is almost absent in mutant animals as compared to control animals. Thus, the loss of mechanoreceptor innervation at least explains the behavioral deficits. Defects in motor neurons could also contribute to the phenotype. Preliminary counts of lumbar motor neurons, however, revealed no loss.

Similar results were obtained when hair follicle mechanoreceptors were examined with the same antibody. Meissner's corpuscles are rapidly-adapting sensory receptors and are innervated by (highly myelinated) Aα neurons while hair follicles are innervated by Aβ neurons. It has been suggested that the survival of mechanoreceptor neurons is dependent on neurotrophins other than NGF such as BDNF, because BDNF is expressed in deeper skin layers. Our results therefore provide evidence that p75 is important for the development of some NGF-dependent and NGF-independent sensory neurons.

NEUROTROPHIC FACTORS: BIOLOGIC EFFECTS IX

421.1

NEUROTROPHIC FACTORS INCREASE AXONAL GROWTH AFTER SPINAL CORD LESIONS AND TRANSPLANTS IN ADULT RATS B.S. Bregman* and M. McAtee, Georgetown University, School of Medicine, Washington, DC 20007.

The capacity of CNS neurons for axonal regrowth after injury decreases as the age of the animal at time of injury increases. After spinal cord lesions and transplants at birth, there is extensive regenerative growth into and beyond a transplant of fetal spinal cord tissue placed at the injury site. After injury in the adult, however, although host corticospinal and brainstem-spinal axons project into the transplant, their distribution is restricted to within 1-2mm of the host/transplant border. The aim of this study was to determine if the administration of neurotrophic factors could increase the capacity of mature CNS neurons for regrowth after injury. Spinal cord over-hemisection lesions were made at T6 in adult rats. Transplants of E14 fetal spinal cord tissue were placed into the lesion site. BDNF, NT-3, NT-4, CNTF or vehicle alone were administered at the site of the transplant at the time of injury and transplantation. After 1-2 months survival, neuroanatomical tracing and immunocytochemical methods were used to examine the growth of host axons within the transplants. NT-3 increased both the density and extent of serotonergic axonal ingrowth into the transplants dramatically, but had relatively little effect on the growth of noradrenergic axons. BDNF, like NT-3, also substantially increased the growth of serotonergic axons into the transplants. Noradrenergic axons increased their projection in response to BDNF and NT-4. Although NT-4 influenced noradrenergic axons, it had no effect on the growth of serotonergic axons. The influence of the administration of the neurotrophins on the growth of injured CNS axons was not a generalized effect of growth factors per se, since the administration of CNTF had no effect on the growth of any of the descending CNS axons tested. These results indicate that in addition to influencing the survival of developing CNS and PNS neurons, neurotrophic factors are able to exert a neurotropic influence on injured mature CNS neurons by increasing their axonal growth within a transplant. The influence of the neurotrophins on mature injured CNS neurons is not uniform. Rather, particular populations of neurons appear to respond preferentially to particular members of the neurotrophin family. Supported by NIH-NINDS NS 19259; neurotrophins supplied by Regeneron.

421.2

TROPHIC FACTOR ENHANCED REGENERATION BY CHRONICALLY INJURED SUPRASPINAL NEURONS FOLLOWING CERVICAL SPINAL CORD INJURY J-H. Ye* and J.D. Houle, Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR. 72205

Whether the regenerative response of chronically injured neurons could be enhanced by exposure to specific trophic factors was examined. Supraspinal neurons were retrogradely labeled with True Blue (TB) at the time of a C3 hemisection lesion of the adult rat spinal cord. Four weeks later gel foam soaked with insulin-like growth factor 1 (IGF-1), combined brain derived neurotrophic factor and neurotrophic factor 3 (BDNF/NT-3) or saline as a control was placed into the lesion cavity. The gel foam was replaced after 3 days with fresh factor and 4 days later (35 days after injury) an autologous peripheral nerve graft (PNG) was apposed to the rostral surface of the cavity. The distal end of the PNG was exposed to Nuclear Yellow (NY) 4 weeks after grafting. Animals were sacrificed 2 days later and serial vibratome sections examined for the presence of regenerative neurons labeled with TB/NY or NY only.

The mean total number of regenerating neurons significantly increased over control values with BDNF/NT-3 (353 vs 256) or IGF-1 (555 vs 256) treatment. The percentage of regenerating neurons that were chronically injured was: BDNF/NT-3 (75%), IGF-1 (61%), control (55%). Enhanced regenerative capacity was greatest within the reticular formation, lateral vestibular nucleus and hypothalamus with either BDNF/NT-3 or IGF-1. Treatment with BDNF/NT-3, but not IGF-1, increased the mean number of regenerating neurons within the locus coeruleus and red nucleus. No effects were found on raphe or spinal trigeminal tract neurons with either factor. These results demonstrate the potential to increase the regenerative response of specific chronically injured supraspinal neurons with a short term exposure to BDNF/NT-3 or IGF-1. BDNF and NT-3 were supplied by Regeneron Pharmaceuticals, Inc. Supported by NIH Grant NS 26380.

421.3

BDNF REVERSES THE ATROPHY OF CHRONICALLY INJURED RAT RUBROSPINAL NEURONS AFTER CERVICAL (C3) AXOTOMY. N. R. Kobayashi*, A. M. Bedared and W. Tetzlaff Dept. of Physiology and Neuroscience, Univ. of Ottawa, Ottawa, Ontario, K1G 8M5, Canada

Rat rubrospinal neurons undergo severe atrophy during the second week after axotomy at the cervical level (C3) of the spinal cord. This atrophy is accompanied by a decrease in the expression of regeneration associated genes i.e. tubulins, actin and GAP-43 (Tetzlaff et al., J. Neurosci. 11:2528-2544, 1991). We have previously shown that the application of BDNF (500 ng/μl/ hr) into the vicinity of the red nucleus from day 7 to 14 fully prevented this atrophy and sustained GAP-43 mRNA expression. (Soc. Neurosci. abtr., Vol 19, 455.7). We have now tested whether BDNF can reverse the atrophy of these neurons after they had been chronically injured. We allowed rubrospinal neurons to undergo atrophy for 5, 7, 16, 24, and 52 weeks after cervical axotomy. Subsequently, BDNF (500 ng/μl/ hr) was infused for 7 days i.e. during the 6th, 8th, 17th, 25th and 53rd week, respectively. The mean cross sectional soma area of the BDNF treated group was 88% of contralateral at 6 weeks, compared to 58% in the vehicle treated animals. After 17 weeks, the neurons of the BDNF treated group showed the cross sectional area of 72% of contralateral, while the vehicle treated group was found at 49% of contralateral. Furthermore, our preliminary *in situ* hybridization results indicate that GAP-43 mRNA is re-expressed and the decrease in Tα1-tubulin expression is reversed in the BDNF treated group, which is not seen after vehicle treatment only. It remains to be shown whether this approach can be exploited to promote regeneration after chronic injury.

BDNF was kindly supplied by Regeneron Pharmaceutical Inc.. Supported by the Medical Research Council of Canada (W.T.), the American Paralysis Association, USA (W.T.), and the Government of Canada Award (N.R.K.).

421.4

BDNF AND NT-3 EXERT DIFFERENTIAL AND OVERLAPPING EFFECTS ON GAP-43 AND Tα1-TUBULIN EXPRESSION IN AXOTOMIZED CORTICOSPINAL NEURONS OF THE RAT.

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In the peripheral nervous system, regeneration is correlated with increased expression of regeneration associated genes (RAG), e.g. GAP-43 and Tα1-Tubulin. In contrast, RAG expression is decreased in corticospinal neurons (CSN) after axotomy (Giehl et al., 1993, Soc. Neurosci. Abstr. 283.9). CSN, retrogradely labelled with Fast Blue, were axotomized at the internal capsule and the neurotrophins BDNF and NT-3 were applied intracortically via an osmotic minipump for 7 days. As shown with *in situ* hybridization, both BDNF and NT-3 increase Tα1-Tubulin expression in axotomized CSN. This is consistent with the expression of trkB and trkC in these neurons (Giehl and Tetzlaff, 1994, Soc. Neurosci. Abstr. 253.14). However, only BDNF but not NT-3 increases GAP-43 expression. In agreement with the lack of trkA expression in CSN, NGF did not have an effect. Thus, BDNF and NT-3 have overlapping as well as differential effects on RAG expression in axotomized CSN.

421.5

NEUROTROPHIN-3 (NT-3) PREVENTS DEATH OF CLARKE'S NUCLEUS NEURONS IN ADULT RATS. M.Shibayama[1,2] S.Hattori[1,2*] B.T.Himes[1,3] N.Matsui[2] M.Murray[1] and A.Tessler[1,3] [1]Dept. of Anatomy & Neurobiology, Medical College of Pennsylvania and Hahnemann University, 3200 Henry Ave. Philadelphia, PA.19129. [2]Dept of Orthopedic Surgery, Nagoya City Univ.School of Medicine, Nagoya, Aichi,Japan.[3]Philadelphia VA Hospital

Fetal transplants placed into the site of hemisection in adults as well as neonates rescue all Clarke's nucleus (CN) neurons destined for retrograde cell death. One reason for retrograde cell death after axotomy is loss of neurotrophic factors. It has been proposed that transplants provide these neurotrophins.We studied whether NT-3 delivered exogenously to adult rats with spinal cord injury can rescue axotomized CN neurons otherwise destined to die. Female Sprague-Dawley rats (250-300g) underwent laminectomy at the mid-thoracic level with interruption of the right dorsal spinal spinocerebellar tract by spinal cord hemisection. This injury transects the axons of the right CN neurons and spares the neurons of the left CN as an intact control. At the time of the hemisection rats had either gelfoam soaked in NT-3 (10 µg, generously provided by Regeneron) or gelfoam soaked in saline placed into the hemisection cavity. Two months after surgery the rats were perfused, and CN neurons at the L1 segment were counted and measured. Rats receiving saline showed 30% loss of CN neurons ipsilateral to the hemisection; average cell area was reduced by 60%. Rats receiving NT-3 showed 15% neuron loss and greater average neuron area than after saline alone. Bioassay of the gelfoam in additional rats showed NT-3 activity at 1 day after surgery but not at 3 days. These results indicate that brief exposure of axotomized CN neurons to NT-3 suffices to produce long-term rescue of 50% of axotomized CN neurons that were destined to die in adult rats; to determine whether continuous availability of neurotrophic factor increases the number of rescued cells, we are administering NT-3 into the hemisection site by Alzet Osmotic Mini-pump. Supported by the VA Medical Research Service and NS 24707.

421.6

SPINAL CORD GRAFTS IN OCULO: EFFECTS OF TREATMENT WITH BDNF, CNTF, GDNF OR NT-3. K. Trok*, B. Holfer and L.Olson. Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden.

Intraocular transplantation of neural tissue provides a method to examine *in vivo* effects of trophic factors. We have previously demonstrated that fetal spinal cord tissue survives grafting to the anterior chamber of the eye. Such transplants receive functional inputs from cografts of brain stem areas containing serotonin or noradrenaline neurons. Intraocular spinal cord tissue also becomes functionally connected with cortex cerebri, spinal cord and skeletal muscle cografts. Because spinal cord maturation and connectivity involve the influence of trophic factors, in the present study we have investigated the influence of brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), glial cell line-derived neurotrophic factor (GDNF) and neurotrophin-3 (NT-3) on such grafts. Spinal cord tissue from E14, E18, P0–1, P7 and P14 rats was transplanted to the anterior eye chamber of adult host rats after incubation in 100 µg/ml of BDNF, CNTF, GDNF, NT-3, or cytochrome C (cyt C) in PBS or vehicle. On days 5, 10, 15, 20 and 25 postgrafting 5 µl of a similar solution was injected into the anterior eye chamber. Treatment with BDNF augmented growth of transplanted spinal cord of all donor stages compared to controls, although this effect appeared transient. Treatment with CNTF enhanced growth of grafts from P1 and P14 donors in an apparantly permanent manner. Effects of NT-3 on postnatal spinal cord grafts appeared to be smaller than those of BDNF, and although they manifested later in graft maturation, they appeared to be more permanent. GDNF-treated P1 grafts grew to volumes larger than their initial size at grafting. P7 and P14 grafts treated with GDNF also responded with increased growth compared with controls but did not exceed their initial size at transplantation. Morphological characterization of grafted spinal cord tissue will be described.

421.7

CLONING AND EXPRESSION OF BRAIN-DERIVED NEUROTROPHIC FACTOR IN BRAIN AND SPINAL CORD OF NEONATAL OPOSSUM. H.A. Vischer * and E. Reinhard. Dept. Pharmacology, Biozentrum, University of Basel, 4056 Basel, Switzerland.

Crushed or cut spinal cord of neonatal opossum Monodelphis domestica has the ability to repair morphologically and physiologically in vivo and in vitro (Woodward et al., 1993; Saunders et al., 1995). In order to investigate the possible role of neurotrophins in this process, we partially cloned the opossum brain-derived neurotrophic factor (BDNF). BDNF has been shown to prevent cell death of motor neurons in vivo and in vitro in the brain and spinal cord (Oppenheim, 1992; Yin, 1994) and might therefore be upregulated during the period of spinal cord repair. Opossum BDNF was cloned by RT-PCR using primers to highly conserved regions of BDNF of different vertebrate species. A 266 bp long fragment was obtained that showed 83% identity on the nucleotide level to chick BDNF, 82% to human BDNF, 81% to pig BDNF and 77% to rat BDNF. Due to this high homology we used a full-length rat BDNF cDNA (gift from Regeneron) as a probe to determine the sites of expression in opossum. Whole-mount *in situ* hybridization studies were performed on isolated CNS preparations, using a digoxigenin-labeled single-stranded BDNF cRNA-probe. In those preparations where the spinal cord was crushed, BDNF was expressed at higher levels in the medulla oblongata. No BDNF was expressed at the crush site. We are currently studying the presence of other neurotrophic factors and their corresponding receptors during spinal cord repair.

421.8

WITHDRAWAL OF TRANSIENT SEROTONERGIC PROJECTIONS FROM NON-TARGET TRANSPLANTS IS REGULATED BY THE AVAILABILITY OF NEUROTROPHIC FACTORS. H. Bernstein-Goral* and B.S. Bregman. Georgetown University, School of Medicine, Washington, DC 20007.

Axotomized serotonergic (5HT) raphe-spinal neurons permanently regenerate axons into target-specific fetal spinal cord transplants placed into either a spinal cord hemisection or transection in the newborn rat. While target-specific transplants permit regeneration, non-target transplants placed into a spinal cord lesion support only transient ingrowth of 5HT axons that is completely withdrawn from the transplant by one month after transplantation. This transient innervation arises from collateral sprouting of intact 5HT axons from the host spinal cord. Transient 5HT axons form synapses within the non-target transplant that are indistinguishable from those in appropriate targets. This suggests that synaptogenesis within the transplant does not regulate the maintenance of innervation. Signals arising from the non-target transplant itself appear to direct the withdrawal of axon collaterals. We hypothesize that specific neurotrophic factors that are critical for the maintenance of axonal ingrowth into the transplant are lacking in the non-target tissues. To test this, non-target transplants (fetal rat hippocampus) placed into a neonatal thoracic spinal cord hemisection, were supplemented with exogenous neurotrophins BDNF (n= 11) or NT3 (n=8) or with saline (n=4). At one month post-transplantation, 5HT axonal ingrowth into the non-target transplant is maintained in animals that received either exogenous BDNF or NT3, but not in controls with non-target transplant alone. The pattern and density of ingrowth into non-target transplants with either exogenous BDNF or NT3 is comparable to the permanent regenerative growth of axons into target-specific transplants. Administration of neurotrophins also appears to affect the development of the hippocampus transplants directly; transplants are strikingly well differentiated and larger in the presence of neurotrophins than in transplants without additional neurotrophins. Our studies suggest that the availability of specific neurotrophins within the transplant may 1) enhance axonal elongation after CNS injury and 2) promote the long-term maintenance of newly formed pathways. Supported by NIH-NINDS NS 19259.

421.9

BDNF PROMOTES THE SPROUTING OF NEUROTOXIN-LESIONED SEROTONERGIC AXONS IN RAT BRAIN. L.A. Mamounas*, M.E. Blue, K.J. Axt and C.A. Altar. National Institute on Aging, GRC, Baltimore, MD 21224; Kennedy-Krieger Inst., Baltimore, MD; Johns Hopkins Univ. Sch. of Med., Baltimore, MD; and Regeneron Pharm., Tarrytown, NY.

Brain-derived neurotrophic factor (BDNF) was recently found to prevent the severe degenerative loss of serotonergic (5-HT) axons normally caused by the selective 5-HT neurotoxin p-chloroamphetamine (PCA) in adult rat neocortex (Mamounas et al., 1995). In our initial study, continuous three-week intracortical infusions of BDNF were started one week before subcutaneous administration of PCA, and continued for another two weeks after treatment with the neurotoxin. Thus, it remained to be determined whether BDNF 1) prevents the degeneration of 5-HT axons normally caused by PCA or 2) dramatically facilitates and accelerates the endogenous sprouting of 5-HT axons after PCA-induced degeneration, or both. The present study evaluated this question by manipulating the temporal parameters of BDNF and PCA delivery, followed by immunocytochemical staining of 5-HT axons using antisera directed against serotonin or the serotonin transporter. Two-week intracortical infusions of BDNF (12 µg/day) started four days after PCA administration (10 mg/kg, s.c.) caused a marked increase in 5-HT axon density near the BDNF infusion cannula when compared to control infusions, suggesting that BDNF accelerates and enhances the normally slow sprouting of 5-HT axons after PCA-induced degeneration. When BDNF infusions were started one week before PCA administration and terminated one day after PCA, an evaluation of the 5-HT innervation 2 days later revealed only a partial sparing of 5-HT axons near the BDNF infusion cannula. Thus, BDNF only partially prevents the PCA-induced degeneration of serotonergic axons, but causes a robust sprouting of 5-HT axons when administered after degeneration by PCA. These results support our initial finding that BDNF causes sprouting of uninjured 5-HT axons in intact (non PCA-lesioned) animals and suggest that BDNF given *after* injury has a tropic role in promoting serotonergic axonal growth in the adult nervous system.

421.10

EXOGENOUS BDNF AND NT-4/5 UPREGULATE EXPRESSION OF BDNF mRNA IN MIDBRAIN NEURONS. K.D. Anderson*, J.-Y. Cho, T.L. Corcoran, R.M. Lindsay, and S.J. Wiegand. Regeneron Pharmaceuticals, Inc., Tarrytown, NY 10591.

Intracerebral delivery of neurotrophins induces changes in neuropeptide levels and electrical activity of neurons, augments neurotransmitter turnover, and alters behavior. To investigate potential mechanisms whereby intracerebral delivery of neurotrophins influences neuron function, we determined the effect of exogenous neurotrophins on the expression of BDNF and NT-3 mRNAs by *in situ* hybridization in adult male rats. Neurotrophins were delivered intracerebrally for 12 days by an osmotic pump connected to a cannula implanted into the right mesencephalon and extending to the center of the substantia nigra pars compacta. Continuous infusion of BDNF (12 µg/day) or NT-4/5 (7.2 µg/day), but not NT-3, NGF (each at 12 µg/day) or saline (12 µl/day), induced a unilateral increase in neuronal expression of BDNF mRNA, but only in structures normally expressing it in untreated rats. Structures exhibiting neuronal BDNF mRNA upregulation included the superior colliculus, the central gray, ventral tegmental area, the substantia nigra pars compacta, the peripeduncular nucleus, and the red nucleus. An increase in NT-3 mRNA was not detected after infusion of BDNF, NT-3, NGF or saline. These results suggest that BDNF mRNA production may be regulated by a TrkB-mediated positive feedback loop. However, enhanced production of BDNF mRNA by NT-3, or enhanced production of NT-3 mRNA by BDNF, are not part of the mechanisms involved in the regulation of neuron function by exogenous neurotrophins.

421.11

THE NEUROTROPHIC ACTIONS OF BDNF IN THE DEVELOPING PONTINE-CEREBELLAR SYSTEM. S.A. Rabacchi, S.L. Meyer, D.H. Baird, and J.E. Springer*, Department of Anatomy, Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA. 19129, and Cephalon, Inc. West Chester, PA. 19380 (SLM)

The cellular events involved in regulating neuron survival, neurite outgrowth, and target contact rely, in part, on the expression of target-derived neurotrophic factors. One family of neurotrophic factors, the neurotrophins, have been shown to play a role in the development and survival of several PNS and CNS neuronal populations. In the developing cerebellum, granule cells have been shown to both synthesize and respond to BDNF, and express trkB. Granule cells are the major target of pontine-derived mossy fibers that innervate the cerebellum in early postnatal stages of development. In the present study we tested the effects of BDNF on trkB mRNA expression in granule cells, as well as the ability of BDNF to promote the outgrowth of mossy fibers in pontine explants. In the first experiment, purified granule cells were prepared from postnatal day 6 rats, cultured in serum-free conditions, and exposed to 1, 10, or 100 ng/ml of BDNF for a period of 4 or 24 hours. Using in situ hybridization, and increase trkB mRNA expression was observed in individual granule cells within 4 hr following treatment with 100ng/ml of BDNF. The levels of trkB mRNA were still elevated in granule cells treated for 24 hours, although the effect was not as great as that observed at 4 hr. In the second experiment, pontine explants from neonates were treated with 1, 10, or 100 ng/ml of BDNF, NGF, or NT-3 for 2 days. BDNF, but not NGF or NT-3, increased the amount of neurite outgrowth from pontine explants at the 10ng/ml concentration. This effect of BDNF is consistent with the expression of trkB mRNA in the pontine explants as determined using PCR analysis. Furthermore, BDNF treatment increased growth cone area by 2-fold. We propose that, in addition to acting as an autocrine/paracrine survival factor for the granule cells, BDNF also plays a role as a target-derived promoter of mossy fiber extension. Supported by NIH grants NS33214 (DHB) and NS30502 (JES).

421.12

BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) STIMULATES RAT CEREBELLAR GRANULE NEUROBLAST MITOSIS IN CULTURE AND *IN VIVO*. E. DiCicco-Bloom*, J. P. Wagner, & J.B. Black. Dep't Neurosci. & Cell Biol, UMDNJ/Rob. Wood Johnson Med. Sch. Piscataway, NJ.

While neurotrophins play critical roles in target-dependent survival, expression in developing brain suggests that the factors regulate neurogenesis. Recent neurogenetic studies *in vitro* indicate two distinct mechanisms by which signals enhance proliferation, including (i) directly stimulating neuroblasts to enter the mitotic cycle (mitogenesis) and (ii) promoting survival of dividing precursors (trophism). We previously defined a trophic role for NT3 in stimulating sympathoblast proliferation. We now characterize mitogenic actions of BDNF in cerebellar granule neurogenesis.

We employed on a highly enriched population of postnatal day 7 rat cerebellar granule neuroblasts cultured in defined medium, and assessed [^{3}H]thymidine ([^{3}H]dT) incorporation, a marker of DNA synthesis, and cell number. BDNF increased [^{3}H]dT incorporation 3-fold at 24 hr. with an EC_{50} of ~0.08 ng/ml and peak effects at 0.3-1 ng/ml. Enhanced DNA synthesis reflected a 50% increase in the proportion of neuroblasts in the mitotic cycle, revealed by [^{3}H]dT autoradiography (labeling index, LI: Con=11±0.8%, BDNF=17±0.4%; $p<0.003$). In addition, BDNF increased cell number by 80%, though only at higher doses (30-100 ng/ml). To examine mitogenesis in the absence of trophism, cultures were assessed prior to cell death, at 6 hr, when numbers of trypan blue excluding cells were equal among groups. BDNF (3 ng/ml) increased the LI by 40%, indicating that the factor directly stimulated granule neuroblast mitosis, independent of trophic action.

To define activity *in vivo*, animals received 100 ng BDNF intracisternally, and after subcutaneous [^{3}H]dT injection at 6 hr, were sacrificed at 8 hr. BDNF increased DNA synthesis 4-fold in isolated neuroblasts, suggesting that the factor directly stimulates mitogenesis in the brain. Our observations indicate that neurotrophins exert both mitogenic and trophic regulation of neurogenesis, acting in population-specific fashion. (Support: NICHD23315; Trophix Pharm.)

421.13

THE BIOLOGICAL EFFECTS OF BDNF OVEREXPRESSION IN DOPAMINE-β-HYDROXYLASE:BDNF TRANSGENIC MICE

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We have examined transgenic mice that express BDNF from the dopamine-β-hydroxylase (DBH) promoter which directs gene expression to adrenergic and noradrenergic neurons. Transgenic lines appropriately overexpress BDNF mRNA in the superior cervical ganglia (SCG), adrenals, and brainstem. Using an anti-BDNF antibody, BDNF protein is overexpressed in neurons of the SCG and locus coeruleus (LC). Innervation density within the SCG derived from cholinergic preganglionic inputs was increased as demonstrated by ChAT and synapsin immunostaining. *trk*B immunoreactivity has been localized to preganglionic neurons, and there is an increase in cell size in a preganglionic subpopulation. In the CNS, neurons of the LC are hypertrophied 40% relative to control which is accompanied by an increase in TH mRNA expression. Immunocytochemistry for DBH in the hippocampus shows an increase in DBH-positive fibers and preliminary evidence indicates changes in catecholamine content within discrete brain regions as demonstrated by HPLC measurements. The observed changes in catecholamine content in regions that contain trk B suggests these neurons may be responding to BDNF secreted from the LC. Based on these results we have hypothesized that sympathetic neuron-derived BDNF regulates its input innervation density via a feedback mechanism whereas, in the CNS, BDNF regulates the growth and survival of LC neurons via a potential autocrine mechanism.

421.14

MAPPING BDNF-RESPONSIVE NEURONS IN THE MATURE CNS: USE OF Tα1:LACZ TRANSGENIC MICE

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We have previously demonstrated that 1.1kb of 5' upstream region from the Tα1 α-tubulin gene was sufficient to direct expression of a nuclear ß-galactosidase marker gene to neurons of the peripheral and central nervous systems (Gloster et al., 1994). Moreover, we demonstrated that the absolute levels of expression of this transgene were regulated as a function of morphological growth in both developing and mature neurons. Interestingly, in the mature nervous system, the Tα1:nlacZ transgene was not expressed homogeneously in a panneuronal fashion. To examine this phenomenon further, we have characterized the distribution of the Tα1:nlacZ transgene in the mature central nervous system, and have compared its expression to that of the endogenous Tα1 α-tubulin mRNA. Our results show that there is a strong correlation between the pattern of transgene expression and the pattern of Tα1 α-tubulin mRNA expression indicating that even in the mature nervous system, the 1.1 kb Tα1 promoter fragment is sufficient to drive transcription as a function of neuronal growth potential. When Tα1:lacZ mice were crossed with a mouse line that overexpresses BDNF from the DBH promoter, ß-galactosidase expression in distinct regions of the brain increased dramatically. This indicates that the 1.1 kb Tα1 promoter fragment contains elements neccesary for BDNF-responsiveness. This system enabled us to rapidly map BDNF-responsive neurons in the mature mouse brain, and provides an ideal sysyem to examine the function of BDNF in the brain.

421.15

CHANGING RESPONSIVENESS OF DEVELOPING MIDBRAIN DOPAMINERGIC NEURONS FOR EXTRACELLULAR GROWTH FACTORS. J. Engele*, Dept. Anatomy and Cell Biology, University of Ulm, 89069 Ulm, Germany.

Previous studies have demonstrated that a vast array of growth factors support the survival of dopaminergic neurons in cultures of the early embryonic mesencephalon by either a direct or an indirect, glial-mediated, mechanism. To further characterize the functional role of these multiple growth factor influences for dopaminergic cell development, various purified growth factors as well as mesencephalic glial-conditioned medium (CM) were screened for their effects on dopaminergic cell survival in serum-free low-density cultures of the dissociated embryonic day (E)15 and E17 rat mesencephalon. Treatment of E15 mesencephalic cultures with FGF-2 (25 ng/ml), TGFα (50 ng/ml), IGF-1 (100 ng/ml), BDNF (50 ng/ml), PDGF-BB (30 ng/ml), interleukin-6 (25 U/ml), GDNF (1ng/ml), and glial-CM (50%) all increased the number of surviving tyrosine hydroxylase-immunoreactive dopaminergic neurons at 6 days when compared to controls. In contrast, with E17 mesencephalic cultures, survival promoting effects on dopaminergic neurons were restricted to FGF-2, BDNF, and GDNF.

These findings demonstrate that, during embryonic development, dopaminergic cell survival sequentially depends on different sets of growth factors. The concomitant loss of sensitivity of developing dopaminergic neurons for mesencephalic glial-CM as well as TGFα, IGF-1, PDGF-BB, and interleukin-6 further suggests that the survival promoting effects of these growth factors on early embryonic dopaminergic neurons are indirectly mediated through resident mesencephalic glia. Supported by DFG, En 187/2-2.

421.16

ROLE OF NEUROTROPHIC FACTOR SIGNAL TRANSDUCTION PATHWAYS IN THE ACTIONS OF DRUGS OF ABUSE IN THE MESOLIMBIC DOPAMINE SYSTEM. MT Berhow* and EJ Nestler, Laboratory of Molecular Psychiatry, Yale Univ Sch of Med, Dept of Psychiatry, New Haven, CT

Chronic morphine and cocaine treatments have been shown to regulate levels of tyrosine hydroxylase (TH) and other proteins in the ventral tegmental area (VTA) of the adult rat brain. Recent research has demonstrated that direct infusion of BDNF or NT4 (2.5 µg/day) midline into the VTA could prevent as well as reverse these biochemical changes associated with chronic morphine and cocaine treatments. In contrast, CNTF (1.5 µg/day) infusions mimicked these biochemical changes (Berhow et al, Neuroscience, 1995, in press).

Present studies are focusing on the possibility that morphine and cocaine regulate TH and other target proteins via actions on neurotrophic factors signaling pathways within the VTA. One major pathway involves the activation of ERKs (extracellular signal regulated kinases). We are using an antisense oligonucleotide strategy to study the role of these kinases in drug action. Infusion of ERK1 antisense oligonucleotide (10 µg/day) into the VTA for seven days resulted in a 60% decrease in ERK1, with no effect on ERK2. This treatment led to a 45% decrease in TH levels in the VTA, however numerous cytoskeletal and signaling proteins showed no change. The possibility that the ERKs mediate morphine and neurotrophin regulation of TH expression is under current investigation. A second major pathway involves activation of the JAK-STAT cascade. To date, we have found that chronic cocaine treatment increases JAK immunoreactivity by 40-50% in the VTA, whereas chronic morphine treatment produces no change in JAK levels. Gel shift assays are currently underway to determine whether there is also an alteration in STAT DNA binding activity with cocaine treatment. These studies promise to elucidate novel mechanisms by which drugs of abuse induce biochemical adaptations in the VTA potentially involved in addiction.

421.17

MORPHOLOGICAL EVIDENCE OF CHANGES INDUCED IN THE VENTRAL TEGMENTAL AREA (VTA) BY CHRONIC MORPHINE TREATMENT. L. Sklair-Tavron,* W.-X. Shi, H.W. Harris, B.S. Bunney, and E.J. Nestler. Laboratory of Molecular Psychiatry, Depts of Psychiatry and Pharmacology, Yale Univ School of Medicine, New Haven, CT 06508.

The VTA and its dopaminergic projections to the limbic forebrain have been implicated in the reinforcing properties of opiates and other drugs of abuse and may be a site where the drugs produce long-term adaptations that underlie aspects of addiction. For example, we have shown that chronic morphine treatment increases levels of tyrosine hydroxylase (TH) and glial fibrillary acidic protein (GFAP), and decreases levels of neurofilament proteins, in the VTA. We have proposed, based on these biochemical findings, that prominent structural changes may occur within this brain region in the chronic morphine-treated state (Neuron 11, 995-1006, 1995). The aim of the present study was to investigate this possibility directly. Immunocytochemical studies revealed an increase in leveis of GFAP immunoreactivity specific to the VTA. Higher magnification revealed a dramatic increase in the number of astrocytes in this brain region. Immunocytochemical analysis also revealed no difference in the number of TH+ neurons following chronic morphine treatment. Morphometric analysis was used to evaluate whether chronic morphine treatment is associated with alterations in the shape and size of dopaminergic neurons in the VTA. Neurons in fixed brain slices were injected with lucipher yellow, and TH+ cells were identified by immunocytochemistry. Preliminary studies have revealed a significant reduction in the mean area and perimeter of VTA dopaminergic (TH+) neurons in slices from chronic morphine-treated rats. In contrast, such changes were not apparent in non-dopaminergic (TH-) neurons. These findings support interpretations of our biochemical data that chronic morphine treatment is associated with prominent structural changes in the VTA, which may underlie some of the long-term behavioral effects of this drug.

421.18

NEUROTROPHIC EFFECT OF NEONATAL PROLACTIN (PRL) TREATMENT ON GROWTH HORMONE-RELEASING HORMONE (GHRH) EXPRESSION IN HYPOTHALAMIC NEURONS OF NORMAL AND AMES DWARF MICE. M.I. Romero* and C.J. Phelps. Neuroscience Training Program and Department Anatomy, Tulane University School of Medicine, New Orleans, LA 70112.

The absence of pituitary GH and PRL in Ames dwarf mice coincides with reduction in the number of dopaminergic neurons (catecholaminergic area A12; Phelps et al., J. Neuroendocrinology, 1994) and an increase in the number of GHRH-expressing neurons (Phelps et al., Endocrinology, 1993) which project to hypophysial portal vessels to inhibit PRL and stimulate GH secretion. The increase in GHRH and the reduction in A12 neuron number in dwarfs both occur in the hypothalamic arcuate nucleus (ARC). This observation, combined with evidence in retrograde tract-tracing studies that the total number of ARC projection neurons is not reduced in dwarfs compared to normal mice, led to the hypothesis that ARC neurons that are originally dopamine (DA)-producing switch phenotype during early postnatal development to GHRH-producing cells. In order to test this theory, the present experimental design took advantage of the previous finding that the A12 deficiency can be prevented by treating neonatal dwarfs with PRL (Romero and Phelps, Endocrinology, 1993). This regimen of PRL replacement was repeated, in the expectation that the GHRH population would be reduced by preservation of the A12 cells. Alternatively, increased co-expression of DA and GHRH could occur, and this possibility is also being examined. Dwarf and normal siblings were treated beginning at 12 days postnatally with oPRL (50 µg/day) and brains were evaluated by formaldehyde-induced catecholamine fluorescence for DA and immunostaining for mGHRH after i.c.v. colchicine (1.5 µg/g). As previously reported, perikaryal fluorescence in A12 was comparable to that in normal mice. However, the number of GHRH+ cells was significantly ($p < 0.005$) increased in both normal (913±61) and dwarf (1703±158) mice, when compared to those observed previously in untreated normal (343±54) and dwarf (976±123) mice. This effect appeared to regress 15 d after PRL withdrawal. These results imply that PRL may be neurotrophic for GHRH as well as DA expression in neurons in the hypothalamic ARC. Supported by PHS grant NS25987.

421.19

IL-1β, IL-6, AND TNFα DIFFERENTIALLY REGULATE THE SURVIVAL OF FETAL SEROTONIN AND DOPAMINE NEURON *IN VITRO*. J.H. Gilmore*, H. Xiao, M.B. Wilkie, J.M. Lauder. Depts. of Psychiatry and Anatomy and Cell Biology, University of North Carolina School of Medicine, Chapel Hill, NC 27599

In utero exposure to infection is associated with a variety of neurodevelopmental diseases, including schizophrenia. It is hypothesized that this association is mediated in part by cytokines, as it has been shown that cytokines can regulate neuronal survival and differentiation. The effects of IL-1β, IL-6, and TNFα on neuronal survival were studied as these cytokines have systemic effects and their concentrations in maternal and fetal blood, as well as in amniotic fluid, are altered in pregnancies complicated by infection. Dissociated cell cultures of rat embryonic day 14 rat substantia nigra (SN) and rostral raphe (RR) were grown for two days in the presence of three concentrations of human or murine rIL-1β or rIL-6 (1,10, 100 units/ml) or rTNFα (0.1, 1.0, 10 ng). mrIL-1β and mrTNFα significantly decreased the survival of tyrosine hydroxylase (TH) immunoreactive neurons in SN cultures and 5-HT immunoreactive neurons in RR cultures, while mrIL-6 significantly enhanced survival of both neuron phenotypes. All murine cytokines had effects at the lowest concentrations studied. In contrast, human cytokines had no effects on 5-HT neurons and required higher concentrations for effects on TH neurons. These finding suggest that human and murine cytokines have different effects on fetal neurons derived from rats. The results also indicate that these cytokines can regulate the survival of the monoaminergic neurons at physiologically relevant concentrations and provide support for the hypothesis that cytokines play a role in the altered neurodevelopment found in schizophrenia.

NEUROTROPHIC FACTORS: RECEPTORS AND CELLULAR MECHANISMS II

422.1

BDNF-MEDIATED DOWN-REGULATION OF TRK-B RECEPTOR PROTEIN AND mRNA IN CULTURED HIPPOCAMPAL NEURONS. L. Frank, R.M. Lindsay, R. Ventimiglia and J.S. Rudge*. Regeneron Pharmaceuticals Inc., Tarrytown, New York 10591.

Given the interest in the clinical potential of peptide growth factors to ameliorate neurodegenerative disease, it is important to gain further knowledge about the effects of using such factors at pharmacological doses on CNS neurons. We decided to investigate how the neurotrophins brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5) affected the expression of the neurotrophin receptors TrkB and TrkC in responsive neurons. Regulation of Trk receptors was investigated in dissociated cultures of embryonic day 18 (E18) rat hippocampal neurons. Cultures were exposed to 50 ng/ml BDNF, NT-3 or NT-4/5 for 24 h upon plating followed by factor washout. As determined by immunohistochemical staining and phosphotyrosine blotting, the functional responses to acute stimulation with BDNF, NT-3 and NT-4/5, including c-Fos induction and phosphorylation of Trk and ERK proteins, were significantly decreased after 6 days in culture by prior exposure to BDNF. As determined by Western and Northern blot analysis, respectively, there was a parallel down-regulation of TrkB protein as well as trkB and trkC mRNA levels in BDNF pretreated cultures. Exposure to NT-3 or NT-4/5 at the same concentrations as BDNF did not down-regulate any of the measured cellular responses or TrkB protein and/or trkB and trkC mRNA levels. None of these effects were accompanied by any change in total cell number or percentage of NSE positive cells in our cultures, indicating that BDNF has no effect on gross survival of late embryonic hippocampal neurons. The present data show that exposure of developing hippocampal neurons in vitro to BDNF produces a long-term functional desensitization to the BDNF, NT-3 and NT-4/5, possibly resulting from down-regulation of TrkB protein and mRNA expression as well as down-regulation of trkC mRNA.

422.2

CNTF INDUCES DOWN-REGULATION OF ITS RECEPTOR AND SIGNAL TRANSDUCTION PATHWAYS IN VIVO: NON-EQUIVALENCE WITH PHARMACOLOGICAL ACTIVITY. P.S. DiStefano*, T.G. Boulton, J. L. Stark and R.M. Lindsay. Regeneron Pharmaceuticals, Tarrytown, NY 10591.

A hallmark of polypeptide growth factors is ligand-induced down-regulation of cell surface receptors. We addressed this issue with the neurotrophic molecule, ciliary neurotrophic factor (CNTF), in vivo. A single injection of CNTF (1.0 mg/kg, s.c.) increased tyrosine phosphorylation of LIFRß and gp130, and induction of the immediate-early gene, *tis*11, in skeletal muscle. Continued administration resulted in down-regulation of CNTFRα mRNA, as well. Injections of CNTF 3-7 hr after an initial injection failed to re-stimulate receptor tyrosine phosphorylation and *tis*11 induction, indicative of a biochemical desensitization of the immediate-early responses to CNTF. When injections were spaced 24 or 48 hr apart, the full induction of immediate-early responses was once again obtained. Cross-desensitization experiments showed that a prior exposure to LIF, which does not utilize CNTFRα, resulted in desensitization to stimulation with CNTF in muscle and DRG; however, CNTF did not cause desensitization to LIF, suggesting that biochemical desensitization mechanisms may lie downstream from the specific CNTF receptor (CNTFRα). To determine if the CNTF-induced down-regulation of CNTF receptor and immediate-early responses resulted in a functional desensitization, we assessed the ability of multiple daily injections of CNTF to prevent the denervation-induced atrophy of skeletal muscle (Helgren et al, 1994). Surprisingly, injections of CNTF every 6 hr, which falls within the putative refractory period for biochemical responses, resulted in equal or greater efficacy compared to once-daily injections. These results suggest that, although CNTF down-regulates its entire signal transduction system, biological signals continue to be recognized and interpreted by the cell.

422.3

TRUNCATED TRKB RESTRICTS BDNF AVAILABILITY DURING DEVELOPMENT: EXPRESSION PATTERN AND BIOCHEMICAL PROPERTIES. S. Biffo*, B. Carter, N., Offenhäuser, and Y.-A. Barde. DIBIT, Istol. Molec., V. Olgettina 58, 20132, Milano, ITALY; MPI. Dept. Neurobiochem. 82152 Martinsried, FRG.

BDNF (Brain-derived neurotrophic factor) plays an important role in neuronal survival. Its biological effect is mediated by the tyrosine kinase receptor trkB that is expressed by a variety of neurons during development. Truncated trkB molecules lacking the tyrosine kinase domain have also been described, but their functions remain elusive. In order to gain insight into their role, we studied the pattern of expression and properties of these truncated receptors in the chick embryo. Truncated trkB m-RNA was detected already early during neurogenesis, and in situ hybridisation experiments indicated that the expression was in non-neuronal cell. Ependymal and leptomeningeal cells expressing truncated trkB completely surrounded the developing brain and the spinal cord throughout development. In the otic vesicle, mesenchymal cells expressing truncated trkB surround cells producing BDNF, as well as neurons expressing trkB with its tyrosine kinase domain. Non-neuronal cells were found not to express trkB mRNA coding for the tyrosine kinase domain. Studies with radioiodinated BDNF performed on frozen sections of the chick embryo and on freshly dissociated leptomeningeal cells revealed that non-neuronal cells expressing truncated trkB bind BDNF with high affinity and selectivity. In addition, experiments with dissociated leptomeningeal cells showed that BDNF binding is rapidly followed by its internalisation. Finally, the cytoplasmic sequence of chick truncated trkB is highly homogous to the mammalian, thus suggesting a common signal transducing pathway. The yeast interaction trap/2-hybrid system is currently being used to detect possible interactors of truncated trkB. Taken together, our results suggest that truncated trkB forms an efficient and selective barrier preventing the diffusion of BDNF and eliminating it by internalisation. This barrier might be necessitated by the multiplicity of developing structures producing BDNF, and by the large number of diverse neuronal populations responding to it.

422.4

EXPRESSION OF CNTF RECEPTOR α IN NORMAL, DENERVATED, AND DYSTROPHIC HUMAN SKELETAL MUSCLE. ***J. Weis*[1], D. C. Lie[1], P. C. Heinrich[1], H.-G. Joost[1], G. D. Yancopoulos[2], J. M. Schröder[1]. Technical University[1], Aachen, Germany, and Regeneron Pharmaceuticals[2], Tarrytown, NY.***

Ciliary Neurotrophic Factor (CNTF) is a trophic factor for a variety of cell types including motor neurons and a candidate molecule for the treatment of human motor neuron diseases. Within the tripartite CNTF receptor complex, CNTFRα is the component specific for CNTF. CNTFRα can be released from the cell surface; soluble CNTFRα is still bioactive. CNTFRα is crucial for the effects of CNTF. However, its expression pattern in normal, denervated and dystrophic human muscle is unknown.

In muscle biopsies from 5 normal subjects, 12 cases of neurogenic muscular atrophy, and 7 cases of Duchenne muscular dystrophy, CNTFRα mRNA levels were determined by Northern blotting. Transcript levels were markedly increased in denervated muscle compared to controls and dystrophic muscle.

These results suggest that the expression of CNTFRα is regulated by innervation. This regulation appears to be selective, because CNTFRα mRNA was not increased in dystrophic human muscle. Increased CNTFRα could confer higher sensitivity to CNTF on denervated muscle fibers and other cell types involved in nerve fiber regeneration.

422.5

REGULATION OF CEREBELLAR GRANULE CELL DEVELOPMENT BY BDNF AND DEPOLARISATION. E. T. Coffey*, K. E. O. Åkerman and M. J. Courtney. Dept. of Biochemistry and Pharmacy Åbo Akademi University, Turku, Finland.

Cerebellar granule cells in serum-based culture are a much exploited system for the study of neuronal development. They are particularly suited for studying the intracellular signalling pathways underlying neuronal development as they can be prepared in large quantities; however, this aspect has been little studied. Their survival is known to be regulated by elevation of intracellular calcium levels by, for example, depolarising culture conditions. We are investigating the regulation of neurotrophic factors and depolarisation on neuronal development and the signalling cascades involved. Development is measured at the level of (i) cell survival and (ii) expression of neurite and synaptic proteins. Cell survival is measured by cell permeant and impermeant DNA dyes, and expression of proteins such as synaptophysin, β-tubulin, tau, GAP-43 and c-Fos and tyrosine phosphorylation levels are measured by immunoblotting.

We have observed that death of cells grown in non-depolarising conditions, which is reported to be apoptotic, is not prevented by the presence of BDNF, NGF, or NT-3; glutamate-evoked neuronal death, however, is reduced by BDNF. Moreover, BDNF increases synaptophysin expression. We have also observed that BDNF evokes rapid tyrosine phosphorylation of a number of proteins and elevated expression of the immediate early gene, c-Fos. The basal level of expression of c-Fos in these cells appears to be developmentally regulated, peaking at 3 days in vitro; This peak is further increased by the presence of BDNF.

We conclude that BDNF modulates neuronal development in a specific manner, affecting the levels of a component of synaptic machinery and certain forms of cell death, but not apoptotic cell death.

422.6

REGULATION OF CILIARY NEUROTROPHIC FACTOR RECEPTORS ON DIFFERENTIATING NEUROBLASTOMA CELLS. R. Malek* and S.W. Halvorsen. *Dept. of Biochem. Pharmacol. State Univ. of New York at Buffalo, Buffalo, NY 14260.*

The cytokine, CNTF, is a putative injury response/target derived trophic factor for motor, CNS, sensory and autonomic neurons. The SH-SY5Y neuroblastoma cell line serves as an *in vitro* model for studying the regulation of CNTF receptor expression during neuronal differentiation. We used retinoic acid, 12-O-tetradecanoyl-phorbol-13-acetate (TPA) or cAMP to differentiate SH-SY5Y cells. We found differences between retinoic acid and TPA induced differentiation manifested in the regulation of ^{125}I-CNTF binding whereas cAMP treatment had no effect on CNTF binding. Retinoic acid caused a two-fold increase in CNTF binding after 24 hours while TPA induced a transient 30% decrease in CNTF binding after 3-5 hours that recovered to control levels after 7 hours. In addition, the retinoic acid induced increase in CNTF binding could be prevented by either cycloheximide or actinomyin D, whereas neither inhibitor prevented the TPA induced decrease in CNTF binding. However, the PKC inhibitor, bisindoylmaleimide, prevented the TPA induced decrease in CNTF binding, consistent with PKC involvement in regulating CNTF binding levels. These results suggest retinoic acid and TPA regulate CNTF binding by different mechanisms. We are further analyzing retinoic acid and TPA effects to determine if the molecular mechanisms involve changes in expression of the receptor subunits, CNTFRα, LIFRβ and gp130 at either the protein or mRNA level.

422.7

TRK B ACTIVATION IN SCIATIC NERVE DETECTED WITH PHOSPHORYLATION STATE-SENSITIVE ANTIBODIES TO SH2 RECOGNITION SITES. A. Bhattacharyya*, R.A. Segal, L. Rua, K.S. Thress, S.L. Pomeroy and C.D. Stiles. Dept. Micro. & Molec. Genetics, Harvard Medical School and Dana-Farber Cancer Institute; Dept. Neurology, Harvard Medical School, Beth Israel Hospital and Children's Hospital, Boston MA 02115.

Using synthetic tyrosine phosphopeptides as immunogens, we generated a panel of antibodies that report the phosphorylation state of four tyrosines common to the cytoplasmic domain of the high affinity neurotrophin receptors, Trks A, B and C. The targeted tyrosines are known autophosphorylation sites for Trk A *in vitro*. Two of them (corresponding to Y674/675 in Trk A) are within the catalytic domain. The other two (Y490 and Y785 in Trk A) flank that domain and, in the phosphorylated state, serve as recognition sites for SHC and PLC-gamma.

These positionally specific antiphosphotyrosine antibodies allow us to ask fundamental questions about Trk signaling in primary neuronal cell cultures and complex nervous tissue such as sciatic nerve that cannot be resolved using conventional antibodies and labeling protocols. By immunoprecipitating normal sciatic nerve extracts from adult rats with anti-Y490, we can detect Trk B phosphorylated at the SHC recognition site. When BDNF is injected into the hindleg muscle of these rats, we detect an increase in Trk B phosphorylation at the SHC recognition site. In nerves that are cut prior to BDNF injection, the Trk B phosphorylation is decreased, verifying that the Trk B activation is due to signals coming through the nerve.

422.8

SIGNAL TRANSDUCTION OF TRKB IN PC12 CELLS. Y. Iwasaki and S. Koizumi*. Bio-organics Res. Dept., International Research Laboratories, CIBA-GEIGY (Japan) Ltd., Takarazuka 665, Japan.

A cell line expressing mouse *trkB* was established from PC12 cells to investigate the signal transduction of TrkB, a receptor of brain-derived neurotrophic factor (BDNF). The TrkB/PC12 cells responded to BDNF as well as nerve growth factor (NGF) by neurite outgrowth. However, the BDNF-induced morphological changes of TrkB/PC12 cells were markedly different from that induced by NGF. Cells treated with BDNF for 48 hr exhibited intensive somatic hypertrophy with longer and thicker neurites compared with NGF-treated cells at the same time point. This is not due to the overexpression of TrkB because the extent of BDNF-induced TrkB autophosphorylation was almost the same as NGF-induced TrkA autophosphorylation in this cell line. The tyrosine phosphorylation of signal proteins such as PI3 kinase, PLCγ, Shc and MAP kinase was stimulated by both BDNF and NGF treatments. However, a 38 kDa tyrosine-phosphorylated protein which was co-immunoprecipitated with TrkA after NGF treatment of the cells was not associated with TrkB stimulated by BDNF. These data suggest that the intracellular signals from TrkB are not completely the same as TrkA, even though they share the most of known signal transduction pathways.

422.9

NATURALLY-OCCURRING TRUNCATED TRKB RECEPTORS HAVE DOMINANT INHIBITORY EFFECTS ON BDNF SIGNALLING. F.F. Eide*, B.L. Eide, K. Zhang, X.-Y. Wang and L.F. Reichardt. Dept. of Physiology, U. of California, San Francisco, San Francisco, CA 94143.

A Xenopus oocyte microinjection assay was used to study the signalling properties and interactions of gp145trkB, and its naturally-occurring kinase-deficient isoforms, trkB.T1 and trkB.T2. When either trkB.T1 or trkB.T2 were co-expressed with gp145trkB, they inhibited BDNF-induced 45Calcium efflux responses. Quantitative tyrosine phosphorylation studies revealed that the truncated isoforms inhibited the BDNF signal by forming non-functional heterodimers with full-length receptors. An ATP binding mutant of gp145trkB had similar dominant inhibitory effects.

Our data suggest that naturally-occurring truncated trkB receptors function as inhibitory modulators of neurotrophin responsiveness. Furthermore, the homodimerization of gp145trkB appeared to be an essential step in the activation of the BDNF signalling cascade.

422.10

Functional importance of the amino termini of neurotrophins. Gene Burton*, Charles Schmelzer, Mike Sadic, Franz Hefti, James Treanor Department of Neuroscience, Department of Recovery Process, Bioanalytical Technology, Genentech, 640 Pt San Bruno Blvd, S.San Francisco CA 94080

The neurotrophin family consists of a group of highly conserved small basic proteins, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT4/5). These molecules are critical for the development and maintenance of a wide range of neuronal populations in both the peripheral and central nervous system. The proteins exert their biological effects through interactions with two types of cell surface receptor, a family of selective tyrosine kinase (trk) receptors and a promiscuous 75kDa receptor (p75). Physical and mutagenesis studies have identified regions of NGF and NT-3 important in their receptor interactions.

This study attempts to investigate the role of the amino terminus by analyzing a battery of proteolytically cleaved or heterodimeric neurotrophin molecules. Neurotrophins contain a conserved proteolytic cleavage site several residues from the NH_2-terminus. NH2-terminally truncated NGF (Δ5-NGF and Δ9-NGF), BDNF (Δ6-BDNF), NT-3 (Δ5-NT3 and Δ7-NT3) and NT-4/5 (Δ10-NT4/5) were generated. Heterodimers of NGF and Δ9-NGF, NT4/5 and Δ10-NT4/5 and Δ9-NGF and NT4/5 were also made. These hybrid molecules were analyzed and compared to wild-type homodimeric neurotrophins in their interactions with both their cognate trk receptor and the p75 receptor. Phosphorylation of trk receptors by these molecules were also analyzed using a novel kinase-immunosorbant receptor assay (KIRA). The results suggest that the amino terminus of all neurotrophins is a critical binding domain for tyrosine kinase receptor but not p75 receptor interactions. The presence of a single intact amino terminus within the confines of the native dimer is sufficient to endow the hybrid molecule with almost wild type trk receptor affinity and signalling capability.

422.11

Identification and characterization of receptor recognition sites of ciliary neurotrophic factor (CNTF)

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Human ciliary neurotrophic factor (hCNTF) was subjected to tertiary structure modeling and site-directed mutagenesis to analyze its structure-function relationship. Cell survival activities and receptor binding abilities of hCNTF mutants, constructed from the PCR mutagenesis, were determined in an assay system of chick DRG neuron and human neuroblastoma cells (SH-SY5Y), respectively. The substitution of D1 cap region (around the boundary between the CD loop and helix D) of hCNTF, including both E153 and K155, resulted in drastic change of cell survival activity[1] but not of receptor binding ability reflecting low-affinity binding. K155 could not permit any substitutions for cell survival activity, and some E153 mutants had increased biological activity. On the other hand, the substitution of Q63 to R resulted in increment of both cell survival activity[2] and low-affinity receptor binding ability. That is, "siteI" around Q63 residue and "siteII" around the D1 cap region participates in low-affinity receptor binding and high-affinity receptor binding, respectively. Whereas, V170 in helix D also participates in receptor binding for its hydrophobic character. Many regions of hCNTF are responsible for receptor binding. Those were grouped and expected to be the binding sites correspond to respective receptor components.

1. Inoue, M. *et al.*, submitted.
2. Panayotatos, N. *et al.*, (1993) *J.B.C.*, **268**, 19000-19003.

422.12

EFFECT OF p140^{trkA} SUPPRESSION ON NGF AND BDNF RESCUE OF APOPTOTIC PC12 CELLS. G. Taglialatela*, K. Werrbach-Perez, R. Perez-Polo. Dept of HBC&G, Univ. of Texas Med. Branch at Galveston, TX 77550.

Programmed cell death, the intrinsic form of apoptosis, plays an integral role in those neurodegenerative events associated with age-related neuropathology. Neurotrophins (NT) such as nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), and NT-3 are required for survival of certain CNS neurons, although mechanistic descriptions for NT cell rescue from apoptosis are not definitive. Here we isolated the individual actions of high-affinity tyrosine kinase (Trk) receptors and p75^{NGFR}, the common low-affinity NT receptor, in NT rescue of apoptotic PC12 cells. Our results showed that the inhibition of Trk receptor phosphorylation abolishes NGF rescue of serum-deprived PC12 cells from apoptosis, but that TrkA suppression with antisense oligonucleotides did not. Also, while BDNF did not rescue naive serumless PC12 cells which lack the BDNF-specific trkB receptor, it significantly increased survival of TrkA-suppressed serum-starved PC12. These data support the hypothesis that while binding of any neurotrophin to Trk-free p75^{NGFR} bearing PC12 cells blocks apoptosis, reducing cognate ligand binding or Trk phosphorylation in Trk-bearing PC12 cells inhibits cell rescue. Supported by the AFAR and by NIH grant NS18708. This is publication no. 34A of the PPG AG10514 awarded by NIA.

422.13

IDENTIFICATION OF FUNCTIONAL CILIARY NEUROTROPHIC FACTOR RECEPTORS ON CHICK CILIARY GANGLION NEURONS. S. Koshlukova*, T. Finn, R. Nishi and S. W. Halvorsen. Dept. Biochem. Pharmacol., SUNY at Buffalo, Buffalo, NY 14260 and Dept. of Cell Biol. and Anat., OHSU, Portland, OR 97201.

Embryonic ciliary ganglion neurons in culture are the fundamental system used to define the ciliary neurotrophic factor (CNTF)/growth promoting activity (GPA) family of factors, yet despite extensive efforts, their receptors have not been detected nor have their biological mechanism(s) been elucidated on these cells. We have examined the interaction of CNTF with its receptors by performing binding studies on ciliary ganglion neurons and have begun to characterize the intracellular pathways activated. ^{125}I-rat CNTF binding indicate that rat CNTF and avian GPA bind to neurons with a single affinity, while human CNTF recognizes the same sites with both a high and a low affinity. Comparison of the relative potency in biological assays indicates that GPA is 3-5 times more active than human CNTF in promoting survival and in regulating acetylcholine receptors. The binding of CNTF is specific, sensitive to phosphatidylinositol-specific phospholipase C (PI-PLC) and partially inhibited by leukemia inhibitory factor but not by other members of the cytokine/neurokine family including interleukin-6, interleukin-11 and oncostatin M. Cross-linking of ^{125}I-rat CNTF to ciliary neurons results in the specific labeling of three proteins with estimated molecular masses of 153, 81 and 72 kDa. The 81 kDa component was released from the cells after treatment with PI-PLC, suggesting a membrane attachment via a glycosylphosphatidylinositol linkage. Stimulation with CNTF or GPA for 5 minutes results in the tyrosine phosphorylation of a 90 kDa protein. Consistent with the binding and cross-linking data the phosphorylation is inhibited by pretreatment with PI-PLC and is not stimulated by the other cytokines. In conclusion, we report here pharmacological and functional properties of CNTF receptors on embryonic ciliary ganglion neurons. Our results provide the means for using chick ciliary ganglia for elaborating the molecular mechanisms of CNTF/GPA action and understanding their physiological role in a defined neuronal population.

422.14

EFFECTS OF GM1 GANGLIOSIDE ON Trk B RECEPTOR EXPRESSION IN MPTP-TREATED MICE. Carl Chiang* and J.S. Schneider Depts. of Anat. and Neurobiol. and Neurology, MCP and Hahnemann University, Philadelphia, PA 19102.

While previous work in our laboratory has shown that GM1 ganglioside treatment promotes biochemical and behavioral recovery in MPTP models of Parkinson's disease, the mechanism through which GM1 exerts its effect is unknown. One hypothesis suggests that GM1 may influence the nigrostriatal dopamine (DA) system indirectly through regulation of neurotrophic factors and/or their receptors. Specifically, brain-derived neurotrophic factor (BDNF) has been shown to increase the survival of DA neurons in vitro, both alone and in concert with GM1. In the present study, we used in situ hybridization (^{35}S-labeled cRNA probe) to examine GM1 effects on the BDNF receptor, Trk B, in the mouse model of MPTP-induced parkinsonism. Mice were divided into three groups: MPTP (20 mg/kg s.c., 4x/day, 1 day) and subsequent GM1 (30 mg/kg i.p., 1x/day, 7 days), MPTP/saline, and untreated controls. Our preliminary data shows that, compared to controls, Trk B mRNA is almost absent in cells of the dorsomedial and ventromedial caudate and downregulated in the medially adjacent septal nucleus of MPTP/saline mice. In contrast, MPTP/GM1 mice showed an upregulation of Trk B mRNA in these three areas compared to control, indicating that GM1 may upregulate Trk B expression above normal levels after MPTP lesions. Interestingly, sparse labeling for Trk B was seen in the substantia nigra pars compacta (SNc) of normal and lesioned mice, unlike data reporting high levels of expression in normal rat SNc. It is possible that GM1 effects on Trk B expression in the striatum may relate to BDNF rescue of DA neurons and/or normalization of striatal neuronal function as a consequence of GM1 treatment. Further studies are ongoing to elucidate the nature of GM1's influence on BDNF and its receptor and the nigrostriatal DA system. Supported by NIH grant AG 10280.

422.15

DEVELOPMENTAL EXPRESSION AND DOWNREGULATION OF THE α COMPONENT OF CHICK CNTF RECEPTOR IN DENERVATED SKELETAL MUSCLE. F. C. F. Ip, A. K. Y. Fu, K. W. K. Tsim and N. Y. Ip*. Department of Biology, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong.

A full length cDNA clone encoding for the chick CNTFRα (362 amino acids) was isolated by screening an embryonic day 13 chick brain cDNA library with a rat CNTFRα probe. The designation of this clone as chick CNTFRα was based on its relatively high homology in amino acid sequence (~70%) with the rat and human CNTFRα. While the carboxyl terminus of the chick CNTFRα exhibits the least degree of identity as compared to mammalian CNTFRα, the chick CNTFRα also lacks the cytoplasmic domain. The expression of chick CNTFRα was examined in various tissues at different developmental stages of the chick by Northern blot analysis. A single transcript encoding for the chick CNTFRα was detected in neural tissues such as brain and spinal cord, and in the skeletal muscle of the periphery. The level of the chick CNTFRα transcript in these tissues was found to be developmentally regulated. In addition, the expression of the chick CNTFRα in skeletal muscle was dramatically decreased by ~10-fold at 36 hours after denervation. Downregulation of the chick CNTFRα transcript was observed in different types of leg muscle examined, albeit with different time course. This is in sharp contrast to the result previously obtained with CNTFRα in denervated rat muscle.

422.16

INCREASED EXPRESSION OF *trk*B mRNA IN RAT CAUDATE-PUTAMEN FOLLOWING 6-OHDA LESIONS OF THE NIGROSTRIATAL PATHWAY. S. Numan* and K.B. Seroogy. Department of Anatomy & Neurobiology, University of Kentucky, Lexington, KY 40536.

The tyrosine kinase receptors *trk*B and *trk*C are high affinity receptors for members of the neurotrophin family, including brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), respectively. Both neurotrophin receptor mRNAs are broadly distributed throughout the caudate-putamen. Loss of dopaminergic nigrostriatal afferents has been shown to alter the expression of peptides, neurotransmitter-synthesizing enzymes, and receptors in striatal neurons. To determine if expression of *trk*B and *trk*C mRNAs is also regulated by the integrity of dopaminergic afferents, adult rats received a unilateral injection of 6-hydroxydopamine (6-OHDA), a selective catecholamine neurotoxin, or vehicle into the ascending medial forebrain bundle. Following a two-week survival period, *in situ* hybridization with ^{35}S-labeled cRNA probes for *trk*B and *trk*C mRNAs was performed in striatal sections. A significant increase in the hybridization density for *trk*B mRNA was observed in the caudate-putamen ipsilateral to the 6-OHDA injection, as compared to the uninjected control side ($P<.001$). In contrast, no alteration in the hybridization density for *trk*C mRNA was observed in the striatum of 6-OHDA-treated rats. Furthermore, no alterations in *trk*B or *trk*C mRNA levels were observed in the striatum of vehicle-treated animals. These data suggest that midbrain dopaminergic afferents selectively regulate the expression of *trk*B mRNA in the caudate-putamen. Alternatively, since BDNF is expressed by midbrain dopamine neurons, the increased expression of *trk*B mRNA in the striatum may reflect a compensatory response of striatonigral projection neurons due to a loss of target-derived trophic support. Supported by NIMH fellowship MH10806 (S.N.) and the National Parkinson Foundation (K.B.S.).

422.17

INDUCTION OF FULL-LENGTH TrkB AND TrkC EXPRESSION IN THE ADULT SPINAL CORD. Connor, H., Palm, K., Neuman, T., Nornes, H.O*. Department of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523.

The neurotrophic factor system is downregulated in the adult nervous system in comparison to its expression during development. In regions of low plasticity, such as in the spinal cord, a truncated form of TrkB is expressed, and TrkC is expressed at low levels. Since lack of functional receptors for neurotrophic factors could contribute to lack of regeneration of the adult CNS, we studied the possibility of inducing TrkB and TrkC in the adult spinal cord. Analysis of promoter sequences of BDNF and TrkB and transient CAT assays demonstrated that these genes have response elements to retinoids, cyclic AMP and elevated levels of Ca2+. All these response elements function in different types of neuronal cells to stimulate expression of BDNF and TrkB genes. Based on this molecular analysis, we treated the adult spinal cord with the combination of all-trans retinoic acid (10 µM), dibutyryl cAMP (2mM) and KCl (50mM) using intraspinal cannulation at a flow rate of 5 µl/hr. Eight hours posttreatment, RNAse protection analysis shows a 10x increase in full length TrkB and a 35x increase in TrkC expression. Experiments are in progress to determine if the induction of the neurotrophic factor receptors promotes survival and regeneration of lesioned neurons.(Supported by the Spinal Cord Society).

422.18

SITE DIRECTED MUTAGENESIS OF THE TRKB RECEPTOR TYROSINE KINASE. Joseph H. McCarty and Stuart C. Feinstein*. Neuroscience Research Institute, University of California, Santa Barbara, CA 93106.

The trkB protein tyrosine kinase is a high affinity receptor for the neurotrophins BDNF and NT-4/5. Neurotrophin binding to the trkB extracellular region leads to activation of the receptor's intracellular tyrosine kinase domain, resulting in the rapid and transient autophosphorylation of specific tyrosine residues. This activation initates a series of poorly understood cytoplasmic and nuclear signal transduction events, which lead to differentiative or proliferative responses, depending on the cellular context.

In order to assess the functional roles of known autophosphorylation sites, we have constructed a panel of tyrosine to phenylalanine substitutions within the intracellular region of trkB. Specifically, substitutions were introduced at the single juxtamembrane phosphorylation site (Y484F), within the proposed "regulatory region" of the kinase domain (Y670/674/675F), and at the lone carboxy terminal tyrosine residue (Y785F). Transfectants stably expressing each of these mutant constructs have been generated. The functional consequences of each point mutation on specific gene inductions will be presented. Supported by American Heart Association #94-421.

422.19

BONE MORPHOGENETIC PROTEIN-2 AND RETINOIC ACID SYNERGISTICALLY INDUCE TrkC RECEPTOR IN CULTURED SYMPATHETIC NEURONS. I. Matsuoka*, M. Kobayashi, and K. Kurihara. Faculty of Pharm. Sci. Hokkaido Univ. Sapporo 060 Japan

Sympathetic neurons undergo a developmental switch in trophic factor dependence from NT-3 to NGF by the sequential expression of trkC and trkA receptors. Recently, it was reported that both NT-3 and NGF promote the switching of neurotrophin dependence. However, the mechanism controlling the expression of trkC receptor at the initial developmental stage has not been clarified. Our previous work indicated that retinoic acid (RA) induces functional trkB receptor while it suppresses the trkA receptor expression in cultured sympathetic neurons isolated from superior cervical ganglia (SCG) of newborn rats. In the present study, by using RT-PCR, we examined further the effects of RA and members of TGFβ super family on the expression of trk receptors of the SCG neurons in culture. Treatment of the SCG neurons with BMP-2 (bone morphogenetic protein-2, 5 ng/ml) in the presence of NGF for 6 days induced the expression of trkC-mRNA without affecting the trkA-mRNA level. RA (100 nM) and activin A (5 ng/ml) had smaller effects on the induction of trkC-mRNA, while bFGF and TGFβ had no effect. RA enhanced the effect of BMP-2 on the trkC-mRNA induction 6-fold, while BMP-2 enhanced the RA-suppression of the trkA-mRNA level. Induction of functional trkC receptor was confirmed by the increase in the number of neurons surviving on NT-3 from 3 % to 64 % of the original population after the BMP-2/RA-pretreatment. These results suggest that BMP and RA *in vivo* synergistically induce the NT-3-dependence of developing sympathetic neurons.

422.20

DISTINCT BIOCHEMICAL PROPERTIES AND SURVIVAL EFFECTS BDNF AND NT-3 ON CULTURED CEREBELLAR GRANULE CELLS T. Nonomura, K. Shimoke, T. Oka, M. Yamada, T. Kubo and H. Hatanaka*. Institute for Protein Research, Osaka Univ., 3-2 Yamadaoka, Suita, Osaka 565, Japan.

Brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), interact with receptor tyrosine kinases, TrkB and TrkC, leading to various intracellular signaling events and the trophic actions. We investigated the signaling pathways exerted by BDNF and NT-3 in relation to their survival promoting effects on dissociated cultures of cerebellar granule cells prepared from postnatal 9-day-old (P9) rats. The granule neurons in culture were strongly supported for their survival by BDNF, but not significantly by NGF nor NT-3. BDNF and NT-3 treatments resulted in not only respective autophosphorylation of TrkB and TrkC receptors but also tyrosine phosphorylation of SHC, a protein involved in controlling p21^{ras} activity, and p42 and p44 proteins of mitogen-activated protein kinases (MAPKs). NT-3 induced the tyrosine phosphorylation of these proteins to a lesser extent than BDNF did, which might reflect the difference of expression level between *trk*B and *trk*C. We also observed transient induction of c-*fos* mRNA by BDNF and NT-3. However, NT-3 did not absolutely induce the tyrosine phosphorylation of phospholipase Cγ (PLCγ), while BDNF promoted the phosphorylation of PLCγ.

The characteristics of signaling and survival effects of NT-3 on the granule neurons indicate a correlation with biochemical properties of insert-containing forms of TrkC (TrkC(ki)) when expressed in cell lines. Our present studies may provide the first evidence for the inhibitory function of TrkC(ki) that was demonstrated in primary CNS neurons.

422.21

CROSS-TALK BETWEEN THE PTPase SH-PTP2 AND TrkB. B.A. Goldsmith*, N. Okada and S. Koizumi. Bio-organics Res.Dept., Int'l Research Laboratories, Ciba Geigy (Japan) Ltd., Takarazuka 665, Japan.

Increasing evidence suggests that protein tyrosine phosphatases (PTPases) play important roles in regulating signal transduction by growth factor receptors. One class of PTPases contains *Src* homology 2 domains (SH2) which may target these PTPases to activated growth factor receptors. Such localization may result in specific dephosphorylation of receptors or associated tyrosine-phosphorylated proteins. Localization may also result in tyrosine phosphorylation of PTPases by activated receptors, thus modulating PTPase activity. The SH2-containing PTPase SH-PTP2 has been found to associate with activated growth factor receptors and is tyrosine phosphorylated by receptor activation. However, interaction of SH-PTP2 with the Trk receptor family has not been characterized.

We have examined the interaction between SH-PTP2 and TrkB by utilizing an expressed intracellular kinase domain of TrkB (ICD-TrkB) and a GST fusion protein of SH-PTP2. We found that autophosphorylated ICD-TrkB (pICD-TrkB) associates *in vitro* with the SH-PTP2 fusion protein. In the presence of ATP and Mg^{2+}, SH-PTP2 is rapidly phosphorylated on tyrosine residues by pICD-TrkB. In the absence of ATP and Mg^{2+}, pICD-TrkB is rapidly dephosphorylated by SH-PTP2. Additionally, SH-PTP2 phosphorylated by pICD-TrkB can undergo autodephosphorylation to reverse tyrosine phosphorylation. Our results demonstrate that TrkB effectively phosphorylates SH-PTP2 and that pICD-TrkB is a target of SH-PTP2 activity. Phosphorylation of SH-PTP2 may modulate the ability of SH-PTP2 to dephosphorylate or associate with its targets. Our evidence suggests that cross talk between TrkB and SH-PTP2 may play a role in modulating signal transduction by TrkB.

HORMONES AND DEVELOPMENT II

423.1

NO SUPPRESSION OF GROWTH HORMONE PULSATILITY IN EXERCISING PREGNANT HAMSTERS. S. Yeo, K. Borer*, G. Beherent, and D. Mauger The University of Michigan, Ann Arbor, MI 48109-0482

In human pregnancy, pulsatile secretion of pituitary growth hormone (GH) ceases by mid gestation due to progressive increase in tonic secretion of a placental growth hormone variant. This hormonal adaptation may interfere with phenomena induced by exercise or food restriction. Pituitary GH takes a critical role linked to these phenomena. Previously we reported that Golden Syrian hamsters expressed somatic growth due to increased exercise-induced GH secretion in the presence of abundant food. When food was restricted, expression of exercise-induced growth was not observed. To determine whether pregnancy in hamsters blocks pituitary GH secretion, forty-seven Golden Syrian female hamsters were assigned to eight groups differing in three variables: pregnancy, food availability, and exercise. Voluntary exercise started 10 days prior to mating and continued through gestation (about 15 days). Food was restricted in REST group by 10 % until mating, and by 20% through the remainders of gestation. Lighting was off at 14:00 h and on at 00:00 h. Hamsters were cannulated on day 13 of gestation and 24 hours blood sampling was performed on day 15. During the 24 h sampling, 20 µl of blood was collected every 15 min. GH concentrations were measured in the homologous RIA for hamster GH. Cluster analysis and ANOVA were applied to log-transformed GH data. We found that exercise increased average GH concentration in both pregnant and non-pregnant hamsters (p=0.08). Increase in GH pulsatility was expressed most strongly when exercising hamsters were fed ad libitum (p=0.0563) than when their food was restricted (REST) (p=0.8). Exercising hamsters indicated a higher increase (22.96 ng/ml) than sedentary animals(17.42 ng/ml) of GH concentration. We conclude that in the hamsters, pregnancy does not block exercise-induced increased in the GH pulsatility. However unlimited food intake is necessary to induce GH increases. Thus secretion of a placental GH variant probably does not occur in pregnant hamsters. (Supported partially by the Office of Vice President for Research University of Michigan and NIH, 1K07NR00047-01A1).

423.2

EFFECTS OF PERINATAL HYPOTHYROIDISM ON GENE EXPRESSION OF CYTOCHROME C OXIDASE IN THE RAT CEREBELLUM. N. Koibuchi*, S. Matsuzaki, H. Ohtake and S. Yamaoka. Dept. of Physiology and Biochemistry, Dokkyo Univ. Sch. Med., Mibu, Tochigi 321-02, Japan.

Neonatal hypothyroidism has been known to dramatically affect neurogenesis of the rat cerebellum. To identify the genes whose expressions are changed in association with perinatal hypothyroidism, we have applied the differential plaque screening method using cDNA library of 15 day-old newborn rat cerebellum. Although we could not isolate novel gene, we cloned cytochrome c oxidase subunit I (COX I) gene, which is located in mitochondrial DNA. Northern blot analysis showed that the expression of COX I gene was decreased in the cerebellum of the rats, which had been rendered hypothyroid by administering 0.05 % methimazole in drinking water to their mothers. This decrease was restored by daily subcutaneous administration of thyroxine (2 µg/100 g body weight). Then we have applied quantitative *in situ* hybridization using ^{35}S-labeled RNA probe for COX I mRNA to examine the effect of perinatal hypothyroidism on COX I gene expression within each area of the cerebellar cortex. A marked change in COX I gene expression was observed not only in the external granule cell layer (EGL) and internal granule cell layer (IGL) but also in the molecular layer (ML). In the EGL of the control animals, a significant rise in COX I gene expression in 5 day-old rats and a gradual decrease in its expression in 10 and 15 day-old rats were observed. Such marked change was not observed in the hypothyroid rats. In the ML of control animals, hybridization signals were concentrated within the lower half, whereas in the hypothyroid animals, the signals were distributed evenly. In the IGL of the hypothyroid rats, a marked decrease in COX I gene expression within the Purkinje cells was observed. So far, because thyroid hormone treatment does not alter oxygen consumption of brain slices, oxidative phosphorylation in the central nervous system is thought to be unresponsive to thyroid hormone. However, the results of present study have demonstrated that thyroid hormone is involved in regulating COX I gene expression.

423.3

DEVELOPMENTAL CONSEQUENCES OF *IN UTERO* EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD): COMPARISONS WITH THE THYROID HORMONE INHIBITING AGENT PROPYLTHIOURACIL (PTU) AND THE ALKYLATING AGENT BUSULFAN (BU) IN THE LONG-EVANS RAT. D.B. Miller*, J.P. O'Callaghan and L.E. Gray. U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Recent work (Gray et al., Toxicol. Appl. Pharmacol. 131, 1995; Mably et al., Toxicol. Appl. Pharmacol. 114,1992) indicates marked reproductive abnormalities are produced in offspring of dams exposed to TCDD on gestational day (GD) 8 or 15 but there is little information on the consequences of these treatment regimens for brain development. To determine the impact of GD15 exposure to TCDD on brain development, offspring of TCDD dams were compared to offspring of dams treated with agents known to compromise development of the CNS by interfering with the actions of thyroid hormone (PTU) or by direct cytotoxicity to dividing cells (BU). Long-Evans dams received TCDD (1ug/kg) on GD-8 or 15, BU (5 or 10 mg/kg) on GD15 or PTU (0.8 or 8.8 mg/kg) in the drinking water from GD-15 through PND21. All 3 agents reduced body size. BU and PTU produced major reductions in brain size. TCDD produced minor decreases in brain size. Measurement of GFAP, the astrocyte-localized protein was used to evaluate the ontogeny of astrocytes as well as determine if these compounds would produce reactive gliosis, a reaction indicative of injury. GFAP changes were evident in offspring of dams treated with PTU and BU but not TCDD. Delayed synaptogenesis, as measured by the synapse-localized protein, synaptophysin, was evident in PTU offspring. Alterations in general motor activity were found with BU but not PTU or TCDD. Decreased cognitive ability, as measured by performance in the Morris water maze, was found with PTU or BU but not TCDD. Gestational exposure to TCDD does not appear to alter brain development in a manner similar to the changes produced by alterations in thyroid hormone or cytotoxicity to dividing cells.

423.4

COMPARISON OF THYROTROPIN-RELEASING HORMONE (TRH) RECEPTOR KINETICS IN THE ADULT AND DEVELOPING AMPHIBIAN BRAIN. C.L. Mitchell* and S.K. Boyd. Dept. of Biological Sciences, Univ. of Notre Dame, Notre Dame, IN 46556.

Quantitative receptor autoradiography was used to characterize binding sites for thyrotropin-releasing hormone (TRH) in the brain of adult and developing *Xenopus laevis*. Frozen brain sections (18µ) from adult males, and from representative stages of premetamorphic, prometamorphic and climax tadpoles, were incubated with ^{3}H-MeTRH (5nM) to determine total binding. Alternate sections were incubated with addition of 2000-fold excess unlabelled TRH for non-specific binding. Sections were exposed to film for 8 to 12 months then developed and quantified using Image 1. Kinetic studies of hypothalamic sites showed that binding of ^{3}H-MeTRH was specific, saturable and reversible at all developmental stages tested. Equilibrium dissociation constants (K_D) were similar at all stages, suggesting that the same receptor is present through development, but association (K_{+1}) and dissociation (K_{-1}) rate constants differed between adults and tadpoles. Compared to adult receptors, TRH dissociation in all stages of tadpoles was significantly faster, and association significantly slower, suggesting some primary difference in receptor binding during development. Most notably, binding was differentially temperature-dependent; in the adult hypothalamus, maximal specific binding at 4°C exceeded that at 23°C by two-fold, while in tadpoles, maximal specific binding at 4°C was reduced to 50 to 70% of that at 23°C. This altered binding may reflect a role for TRH in regulation of rate of metamorphosis, since low temperature significantly delays development.

423.5

LOCAL DEGENERATION WITHIN MUSCLE DEO1 TRIGGERS LOCAL ENDPLATE LOSS AND AXON RETRACTION IN THE MOTH, *MANDUCA SEXTA*.
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During metamorphosis in the moth, *Manduca sexta*, the larval abdominal body-wall muscle DEO1 is remodeled to form the adult muscle DE5. The motoneuron innervating DEO1 prunes its axon arbor over most of the degenerating muscle, while maintaining two contact sites. It is from these sites that axon branches sprout over the developing adult muscle DE5 (Truman and Reiss, 1995).

Using ligation between thorax and abdomen to remove the prothoracic glands, the source of endogenous ecdysteroids, we have shown that muscle degeneration requires the pre-pupal peak of ecdysteroids. In ligated animals, local application of the ecdysteroid analog RH 5992 results in a gradient of degeneration within the muscle. This local degeneration results in endplate loss and branch retraction from the affected region. We conclude that a cue released by the degenerating muscle triggers local pruning of the axon.

423.6

LONG TERM EFFECT OF INCREASE OF MATERNAL CORTICOSTERONE DURING LACTATION ON HIPPOCAMPAL CORTICOSTEROID RECEPTORS, STRESS RESPONSE AND LEARNING IN THE OFFSPRING.
A. Catalani, P. Casolini, G. Cigliana, F.R. Patacchioli, M.R. Domenici, S. Sagratella, S. Scaccianoce, S. Kent*† and L. Angelucci, Farmacologia 2, Università "La Sapienza" Medical Faculty, Rome 00185, Italy. † INSERM U.394, 33077 Bordeaux, France.

There is a frequent association between a deregulation of glucocorticoid (GC) secretion and affective disorders in humans, cognitive impairment during ageing and self drug administration in animal research. As GCs are an important link between events occurring during the early stages of life and maturation of CNS, we investigated the influence of the increase (4.3±0.5 to 9.5±.8 µg/100ml) of maternal corticosterone (B) during lactation on the hippocampal corticosteroid receptors, hypothalamo-pituitary-adrenal axis (HPAA) activity and behavior in rat progeny from 11 days to 1 year of life. We added the hormone (200µg/ml) to the dams' drinking water, thus avoiding any interference with the mother-pups relationship. The results were: a) lower stress-induced B secretion at 12 months, but not at 11 and 16 days; b) increase of hippocampal type I receptors at 1 and 15 months, but not at 11 and 16 days; c) better learning ability at 12 months in active avoidance, but not at 16 days in the water maze; d) changes in the expression of in vitro long-term potentiation at 2 months. Our results: i) indicate that the effects of the increase of maternal B is apparent only after weaning; ii) suggest that the increase of corticosteroid receptors may at least in part, be responsible for the lower stress response and the better learning ability of the progeny; and iii) confirm our previous data on stress response and learning observed at 1-3 months and extend them up to one year of life. It is tempting to find similarities between the endocrine and cognitive profile of our controls and some characteristics of HPAA and learning observable in certain human psychoaffective disorders. Our results suggest that the lack of arousal in infancy may have a negative influence in shaping some characteristics of the CNS of the future individual. Supported by CNR 94.00479.PF40 grant.

423.7

POLYCLONAL ANTIBODY TO THE RAT GLUCOCORTICOID RECEPTOR EXPRESSED IN *E. COLI* USING GST GENE FUSION SYSTEM
N. Morita*, M. Morimoto and M. Kawata, Dept. Anat. and Neurobiol., Kyoto Pref. Univ. Med., Kyoto 602, Japan

To study the development and differentiation of glucocorticoid receptor-immunoreactive cells in the nervous system, we made trials to produce a specific polyclonal antibody to the glucocorticoid receptor, which would be used for highly sensitive and multiple histochemical observation.

The rat liver glucocorticoid receptor cDNA fragment, which encodes 173 amino acids in the transcription modulation domain of the receptor protein, was inserted to the pGEX vector to express a rat glucocorticoid receptor-GST fusion protein in *E. coli*. After the purification of the fusion protein, the receptor protein domain was cleaved out. By immunization with the purified protein, a polyclonal antibody was raised in a rabbit. In immunoblot analysis of rat liver and brain homogenates, a single band of Mr 97000, the size deduced from the rat glucocorticoid receptor, was detected by this antibody. Moreover, the antibody clearly revealed the localization of immunoreactive neurons and glial cells in specific regions of the rat CNS with having low background.

These results demonstrate that the polyclonal antibody obtained in this study has high specificity and immunoreactivity to the glucocorticoid receptor, and that it would be particularly useful for our neuroanatomical pursuits.

423.8

DISTRIBUTION OF MRNA AND PROTEIN OF GLUCOCORTICOID RECEPTOR IN RAT CENTRAL NERVOUS SYSTEM.
M. Morimoto, K. Yokoyama, D.T.M. Visser, N. Morita and M. Kawata*, Dept. of Anat. and Neurobiol., Kyoto Prefec. Univ. Med., Kyoto 602, JAPAN.

Glucocorticoids play an important role in the development and the differentiation of the nervous system. The effects of glucocorticoids are mainly mediated by the glucocorticoid receptor (GR), which is distributed in various regions of the central nervous system. In this study, the cellular localization of GR mRNA and GR immunoreactivity in the adult rat brain was examined by *in situ* hybridization and immunohistochemistry. In the hybridization study, fluorescein-labeled RNA probes, complementary to about 500 base pairs coding the 5' untranslated region and a part of the transcription modulation domain in the rat GR cDNA were used. In immunohistochemistry, polyclonal antibodies against the transcription modulation domain of rat GR were used. Those have been obtained from rabbits, immunized with the GR protein produced by a gene fusion vector.

The expression of GR mRNA was detected in the cytoplasm and GR immunoreactivity was predominantly localized in the nucleus. GR mRNA-expressing and GR-immunoreactive cells were observed in various regions of the rat brain, especially in the tel- and diencephalon and the cerebellar cortex. The distributional pattern of GR mRNA in many regions of the rat brain was correlated with the distribution of GR immunoreactivity but in certain areas different localization could be found.

423.9

A NOVEL MEMBER OF THE STEROID HORMONE RECEPTOR FAMILY IS REGULATED BY SYNAPTIC ACTIVITY AND IN THE DEVELOPING CENTRAL NERVOUS SYSTEM.
K.I. Andreasson*, A. Lanahan, P. Brakeman, C.A.Barnes and P. F. Worley, Depts of Neurology and Neuroscience, Johns Hopkins Univ. Sch. of Medicine, Baltimore, MD 21205 and Depts. of Neurology, Psychology and Div. of Neural Systems, Univ. of Arizona, Tuscon, AZ 84724.

We have utilized differential cDNA cloning to identify genes regulated by activity in the rat hippocampus. One of the cDNAs isolated has an open reading frame that encodes a predicted protein which shows homology to the steroid receptor transcription factors. Northern blotting, analysis of cDNA clones, and PCR analysis reveal that alternative processing of the RNA transcript results in the presence of at least two forms of the mRNA in the hippocampus following stimulation. The mRNA for IEG59 is regulated by a variety of natural stimuli in developing and adult rats. Four hours following a maximal electroconvulsive seizure expression is induced by Northern analysis and *in situ* hybridization in the dentate gyrus. IEG59 mRNA is induced in NMDA dependent synaptic stimulation. In chronically implanted animals, IEG59 activin mRNA was strongly induced in hippocampal granule cells ipsilateral to high frequency LTP stimulus four hours after treatment. This induction was eliminated with pretreatment of the animals with MK-801. During fetal and post natal development, IEG59 is expressed in discrete patterns in developing cortex and spinal cord. Supported by M206826, MH53608, AG9219, and M442187.

423.10

EFFECT OF PROGESTERONE AND TESTOSTERONE METABOLITES ON GFAP GENE EXPRESSION IN TYPE 1 ASTROCYTES.
R.C. Melcangi°, M.A. Riva§, F. Fumagalli^, V. Magnaghi°, G. Racagni^ and L. Martini°*, ° Dep. Endocrinology, Univ. of Milan; § Di.Bi.T., San Raffaele Hospital, Milan;^ Center of Neuropharmacology, Inst. of Pharmacol. Sci., Univ. of Milan, Italy.

Recent data obtained in this laboratory have indicated that in the central nervous system, the enzyme 5α-reductase which converts testosterone (T) and progesterone (P) into their respectives 5α-reduced metabolites, is preferentially localized in neurons, but is present also in type 1 astrocytes (Melcangi et al. Endocrinology, 132:1252-1259, 1993; Brain Res. 639:202-206, 1994). On the basis of these observations, it was deemed of interest to analyze whether the original hormones T and P, and their 5α-reduced metabolites dihydrotestosterone (DHT) and dihydroprogesterone (DHP) might exert some effects on the expression of the most typical astrocytic marker, i.e. the glial fibrillary acidic protein (GFAP). Cultures of rat type 1 astrocytes were exposed to the various steroids for 2, 6, and 24 hours, and the variations of GFAP mRNA were measured by Northern blot analysis. A significant elevation of GFAP mRNA levels was observed after exposure to either P or DHP; the effect of DHP appeared more promptly (at 2 h) than that of P (at 6 h), a result which suggests that the effect of P is linked to its conversion into DHP; this hypothesis has been confirmed by showing that the addition of finasteride (a specific blocker of the 5α-reductase) is able to completely abolish the effect of P. After exposure to either progestagen, a decrease of GFAP gene expression was observed at later intervals (24 h). In the case of androgens, the data indicate that T does not change GFAP expression at any time of exposure, while DHT produced a significant decrease of GFAP mRNA only after 24 hours of exposure. The data suggest that beyond P, the 5α-reduced metabolites of T and P may modulate the expression of GFAP in type 1 astrocytes. (Supported by CNR through BTBS n. 93.01103.PF70 and ACRO n. 94.01162.PF39).

423.11

THE SEXUALLY DIMORPHIC BEHAVIOR OF SWEET SOLUTION CONSUMPTION IS MEDIATED BY TESTOSTERONE.
Pizzi*, W., Poskozim, J., Bellucci, C., Andrews, R. Northeastern Illinois University, Chicago, IL.

Two experiments were conducted to elucidate the hormonal mechanism controlling the sexually dimorphic behavior of sweet solution consumption. In exp. 1, cyproterone (cyp) 2 mg/kg, sc. in oil was administered to pregnant rats from gestation days 15-17. The offspring did not show differences in saccharin preference (SP) when tested prior to puberty. After puberty the vehicle control (VC) males showed a significant reduction in SP, while the cyp-exposed males did not show a reduction. The cyp-exposed females showed a dramatic increase in SP. A reproductive screen showed the cyp-exposed males to be impaired ($p<0.05$). A necropsy showed reduced testis ($p<0.02$) and seminal vesicle ($p<0.001$) weights. Exp. 2 was conducted on normal adult male rats treated with a single injection of cyp (5 mg/kg, sc), or female rats treated with tamoxifen (1 mg/kg, sc). Cyp-treated males showed a decreased SP over a 5-day period ($p<0.05$), while tamoxifen-treated females were unaffected. These results suggest that the sexually dimorphic behavior of sweet-solution consumption is mediated by testosterone.

423.12

THE GROSS SIZE OF THE SPLENIUM OF THE RAT CORPUS CALLOSUM : SEX DIFFERENCES, HORMONES AND POSSIBLE CORRELATES WITH AXON NUMBER
J. L.Nunez*, J. H.Y. Kim and J .M.Juraska Neuroscience Program and Department of Psychology, University of Illinois at Champaign, Urbana Champaign, IL 61820

Previously, we have not found significant sex differences in the gross size of the rat corpus callosum or the splenium (posterior 1/5) with n's of 8-10 per sex, but males have consistently had larger means than females. Here we found that by expanding our sample to 15-24 per sex, males have a significantly larger splenium and corpus callosum overall. Neither neonatal castration of the males nor neonatal testosterone injections of females altered the size of the splenium or the total corpus callosum. There were some preliminary indications that control manipulations for the hormone groups increased callosa in comparison to undisturbed litters. One must be cautious in interpreting measures of callosal size: in spite of males having a larger splenium, there are no sex differences in axon number when the splenium is thoroughly sampled (means favor females); nor does splenial size consistently correlate with the number of axons within it across age and sex.
Supported by NSF IBN 9310945 and NIGMS 1 F31 GM 17209-01

423.13

DEVELOPMENTAL PROFILE OF ANDROGEN RECEPTOR (AR) mRNA EXPRESSION IN THE FOREBRAIN OF PERINATAL RATS.
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Testosterone (T) is essential for the normal masculinization of the rat brain. The primary metabolite of T responsible for masculinization is estrogen (E), acting via estrogen receptors. However, AR antagonists partially disrupt sexual differentiation, suggesting that T and its androgenic metabolites exert additional masculinizing effects via ARs. To determine the sites and magnitude of sensitivity to androgens during sexual differentiation of the forebrain, we used in situ hybridization to investigate the development of AR mRNA expression in regions prominent in sexually differentiated functions. Perinatal male and female rats were sacrificed on embryonic day 20 (ED-20; ED-0=day of conception), and postnatal days 0, 4, 10 or 20 (PND; PND-0=day of birth). In situ hybridization was performed on brain sections using an ^{35}S-labeled riboprobe for rat AR mRNA. The lateral septum (LS), bed nucleus of the stria terminalis (BNST), medial preoptic area (MPOA), ventromedial (VMH) and arcuate (ARC) hypothalamic nuclei were analyzed. AR mRNA was present in all cell groups by ED-20. Relative amounts of ARmRNA followed the general gradient: caudal BNST>> MPOA> rostral BNST=LS=VMH. AR mRNA was not different on ED-20, PND-0 and PND-4 (although levels were slightly higher on PND-4). AR mRNA levels were significantly greater on PND-10 and PND-20 than at earlier ages. Furthermore, there was a sex difference at ages PND-4, 10, and 20, with males having higher levels of AR mRNA in the MPOA and BNST than females. This ontogenetic profile of AR mRNA expression parallels previous steroid binding studies (Lieberburg et al, Brain Res, 1980; Vito and Fox, Dev Brain Res, 1982). The presence of AR mRNA in these forebrain regions provides the requisite transcriptional machinery for T and its metabolites to play a role in sexual differentiation. Supported by NIMH grant MH48794.

423.14

REGIONAL LOCALIZATION OF 5α-REDUCTASE TYPE 1 mRNA IN THE DEVELOPING RAT BRAIN.
M.E. Lauber and W. Lichtensteiger*. Institute of Pharmacology, University of Zürich, Switzerland.

Steroid hormones are essential for normal development and function of the central nervous system (CNS). In the adult rat brain, androgens are involved in the activation of male typical behaviors and the regulation of food intake. In order to precisely elucidate the molecular basis of such androgen action and to examine potential effects of these hormones in CNS development, we studied 5α-reductase type 1 mRNA expression in fetal, postnatal and adult rat brain by means of *in situ* hybridization. Our results indicate that 5α-reductase mRNA is expressed in two distinct patterns depending on the developmental stage. In early postnatal (PN15) and older animals, 5α-reductase mRNA is detected predominantly in white matter structures, such as optic chiasm, lateral olfactory tract, corpus callosum and internal capsule. Specific mRNA is also detected in thalamic regions and low levels of mRNA appear to be distributed uniformly in the entire brain. These findings are in agreement with previous studies reporting 5α-reductase activity in many regions of the rat brain including myelinized structures. A completely different pattern of 5α-reductase mRNA expression was detected in the rat brain during fetal and early postnatal development (gestational day 14 to PN6). At these stages, high levels of 5α-reductase mRNA were present in the neuroepithelium of many regions, including cortex, basal telencephalon, retina and spinal cord. Abundant mRNA was also detected in the pituitary, nasal epithelium, liver and lens of the developing eye. These results suggest that 5α-reduction may serve different functions in the developing and adult nervous system.

423.15

FRONTAL CORTICAL BRAIN AROMATASE ENZYME ACTIVITY IN MALE AND FEMALE RATS DURING PERINATAL DEVELOPMENT.
E. D. Lephart and R. W. Rhees*. Dept. Zoology, Cellular Biology Division, Brigham Young Univ., Provo, UT 84602.

The conversion of androgens to estrogens is catalyzed by an enzyme complex termed aromatase. The presence of the aromatase enzyme in brain is thought to be responsible for the establishment of sexual dimorphic patterns in diencephalic and limbic structures during perinatal development. However, the presence and function of the aromatase enzyme in cortical tissues is not as well characterized. In this study, frontal cortical brain aromatase enzyme activity was measured in male and female Sprague-Dawley rats during perinatal development [from gestational day (GD) 17 to postnatal day 6]. In preliminary studies, brain aromatase activity was linear with time of incubation and protein content of the assayed tissue. The results from the 'tritiated water' release assay were confirmed by the estrogen product isolation assay. In both males and females the activity was highest (@ 320 fmol/hr/mg protein) at GD 17. In general, the aromatase levels declined in a linear fashion from GD 17 to GD 21 (@ 280 fmol/hr/mg protein) and continued to decrease by postnatal day 6. These results provide evidence for the presence of the aromatase enzyme in cortical tissue. Furthermore, the pattern and profile displayed in cortical tissue is different compared to hypothalamic aromatase activity which suggests that an alternative mechanism may regulate the aromatase enzyme in frontal cortical tissue during perinatal development.

423.16

SEX DIFFERENCE IN THE DISTRIBUTION OF ESTROGEN RECEPTOR AND AROMATASE IN THE RAT BRAIN.
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Distribution patterns of the neurons containing estrogen receptor (ER) and/or aromatase (AROM) immunoreactivity were studied in the newborn and adult rat brain by immunohistochemistry. In the newborn rat, AROM and ER were found in the anterior medial preoptic nucleus (AMPN), the dorso-caudal region of medial preoptic nucleus and its continuation to the bed nucleus of the stria terminals (MPNdc-BST), the ventro-lateral part of the ventromedial hypothalamic nucleus (VMHvl) and the medial amygdaloid nucleus (MEA). In the VMHvl, most of the AROM immunoreactive (-IR) cells contained ER-IR in their cell nuclei. In the adult brain, AROM-IR in MPNdc-BST and MEA stayed at high level, while that in AMPN and VMHvl was diminished. Moreover, in both newborn and adult, sex difference in AROM- and ER-IR was found in MPNdc-BST: intensity of AROM-IR, female < male; density of ER-IR, female > male. These results suggest that, (I) AMPN and VMHvl seem to be actively involved in the sex differentiation of the brain during the neonatal life, (II) In VMHvl, aromatization and binding of estrogen to its cognate receptor seem to occur in the same neurons, (III) Active aromatization and estrogen binding occur also in MPNdc-BST. This intimate relationship between AROM- and ER-IR in these regions is likely related to reproductive functions.

423.17

3β-HYDROXYSTEROID DEHYDROGENASE/Δ^5-Δ^4 ISOMERASE. A KEY ENZYME IN THE BIOSYNTHESIS OF NEUROSTEROIDS. IS EXPRESSED IN THE RAT NERVOUS SYSTEM. R. Guennoun*, M. Schumacher, M. Gouézou, F. Robert, K. Shazand, P. Robel, E-E. Baulieu. INSERM U33, 94276 Le Kremlin-Bicêtre Cedex, France.

The 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD) enzyme, can convert pregnenolone (PREG) into progesterone (PROG). We here show, by reverse transcriptase- polymerase chain reaction (RT-PCR), *in situ* hybridization and immunohistochemistry, for the first time, that sensory neurons of rat peripheral nervous system (PNS) express 3β-HSD. 3β-HSD is expressed in dorsal root ganglia (DRG) neurons during embryonic development and in adulthood. Sensory neurons cultures prepared from embryonic (E15) rat DRG can convert [^{3}H]PREG into [^{3}H]PROG. [^{3}H]PROG is further converted to its 5α-reduced metabolite 5α-pregnane-3,20-dione (5α-DH PROG). Cultures of sensory neurons on a microporous membrane on top of a feeder layer of Schwann cells results in three- fold increase of the [^{3}H]PREG→[^{3}H]PROG conversion. The identity of both [^{3}H]PROG and [^{3}H]DH-PROG was confirmed by high pressure liquid chromatography (HPLC) and crystallizations to constant specific activity after reverse isotopic dilution.

In situ hybridization showed that 3β-HSD mRNA is also expressed in neurons of defined regions of the central nervous system (CNS). RT-PCR amplification and nucleotide sequencing of the amplified products are in progress, to identify the isoform(s) of 3β-HSD expressed in the CNS.

In conclusion, our results give evidence of the CNS and PNS capacity to synthesize progesterone, an active neurosteroid, which may be involved in the control of myelin synthesis.

423.18

LOCALIZATION OF ESTROGEN RECEPTOR MRNA IN THE DEVELOPING BRAZILIAN OPOSSUM AND RAT BRAIN. M.C. Kuehl-Kovarik,* M.R. Bollnow,# R.J. Handa,# and C.D. Jacobson. Dept. of Veterinary Anatomy and Neuroscience Program, Iowa State University, Ames, IA 50011, and #Dept. Cell Biology, Neurobiology, and Anatomy, Loyola University, Chicago, IL 60153.

Estrogen receptor-like immunoreactivity (ER-LI) has been reported in the facial motor nucleus of rats from one to eleven days of postnatal age, but not in adults. We have not detected ER-LI in the facial motor nucleus of the Brazilian opossum at any age, although other receptor types are transiently expressed in this nucleus. In this study, we have used *in situ* hybridization to detect estrogen receptor mRNA in the developing rat and opossum brain. An ^{35}S-labeled cRNA probe against the rat estrogen receptor (nucleotides 1542-1713) was utilized to detect estrogen receptor mRNA in both rat and opossum brain slices. Estrogen receptor mRNA (ER mRNA) was not detected in the midbrain or hindbrain of the embryonic day 19 rat. However, by eight days postnatal, ER mRNA was abundant in the facial motor nucleus. By 25 PN, no ER mRNA was detected in the rat facial, although it was abundant in forebrain areas. Estrogen receptor mRNA was not detected in the 5 or 10 PN Brazilian opossum brain. By 25 PN, ER mRNA was detected in the opossum forebrain and midbrain. At 70 PN, ER mRNA was detected in multiple forebrain areas, including the lateral septum and medial preoptic area. However, no estrogen binding sites were detected in the opossum brainstem at any age studied. These results agree very well with what our laboratory and others have found using immunohistochemistry, demonstrating that our rat probe effectively recognizes opossum mRNA. The estrogen receptor mRNA and protein are expressed transiently in the facial motor nucleus of the neonatal rat. However, neither the mRNA or the protein are detected in the facial motor nucleus of the Brazilian opossum at any age we have studied so far. The significance of transient ER expression in the neonatal rat facial motor nucleus remains to be elucidated.

423.19

PRENATAL EXPOSURE TO ETHANOL DECREASES THE DEVELOPMENTAL EXPRESSION OF ESTROGEN RECEPTOR mRNA IN THE MEDIAL PREOPTIC AREA OF THE RAT. Y.B. Li*, L.L. DonCarlos, D. Stancik and R.J. Handa. Dept. of Cell Biology, Neurobiology and Anatomy, Loyola Univ. Chicago, Maywood, IL. 60153.

Fetal alcohol exposure disturbs the development of the hypothalamo-pituitary-ovarian axis and results in neuroendocrine disorders in adult female offspring. To determine if these neuroendocrine deficits arise as a result of changes in estrogen sensitivity, we examined the effect of fetal exposure to ethanol on the development of estrogen receptor (ER) mRNA in the rat medial preoptic area using in situ hybridization histochemistry. Timed pregnant dams were fed a liquid diet with 35% ethanol derived calories from gestational day 14 to parturition. Control animals were either pair fed an isocaloric diet with sucrose substituted for ethanol or maintained on regular laboratory chow. On postnatal days 0 (day of birth), 4, 10, or 21, animals were sacrificed by nembutal and intracardiac perfusion with 4% paraformaldehyde. Brains were cut on a cryostat. In situ hybridization was performed using a ^{35}S - labelled cRNA probe for rat ER mRNA. The levels of ER mRNA in the medial preoptic area were highest on postnatal day 0,4 and 10, and underwent a dramatic decline after postnatal day 10 to adult levels by postnatal day 21 ($p<0.05$). As previously observed, ER mRNA expression in the medial preoptic area was higher in females than males on postnatal day 0, 4 and 10 ($p<0.05$). ER mRNA levels in the medial preoptic area were reduced by prenatal alcohol exposure both in male and female rats on postnatal day 0, 4 and 10 ($p<0.05$). The reduced expression of ER mRNA in the preoptic area by prenatal alcohol exposure may underlie the observed neuroendocrine disorders which appear later in life in female rats. *Supported by USPHS AA08696, NSF IBN 94-08890 and MH 48794*

423.20

Identification of genes regulated by estrogen in adult forebrain neurons using PCR-differential display. F. Sohrabji* and R. C. Miranda. Department of Human Anatomy and Medical Neurobiology, Texas A&M University, College Station, TX 77843.

We are interested in the role of estrogen on the survival and maintenance of neurons in the basal forebrain cholinergic system and its forebrain targets. In adult animals, estrogen rapidly regulates the expression of BDNF mRNA in two forebrain targets, the cerebral cortex and olfactory bulb, suggesting that the expression of this neurotrophin gene may be an early event in an estrogen-stimulated cascade regulating cellular function. Since the estrogen receptor is a ligand-dependent transcription factor, the present study analyzed the expression of other novel downstream genes regulated by estrogen, using a PCR-based differential display technique. Cortical and olfactory bulb RNA was obtained from adult ovariectomized females exposed to either estradiol or vehicle and sacrificed 4 or 52 hours later. DNase-treated RNA was reverse transcribed and amplified using a modification of the previously-described technique (Liang and Pardee, 1992). While the majority of amplified transcripts were present in all groups, a doublet of approximately 1 kb and two smaller (450 and 300 bp) transcripts were regulated by estrogen. While the 1 kb transcript(s) had an early onset and stable expression in the olfactory bulb, its expression in the cerebral cortex was comparatively delayed. The smaller transcripts (450 bp in the cortex, 300 bp in the olfactory bulb) were expressed only in the group that received a 4 hour exposure to estrogen, and may represent genes that are transiently stimulated by estrogen. Characterization of these differentially-regulated genes may yield insight into the actions of estrogen, and also shed light on the downstream effects of estrogen-dependent stimulation of the neurotrophins.

NEURONAL DEATH V

424.1

IDENTIFICATION OF GENES DISPLAYING ALTERED EXPRESSION IN CEREBELLAR GRANULE NEURONS DURING APOPTOSIS. S.R. D'Mello[1] *, A. Yan[1], K. Borodezt[1], G. Rompato[1], and M. Cartwright[2]. Dept. of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06269 [1], and Section of Biomolecular Medicine, Boston University Medical Center, Boston, MA 02118 [2].

Cultured cerebellar granule neurons undergo apoptosis when switched from a medium containing high K+ (25 mM) to one containing a low K+ concentration (5 mM). Death resulting from the lowering of extracellular K+ can be prevented by IGF-I, cyclic AMP, lithium, and the calcium-ATPase inhibitor thapsigargin. Apoptosis can also be inhibited by transcriptional and translational inhibitors suggesting the necessity for newly synthesized gene in the death process. Work in other systems has suggested that in addition to "killer genes" that are induced during cell death, apoptosis results from the downregulation of "survival genes" that normally serve to suppress cell death. In an effort to identify genes involved in the induction or suppression of apoptosis in granule neurons we have used the PCR-based differential display technique. Total RNA from granule neurons switched to medium containing low K+, high K+, IGF-I, or forskolin (adenylate cyclase activator) was analyzed. Complementary cDNAs representing several mRNA were detected that showed altered expression in low K+ treated cells (primed to die), as compared to the cells maintained in one of the three survival promoting factors. That the expression of these mRNAs is altered during cell death is presently being confirmed by Northern blot analysis. Results concerning the characterization of these putative killer and survival genes will be presented (Supported by grants from the Whitehall Foundation and the A-T Children's Project).

424.2

DIFFERENTIAL DISPLAY INDICATES NOVEL ACTIONS OF PROSTAGLANDINS ON CENTRAL NEURONS. M. Cartwright[1,3], P. Isackson[4], S. D'Mello[5], and G. Heinrich*[2,3]. 1 Dept. of Pharmacol. and 2 Dept. of Med., Boston Univ. Med. Center, Boston, MA. 3 GRECC, VAH., Bedford, MA. 4 Mayo Clinic, Jacksonville, FL. 5 Dept. Neurobiol. and Physiol., UConn., Storrs, CT.

The predominant prostaglandins (PGs) synthesized in the brain are PGD_2, PGE_2, and $PGF_{2\alpha}$. The rate-limiting enzyme in PG synthesis is cyclooxygenase (COX). Using Northern and Western blot analyses, we showed that kainic acid upregulates COX2 mRNA and COX2 in central neurons. PGs act via G-protein linked seven transmembrane domain receptors. Three PGE_2 receptor subtypes have been cloned (EP1, 2, and 3). Four variants of the EP3 subtype (A through D) arise via differential splicing of EP3 transcripts. These EP3 variants are coupled to different G-proteins.

We used RT-PCR assays to show EP1 and EP3A and B mRNAs in intact rat cortex as well as cultured cortical and cerebellar neurons. The identity of each mRNA was confirmed by restriction digest or cloning followed by nucleotide sequence analysis of the PCR products. Using RT-PCR we could not detect EP2 mRNA nor the mRNA that encodes the $PGF_{2\alpha}$ receptor (FP) in the intact cortex or cultured neurons.

The expression of EP1 and EP3 mRNAs by neurons raised the possibility that PGE_2 may regulate transcription of neuronal genes. To address this possibility cultured cortical and cerebellar neurons were treated with 1 μM PGE_2 for 1h or 6h. Rats received intra-ventricular(iv) injections of PGE_1 and PGE_2 for 3h. Total RNA was extracted from cultured neurons, and rat hippocampus and cortex and analyzed by differential display. 14 of the visualized mRNAs differed between treated and untreated cells and animals. The corresponding cDNAs were cloned. None of 14 clones matched known nucleotide sequences. A clone downregulated by iv PGE_2, derived from rat cortex, hybridized to an abundant, 2.3 kb mRNA on Northern blots.

These results indicate that PGR mRNAs are expressed in neurons and that PGs regulate mRNA levels in neurons. Future experiments will study these regulated mRNAs to further understand the molecular mechanisms of PG action in the brain.

424.3

MULTIPLE GENES ARE REGULATED BY THE INDUCTION AND SUPPRESSION OF APOPTOSIS IN CEREBELLAR GRANULE NEURONS.

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Activity dependent modification of neural functions and neuronal differentiation are considered to be cellar bases of developmental plasticity. One of the activity dependent phenomenon during the neural network formation is the activity dependent regulation of survival and death of neuronal cells. The primary culture of cerebellar granule neuron provides an excellent model for studying the regulatory mechanisms of the activity dependent survival and death. In the absence of depolarization reagent(s) such as high concentration of KCl or NMDA in the culture medium, the granule neurons gradually undergoes to die with apoptosis within a few days. On the other hand, the addition of these reagents to the culture medium before 2.5 days *in vitro* (DIV.) suppresses the apoptosis of this neuron but does not suppress it if they are added after 3.0 DIV. We have been interested in gene regulation during this critical period of induction / supression of the apoptosis in this system. In order to search regulated genes during this critical period, we have employed mRNA differential display technique and found that multiple genes are regulated at this critical period of induction / suppression of the apoptosis.

424.4

ISOLATION OF BCL-Y, A NOVEL BCL-2 HOMOLOGUE, FROM RAT AND HUMAN BRAIN.

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The bcl-2/ced-9 gene family is composed of ten known genes which play a critical role in the regulation of cell survival. Some members of this family (such as bcl-2, ced-9 and bcl-x) are cell death blockers, while others (such as BAX and BAK) are cell death promoters. In order to understand better the molecular basis of cell death in the nervous system, we have used degenerate RT-PCR to search for additional members of this family in the brain. Using this approach, we have identified a novel bcl-2 homologue that is expressed in both rat and human brain.

Degenerate oligonucleotides were designed based on conserved sequences in bcl-2 homology domains 1 and 2 (BH1 and BH2). These oligos were used as primers in the PCR with rat brain cDNA and rat genomic DNA templates. PCR products of the predicted size were cloned and sequenced. Among these products were DNA fragments corresponding to bcl-2, bcl-x and BAX, all of which are known to be expressed in the nervous system. In addition, a novel sequence, with approximately 70% homology to bcl-2 and bcl-x at the amino acid level, was identified. This PCR product was designated bcl-y and was used to isolate full-length rat and human cDNA clones. Bcl-y is homologous to all known members of the bcl-2 family and is most similar to bcl-2 and bcl-x. Significant homology is observed throughout the length of the protein, but is most pronounced in the C-terminal half, particularly in BH1 and BH2. The C-terminal end of the protein contains residues predicted to form a mitochondrial membrane targeting sequence, suggesting that, like bcl-2 and bcl-x, bcl-y may be located at organelle membranes. We are in the process of constructing stable cell lines expressing bcl-y and producing polyclonal antibodies to the recombinant protein in order to establish the function and cellular distribution of this molecule.

424.5

NEUROLOGIC EFFECTS OF BCL-2 GENE ABLATION IN MICE.

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Bcl-2 and its homologs have been shown to play a key role in the control of cell survival, from *C. elegans* to man. Over-expression of bcl-2 has been shown to protect a number of different cell types from programmed cell death. Within the vertabrate nervous system, bcl-2 is widely expressed during the period of neurogenesis, and this expression continues within restricted neural populations postnatally. In order to better understand the properties of bcl-2 *in vivo*, we have examined the nervous system of mice which are homozygous for a null mutation in the bcl-2 (alpha/beta) gene. These animals proceed through development normally and all major organ systems are functional at birth. However, they are noticably smaller than their heterozygous littermates, and exhibit a substantially reduced lifespan.

With respect to the nervous system, bcl-2 -/- mice exhibit all major neuroanatomic structures. However, they exhibit a disproportionate reduction in the volume of their neocortex and cerebellum compared to heterozygous littermates. Preliminary analysis of the constituitively proliferating subependymal cells within the rostral forebrain indicate a reduction of this population. In addition, morphometric analysis of motor axons in these mice (L4 ventral root versus facial nerve) indicates that bcl-2 -/- animals undergo a dramatic reduction their population of gamma motor neurons, in addition to an overall reduction in motor axon caliber. In contrast, the L4 dorsal root and optic nerve of these animals do not show marked alterations. These results demonstrate that loss of bcl-2 activity has subtle, yet specific, functional consequences *in vivo*.

Work supported by the Rick Hansen, Man in Motion foundation.

424.6

TRANSCRIPTION FACTOR INTERACTIONS FOLLOWING STRIATAL INJURY.

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Acute neuronal injury is characterised both by the rapid induction of a range of immediate early genes (IEG) and by the concomitant activation of the glucocorticoid receptor (GR) by increased circulating steroids. In this study, we investigated the induction of IEG of the AP-1 family (c-Jun, Jun-B, Jun-D, c-Fos, Fos-B, Fra-1, Fra-2) in the rat striatum by quinolinic acid (QA) injection *in vivo* and the effects of GR activation or antagonism on the expression of these IEG.

Induction of c-Fos and Jun-B mRNA and protein is rapid and transient at two hours post-lesion and distributed around the medial and ventral margins of the striatum. A delayed, extended upregulation of c-Jun, Jun-D, and Fos-B message and protein occurs in the dorso-central portion of the striatum. We additionally show the expression of ΔFos-B, an alternatively spliced truncated form of Fos-B, which is the predominant isoform in the striatum. Results show Fra-1 and Fra-2 are constitutively expressed and never induced by striatal QA lesion. We also observe three previously unreported Fos-specific proteins which are also constitutively expressed. Data shown here illustrates that ΔFos-B is involved in an AP-1 complex that binds to a nerve growth factor AP-1 DNA binding site in increased levels following striatal QA lesion. Interestingly, co-injection of either the synthetic glucocorticoid dexamethasone or the GR-antagonist Ru38486 with QA does not significantly alter the temporal and spacial expression patterns or levels of Jun and Fos IEG. The ability of glucocorticoids to markedly enhance glutamate-induced neuronal death may therefore be due to direct protein-protein interactions between GR and AP-1, which may reduce the transcriptional activation capacity of AP-1, rather than have any direct effect on AP-1 expression.

This work is funded by the MRC and BBSRC

424.7

DEREGULATED INSULIN-LIKE GROWTH FACTOR-II EXPRESSION IN HUMAN NEUROBLASTOMA CELLS.

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Insulin-like growth factor-II (IGF-II) promotes autocrine growth in human neuroblastoma cells via the type I IGF receptor. SK-N-SH human neuroblastoma cells have been cloned into two distinct cell lines: SH-SY5Y cells and SHEP cells. Autonomous growth in SH-SY5Y cells is dependent on IGF-II mediated autocrine growth. In contrast, SHEP cells lack IGF-II gene and protein expression and require serum for growth. In the current study, we determined whether deregulated expression of IGF-II alters the phenotype of SHEP cells and permits autonomous neuronal growth.

An 854 bp IGF-II cDNA was inserted into pSSFV plasmids containing a single copy of IGF-II were selected by *sal*I digest prior to SHEP cell transfection with lipofectin. Neomycin resistant transfectants were selected and screened for IGF-II production by enhanced chemiluminescence Western blotting. Four stable transfectants expressed the 2.2 kb IGF-II mRNA transcript and immunoreactive IGF-II. Cell growth was measured after 1, 2 and 3 days using a colorimetric assay that detects reduction of the tetrazolium salt MTT. Unlike SH-SY5Y cells, IGF-II transfected SHEP cells remained dependent on serum for growth in spite of autocrine IGF-II production. These results imply that 1) IGF-II alone is insufficient to support autocrine neuronal growth, and 2) an additional permissive factor present in either serum or the extracellular matrix is required for growth.

This work was supported by R29 NS32843.

424.8

IN VIVO CELL MIXING EXPERIMENTS DEMONSTRATE THE CELL AUTONOMOUS ACTION OF THE weaver GENE ON CEREBELLAR GRANULE CELL SURVIVAL.

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The cellular event that defines the weaver mutation is the death of nearly all of the midline cerebellar granule cells. We are quantitatively re-examining adult chimeric cerebella and examining early postnatal chimeric cerebella (days 7 and 15) to determine if increased numbers of +/+ cells help genetically *wv* cells to survive. The genotype of IGL and EGL cells was determined using strain specific DNA probes and in situ hybridization. In adult weaver chimeric cerebella, there are regional differences in the numbers of granule cells in the internal granule layer (IGL) that strongly correlates with the percentage of chimerism in the animal. In these weaver chimeras IGL granule cells are always predominantly of the +/+ genotype indicating that the variation in granule cell number in different regions of a chimeric cerebellum is due to the variable donation of +/+ granule cells. Surviving weaver granule are present in numbers similar to that found in a weaver cerebellum. Thus, there is little enhanced survival of weaver granule cells in regions where many +/+ granule cells have colonized the IGL.

In postnatal day 7 and 15 weaver chimeras, the external granule layer (EGL) shows a regionally graded mixture of genotypically mutant and wild-type cells, from a preponderance of +/+ cells to a predominance of *wv/wv* cells to percentage mixtures in-between. In all instances, however, the vast majority of granule cells in the IGL is of the +/+ genotype with only a few surviving *wv* granule cells. Thus genotypically *wv* EGL cells do not successfully translocate to the IGL. In addition, an increased presence of pyknotic nuclei in the EGL, a characteristic of the weaver cerebellum, is found in the chimeric cerebellum.

We conclude that a wild-type environment does not promote increased survival of *wv* granule cells. Thus while other features of the wv granule cell may be ameliorated, such as axonal outgrowth (Gao & Hatten), the defining phenotype of the *wv* granule cell -- that of cell death -- is unaltered. The conclusion from these in vivo cell mixing studies is that the *wv* gene not only intrinsically targets granule cell survival, but this action is cell-autonomous. Supported by NINDS.

424.9

DIFFERENTIAL REGULATION OF BAX AND BCL-X EXPRESSION DURING DIFFERENTIATION OF PC12 CELLS. K. Vekrellis, L.L. Rubin* and J. Ham. Eisai London Research Laboratories, Bernard Katz Building, University College London, Gower Street, London WC1E 6BT, UK.

We are studying the pattern of expression of members of the Bcl-2 family during neuronal differentiation and neuronal cell death. Bcl-2 and Bcl-xL have been shown to suppress apoptosis in a variety of cell types including sympathetic neurons deprived of survival factors. Bcl-x, in particular, plays an important role in the nervous system in that disruption of the Bcl-x gene in mice by homologous recombination leads to extensive cell death in the developing nervous system. In contrast to Bcl-2 and Bcl-xL, the Bax protein antagonizes Bcl-2 and accelerates cell death. To study the pattern of expression of these proteins in the nervous system, we have developed antibodies that specifically recognize Bcl-x and Bax. As a simple model system for studying neuronal differentiation and survival we have used the rat PC12 cell line, which can be induced by nerve growth factor (NGF) treatment to differentiate into cells that resemble sympathetic neurons. NGF also promotes PC12 survival.

We have found in western blotting experiments that PC12 cells express Bcl-x and Bax, but not Bcl-2. We have also demonstrated in co-immunoprecipitation experiments that, like Bcl-2, Bcl-x also interacts with Bax. When PC12 cells are treated with NGF, Bax protein levels decrease by at least 5 fold, whereas Bcl-x levels are unaffected. Downregulation of Bax occurs within 8 hours of NGF treatment. Other agents which promote neurite formation and survival of PC12 cells, such as CPT-cAMP, also cause a specific decrease in Bax levels. In contrast, serum or insulin, which promote PC12 survival but do not cause differentiation, do not affect the level of Bax. These results suggest that Bax downregulation is associated with PC12 differentiation. We have also observed that Bax is preferentially downregulated during development of the brain and cerebellum. We are currently studying the molecular mechanism by which NGF causes Bax levels to decrease. We are also investigating the biological consequences of the change in the ratio of Bax to Bcl-x that occurs during PC12 differentiation.

424.10

BCL-2 SUPPRESSES APOPTOSIS AND ACTIVATION OF NF-KAPPA B INDUCED BY SINDBIS VIRUS INFECTION. K-I Lin, J.M.Baraban, J.M.Hardwick, M.Veliuona, B.Levine, R.Narayanan and R.R.Ratan* Johns Hopkins Univ., Baltimore, MD 21205

Infection of mice with Sindbis virus (SV) results in encephalitis and has thus been used to model human encephalitides due to alphaviruses. SV infection causes apoptosis in most cultured cell lines and primary neurons; overexpression of the human proto-oncogene bcl-2 suppresses SV-induced apoptosis. In previous studies, we have shown that NF-kappa B activation is necessary for SV-induced apoptosis in AT3 Neo prostate carcinoma cells. We now report that the activation of NF-kappa B is suppressed in AT3 Bcl-2 cells infected with SV as well as AT3 Neo cells infected with a recombinant SV carrying the *bcl-2* gene. These observations suggest that Bcl-2's anti-apoptotic action may derive from inhibition of an NF-kappa B signaling pathway. Current studies are underway to define the precise mechanism by which Bcl-2 inhibits SV-induced NF-kappa B activation.

424.11

GENERATION OF TRANSGENIC MICE OVEREXPRESSING BCL-2 OR BAX UNDER THE MOUSE CHOLINE ACETYLTRANSFERASE (ChAT) PROMOTER J.D.Cooper*, T.Michaelidis, G.Tzimagiorgis, D.Lindholm and H.Thoenen Department of Neurochemistry, Max-Planck-Institute for Psychiatry, 82152 Martinsried, Germany.

Although bcl-2 and bax appear to play a key role in respectively suppressing or enhancing the process of apoptotic cell death, the role of these molecules in the CNS remains unclear. To study the effect of overexpressing bcl-2 or bax in defined populations of neurons we have generated transgenic mice in which expression of these molecules is driven by the promoter region of the murine choline acetyltransferase (ChAT) gene. We have previously shown that a 4kb EcoRI/HindIII fragment of the 5' flanking sequence of the murine ChAT gene confers NGF-inducible expression of reporter genes in transiently transfected PC12 cells but not NIH 3T3 fibroblasts. This 4kb ChAT promoter fragment was linked to a murine bcl-2 mini-gene containing both coding exons of bcl-2. The same ChAT promoter fragment was also linked to a construct containing coding and endogenous intronic sequences of the mouse bax gene. Pronuclear injections of these constructs have generated several lines of ChAT-bcl-2 and ChAT-bax transgenic mice, some of which also express the reporter gene LacZ under the ChAT promoter. RT-PCR of F1 generation ChAT bcl-2 mice indicates that the region of the murine ChAT promoter used in our constructs is capable of driving transgenes within particular regions of the CNS. We are currently mapping the pattern of transgene and reporter gene expression to determine their specificity and the relative levels of transgenic vs. endogenous expression of bcl-2 or bax. ChAT-bcl-2 and ChAT-bax mice will subsequently be used in studies of naturally occurring developmental cell death and the assessment of the mechanism of cell death after neurotrophin withdrawal or neurotoxic insult in defined *in vitro* and *in vivo* manipulations.

424.12

POSTNATAL OVEREXPRESSION OF HU-BCL-2 GENE IN LURCHER MUTANT MICE PROTECTS INFERIOR OLIVARY NEURONS FROM TARGET-RELATED CELL DEATH. H. Zanjani*, J.C. Martinou, N. Delhaye-Bouchaud, and J. Mariani. Institut des Neurosciences, UPMC and CNRS, Paris, France and Glaxo Imb., Geneva, Switzerland.

It has been shown that the human proto-oncogene Bcl-2 can protect cells from apoptosis. Overexpression of Hu-Bcl-2 in transgenic mice protects both Purkinje cells (PC) and inferior olivary neurons (ION) from naturally occurring cell death (Zanjani et al., 1994). In the *lurcher* mutant, almost 100% of the PCs and 75% of the IONs die due to the intrinsic degeneration of the +/Lc PCs. To study whether Bcl-2 protects neurons from pathological death we introduced this gene in *lurcher* mutant mice by crossing NSEb-Hu-Bcl-2 transgenics with + + /*Lc* Mi^{wh} mutant mice. Hu-Bcl-2 expressing +/Lc offspring were identified by PCR for Hu-Bcl-2 gene expression and by the presence of a white coat spot indicating linkage to the *Lc* locus. +/Lc genotype was also confirmed histologically. We have analyzed four adult mice that overexpressed the Hu-Bcl-2 gene and are +/Lc in genotype. ION numbers were analyzed in serial coronal paraffin sections and the counts compared to ION numbers in Hu-Bcl-2 transgenic mice and +/Lc mutant mice. The number of IONs in the Hu-Bcl-2 overexpressing +/Lc mutants was similar to C57BL/6J wild type controls and increased 78% compared to +/Lc mutant mice. The number of rescued IONs was 15% lower than the number of IONs in Hu-Bcl-2 transgenic mice (t-test; $p < 0.02$). Hu-Bcl-2-+/Lc cerebella resemble +/Lc cerebella so postnatal Hu-Bcl-2 overexpression did not prevent the death of +/Lc Purkinje cells. We suggest that the genetic pathway for +/Lc PC death may be initiated prenatally as in Lc/Lc mice and/or is independent of Hu-bcl-2 gene action. The rescue of IONs in Hu-Bcl-2-+/Lc mice shows that bcl-2 gene overexpression can prevent target related cell death.

424.13

NUMERICAL MATCHING OF GRANULE CELLS WITH PURKINJE CELL TARGET SIZE IN TRANSGENICS OVEREXPRESSING THE BCL-2 ONCOGENE. M. W. Vogel*, H. S. Zanjani, J.C. Martinou, N. Delhaye-Bouchaud, and J. Mariani. MPRC, Univ. MD Med. School., Baltimore, MD 21228, Inst. des Neurosciences., UPMC and CNRS, Paris, France and Glaxo Imb., Geneva, Switzerland.

Naturally occurring cell death is an ubiquitous feature of neuronal development involving the matching of neuronal numbers with trophic factor support. The proto-oncogene Bcl-2 has been shown to protect neurons from programmed cell death by blocking apoptosis. Zanjani et al. (1994) have shown that Purkinje cell (PC) numbers are increased by 50% in transgenics overexpressing Hu-Bcl-2 embryonically, while normal PC numbers are found in Hu-Bcl-2 transgenics with postnatal overexpression. The Hu-Bcl-2 transgene is overexpressed in PCs, but not in granule cells (GC). To determine the effect of overexpression of Hu-Bcl-2 in target neurons and larger target size on the survival of afferent neurons, we have analyzed GC numbers in both the embryonic and postnatal expressing lines of Hu-Bcl-2 transgenic mice. GC numbers were counted in serial sagittal paraffin sections of the fixed transgenic and control brains that had previously been analyzed for PC numbers. The number of GCs is increased by 28% (t-test; $p<0.05$) in the transgenic line overexpressing Bcl-2 embryonically, while GC numbers are normal in the postnatally overexpressing line. The ratio of GCs to PCs in both lines is similar to controls. The results indicate that the increased target size in the early expressing line provides for increased numbers of granule cells, but overexpression of the Bcl-2 oncogene in target neurons alone does not rescue afferent neurons.

424.14

AXOTOMY-INDUCED MOTONEURON DEATH IN Cu\Zn SOD KNOCKOUT MICE. J.L. Elliott[1]*, J. Harding[1], A. Reaume[2], E.K. Hoffman[2], R. Scott[2], and W.D. Snider[1]. Dept. of Neurology[1], Washington University, St. Louis, Mo, 63110; Cephalon Inc[2]., N.J.

Missense mutations in the gene encoding Cu\Zn superoxide dismutase (SOD1) are responsible for one form of familial amyotrophic lateral sclerosis(FALS). Unclear however, is the mechanism by which abnormalities in SOD1 lead to motoneuron loss. Experiments using transgenic mice overexpressing the mutant human SOD1 provide support for a toxic gain of function model. An alternative hypothesis is that motoneuron survival is dependent on levels of SOD1 and that decreases in overall levels of SOD1 activity lead to motoneuron loss. Previously this hypothesis has been difficult to test in vivo, but we have now gene-targeted mice heterozygous and homozygous for deletions in the murine SOD1 gene.These mice have 50% and 0-5% of normal SOD1 activity respectively and are ideal for definitively addressing this hypothesis. At 2.5 months of age, gene-targeted mice heterozygous for a SOD1 appeared phenotypically normal. To examine the vulnerability of adult motoneurons in adult SOD1 deficient mice, a right facial axotomy was performed on female (+\-) knockout and age matched (+\+) controls. At 5 weeks post-axotomy, the degree of facial motoneuron loss in control and heterozygote mice was similar (37% in control vs. 42% in heterozygotes). At 10 weeks post axotomy there was a trend toward more significant loss of facial motoneurons in heterozygotes (68%) than control (59%) but the results did not achieve statistical significance. Interestingly the degree of facial motoneuron loss in control (+\+) mice following axotomy was considerably higher than has been previously observed in adult rats. Experiments to date suggest that gene-targeted mice with 50% of normal SOD1 activity do not exhibit increased motoneuron loss following axotomy compared to controls, at least during the time frame studied. The consequences of further lowering SOD1 activity on motoneuron survival are now being addressed with similar experiments in mice homozygous for the SOD1 knockout.

424.15

NEURONAL CELL DEATH IN WEAVER SUBSTANTIA NIGRA IS NON-APOPTOTIC. TF Oo[1], C Henchcliffe[1], SWM Harrison[2], SK Roffler-Tarlov[2], RE Burke[1]*. [1]Dept Neurology, Columbia University, NYC, NY; [2]Dept of Neuroscience and Dept of Anatomy & Cell Biology, Tufts University, Boston, Mass.

Weaver (wv/wv) is a murine mutation which results in the spontaneous loss of several cell populations during postnatal development including dopamine neurons in the substantia nigra (SN). The mechanism of cell death is unknown. We have shown in rat that developmental cell death with the morphology of apoptosis occurs in SN. We therefore sought to determine whether augmented apoptotic cell death may occur in wv/wv. We used silver staining to identify dying cells in wv/wv, wv/+ and +/+ mice at postnatal days (PNDs) 7, 14, 24/25 and adults. As in normal rats, natural cell death with the morphology of apoptosis was observed in SN in all mouse genotypes during postnatal development. However, we did not observe augmented apoptotic cell death in wv/wv. At PND 24/25 wv/wv = 4.4±0.9 (N=3), wv/+ = 3.2±0.5 (N=4), +/+ = 3.1±0.6 (N=4) apoptotic cells/SN section. Unique to the wv/wv genotype was the occurrence of non-apoptotic cell death in SN. Degenerating cells appeared neuronal, with an argyrophilic nucleus and cytoplasmic silver deposits. These cells appeared on PND 14, were most numerous on PNDs 24/25, and were very rare in adult animals. Rarely degenerating cells were observed in wv/+ mice on PND 24. Degenerating cells could also be identified by abnormal nuclear Nissl stain, but they were not TH-positive, possibly due to loss of phenotype expression. In situ 3'-end labeling of midbrain sections from wv/wv revealed no evidence for apoptotic cell death. In conclusion, the morphology of cell death in wv/wv SN is non-apoptotic and it remains to be determined whether it is necrotic or an alternate morphology of programmed cell death. NS26836, NS20181, PDF.

424.16

APOPTOTIC AND NON-APOPTOTIC CELL DEATH IN THE MOUSE MUTANT WEAVER. S.M.W. Harrison* and S. Roffler-Tarlov. Dept. of Neuroscience & Dept. of Anatomy and Cell Biology, Tufts Univ. Sch. of Med. Boston, MA 02111.

The postnatal development of at least four cell populations are affected by the murine autosomal recessive mutation *weaver*. Large numbers of dopamine-containing neurons in the midbrain, granule and Purkinje cells in the cerebellum, and male germ cells fail to complete terminal differentiation and many die. To better understand the consequences of the *weaver* mutation, we have begun to study the death of the affected cell populations.

The death of a cell can be generally categorized as apoptotic, non-apoptotic non-necrotic, or necrotic by morphological characteristics. One of the properties that most consistently defines an apoptotic cell death is the fragmentation of the cell's DNA into nucleosomal sized pieces. Apoptotic cells can be detected in tissue sections by *in situ* end-labeling (ISEL) of the free DNA ends generated by this type of fragmentation. We have used this technique to determine whether the cells vulnerable to the actions of *weaver* die by apoptosis. We have also examined the morphological details of weaver cell death using standard microscopic techniques.

Cell death in the brain of the homozygous weaver (*wv/wv*) takes place within the first few postnatal weeks and after postnatal day (P) 28 in the testis. We have been unable to detect apoptotic cells by ISEL in the midbrain of P14 *wv/wv* mice. ISEL has, however, revealed extensive apoptosis within the *wv/wv* granule cell population at P7 and P14. Germ cells in the *wv/wv* male appear to undergo both apoptotic and non-apoptotic, non-necrotic deaths as determined by ISEL, thin-section light microscopy, and EM. Necrosis was not observed in any of the affected cell populations. These results suggest that lack of the normal weaver gene product provides a signal that leads to apoptotic and non-apoptotic modes of cell death but not necrosis. The program of cell death followed may differ between cell types as well as within a single cell type. NIH NS 20181.

424.17

APOPTOSIS IN WEAVER AND LURCHER MUTANT MICE

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Few *in vivo* data are available on apoptosis in neurodegenerative disease and an appropriate animal model is lacking. Apoptotic programmed cell death in the developing nervous system is associated with a characteristic DNA fragmentation. We used in situ end labeling to study apoptosis in mutant weaver (wv) and lurcher (lc) mice. Lc is an autosomal dominant mutation that resembles adult dominant ataxia in its histological and clinical features; Purkinje cells degenerate from P6 onwards and affected animals show symptoms at about P12. Apoptosis occurred in Purkinje and granule cells of affected lc mice; labeled cells were also found in brain stem nuclei. Wv is an autosomal recessive mutation leading to cerebellar granule cell loss and - in homozygous animals - to impairment of the nigrostriatal system. A single litter of wv mice (heterozygous parents) at P9 segregates in two groups on the basis of the number of apoptotic granule cells (average number of positive cells per midsagittal 8µm section: 94±5 vs. 24±7). Purkinje cells appeared to be affected to a much lesser extent.

424.18

IDENTIFICATION OF GENES THAT CHANGE EXPRESSION IN DYING MOTONEURONS DEPRIVED OF TROPHIC SUPPORT.

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One population of neurons in which cell death has been extensively studied is spinal motoneurons in the developing chick spinal cord. The difficulty in studying molecular changes in this tissue, or any complex tissue displaying cell death, is that condemned cells are interspersed among viable ones. For these studies, we have used and characterized a primary tissue culture system that yields a pure population of motoneurons that survive in the presence of trophic support (muscle extract, MEx) and die when deprived of it. RNA was collected from motoneurons 16-18 hours after plating with or without MEx, a time when cells deprived of MEx are committed to die. After amplification of antisense RNA and conversion to double stranded cDNA, messages that are potentially up- or down-regulated in death-committed motoneurons were cloned using a PCR based subtractive hybridization paradigm. After the first round of subtraction several up-regulated messages have been identified. These appear to include a novel sequence, a "DEAD" box gene, and a Bcl-2 family member. Identification and characterization of the genes involved in motoneuron cell death will contribute to the understanding of normal developmental processes as well as the pathological cell death of motoneurons.

This work was supported by a grant from the Spinal Cord Research Foundation to CEM, NS20402 to RWO and GM40458 to LMS.

424.19

A MUTATION OF THE pRB-BINDING REGION OF SV40 T-ANTIGEN (Tag) ALTERS TARGETED PURKINJE CELL DEATH IN TRANSGENIC MICE

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We have shown previously that intact SV40 Tag and the T147 form of Tag with the pRB-binding region intact result in Purkinje cell (PC) death and ataxia when their expression is directed transgenically to murine PCs by the *pcp-2* promoter. To test whether other portions of the Tag molecule are sufficient to induce PC death, transgenic mice were made using the PVU-Tag mutation (Tag unable to bind pRB) with the *pcp-2* promoter. Six PVU lines have been generated, four of which develop ataxia by three weeks of life. These mice differ from our previous Tag transgenic lines in that the PVU mice have minimal loss of PCs at the onset of ataxia. There is some loss of PCs over time in most lines, but not as severe as seen in lines made with intact Tag or T147. Two PVU-expressing lines are not ataxic and have no PC loss. One of these nonataxic lines has a single copy of the transgene which has a 3'-end deletion which may affect the ability of the Tag to bind p53. The other nonataxic line has expression of Tag in only some PCs. Although there is loss of PCs in the ataxic PVU lines, it occurs late in the disease course and cannot account for the onset of ataxia, particularly when compared to the degree of PC loss necessary for ataxia in the other Tag transgenic lines. The PC loss appears to be apoptotic but differs from that in the other Tag-transgenic mice in that cell death is not preceded by DNA synthesis. In one ataxic PVU line, P03, there is a decrease in 2-deoxyglucose uptake in the cerebellar cortex and deep cerebellar nuclei after the onset of ataxia but prior to significant loss of PCs. There is evidence that there is a failure of maturation of the cerebellar cortex in the ataxic PVU-transgenic mice. We conclude that in transgenically targeted PCs, SV40 Tag without its pRB-binding activity can induce sublethal alterations in PC development/function sufficient to produce ataxia. In PVU mice onset of ataxia is independent of PC loss, while in Tag transgenic lines with intact pRB binding sites, ataxia occurs only after significant PC loss. (Supported by PO1 NS31318)

425.1

INTRACELLULAR SIGNALLING MECHANISMS MEDIATING MICROGLIAL MOTILITY INDUCED BY COMPLEMENT 5A (C5a) Ch. Nolte, Th. Möller, T. Walter, H. Kettenmann* Cellular Neurosciences, Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Str.10, D-13125 Berlin, FRG.

Microglial cells are considered as the resident macrophages of the central nervous system. They respond to most pathological events by rapid transformation from a quiescent into an activated phenotype being characterized by increased cytotoxicity and motile activity. To search for motility controlling factors, we tested the complement factor C5a by time lapse videomicroscopy and a computer-based motility assay. Upon stimulation with C5a microglial cells responded by transient motility enhancement as characterized by intense ruffling of the cytoplasmic membrane and spreading on the substrate. This process was driven by actin polymerisation since cytochalasin B was able to inhibit the C5a-induced motility. C5a-receptor activation induced the release of Ca^{2+} from IP3-sensitive intracellular stores as recorded by a Fluo-3 based imaging system. However, conditions mimicking intracellular Ca^{2+}-transients like incubation with thapsigargin or addition of the calcium ionophore A23187 were not able to induce the motility reaction in microglia suggesting that Ca^{2+}-changes are not necessary for, but merely associated with, microglial motility. Both Ca^{2+} transients and motility were sensitive to preincubation with pertussis toxin (PTX) suggesting that a PTX-sensitive G-protein is involved in intracellular signalling after C5a-receptor activation. However, the C5a-induced motility reaction was markedly attenuated by Wortmannin, an inhibitor of PtdIns 3-kinase (PI3-K), but the Ca^{2+}-transients were not affected by this treatment. PI-3K, activated by G-protein subunits, may therefore play a role in actin rearrangement via its products. These data imply that the signal transduction pathways for motility/chemotaxis and Ca^{2+}-regulated cellular responses, respectively, diverge downstream the C5a-receptor associated G-protein. Since complement factors are released at pathological sites, the PI3-K dependent signal cascade could serve to direct microglial cells to the lesioned or damaged brain areas.

425.2

SELECTIVE BINDING OF TERMINAL N-ACETYL-GALACTOSAMINE-SEPCIFIC LECTINS (SBA AND VVA) TO MICROGLIA IN EMBRYONIC MOUSE BRAIN. I. Nagata[1] and K. Ono[2]* [1]Dept. of Cell Biology, Tokyo Metropo. Inst. Neurosci., 2-6 Musashidai, Fuchu 183; [2] Dept. of Anatomy, Okayama Univ. Med. Sch., Shikadamachi, 2-5-1 Okayama 700, Japan

To obtain specific surface markers on immature glia, embryonic mouse brains were immersed in fixatives containing glutaraldehyde. Frozen sections were made and stained with various fluorescein-conjugated (F) lectins. F-SBA and -VVA (Vector, 1:100), both of which react to the terminal N-acetylgalactosamine, bound specifically to "amoeboid" and "ramified" types of cells presumed to be microglia. They were localized mainly in the subventricular zone and gray matter in the cerebral cortex, respectively, and emerged from ca. embryonic day 14, increased during the prenatal stage, decreased at the postnatal stage and disappeared at ca. postnatal day 10. In the EM study, these labeled cells showed features characteristic of microglia. Thus, such spatially and temporally defined bindings of SBA and VVA to microglia in embryonic brain may serve as good markers for elucidating the origin and differentiation of microglia.

425.3

MICROGLIA EXPRESS NEUROTROPHINS AND RESPOND TO BDNF AND NT-3. S. Elkabes*, E.M. DiCicco-Bloom and I.B. Black, Neurosci. Cell Biol., RWJMS/UMDNJ, Piscataway, NJ 08854

Although microglia-mediated cytotoxicity has been extensively investigated, little is known about the potential microglial role in neuronal and glial support. Characterization of trophin elaboration by microglia and identification of responsive populations may define novel functions. We now report that microglia/brain macrophages express neurotrophins of the NGF gene family *in vitro* and *in vivo*, suggesting that these cells promote development and normal function of neurons and glia. Moreover, neurotrophins appear to act on microglia themselves, promoting mitosis and survival *in vitro*.

Our observations indicated that microglia express neurotrophins in a region-specific manner and that within any region only subpopulations elaborate trophins. Using an antiserum specific for NT-3 with the microglial/macrophage marker OX-42, we identified double-labelled cells in microglial subpopulations of the cerebral cortex, globus pallidus and medulla; NT-3 was undetectable in OX-42 positive cells in the ependyma, external capsule, meninges and choroid plexus. *In situ* hybridization studies on purified microglial cultures suggested that neurotrophins are synthesized by microglia. They also confirmed that only subpopulations express the NGF and NT-3 genes, substantiating the notion of microglial heterogeneity.

PCR analysis indicated that trk tyrosine kinase receptor genes are expressed by microglia *in vitro*, implicating responsiveness to neurotrophins. Indeed, BDNF and NT-3 increased ^{3}H-thymidine incorporation two-fold in cultures grown in serum free medium suggesting stimulation of microglial mitosis. Moreover, in serum containing cultures, NT-3 increased total cell number, indicating synergism between factors present in serum and NT-3 to promote proliferation. NGF was ineffective. Our results indicate microglial expression of select neurotrophins which in turn differentially regulate microglial proliferation.

425.4

THREE-DIMENSIONAL MORPHOLOGY OF RESIDENTIAL AND REACTIVE MICROGLIA IMMUNOSTAINED BY ANTI-β-THYMOSIN ANTIBODY. K.Tohyama* , T. Morita, N.Sato[2], K.Sano, H.Yaginuma[1] and Y. Uchiyama[2], Dept. of Cell Biology and Neuroanatomy, Iwate Medical Univ. School of Medicine, Morioka, Iwate 020 Japan, 1)Dept. of Anatomy, Institute of Basic Medical Science, University of Tsukuba, Tsukuba , Ibaragi 305 Japan, and 2) Dept. of Anatomy, Osaka Univ. School of Medicine, Suita, Osaka 565 Japan.

We have studied the 3-dimensional morphology of residential and reactive microglia, in reconstructions of confocal laser images taken at 0.2-0.5μm intervals from 50-80μm-thick sections of adult rat spinal cord or facial nucleus, immunostained by anti-β-thymosin antibody (β-Th; Dev. Brain Res. 79:177-185,1994). In normal tissues, residential microglia showed a ramified morphology, with long, slender, immunostained processes, in both gray and white matter. Such morphological characteristics of β-Th-immunoreactive cells correspond with those of OX-42 immunopositive Hortega cells in rat CNS. Reactive microglia immunopositive for β-Th were enormously increased in number in the dorsal and ventral horns of ipsilateral L4-L5 spinal cord or in the ipsilateral facial nucleus following sciatic nerve or facial nerve transection. However, these peripheral nerve injury (PNI)-induced microglia did not show ameboid morphology; most continued to display the ramified morphology of residential microglia. These morphological characteristics of PNI-induced reactive microglial cells suggest that they possess non-phagocytic properties. These findings raise the question of the non-phagocytic role of microglial cells and also show that the β-Th antibody is a good marker for both residential and reactive microglial cells.

425.5

LIGHT MICROSCOPIC AND ULTRASTRUCTURAL DETECTION OF DNA FRAGMENTATION IN ACTIVATED MICROGLIA: *IN SITU* CELL DEATH AS A POSSIBLE MECHANISM FOR POPULATION CONTROL OF MICROGLIA

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Microglia are the main immuno effector cells of the CNS and it has been suggested that they play a vital role in the neuronal regeneration process. Shortly after injury, microglia begin to accumulate at the lesion site through *in situ* proliferation, as well as in the projection areas of the lesioned neurons. At the peak of their proliferation, approximately four days after lesion, the microglia population has increased four fold and many microglia cells occupy a distinct perineuronal position. However, within three weeks of this dominating presence, the microglial population tapers down once again to a resting state. Previous studies have suggested the possibility that the microglia may migrate away from the affected area and leave the CNS through blood vessels. Another possibility would be that the microglia population is regulated by programmed cell death. We investigated the possible role of programmed cell death in the down regulation of the microglial population.

Facial nerve axotomy or the injection of toxic ricin into the facial nerve was performed on adult Wistar rats, with survival periods from 1 to 120 days. Both coronal sections of the facial nucleus and primary cell culture from microglia were submitted to *in situ* terminal transferase labelling. DNA fragmentation labelling was observed both at the level of light microscopy as well as at the level of electron microscopy. These results were verified by the presence of DNA laddering observed through gel electrophoresis.

We demonstrate, through the use of *in situ* terminal transferase labelling, the specific delayed presence of DNA fragments in the facial nucleus after peripheral transection of the nerve. Our results show that microglia undergo DNA fragmentation and suggest programmed cell death as a mechanism to control activated microglia following injury.

425.6

MACROPHAGE RESPONSES FOLLOWING SCIATIC NERVE INJURY IN TRANSGENIC MICE DEFICIENT FOR IL-1 OR TNF- α RECEPTORS. AT Dailey[1]*, M Carpenter[2], AM Avellino[1], J Peschon[2], M Glaccum[2], M Kliot[1]. [1]Dept. Neurosurgery, University of Washington, Seattle WA 98115, [2]Immunex Corporation, Seattle WA

During Wallerian degeneration in the peripheral nervous system (PNS), there is a pattern of axonal degeneration followed by a monocyte/macrophage (MØ) response beginning on day 1 (Avellino et al, 1994, Griffin et al, 1993). Successful completion of degeneration and possibly regeneration are dependent upon the arrival of peripheral blood monocyte/MØ. The recruitment and activation of monocyte/MØ is controlled by growth factors and cytokines with pleiotropic affects. Previously, we have found differences in monocyte/ MØ response in the osteopetrotic mouse (deficient in macophage colony stimulating factor) during PNS Wallerian degeneration (Dailey et al, 1994). However, the precise mechanisms responsible for the MØ recruitment seen during Wallerian degeneration are not understood.

To further determine the role of specific cytokines in MØ responses during PNS Wallerian degeneration, we employed mice with targeted gene deletions of either the high affinity IL-1 receptor or the p60 and p80 components of the TNF-α receptor. MØ's were identified with the F 4/80 monoclonal antibody. Degenerating sciatic nerve segments were harvested at 3, 7, and 14 days following transection. No differences in MØ counts (expressed as cells / 0.1 mm^2) were observed in uncut nerves (control 13±1 , IL-1 11±1, TNF-α 10±1: p>.05) or 7 and 14 days (control 123±4, IL-1 118±5, TNF-α 115±6: p>.05) following transection. However, at 3 days following transection fewer MØ were observed in both the IL-1 and TNF-α receptor deficient mice as compared to the response in normal control animals (control 30±2, IL-1 22±2, TNF-α 20±2: p<.005).

In addition, sciatic crush was used as a model for regeneration in the three groups. Although axons successfully regenerated in the transgenic mice, there was a decrease in the number of cells staining with antibody to nerve growth factor (NGF) in the distal portions of regenerating nerves in the IL-1 receptor deficient animals. These findings suggest that these cytokines may play a role in cellular responses in axonal degeneration and regeneration. However, the demonstration of only a partial effect points to the importance of other cytokines and their receptors. Supported by a CIDA grant to MK and VA funding.

425.7

SYNERGETIC EFFECTS OF NEURON-DERIVED FACTORS AND COLONY-STIMULATING FACTOR 1 (CSF-1) ON MACROPHAGE PROLIFERATION A. Dobbertin and M Mallat*. INSERM U114, Paris, France.

Infiltration and proliferation of bone marrow-derived macrophages (BMM) in the CNS contribute to growth and reactions of microglia during development or following lesions. In vitro cultures were used to investigate influences of rat or mouse CNS cells on purified BMM derived from adult rodent bone marrow. Survival and proliferation of isolated BMM required addition of CSF-1 to culture medium. A coculture system allowing exposure of BMM to soluble compounds released by CNS-derived cells, indicated that neurons from different developing brain regions produce factors which dramatically enhance mitogenic effect of CSF-1. Using saturating concentration of CSF-1 (10ng/ml of human recombinant CSF-1), the presence of neurons led to a 2-fold increase of the number of BMM within 72h. In contrast, cultured astrocytes or microglia failed to enhance CSF-1 mitogenic effect. ^{3}H-thymidine labeling of synchronized BMM indicated that the neuronal stimulation involved shortening of the cell cycle rather than induction of proliferation of a BMM subpopulation unresponsive to CSF-1 alone. Moreover, neuron-derived factors failed to rescue BMM from death in the absence of exogenous CSF-1. Potentialisation of CSF-1 mitogenic effect by neurons was also observed using cultured ameboid microglia or peritoneal macrophages instead of BMM. Considering that astrocytes can produce CSF-1, our results suggest a cooperation between different CNS cell lineages in promoting microglial growth or reactions .

425.8

A QUANTITATIVE IMMUNOHISTOCHEMICAL DESCRIPTION OF THE MACROPHAGE RESPONSE DURING PERIPHERAL NERVE WALLERIAN DEGENERATION

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Following axotomy in the peripheral nervous system, blood-borne monocytes rapidly invade the site of injury and, after a delay, the distal Wallerian degenerating segment of nerve where they differentiate into activated macrophages that remove degenerating axonal and myelin debris (Friede & Beuche, 1993; Griffin et al, 1992; Perry et al, 1993; Stoll et al, 1989). The mechanisms responsible for this invasion remain incompletely understood.

We characterized quantitatively temporal and spatial aspects of the macrophage response following transection of the rat sciatic nerve. Immunohistochemistry was performed on longitudinal frozen sciatic nerve sections from 50 male Lewis rats sacrificed from 4 hours to 35 days after nerve transection using the commercially available ED-1, ED-2, and Ox-42 as well as the CD11a (ICOS Corp.) monoclonal antibodies. We observed the following: 1) ED-1 positive macrophages began to increase in number in the external epineurium at 4 hours and peaked at 7 days, before declining to baseline control levels at 14 days; 2) a statistically significant two-fold increase in the number of intraneural ED-1 positive macrophages was seen by 1 day in the distal Wallerian degenerating segment of nerve; 3) intraneural ED-1 positive macrophages increased in number synchronously along the length of degenerating nerve over a 21 day period; 4) the morphology of infiltrating intraneural ED-1 positive macrophages changed over time from small slender ramified cells to large elongated multi-vacuolated cells; 5) intraneural Ox-42 positive macrophages increased in number at 2 days and peaked at 14 days before leveling off; 6) intraneural ED-2 positive macrophages increased in number at 2 days and peaked at 14 days, before declining; and 7) intraneural CD11a positive macrophages increased in number at 2 days and peaked at 14 days, before declining.

Differences in the macrophage response as determined by different antibodies may reflect subpopulations and/or functional states of activation. Supported by NIH and VA funds as well as a CIDA grant to MK.

425.9

THE EFFECT OF PINEAL MICROGLIA ON CULTURED PINEALOCYTES

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Microglia play important roles during neural development and damage through expression of trophic factors and adhesion molecules (Piani, Constam et al. 1994). Earlier studies have shown that microglia comprise an important part of the pineal gland in the adult rat (Kelly, Fox et al. 1993; Pedersen, Fox et al. 1993). However, the effects of microglia on pinealocytes have not been studied. The presence of microglia in 1 day Sprague-Dawley (S/D) neonate was verified by DiI-acetylated-LDL uptaking cells in pineal cell suspension and OX-42 immunocytochemistry on pineal sections. In order to investigate the effects of microglia on pinealocyte development, microglia-depleted and microglia-enriched pineal cell cultures were generated from 1-day-old S/D neonate pineal cells by fluorescence activated cell sorting (FACS). Pineal cells ($1X10^5$ cells/coverslip) were plated in the following groups: 1) control pineal cells prepared before FACS, 2) microglia-depleted pineal cells, 3) microglia-enriched pineal cells and 4) combined culture of microglia-depleted and enriched pineal cells. After 7 days of culture (humidified 5% CO_2, 95% air, 37°C), tissues were fixed (4% paraformadehyde) and processed for S-Ag immunocytochemistry followed by morphometric analysis of pinealocyte neurite length. Our data indicate that microglia comprise 5-10% of the total cells in the pineal gland of the neonate. Pinealocytes neurite extension is enhanced in a microglia-depleted environment and inhibited in a microglia-enriched environment (Anova, $p<0.01$). The results of this study suggest that microglia play important roles in pinealocyte development.

425.10

CO-LOCALIZATION OF THE B2-CHAIN OF LAMININ AND AMYLOID PRECURSOR PROTEIN IN THE DEVELOPING AND ADULT CNS OF THE RAT

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The ECM-molecule laminin plays a role in brain development as well as in neurodegeneration and regeneration in the mature CNS. Laminin is increased in glial cells during Alzheimers disease and has binding sides for amyloid precursor protein (APP). Recently we have shown that a synthetic peptide derived from a neurite outgrowth-promoting domain on the B2-chain of laminin modulates the electrical activity of pyramidal cells in adult rat neocortex and increases the excitotoxic vulnerability of neurons. With antibodies against the B2-chain of laminin and APP we show co-localisation of both molecules in the CNS

Cryostat sections were fixed in p-benzochinone and double labelled with antibodies against the B2-chain of laminin or APP and GFAP. The sections were observed with confocal laser scanning microscopy.

Immunoreactivity against both the B2-chain of laminin and APP was co-localized on GFAP-positive radial glial fibers and on astrocytes in the developing cerebellum. Punctuated labelling of antibodies against both antigens could be seen on neurons and in the neuropil. Labelling for both antigens persisted in neurons and the neuropil of adult rats but was confined to perivascular astrocytes, Bergmann glial fibers and GFAP-positive astrocytes in the white matter of the cerebellum. In the corpus callosum and in a subpopulation of GFAP-positive astrocytes in the hippocampus of adult rats, GFAP positive astrocytes displayed immunoreactivity for the B2-chain of laminin but not for APP.

Our results suggest that the B2-chain of laminin and APP are co-localized and widely distributed in different patterns in developing and adult CNS. This may be relevant for the functional role of both molecules in the developing and adult CNS.

425.11

GLIAL CELL LINES DERIVED FROM MX-1-T-ANTIGEN TRANSGENIC MICE EXHIBIT INDUCIBLE ONCOGENE EXPRESSION. J. P. Hammang[1], C. R. Greco[1] W. C. Kisseberth[2], J. Y. Garbern[3] and A. Messing[2]*. 1) CytoTherapeutics, Inc., Providence, RI. 02906, and 2) University of Wisconsin-Madison, Madison, WI. 53706, 3) University of Pennsylvania, Philadelphia, PA. 19104.

The regulation of oncogene expression in neural cell lines offers a valuable mechanism for controlling cellular differentiation. In order to derive neural cell lines with regulatable oncogene expression, we produced a line of transgenic mice in which the mouse Mx-1 promoter directs the expression of the SV40 Large T-antigen. The Mx-1 promoter has been previously shown to be highly inducible by interferon (IF). EGF-responsive neural stem cells that have the capacity to generate astrocytes, oligodendrocytes and neurons *in vitro* were derived from these animals. A portion of the stem cells were forced to differentiate by removing EGF and adding FBS. With the addition of 1000 Units/ml. of alpha/beta IF, clusters of flat, astrocyte-like cells began to proliferate and eventually filled the culture dishes. The cells have been continuously maintained in IF for over 70 passages and have maintained a doubling rate of 24-36 hours over this period. When probed with a panel of neural and glial-specific antibodies, these IF-treated cells were virtually all nestin- and T-antigen-positive but were weakly immunoreactive for glutamine synthetase and were GFAP-negative. Upon removal of the IF, these flat cells rapidly decreased their rate of division, lost T-antigen immunoreactivity and gradually increased glutamine synthetase and GFAP immunoreactivity. These cells survive for several months *in vitro* and little or no proliferation is evident in the continued absence of IF. Interestingly, T-antigen immunoreactivity and cellular proliferation can be re-induced with the addition of IF. Cell lines with the capacity to proliferate or differentiate in a controlled fashion are useful tools for the study of developmental gene expression.

425.12

ELECTROPHYSIOLOGICAL FEATURES OF MÜLLER GLIAL CELLS FROM HEALTHY AND PATHOLOGICAL HUMAN RETINA. T. Pannicke, M. Francke, B. Biedermann, P. Wiedemann#, F. Faude# and W. Reichelt*, Paul-Flechsig-Institute for Brain Research, #Eye Hospital, University of Leipzig, 04109 Leipzig, Germany.

We intended to compare the electrophysiological features of Müller cells (MC) from healthy and pathologically changed human retinas. To this end we performed whole-cell voltage-clamp and patch-clamp experiments on acutely enzymatically isolated MC. The study was approved by the ethic committee of the University of Leipzig.

A clear loss of inwardly rectifying K^+ currents was found in the pathological MC. This caused a dramatic increase of the input resistance (ir). Of the healthy MC 92% had an ir of less than 0.5 GΩ, whereas of the pathological MC 55% and 23% had an ir higher than 1 GΩ and between 0.5 and 1 GΩ, respectively. The transient Na^+ current amplitudes were clearly increased in pathological MC.

Pathological as well as healthy MC possessed a high affinity Na^+/glutamate uptake, but no glutamate receptor currents. The high affinity Na^+/GABA uptake found on MC of other mammals was also present on human MC. However, the human MC additionally displayed $GABA_A$ receptor currents, which could not be found in guinea pig, mouse and horse MC. Interestingly, in a recent paper $GABA_A$ receptor currents were reported for the skate MC [Malchow, R.P. et al. (1986) Proc. Natl. Acad. Sci. USA 86, 4326-4330]. We are not yet able to decide whether or not there is a different density of glutamate- and GABA-transporters as well as $GABA_A$ receptors between healthy and pathological MC.

The loss of inwardly rectifying K^+ currents must severely interfere with the normal MC function, i.e. K^+ spatial buffering to maintain normal $[K^+]_o$. Supported by 0316916A and 01ZZ9103/2.23 (Bundesministerium für Bildung und Forschung, Germany).

425.13

A GLIAL POPULATION IDENTIFIED IN VIVO BY ANTIBODIES TO NG2 PROTEOGLYCAN AND PDGF ALPHA RECEPTOR. Akiko Nishiyama*[1] and William B. Stallcup[2] Dept. of Neurosciences[1], The Cleveland Clinic Foundation, Cleveland, OH 44195 and La Jolla Cancer Res Foundation[2].

We have used antibodies against the NG2 proteoglycan and PDGF α receptor to identify a unique population of cells in the developing and mature rat cerebrum *in vivo*. Both NG2 and PDGF α receptor have previously been shown to be expressed by O2A progenitor cells *in vitro* and down-regulated as they differentiate into mature oligodendrocytes. During late embryonic and early postnatal stages *in vivo*, NG2 and PDGF α receptor are colocalized on cells that share the features described previously for oligodendrocyte precursors. The immunoreactive cells, which are scattered throughout the central nervous system, continue to increase in number until the first postnatal week, which marks a peak in both the density of immuno-reactive cells and the extent of colocalization of the two molecules. The immunoreactive cells decrease in number after the second postnatal week but can still be detected in significant numbers in both gray and white matter of the mature brain. In the mature brain NG2 is localized on both cell bodies and processes, while PDGF α receptor becomes mostly confined to cell bodies. The NG2-positive cells in the mature brain display a complex morphology with multiple processes. Cells in the white matter extend processes parallel to axon tracts, whereas those in the gray matter extend multiple, fine processes in all directions giving them the appearance of astrocytes. Confocal laser microscopy of sections double-labeled for NG2 and GFA reveals no overlap between NG2+ and GFA+ cells but shows that NG2-positive processes are closely apposed to those of GFA-positive astrocytes. The processes from each NG2-positive cell in gray matter surround several neuronal cell bodies. These findings indicate that in addition to their properties as oligodendrocyte precursors, the NG2+ / PDGF α receptor+ cells have a unique physiological relationship between astrocytes and neurons.

425.14

NOVEL ZINC FINGER PROTEINS EXPRESSED IN THE NERVOUS SYSTEM C. Wiese*, J. Kim, A. Warrington and L. Hudson. Lab. of Developmental Neurogenetics, NINDS, National Institutes of Health, Bethesda, MD 20892, U.S.A.

Cells in the early stages of the oligodendrocyte lineage express a novel gene of the zinc finger superfamily, MyTI, that can bind to the cis-regulatory region of the myelin proteolipid protein (PLP) gene. By a random site selection assay, MyTI was found to recognize a G-rich consensus sequence (GGTGGGGPuPuPu) that is present in a number of myelin genes. MyTI contains six fingers of the C2HC class present in two widely separated clusters of two and four fingers each. The four finger cluster is associated with an acidic domain, whereas a large second acidic domain (88% glutamate residues) preceeds the two finger cluster. MyTI is encoded by a single gene, but a family member that is closely related to MyTI was also found to be expressed in early CNS development. This MyTI family member displays 92% amino acid identity with MyTI in the DNA binding domains, although it is missing one finger. But unlike MyTI, the transcripts of the family member are not detectable in the oligodendrocyte lineage cells based on Northern and RT-PCR analysis. The coding regions of the mouse MyTI are highly homologous to the human MyTI (95% amino acid identity). MyTI has been mapped to human chromosome 20 by a Southern analysis of a panel of monochromosomal human/rodent hybrid cell lines, whereas the family member has been localized on chromosome 2. To examine the function of this class of zinc finger proteins in the nervous system, a targeted mutation in MyTI is being created in transgenic mice. A construct carrying the genomic MyTI with the insertion of the lacZ gene and the neo and tk selectable markers has been electroporated into embryonic stem cells and at least one correctly targetted ES clone has been identified by PCR. Further studies should illuminate the role that MyTI plays in the specification and maturation of oligodendroglia cells.

425.15

VIMENTIN EXPRESSION AND INCREASED GFAP IN THE HIPPOCAMPUS AFTER REPEATED SEIZURES. J. L. Stringer* Department of Pharmacology, Baylor College of Medicine, Houston TX 77030

Reactive gliosis is characterized by hypertrophy of the cell bodies and processes of astrocytes and an increase in the expression of glial fibrillary acidic protein (GFAP). The signal that regulates the transition to the reactive state is unknown, as is the role of vimentin. This study used a model of repeated seizures in the hippocampal-parahippocampal circuits of the anesthetized rat to determine the extent and time course of reactive gliosis in response to seizures. The appearance of vimentin after seizures was also examined. Reactive gliosis, detected by immunohistochemistry for the presence of GFAP and vimentin, was present 2-7 days after the repeated seizures. At least 9 seizures, or at least 250 sec of total seizure discharge, were needed to induce reactive gliosis. After seizures, cells staining for vimentin were found in the dentate gyrus molecular layer and hilar region, as well as in the molecular layer of CA1. Fewer cells were stained in the CA3 region. Sections stained for GFAP immunoreactivity showed cell staining throughout the hippocampus. These experiments demonstrate that repeated discrete seizures of the hippocampal-parahippocampal circuits can cause reactive gliosis and can induce expression of a glial protein (vimentin) that is not normally expressed in the adult brain. If the appearance of vimentin protein can be taken as a marker for reactivation of the vimentin gene, then the data demonstrate that seizures can alter gene expression in glia. Supported by NINDS NS28871 and NS01784.

425.16

INFLUENCE OF FUNCTIONAL GLIA ON THE ELECTROPHYSIOLOGY OF PURKINJE CELLS IN ORGANOTYPIC CEREBELLAR CULTURES R. Drake-Baumann[1,2] and F.J. Seil*[1,2,3], Neurology Research[1], VA Medical Center and Departments of Neurology[2] and Cell Biology & Anatomy[3], Oregon Health Sciences University, Portland, OR 97201.

Previous studies have shown that exposure of explant cultures of neonatal mouse cerebellum to cytosine arabinoside (Ara C-Sigma), for the first 5 days *in vitro* (DIV) drastically reduced the granule cell population and compromised glial function (Seil, F.J. *et al.*, Brain Res. 186:393-408, 1980). Myelination was absent and astrocytes failed to ensheath Purkinje cells (PC). More recent studies have shown that exposure of cerebellar cultures to a different formulation of cytosine arabinoside (Ara C-Pfanstiehl) also destroyed oligodendrocytes and the granule cell population but did not affect astrocytes (Seil, F.J. *et al.*, Neuroscience 51:149-158, 1992). These two formulations of Ara C provide the opportunity to examine the role of astrocytes in the development of electrophysiological properties of PCs

Intracellular studies have shown that PCs in normal cultures exhibit spike activity consisting of a mixture of complex and simple action potentials, which is the characteristic of mature Purkinje cells *in vivo* and *in vitro*. By contrast, PCs in cultures exposed to Ara C-Sigma of similar age have a lower input resistance and generate only simple spike activity, reminiscent of the type of activity seen in immature PCs. After two weeks *in vitro*, PCs in cultures exposed to Ara C-Pfanstiehl express membrane conductances and spike activity similar to those of PCs of control cultures. These include TTX sensitive fast and slowly inactivating sodium conductances, and potassium and calcium conductances. These studies suggest that the presence of functional glia and/or astrocytic ensheathment of PCs influences their electrophysiological maturation. (Supported by the U.S. Department of Veterans Affairs and NIH grant NS 17493).

425.17

PHENOTYPIC AND FUNCTIONAL STUDIES OF OLFACTORY NERVE ENSHEATHING CELLS. Isabelle A. Franceschini and Susan C. Barnett*. Depts. of Neurol./Med. Onc., Univ. of Glasgow, Switchback Road, Glasgow G61 1BD, U.K.

Olfactory nerve ensheathing cells (ONECs) are a specialised type of glial cell, residing in the primary olfactory system, which are believed to play a role in the exceptional regenerative capacity of this tissue. We have developed a culture system in which ONECs closely resemble their *in vivo* counterparts and have assessed their ability to support neurite outgrowth as compared to astrocytes and Schwann cells.

ONECs were purified from neonatal rat olfactory bulbs using the O4 antibody and fluorescence activated cell sorting. Their antigenic profile was characterised with a large panel of neural markers under various culture conditions and compared to that observed during development. The serum-free medium (DMEM-BS) but not the serum-containing medium (DMEM-FCS) maintained the cells with a heterogeneous phenotype that closely resembled their *in vivo* counterparts. In the former medium, two classes of ONECs were observed, characterised on the basis of their morphology and antigenic profile using antibodies to polysialic acid and to the low affinity nerve growth factor receptor. Both cell types were detected *in vivo* from embryonic day 14. It is likely that they belong to the same lineage as two cell types of similar morphology and antigenic profile were detected in a retrovirus-infected ONEC cell line shown to be clonal by Southern blot analysis.

ONECs could be expanded in vitro in DMEM-FCS but also with a partially purified and yet unidentified factor(s) present in DMEM-BS conditioned by type 1 astrocytes (ACM). ONECs grown in either media supported greater neurite outgrowth, attachment and/or survival of cerebellar granule neurons than astrocytes or Schwann cells. Although the average neurite outgrowth-promoting activity of ONECs was constant regardless of the culture medium it was found that ONECs grown in ACM supported greater neuronal attachment and/or survival than ONECs grown in DMEM-FCS. This emphasises the importance of optimising the culture conditions for the study of ONEC-neuronal interactions if comparison are to be made with the *in vivo* situation.

425.18

REGULATION OF GLIAL CELL NUMBERS IN OPTIC NERVE OF MICE EXPRESSING A HUMAN *BCL-2* TRANSGENE FROM A NEURON-SPECIFIC ENOLASE PROMOTER. J.F. Burne* and M.C. Raff, MRC LMCB, University College London, London, UK.

We are interested in how the number and proportions of glial cells are controlled in the rodent optic nerve.

In the present study we analysed glial cells in the optic nerves of transgenic mice that express the human *bcl-2* gene from a neuron-specific enolase promoter (Martinou 1994). The *bcl-2* transgene suppresses normal neuronal cell death and, as a result, the mouse contains 70% more RGC axons in the optic nerve. DNA measurements indicate that the nerve also contains about twice as many glial cells. EM analysis of the glial population shows that there are twice as many oligodendrocytes, astrocytes and microglial cells so that the relative proportions of the axons and each glial cell type are preserved. We present evidence that oligodendrocyte numbers are increased by a reduction in normal oligodendrocyte death, whereas astrocyte numbers are increased by an increase in cell division.

Surprisingly, we find that the expression of the *bcl-2* transgene is not confined to neurons but is also expressed in mature oligodendrocytes and astrocytes. We provide evidence however, that the increase in oligodendrocyte and astrocyte number in the transgenic optic nerve is probably not directly related to the expression of the transgene in the glial cells but is probably secondary to the increase in the number of axons.

These findings suggest that complex interactions between axons and developing glial cells ensure that optimal numbers of each type of glial cell are generated in the optic nerve.

425.19

K⁺ CURRENTS IN THE ENDFEET OF MÜLLER (GLIAL) CELLS ISOLATED FROM FROG RETINA. EFFECT OF CUTTING THE OPTIC NERVE.

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Müller cells are radial glial cells which span the whole retina and play a role in transferring K^+ away from regions where it has accumulated due to impulse activity in the neurons. Such a function requires both inward (I_{KIR}) and outward (I_o) currents via K^+ channels in the Müller cell membrane. >95% of these channels are located in the endfeet. To understand the nature of potassium membrane currents in the endfoot of isolated Müller cells from the amphibian, *Rana pipiens*, we used simultaneously whole cell patch clamp applied to the endfoot of Müller cells as well as rapid changes in external K^+ ($[K^+]_o$). Changing $[K^+]_o$ from 3 to 10 mM at E_K for $[K^+]_o$ 3 mM. induced an inward current that was 5.48 (± 0.89 SD, n=9) fold larger than I_o induced by application of 1 mM $[K^+]_o$. The inward current is linear from holding potentials of -140 to -40 mV, while the outward current displays a different behavior and has maximal amplitude at physiological potentials close to -80 mV. Both the outward and the inward currents were completely blocked by blockers of the K^+-inward rectifier, 0.3 mM Ba^{2+} and 5 mM Cs^+. Other K^+-channels did not appear to be present (TEA⁺,30 mM or Cd^{2+}, 2 mM had no effect on the currents). Inward and outward currents in Müller cells appear to flow only through the inward rectifying K^+ channel. 40-60 days after cutting the optic nerve, leaving blood vessels intact, without allowing regeneration, isolated Müller cells appeared normal. However, the ratio of the inward current to the outward current in the endfeet was markedly depressed. In 98 mM K^+ this ratio was 2.86± 1.03 SD n=24 in controls and 1.13±0.59 SD n=21 in Müller cells from denervated retinae. The results suggest that the behaviour of K^+ channels in endfeet depends on the integrity of the closely apposed ganglion cells.

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425.20

CELL IDENTIFICATION OF LONG SURVIVING MURINE SPINAL CORD CULTURES. L.S. Gargan, J.L. Fuchs*, and G.W. Gross. Center for Network Neuroscience, Dept. of Biological Sciences, University of North Texas, Denton, TX 76203.

Our culturing methods have generated neuronal networks that have remained electrically active and pharmacologically responsive up to 312 days in vitro. Although much electrical data have been analyzed through recordings using array techniques, the cellular characterization of these stable culture systems has so far received less attention. We have begun to characterize the cellular components of the carpet layer that is situated between the electrode array and the neurons. Using immunofluorescence and electron microscopy, we found 40% astrocytes, 9% oligodendrocytes, and 4% neurons. Just beneath these identified cells, a "cobble-stone" layer composed of the remaining 47% of the cells, were non-neuronal and did not stain for astrocyte-specific glial fibrillary acid protein or oligodendrocyte-specific galactocerebroside. It is these cells that adhere primarily to the poly-D-lysine and laminin surface of the recording electrode plate. We anticipate that the lower layer of the carpet may consist of confluent fibroblasts, but could also be derived from pia or endothelial tissue fragments. The longevity of these cultures might be attributed to this layer of cells. (Supported by the Communities Foundation of Texas.)

MOLECULAR CHANGES AFTER LESIONS

426.1

THE EXPRESSION AND LOCALIZATION OF A FATTY ACID LIPID BINDING PROTEIN (FABP) IN THE RAT DORSAL ROOT GANGLIA AFTER SCIATIC NERVE TRANSECTION. C.A. Molina†, A.A. Welcher¶, M. De Leon*†. †Dept. of Physiol, Loma Linda Sch. of Med., Loma Linda CA 92350; ¶Amgen Inc., Thousand Oaks, CA 91320.

The present study used immunocytochemistry (ICC) and Western blots analysis to examine the expression and localization of DA11, a rat FABP isolated from a dorsal root ganglia (DRG) cDNA library. The predicted amino acid sequence of DA11 was elucidated and peptide polyclonal antibodies were generated. Adult male Sprague Dawley rats received a left ipsilateral (ipsi) sciatic nerve transection and a right contralateral (contra) sham operation. The L_4-L_5 ipsi and contra DRG were harvested, their respective proteins extracted, electrophoresed in a polyacrylamide gel, electrobloted to nitrocellulose and immunodetected with the DA11 antiserum. The Western blots showed the presence of a single ± 15.1 kd immunoreactive band in protein extracted from the DRG tissue. The DRG ipsi to the sciatic nerve transection showed a two-three-fold elevation in DA11 protein as compared with contra or naive controls. Additional animals were surgically manipulated as described above and processed for ICC. Neurons in contra and naive DRG showed a basal level of cytoplasmic expression of DA-11-like immunoreactivity, with very few neurons exhibiting nuclear staining. In contrast, neurons in the DRG ipsi to the sciatic nerve transection showed a marked DA11-like immunoreactivity in the cell nuclei. Our data indicate that DA11 protein is up-regulated in axotomized DRG neurons after sciatic nerve transection.

426.2

THE EXPRESSION OF A NOVEL FABP (DA11) IN RAT CNS DURING DEVELOPMENT. Y. Liu*, C.A. Molina¶, A.A. Welcher#, M. De León¶. *Dept. Pathol. & Hum. Anat. and ¶Dept. Physiol. Loma Linda Univ., Sch. of Med., Loma Linda, CA 92350; #Amgen Inc., Thousands Oaks, CA, 91320.

The identification of genes whose expression is regulated during neuronal injury and nerve regeneration is one way to study the molecular mechanism associated with neuronal plasticity and axon growth. In previous studies we reported the cloning and characterization of DA11, a full length of cDNA isolated from a rat dorsal root ganglia cDNA library that encodes for a novel rat fatty acid lipid binding protein. The sequence analysis revealed that DA11 is the rat homologue of human psoriasis-associated fatty acid-binding protein (PA-FABP). We now report the expression of DA11 mRNA and protein during the embryonic and neonatal development of the rat central nervous system (CNS). Total RNA was extracted from different rat CNS tissues and analyzed using Northern and Western blots. Our results show that DA11 mRNA is significantly elevated in all the neonatal and embryonic tissues analyzed. DA11 mRNA had a maximum elevation of 159-fold in the cerebellum, 87-fold in the brain stem, 34-fold in the hippocampus and 12-fold in the cerebrum during late embryonic and early post-natal period as compared to adult levels. Similarly, Western blots experiments showed that the DA11 protein was up-regulated in embryonic and neonatal rat CNS, as compared with adult. We conclude that DA11 may play a role during the development of the rat CNS.

426.3

Induction of Embryonic GAD Transcripts and Nestin Following Hippocampal Injury: Recapitulation of Developmental Programs

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Repair of damaged CNS tissue is expected to involve the reactivation of genes necessary for "building" the CNS, i.e. genes usually expressed during development. Nestin is an intermediate filament protein expressed in proliferating stem cells of the CNS but not in terminally differentiated neurons. Several GAD (glutamic acid decarboxylase) 67-derived mRNAs and protein forms, collectively referred to as G67I80/86, are transiently expressed during CNS development in the phase of neurogenesis. Here we have investigated the regulation of nestin and embryonic GADs in injured adult CNS. Systemic treatment of male adult rats with kainic acid (KA), a glutamatergic agonist, results in seizures, excitotoxicity and cell death, accompanied by gliosis and sprouting. We have used a high-sensitivity quantitative RTPCR protocol to analyze the expression of mRNAs for nestin and the known forms of GAD. Induction of embryonic G67I80/86 was measured after 3h, followed by maximal expression at 6h, and a gradual decline at 24h to basal levels at 48h. Nestin mRNA expression increased at 3h, declined at 6h and increased again at 24h and 48h following KA administration. In situ hybridization with ^{35}S nestin cRNA and immunocytochemistry using the 130 nestin polyclonal antibody confirmed that nestin was upregulated at early time points following seizure activation in neuronal regions of the hippocampus and in both neuronal and non-neuronal cell populations at longer time points, corresponding to the onset of kainic-acid induced neurodegeneration. Thus, selective and transient overexpression of nestin and embryonic GAD forms may reflect the activation of repair mechanisms which involve genes previously associated with neurogenesis.

426.4

The level of hemopexin, a glycoprotein absent from the CNS, increases after lesion and inflammation in the rat sciatic nerve. L. Camborieux, N. Madore, J. Smith* and J.-P Swerts. *Centre de Biologie du Développement UMR CNRS 9925. Université Paul Sabatier 31062 TOULOUSE - FRANCE.*

We have previously demonstrated that a serum glycoprotein, **hemopexin (HPX)**, is synthesised in the rat sciatic nerve. After axotomy, HPX levels increase. They remain high in permanently degenerated sciatic nerve but return to normal if regeneration is allowed to occur. Thus the hemopexin content seems closely correlated with the degeneration status of the nerve.

To get further insight into the role of HPX in nerve regeneration, we have studied the distribution of the protein in PNS and CNS. In sciatic (mixed), hypoglossal (motor) and cervical sympathetic trunk (unmyelinated) nerves, HPX labeling is detected around axonal units and blood vessels, partly colocalised with laminin. After lesion, HPX immunoreactivity increases drastically in the distal part of all peripheral nerves analysed, without change in its localisation. In the intact optic nerve and in the brain, HPX is found exclusively in blood vessels. In the transected optic nerve, no modification of the localisation or amount of HPX is observed. These findings indicate that the presence and the accumulation of HPX after injury is a general feature of the PNS, whereas the protein is absent from the CNS even after lesion.

One of the differences between the PNS and the CNS after lesion lies in the importance of the resulting inflammatory reaction. We hypothesised that accumulation of HPX may be dependent on macrophages. This idea is supported by the observation that enhanced HPX immunoreactivity is correlated with the presence of macrophages during inflammation induced by lipopolysaccharide in the sciatic nerve. Thus, as neither axons nor Schwann cells are injured in this process, inflammation alone seems to be sufficient to induce accumulation of HPX in PNS nerves.

426.5

DIFFERENTIAL EXPRESSION OF JAK FAMILY GENES IN RAT HYPOGLOSSAL MOTONEURONS AFTER NERVE INJURY. G. L. Yao, H. Kato S. Shimada* and H. Kiyama. Dept. of Neuroanatomy, Biomedical Research Center, Osaka Univ. Sch. of Med.,Yamadaoka, suita, Osaka 565. JAPAN.

Some lines of studies suggested that growth factors and cytokines might play important role in the peripheral nerve regeneration, therefore it is likely that intracellular signaling pathways following growth factor and cytokine receptors can be crucial for peripheral nerve regeneration. Since it has been demonstrated that JAK family tyrosine kinases (JAK1, JAK2. JAK3 and TYK2) play a crucial role in intracellular signaling particularly downstream the cytokines' receptors, we studied regulation of JAK family genes expression following the peripheral nerve injury by in situ hybridization histochemistry. Unilateral hypoglossal nerve of rat was axotomized, and the mRNA level of these JAK family in the hypoglossal nucleus after post-operative time of 1,3,5,7,14,21,28 and 35 days was examined. Among JAK family, JAK2 and JAK3 mRNAs' level was substantially increased in the operated hypoglossal nucleus after nerve injury, whereas such significant increase was not observed in JAK1 and TYK2. These results suggest that intracellular signaling pathway which involves JAK2 and/or JAK3 could be important for the nerve regeneration.

426.6

NERVE INJURY ENHANCES RAT NEURONAL GLUTAMATE TRANSPORTER EXPRESSION: IDENTIFICATION BY DIFFERENTIAL DISPLAY PCR. S.Kiryu*, G.L.Yao, N.Morita, H.Kato, H.Kiyama. Dept. of Neuroanatomy, Biomedical Research Center, Osaka Univ. Med. Sch., Suita, Osaka, 565 JAPAN.

Using the differential display PCR(DD-PCR), we have compared differentially expressed genes between normal and nerve injured hypoglossal nuclei. Expression of several cDNA fragments were up-regulated on the displayed gel. In situ hybridization using those derived cDNA fragments showed that only five fragments among the candidates were apparently up-regulated in the injured nuclei. The five cDNA fragments were further used as probes to screen a rat cDNA library and isolate their corresponding complementary DNA clones.

Among cloned cDNAs, one clone showed high similarity to rabbit high affinity glutamate transporter (EAAC1). Since it is suggested that CNS injury such as ischemia induces an excess extracellular glutamate accumulation which causes severe neuronal damage, the up-regulation of glutamate transporter expression could be a response against the neuronal cell death caused by the glutamate toxicity. Therefore, activation of the glutamate uptake system might play an important role for damaged neurons to survive.

426.7

UP-REGULATION OF CYCLIN G EXPRESSION IN MOTONEURONS AFTER NERVE INJURY. N. Morita, S. Kiryu and H. Kiyama*. Dept. of Neuroanatomy, Biomed. Res. Center, Osaka Univ. Med. Sch., Suita, Osaka 565, Japan.

The differential display PCR (DD-PCR) technique was used to isolate nerve regeneration associated genes. Unilateral hypoglossal nerve of Wistar rats were cut, and total RNAs from control and operated sides of hypoglossal nuclei were extracted 7 days after the operation. DD-PCR was performed by using single arbitrary primer. The amplified cDNA fragments which were more abundant in the operated side than in the control side were subcloned, and checked by in situ hybridization (ISH) using unilaterally transected section. The positive clones confirmed by ISH were then sequenced. One of the up-regulated gene was identical to the cyclin G. Although the cyclin G belongs to G1-cycline family, it was suggested that the cyclin G was associated with growth stimuli but not with the cell cycle. Present result suggests that the cyclin G was closely associated with nerve regeneration event.

426.8

Immunoreactivity for NGF and low affinity NGF receptor (p75) in the medial septal nucleus: a time course following fimbria-fornix transection

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Many cholinergic neurons in the medial septal nucleus (MS) are able to restore the synthesis of their marker protein choline acetyltransferase (ChAT) after bilateral fimbria-fornix transection (ff-t) without exogenous treatment with nerve growth factor (NGF) (Naumann et al. 1994). It is still unknown whether these axotomized neurons receive NGF from other sources than the hippocampal formation which normally provides them with this trophic factor. To this end we have investigated changes in immunoreactivity for NGF and the low affinity receptor in axotomized cells. We looked at local axonal arborizations of MS neurons after different survival times following ff-t in both immunostained neurons (p75) as well as cells filled intracellularly with biocytin.

50 adult Sprague-Dawley rats received a bilateral ff-t. After defined survival times these animals and age-matched controls were processed for NGF and p75immunocytochemistry. Another group of rats received multiple tracer injections (Fast Blue, Fluoro-Gold, Rhodamine) into different brain regions in order to identify different targets of MS neurons.

3 weeks postlesion the number of NGF positive neurons was decreased to 11% of control. There was no recovery after long survival times (6 months). A similar time course was found for p75-immunoreactive cells in the MS. As reported by others (Sofroniew et al. 1990) septohippocampal neurons have only one axonal target, but p75 immunocytochemistry as well as intracellular biocytin injections revealed extensive local axonal arborizations. We conclude that cholinergic neurons in the MS maintain local axonal arborizations following ff-t. The loss of NGF immunereactivity in MS neurons after ff-t suggests that these cells do not receive NGF from other sources but the hippocampus.

(Supported by the DFG: Leibniz Program and Fr 620/4-2)

426.9

C-JUN EXPRESSION IN AXOTOMIZED MEDIAL SEPTAL NEURONS IS NOT RELATED TO NEURONAL DEGENERATION. C. A. Haas, J. Lübke* and M. Frotscher. Institute of Anatomy, University of Freiburg; 79001 Freiburg, FRG.

The transcriptional control of neuronal regeneration/degeneration is of great interest for the understanding of neurodegenerative disorders. We use the anatomically well-defined fimbria-fornix system as a paradigm to study the molecular events involved in neuronal degeneration. The expression of three immediate early genes, c-fos, c-jun and jun B, was investigated in rat medial septal neurons following bilateral fimbria-fornix transection (FFT). Moreover, the degree of retrograde degeneration was assessed by measuring the DNA fragmentation in these injured neurons. A detailed post-operative time-course was performed ranging from 1 day to 9 weeks following bilateral FFT. A strong increase of c-jun immunoreactivity (IR) was observed in the nuclei of medial septal neurons as early as 2 days postaxotomy. The number of c-jun-positive neurons stayed high over 6 days, followed by a decline at day 12. Nine weeks after FFT c-jun-IR had disappeared. c-Fos or jun B immunoreactivity was neither detectable in unoperated nor in lesioned medial septal neurons. In addition, a massive induction of c-jun mRNA could be localized in the medial septal nucleus by in situ hybridization histochemistry. Experiments using the TUNEL technique (TdT-mediated dUTP nick-end-labelling), designed to detect DNA fragmentation in degenerating neurons complemented this study. Within three weeks postaxotomy medial septal neurons did not exhibit any DNA fragmentation. Taken together, the strong and long-term c-jun expression in axotomized medial septal neurons and the lack of DNA fragmentation in these cells suggests a strong survival capacity of this neuronal population.

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426.10

TENASCIN-C mRNA AND PROTEIN DISTRIBUTION IN THE RAT SPINAL CORD FOLLOWING UNILATERAL DORSAL COLUMN INJURY

Yi Zhang, P. N. Anderson, H. Mohajeri[1], M. Schachner[1], J. Winterbottom and A.R. Lieberman* Dept. of Anatomy and Dev. Biology, University College London, WC1E 6BT and Neurobiology, Swiss Federal Institute of Technology, Zurich[1].

Tenascin-C is a developmentally regulated ECM molecule. In rat spinal cord it is produced by glial cells and by a small subset of neurons. Tenascin-C expression patterns vary in different parts of the CNS after injury and there is controversy as to whether injury-induced tenascin-C inhibits or has the potential to support axonal regeneration. We have studied the expression and distribution of tenascin-C by in situ hybridization and immunofluorescence (with antibodies against tenascin-C, GFAP, OX-42 and the 200KD NF) in the spinal cord after stab wounds of the thoracic dorsal column in halothane anaesthetized adult rats. Between 3 and 30d after injury, lesion areas were largely devoid of GFAP+ astrocytes and often packed with OX-42+ macrophages and GFAP-/OX-42- cells. A zone of diffuse tenascin-C immunoreactivity was found around the lesion site and in the degenerating dorsal column rostral to the lesion. Stronger immunoreactivity, sometimes with a filamentous appearance, was present around cells in and close to the lesion, most of which were GFAP- and OX-42-, and on some capillaries in the same region. Only a few of the GFAP+ astrocyte processes forming the glia limitans bordering the lesion were tenascin-C+. Many NF+ axons were present in the zone of diffuse tenascin-C immunoreactivity; some appeared to traverse this zone and enter the lesion area, but none was tenascin-C+. Tenascin-C mRNA+ cells were present in and around the lesion 3-14 days after injury but had disappeared by 30d, although tenascin-C was still present at this time. A minority of these cells were GFAP+ astrocytes. Thus the distribution of tenascin-C around lesion sites is compatible with it influencing axon regeneration, but sprouting NF+ dorsal column axons, unlike regenerating axons in the thalamus, do not appear to bind the molecule. Tenascin-C is produced at the lesion site by a population of cells which may be meningeal cells and/or Schwann cells, and by a subpopulation of reactive astrocytes. *(Supported by the MRC)*

426.11

QUANTIFIABLE DETECTION OF PROTEIN AND GENE EXPRESSION CHANGES IN ADULT RAT DORSAL ROOT GANGLIA AFTER SCIATIC NERVE TRANSECTION. A.M. Kenney and J.D. Kocsis*. Dept. of Neurology, Yale Univ. Med. Sch., New Haven, CT. 06510, and Neuroscience Res. Ctr., VAMC, West Haven, CT. 06516

Immunohistochemistry and *in situ* hybridization studies of adult rat dorsal root ganglia (DRG) have been used to study genes regulated in response to sciatic nerve axotomy. Increased c-jun protein and mRNA levels in neurons 10-12 hours after injury has been identified as one of the first indications that a molecular program for reacting to axotomy has been initiated. Using the techniques of Western blotting with enhanced chemiluminescent (ECL) detection and northern blotting with radiolabelled probes, we quantified changes in gene expression in DRG following sciatic nerve transection with chronic target disconnection. Anesthetized 200 g adult female Wistar rats underwent unilateral sciatic nerve axotomy and contralateral sham surgery at the mid-thigh level. The nerve stump was drawn into a silicon cuff. From 1 hour to 30 days after surgery, the rats were sacrificed. Protein or total RNA was prepared from L4 and L5 DRGs. Protein was analyzed by SDS-PAGE and Western blotting. Elevated c-jun protein levels were detectable within 5 hours of axotomy, peaked at 5 days, and remained above constitutive levels through 30 days after axotomy. No elevation in c-jun was detected in sham controls. Message regulation was studied by northern blotting. We are using these techniques to measure changes in gene expression after axotomy with various treatments to elucidate signals that regulate the axon reaction and subsequent cell death or neurite outgrowth. Current results indicate that the techniques used here, while not cell specific, are quantifiable and useful for measuring subtle changes in gene expression, as evidenced by the increased c-jun protein levels seen here at 5 hours, rather than 10 hours, after sciatic nerve transection. Supported in part by the VA and the NIH.

426.12

TISSUE PLASMINOGEN ACTIVATOR (TPA) BUT NOT UROKINASE (UPA) IS INDUCED IN PERIPHERAL NERVE INJURY. I.V. Smirnova, J.Y. Ma, D. Hantaï, B.A. Citron* and B.W. Festoff. Neurobiology Research Lab., VA Med. Cent., Kansas City, MO 64128, Dept. of Neurology, Univ. of Kansas Med. Cent., Kansas City, KS 66103, and INSERM U. 153, Paris, FRANCE.

Serine proteases play crucial roles in development, maintenance and regeneration of the nervous system. Neurite outgrowth is dependent on components of the plasminogen-plasmin system. PAs, both tPA and uPA, are critical factors in this system. uPA, but not tPA, is altered in muscle after axotomy. In contrast, tPA, more than uPA, is involved in neurite outgrowth in both ganglionic and CNS neurons in culture. We sought to determine whether tPA or uPA activities are altered in murine sciatic nerve after crush injury. Highly sensitive chromogenic assays with specific protease substrates were employed to measure PA activities in nerve extracts by plasminogen activation to plasmin followed by determination of plasmin activity with the synthetic substrate, S-2251. Addition of fibrin monomer (FM), which modulates tPA activity, allowed us to distinguish tPA from uPA. We found rapid increase of activity (in the presence of FM) in the distal nerve segment after crush from day 1-4, when it peaked, declining by day 9- 21, reaching basal levels on day 30, and remaining at this same level 60 days following injury. Nerve extracts showed no detectable activity in the absence of FM. No uPA activity was found by direct amidolytic assay with the substrate S-2444, relatively specific for uPA. Contralateral controls did not show measurable tPA activity. Our findings demonstrate that tPA, but not uPA, activity is significantly elevated in early stages (day 4) of nerve injury and may precede potential regeneration. (Supported by Association Française contre les Myopathies, INSERM, NATO, the Marion Merrell Dow Foundation/SEP, and the Medical Research Service of the Department of Veterans Affairs)

426.13

MOLECULAR MECHANISMS OF NEURONAL REGENERATION. J. A. Davies*, S. Korneev, A. Fedorov and S. E. Blackshaw[1]. Sussex Centre for Neuroscience, Sussex University, Falmer, Brighton BN1 9QG, U.K and [1]Laboratory of Cell Biology, University of Glasgow, Glasgow G12 8QQ, Scotland.

We are investigating the changes in gene expression that occur during neuronal regeneration in the leech, *Hirudo medicinalis*, by isolating and characterising molecules specific to regenerating neurons. Our previous studies have demonstrated the successful use of PCR technology in the construction of cDNA libraries from individually dissected, identified regenerating and non-regenerating neurons (Korneev et al., Progress in Neurobiology, 42: 339-346). Subtraction hybridisation has been used to create a library enriched in sequences from regenerating Retzius cells and its quality has been confirmed by both differential hybridisation and hybridisation with a subtracted probe. Sequence analysis of the recombinants has identified both known and novel sequences. Homologies include α- and β-tubulin, a calmodulin-like protein and a clone encoding a protein homologous to the CCAAT/enhancer-binding protein (C/EBP) family of transcription factors. Further characterisation of the clones is underway and will enable us to explore the mechanisms which axons use to find and synapse with their target cells during normal and regenerative growth.

426.14

THE AXOTOMY-INDUCED NEUROPEPTIDE GALANIN IS ANTROGRADELY TRANSPORTED IN AXOTOMIZED SYMPATHETIC NEURONS. A. M. Shadiack* and R. E. Zigmond. Dept of Neurosciences, Sch of Med, Case Western Reserve University, Cleveland, OH 44106-4975.

Axotomized sympathetic neurons reduce their production of molecules necessary for transmission and begin producing proteins which may aid in survival or regeneration. Among these newly produced proteins, are certain neuropeptides such as galanin (GAL). After axotomy of the middle and inferior cervical ganglion (MICG), GAL is produced in the axotomized neurons and later found in postganglionic axons in the cervical sympathetic trunk (CST). We have previously found that the amount of GAL-immunoreactivity (IR) in the MICG increases 2-3-fold between 7 and 14 days after axotomy (Shadiack, et al., 1994). It is possible that more neurons begin to produce GAL or the same cells produce more GAL. We now report that this change is not accompanied by a further increase in GAL mRNA. Quantitation of GAL-IR cells colocalized with fast blue, a fluorescent retrograde marker, after axotomy shows no change in GAL+ cell number between 7 and 14 days. We have studied the appearance of GAL-IR in the CST at 2, 4, and 7 days after axotomy with special reference to regionality within the CST. At earlier times, GAL-IR is localized to the region most distal to the ganglion, near the site of transection. At later times, GAL-IR was identified throughout the CST though the highest concentration remains distal to the ganglion. Using confocal laser microscopy, the GAL-IR could be further localized to synaptic vesicle-like structures within fibers. These data taken together suggest that the galanin produced by the axotomized neurons is actively transported toward the site of the transection perhaps to aid in regeneration. Furthermore, the secondary increase in GAL-IR at 14 days post-axotomy may be due to GAL, which would otherwise be transported away from the ganglion, building up in the sympathetic cell bodies due to a saturated transport system.

426.15

BROMODEOXYURIDINE (BRDU)- LABELED MACROPHAGES IN THE SUPERIOR CERVICAL GANGLION (SCG) AFTER AXOTOMY. R. C. Schreiber* S. A. Vaccariello, and R. E. Zigmond. Dept of Neurosciences, Sch of Med, Case Western Reserve University, Cleveland, OH 44106-4975.

Transection of the facial nerve results in proliferation of microglial cells but not astrocytes in the facial nerve nucleus (Graeber et al., 1988). Our laboratory has shown that postganglionic nerve transection results in increased expression of neuropeptides, increased immunoreactivity for glial fibrillary acidic protein (GFAP) and certain macrophage antigens (Bachoo et al., 1992; Schreiber et al., 1995), as well as proliferation of non-neuronal cells (Bachoo et al., 1992) in the SCG. This study examines the extent to which macrophages contribute to the proliferating cell population in the SCG. Rat SCG were axotomized and the animals injected every 6 h for 2 d with BrdU. SCG sections were immunostained for BrdU and GFAP or a cocktail of macrophage markers (ED1, ED2, and OX6). Confocal microscopy confirmed our earlier findings that many of the BrdU+ nuclei are present in GFAP+ cells (Bachoo et al., 1992). In addition, 20% of the BrdU+ nuclei was present in macrophages. Analysis of individual macrophage markers revealed that both the total number of ED1+ cells and the number of these cells containing BrdU+ nuclei increased 4-fold. While the number of OX6+ and ED2+ cells did not change appreciably 2 d after axotomy, there was a 3- and 11-fold increase, respectively, in the number of these cells which contained BrdU+ nuclei. Transecting the afferent input to the SCG (decentralization) results in extensive nerve terminal degeneration with little increase in neuropeptides. Preliminary studies indicate that there is little change in the number of macrophages after decentralization. BrdU studies on decentralized SCG will be reported. The data with colocalization of BrdU and ED2 (which identifies resident macrophages) are evidence that these cells, in addition to elicited (ED1+) macrophages, are affected by axotomy. The difference in the macrophage response to axotomy and decentralization raises interesting questions with regard to the signals affecting macrophages and the physiological functions of these cells in situations involving neural damage.

426.16

EXPRESSION OF SEVERAL GROWTH FACTORS AND DETECTION OF DNA FRAGMENTATION AFTER CORTICAL ABLATION IN RATS. M. Isono*, Y. Wakabayashi, K. Asakuno, M. Fujiki, T. Mori, S. Hori. Dpt. of Neurosurgery, Oita Medical Univ. Oita, Japan, 879-55

To investigate the molecular changes due to deafferentation in vivo, expression of several growth factors and peptides were studied in rat brain after unilateral sensorio-motor cortical ablation. Also, whether DNA fragmentation and apoptosis related peptides might appear was investigated in the same model. Immunoreactivities of GFAP, bFGF, Syntaxin, OX-42, bcl-2 were studied by ABC method and compared with non-lesioned site. DNA fragmentation was detected by TUNNEL method. The observation period was ranged from 3 hrs to 3 months after the ablation surgery. Throughout the whole period, TUNNEL positive cells was not found, although routine staining revealed deformity of the nucleus in certain area. Relatively strong expression of bFGF in ipsilateral cortex was observed one day after the surgery, but only little expression was observed in the target area such as striatum or thalamus, where GFAP was positively expressed, throughout the period. Interestingly, rather strong expression of Syntaxin was observed in ipsilateral site. These results suggest that changes in the target area after deafferentation do not include apoptosis and are only little compared to those in area nearby the primary lesion.

426.17

INJURY-ASSOCIATED CHANGES IN GLYCOSAMINOGLYCANS AND THEIR EFFECTS ON THE REGENERATION OF TRANSECTED SCIATIC NERVES OF GUINEA PIGS. D.K.Y. Shum[1], C.H. Chau[1], K.-F. So[2], Y.S. Chan[3] Depts of Biochemistry[1], Anatomy[2] and Physiology[3], The University of Hong Kong, Hong Kong. (SPON: The Hong Kong Society of Neurosciences)

The glycosaminoglycan (GAG) composition and content of sciatic nerves during recovery from crush-injury were studied in adult guinea pigs and compared with those of untreated controls. The GAGs were recovered from the 1,900 g supernatant and pellet of the tissue homogenate and assayed for hexuronate contents and susceptibilities to hyaluronidase, chondroitinase ABC and nitrous acid. In the untreated controls, GAGs were found only in the pellet which showed heparan sulphate (HS) as a major GAG class. In the 2 weeks post-crush, the GAG extracts indicated progressive increase in hexuronate which was due mainly to additional GAGs in the supernatant where chondroitin sulphate (CS) was the major GAG class found. The pellet fraction of crushed nerves in the same period was however similar to the untreated controls in relative abundance of GAG classes and hexuronate content. At 4 weeks post-crush, although the total hexuronate returned to the control level, a significant proportion of GAGs remained in the supernatant fraction. To study the effects of increased soluble GAGs in the neural environment, an 8-10 mm silicone tube, filled with solutions of CS, HS, or saline, was used to bridge transected sciatic nerves in halothane-anaesthetized animals. The animals were allowed to recover. At timed periods, they were re-anaesthetized for EMG measurements - electrical stimuli were applied at defined positions flanking the bridge on the transected nerve and the difference in latency for EMG to be recorded from the gastrocnemius muscle was used to determine conduction velocity across the bridge. Sections of the bridge were also histologically examined for nerve fibres. With saline as the bridge material, 3-4 weeks were required for histological indications and 9-10 weeks, for electrophysiological indications of regeneration. At these times, indications resulting from application of CS and HS will be compared to reach conclusions regarding their effects on neural regeneration.

426.18

UNILATERAL PERIPHERAL NERVE INJURY IN THE RAT INDUCES TIME-DEPENDENT BILATERAL CHANGES IN mRNA LEVELS OF THE AXONAL GROWTH ASSOCIATED PROTEIN GAP-43 IN MOTONEURONS. E. W. McNally, C. E. Blanco, T. Mosconi, P. E. Micevych and L. Kruger*. Dept. of Neurobiology, UCLA School of Medicine, Los Angeles, CA 90095.

Relative GAP-43 mRNA levels were determined in motoneurons of spinal cord segments L_4-L_6 innervating their targets via the sciatic nerve using in situ hybridization histochemistry. In intact adult male Sprague-Dawley rats, GAP-43 mRNA was undetectable in L_4 motoneurons and expressed at low levels in L_5-L_6 motoneurons. Unilateral axotomy or placement of a loose polyethylene cuff on the sciatic nerve increases the number of motoneurons expressing GAP-43 mRNA in a time dependent, asymmetric bilateral manner. Levels were examined at post-operative intervals of 0, 1, 3, 7, 10, 14 and 28 days. GAP-43 mRNA levels of motoneurons ipsilateral to the axotomy increase and remain elevated to four times normal levels 28 days after injury. The nerve cuff resulted in peak GAP-43 mRNA levels (three times greater than control levels) within seven days in the ipsilateral lateral motor column and decline thereafter. Both sciatic axotomy and nerve cuff result in expression in contralateral L_4 motoneurons, but not in L_5 or L_6.

These results indicate that unilateral peripheral nerve injuries increase the GAP-43 mRNA levels of motoneurons whose axons are directly injured as well as in motoneurons which innervate the contralateral limb. The expression of GAP-43 in uninjured motoneurons suggests the nerve-injury induced changes in weight bearing and in locomotion may underlie axonal outgrowth and synaptic remodeling.

This research was supported by NS-5685, HL-34817 and NS-21220.

426.19

THE ROLE OF C-JUN IN REGULATING VIP AND OTHER PEPTIDES IN SENSORY NEURONS: IMPLICATIONS FOR THE RESPONSE TO AXOTOMY. P. K. Mulderry & S. P. Dobson. SPON: Brain Research Association . MRC Brain Metabolism Unit, Royal Edinburgh Hospital, Edinburgh EH10 5HF, U. K.

Adult rat dorsal root ganglion (DRG) neurons express both VIP and c-Jun in response to axotomy *in vivo* and when the cells are placed in culture. We have used cultured DRG neurons to investigate the role of c-Jun in regulating expression of VIP and other peptides associated with the axotomy response. To demonstrate that VIP expression was dependent on c-Jun, we blocked neuronal c-Jun expression by microinjecting a c-*jun* antisense oligonucleotide. Quantitative immunofluorescence measurements in individual neurons confirmed that the antisense oligo specifically reduced c-Jun levels and also revealed a reduction in VIP immunofluorescence. This effect was selective, since neither substance P nor CGRP levels were reduced. To find sites of interaction between c-Jun and the VIP gene, we performed gel retard experiments. These demonstrated that c-Jun could bind the rat VIP cyclic AMP responsive element (CRE) in the presence of c-Fos, though not on its own, and we therefore tested whether VIP expression in DRG requires transcription factor binding to the CRE. Microinjection of a plasmid containing multiple copies of the CRE sequence, designed to sequester DRG transcription factors occupying the endogenous VIP CRE, selectively and specifically reduced VIP expression. Thus, c-Jun appears to activate VIP gene transcription in cultured DRG neurons by binding to the CRE in combination with other factor(s). We suggest that the same mechanism could account for VIP induction after axotomy *in vivo*. Accordingly, we examined the effects of the c-*jun* antisense oligo on other peptides induced by axotomy. The oligo reduced neuropeptide Y immunofluorescence but left galanin unaffected. We conclude that while c-Jun may mediate the induction of VIP and neuropeptide Y after axotomy, it is not necessarily required for galanin expression.

426.20

GAP-43 mRNA LEVELS ARE UNCHANGED BY PROXIMAL AXOTOMY IN MOUSE CORTICAL NEURONS. E.J. Elliott*, D.A. Parks, P.S. Fishman. Research Service, VA Medical Center, Baltimore, MD 21201.

Neurons in the mammalian cerebral cortex do not regenerate successfully after axotomy. In those neurons of the mammalian CNS which do show some regenerative growth following axotomy, the extent of sprouting and the degree of upregulation of growth-associated proteins and mRNAs are correlated with the proximity of the axonal lesion. Lesions closer to the cell body are more effective in stimulating a regenerative response. We wished to determine whether close axotomy of cortical neurons in the mouse results in upregulation of GAP-43 mRNA.

Cortical pyramidal neurons that project across the callosum were labeled retrogradely by applying the fluorescent dye Fluorogold to the contralateral side of the cortex. A week later, a knife wound was made through the cortex and corpus callosum on the ipsilateral side. This wound divided the fluorescently labelled neurons into a lateral population of cells which were axotomized near their cell bodies and a medial population which had their axons intact.

In situ hybridization experiments, using a ^{33}P-labeled oligo-nucleotide probe, showed no detectable increase in GAP-43 mRNA in the axotomized cortical neurons compared to the un-axotomized cells, at 1 day, 3 days or 7 days after injury. Thus close axotomy alone does not result in biologically significant upregulation of GAP-43 mRNA in these neurons. We are currently investigating the potential of neurotrophic factors to enhance the response of cortical pyramidal cells to axotomy.

426.21

GAP-43 AND SYNAPTIC VESICLE PROTEINS IN RAT AXOTOMIZED SCG NEURONS. Xiu-E Hou* and Annica B. Dahlström Dept. of Anatomy & Cell Biology, Göteborg University, Göteborg, Sweden.

Rat superior cervical ganglia (SCG) were used as a model to study GAP-43 and synaptic vesicle proteins in axotomized adrenergic neurons in the PNS. One, 3 or 8 days after cutting the internal and external carotid nerves, sections were incubated for immunofluorescence. Confocal laser scanning microscopy was used to study and semiquantitate immunoreactivities (IR). One day post axotomy many perikarya, normally GAP-43-negative, became GAP-43 positive, and nerve fibers and terminals around principal neurons became weakly GAP-43-IR, compared with the strong IR in control. Three and 8 days post axotomy GAP-43-IR in perikarya was stronger as compared with 1 day post-axotomy, but limited to neurons located in the middle and caudal parts of the SCG. The GAP-43 IR in the neurons was distributed as small granules in addition to diffuse GAP-43-fluorescence. Nerve fibers in the cranial part were intensily GAP-43 positive, due to accumulations in the cut axons. The neurons in the cranial part appeared to be degenerating, since the normal morphology of the synaptophysin- and synaptotagmin-positive Golgi complex had become diffuse and since they no longer contained detectable GAP-43-IR.

In 1 day irides GAP-43-IR was much decreased, especially in the terminals in the dilator. Synaptophysin-positive nerve fibers and nerve bundles became thinner, but did not disappear. In 3 and 8 days irides the intensity of GAP-43-IR increased, but intensity of synaptophysin-IR did not change.

The results show **1)** that GAP-43-IR is up-regulated in the neurons undergoing regeneration, **2)** that axotomy results in death in neurons close to the cut, **3)** that most GAP-43-IR normally present in the iris terminals, emanate from the SCG; **4)** that GAP-43-IR is upregulated in parasympathetic terminals of the iris after adrenergic denervation. Also, **5)** synthesis of synaptophysin is probably altered, since the normal appearance of synaptophysin-positive Golgi complex disappeared at 3 and 8 days post axotomy.

427.1

A LOW-POWER EPIFLUORESCENSCE MICROSCOPE FOR NEUROPHYSIOLOGISTS. D.R. McPherson[1]* and J.T. Buchanan[2], [1]SUNY Geneseo, Geneseo, NY 14454 and [2]Marquette U., Milwaukee, WI 53233.

For studies of the synaptic organization of lamprey spinal cord, we wish to use fluorescent retrograde labelling to identify and then impale labelled fluorescent neurons in vitro. As inverted microscopes do not work well with the lamprey spinal cord-notochord preparation, we have developed a low power, upright epifluorescent microscope that has long working distance like a stereo dissecting scope (40-80 mm) and light-gathering power comparable to a compound microscope operating at similar magnification. The design uses off-the-shelf modular components, along with a conventional camera lens mounted in reverse. Magnification depends upon the separation between camera lens and microscope nosepiece, and working distance depends upon the choice of camera lens. With a medium format lens, working distance is about 80 mm. Medium-sized labelled neurons are readily distinguishable and their impalement is facilitated by this device. Compared to previously published descriptions of low-power epifluorescent microscopes (Ratzlaff & Grinvald, J.Neurosci. Methods 36:127), our design requires a minimum of specialized machining. A schematic design of the microscope will be available. Supported by NIH NS09111 to D.R.M. and NS28369 to J.T.B.

427.2

Automated Recognition and Mapping of Immunolabeled Neurons in Developing Cortex. *L.S. Hibbard*[1], *J.S. McCasland*[3], *J.E. Brunstrom*[1], *and A.L. Pearlman*[1,2]. *Dept. of Neurology*[1] *and Cell Biology*[2], *Washington University School of Medicine, St. Louis, MO 63110, and Dept. of Anatomy*[3], *SUNY HSC, Syracuse, NY 13210*

During development, neocortical laminae are populated by neurons that have migrated from the ventricular zone. To aid in evaluating experimental perturbations that are applied to an organotypic slice preparation of embryonic cortex, we have developed a digital image analysis system to assess the migration of BrdU labeled neurons quantitatively. Entire cortical sections are digitized as mosaics of 500X fields using a computer-controlled scanning stage microscope, and the cortical zone is identified interactively as a region-of-interest (ROI) in a low magnification mosaic-image. The ROI delimits the parts of all the high magnification tile-images which intersect the cortical zone. All significant stained objects in the tile-images (inside the ROI) are detected by template correlation, and the detected objects are classified as either true neurons or non-neurons by a minimum risk, Bayesian decision rule designed to produce the minimum error (19% currently) on training and test data consisting of several thousand expert-identified cells. Functions of Gabor wavelets formed some of the features used by the decision rule. Plots of detected neuron densities provide a quantitative record of migration patterns that can be used in assessing the molecular signals that guide neuronal migration. (Support: NIH P01 NS 17763)

427.3

TWO-PHOTON IMAGING OF CULTURED RAT HIPPOCAMPAL NEURONS STAINED WITH DII, DIO, DIA, AND BODIPY CERAMIDE. S.M. Potter* and S.E. Fraser, Biological Imaging Center, 156-29, California Institute of Technology, Pasadena, CA 91125

In order to observe morphological changes in single living neurons over time, relatively non-invasive, high-resolution imaging techniques must be employed. Confocal laser-scanning microscopy offers high resolution, but its use has been limited by phototoxicity and by photobleaching of the fluorophore. Two-photon laser-scanning microscopy (TPLSM) greatly reduces these limitations; fluorescence, and thus bleaching and phototoxicity are limited to the focal plane. Due to the lack of a pinhole aperture, and the easy separation of excitation (infrared) and emission (visible) light, TPLSM systems have inherently better signal throughput than standard confocal microscopes. Excitation of UV dyes and molecular uncaging are also possible without UV lasers and special UV-transparent optics.

We have modified a standard confocal microscope (Molecular Dynamics Sarastro 2000) to use excitation light from a pulsed Ti:Sapphire laser (Coherent Mira 900), continuously tunable from 710-970nm. Using pulsed infrared illumination, we have imaged live rat hippocampal neurons in dissociated cultures and in cultured slices, obtaining better-than-confocal resolution, using a variety of neuronal tracers, such as DiI, DiO, DiA, and BODIPY ceramide. Two-photon excitation spectra in these specimens were determined, since the excitation wavelength maxima are not simply twice the one-photon maxima. We have observed little or no photobleaching or phototoxicity with continued scanning of specimens.

Thus, extended time-lapse or long-term 3-dimensional imaging of single living neurons at high resolution using standard neuronal tracers is now feasible, using TPLSM.

427.4

A COMPUTER TECHNIQUE TO ESTIMATE GOLD PARTICLE DENSITIES FOR ELECTRON MICROSCOPE POST-EMBEDDING IMMUNOCYTOCHEMISTRY

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Post-embedding immunocytochemistry employs gold particles to mark the location of antibodies within brain. Counts of these particles can be used to estimate antibody labeling density. We have developed a computer routine which allows the counting of gold particles using image analysis techniques. Images were captured on a JEOL 1210 electron microscope using a Kodak 1K CCD camera and JEOL Temcam 2.3 digital archiving software, transferred across a local area network to a MacIntosh IIci computer, and stored on optical disks. A macro task list developed using NIH Image was employed to count particles and compute particle density. Contrast and brightness were first manipulated using the look up table function in Image. The image was further processed with three enhancement filters: sharpen, convolve, and smooth. A 3 X 3 sharpening matrix was used to sharpen particle edges. A 9 X 9 'mexican hat' convolution was used to isolate particles. A 3 X 3 smoothing matrix was used to blur the background. A freehand selection tool was then used to draw around a profile or other region of interest (ROI) that contained gold particles. A routine then measured the area of the ROI. The isolated gold particles within the ROI were then captured as a binary image by thresholding the particles using the density slice command. Two binary operators (erode and dilate) were then applied to disconnect adjacent particles and a measurement command was employed to count the number of particles and mark their position. We analyzed three profile types in 83 digital micrographs taken from the cat superior colliculus after application of a glutamate antibody. Retinal terminals (RT) with round synaptic vesicles; profiles containing pleomorphic or flattened vesicles (Fs or PSDs); and myelinated axons (A). Analysis revealed that RT terminals contained approximately 2.5 times the density of particles contained in F or PSD profiles. Comparison of automated and manual counts showed a high correlation between the two methods of counting particles. Supported by NIH NEI R01-02973.

427.5

CONFIRMATION OF IMMUNOHISTOCHEMICAL REAGENT PENETRATION IN THICK SECTIONS BY 3D MICROSCOPY

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Iontophoretic injections of biotinylated dextran were made into the auditory cortex of the rat to label somata and neuronal profiles in the ipsi - and contralateral cortex. These tissues as well as skin and egg membrane samples were processed with conventional Streptavidin-biotin and histochemical methods to visualize: biocytin stained structures, keratin and salmonella respectively. These tissues were then examined and photographed using 3 dimensional light microscopic techniques.

The thickness of histological tissue sections vary widely (1 to 800 um) depending on the particular stain protocol. An overiding concern in all methods which require thick section is the question of complete reagent penetration. One approach for confirming reagent penetration is to attempt to visualize stained structures through the entire section. However, as the thickness of tissue sections viewed by conventional light microscopy increases to the point where it exceeds the depth of focus, the plane of focus must be continually adjusted. The 3 dimensional light microsope (Edge Scientific Inst. Corp.) has considerable depth of focus, permitting viewing of sections as thick as 120 um without continuous focus adjustments.

The results showed that this type of microscope is very useful when it is necessary to view microscopic structures which pass through the thickness of the entire section (e.g. axons, dendrites, ducts, vessels). In addition, with conventional light microscopes, linear structures appear as puncta, whereas the 3D microscope reveals theme to be linear structures passing through the tissue section orthogonal to the cut surface. Supported by Kirkegaard and Perry Laboratories and the Veterans Administration.

427.6

HIGH QUALITY ELECTRONIC IMAGES OF STAINED BRAIN SECTIONS.

J. I. Johnson*, W. I. Welker, and R. C. Switzer III, Anatomy Dept. and Neuroscience Program, Michigan State Univ., East Lansing, MI 48824; Dept. of Neurophysiology, Univ. of Wisconsin-Madison, Madison, WI 53706; Neuroscience Associates, Knoxville, TN 37922.

Our purpose was to determine the optimal means of capturing electronic images of stained sections of mammalian brains, at both high and low magnifications(whole sections). Three methods proved optimal, depending on the size of the field to be imaged. 1) For sections or fields over 25 mm in length or width, direct scanning of the tissue in a good quality desktop scanner (e.g. the LaCie Silverscanner II), using the Transparency mode; 2) For those less than 15 mm in length or width, a microscope is used with either a film camera (to make 35 mm slides for subsequent scanning) or a digital camera (in our tests, the Kodak DCS 200). 3) For those between 15 mm and 25 mm, optimal results are obtained using a camera (film or digital), our Leitz series (or their equivalents by Nikon) of "macro" (24 to 80 mm) lenses, and a specially designed, portable, slide holder and illuminator.

Images obtained from the desktop scanner or the digital camera had two advantages over those captured from film and subsequently scanned: 1) much less editing was needed to produce good final results, and 2) the electronic images were immediately available, avoiding the time-consuming processes of film development and subsequent scanning.

It was further determined that for our brain sections, computer image files of 150 pixels per inch, with a maximum dimension of 1050 pixels, stored as PICT files, compressed by the "high-quality" level of JPEG compression included in the PICT file creation module of the Adobe Photoshop program, proved best for economical electronic storage and transmission, for on-screen viewing, and for making good quality prints. An electronic version of this poster is in preparation for viewing on the Internet at http://www.neurophys.wisc.edu/brain/brain.html

(Supported by NSF grants BNS 9111952, IBN 9318819, and BNS 899438.)

427.7

COMPUTER MATCHING HOMOLOGOUS HISTOLOGICAL SECTIONS. F. S. Cohen, Z. Yang, J. Nissanov, Z. Huang & D.L. McEachron*. Computer Vision Center for Vertebrate Brain Mapping, Drexel Univ. Philadelphia, PA 19104.

An objective of computer vision based brain mapping is to develop techniques which will automatically combine images of sectional material derived from different animals. One step in the process is assigning the correct brain level of a given section. We report here on a coarse navigator which matches rat sections to a reference atlas using only external section contours. To assess its performance we employed an atlas consisting of 121 evenly spaced coronal sections extending across the length of one brain and 15 randomly selected coronal sections from each of 10 other brains. A semi-automatic procedure, thresholding followed by a minimum of manual editing, was utilized to generate the section contours. This did not always generate a perfect outline – for many images extraneous extensions from the section proper were also delineated – and as such this generated a realistic contour data set very much of the quality which will be encountered in normal laboratory settings. Identification of the homologous atlas section relied on B-spline modeling of the contours using a similarity measure which is based on the knot points associated with the B-spline curves, and the use of the B-spline affine invariant moments for alignment. The results were compared to classification by a human expert. Despite imprecision in the contour outlines, and variability between the different rat brains, 57% of the test cases were classified 12 sections or less from the correct section. This performance is adequate for a coarse navigator. Finer navigation will require consideration of some internal contours and a 3D reference atlas set. (Supported by NIH award P41RR01638, a Biomedical Technology Resource grant).

427.8

AN INTERACTIVE SYSTEM FOR VISUALIZATION OF THREE-DIMENSIONAL BIOLOGICAL STRUCTURES

D. Hessler, S. J. Young* and M. H. Ellisman. Dept. of Neurosciences, University of California San Diego, La Jolla, CA 92093-0603.

The increasing availability of three-dimensional (3D) digital data on biological structures acquired with electron microscope (EM) tomography or serial sections as well as confocal microscopy (CM) and other biomedical techniques has led to considerable interest in software for 3D analysis and visualization. Understanding 3D biological structures in volume data is an important and a difficult problem. Structures of interest are often spatially complex, intricately textured and may be obscured by other components. To facilitate 3D analysis and visualization, we have recently developed "DUCKY", a flexible and versatile software system for processing, rendering, and animating 3D datasets. DUCKY is based on a run-time interpreter that executes ordinary text scripts expressing a powerful language with features similar to those in C++. An interpreter provides several advantages including rapid modification and debugging of processing and rendering operations as well as improving portability. To maximize speed, precompiled functions are included for compute-intensive operations such as rendering. An interactive menu and window-based visualization environment is provided for executing scripts, manipulating renderings, debugging and modifying scripts. Because the system is interpreter based, changes in script parameters or operations are immediately evident in the 3D rendering. Scripts are provided for many common tasks. Additional scripts can be written to create new interactive environments and processes for unique tasks. Since volume or surface rendering may be optimal for revealing particular structures, both rendering methods are provided and may be combined in a single visualization. DUCKY can create animations of a sequence of different views of a structure either within a 3D volume or across volumes acquired in a CM time-series. Demonstrations will include: (1) combined volume rendering of correlated 3D datasets of spiny dendrites imaged by CM and electron tomography; (2) an animated walk-through of a segment of smooth endoplasmic reticulum from a Purkinje cell dendrite; and (3) combined surface and volume rendering.

427.9

NEUROZOOM: COMPUTER SOFTWARE FOR QUANTITATIVE NEUROANATOMIC MAPPING AND STEREOLOGY. W.G. Young*, E.A. Nimchinsky†, P.R. Hoft, F.E. Bloom, and J.H. Morrison†. Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037. †Fishberg Research Center for Neurobiology, Mount Sinai School of Medicine, NY, NY 10029.

Using SmalltalkAgents®, an object oriented programming environment, we developed NeuroZoom, a platform-independent set of programs designed to map molecular markers across multiple levels of resolution (i.e., from macro to ultrastructural) into 3D coordinate space. NeuroZoom appears as a standard Macintosh application with multiple document windows, floating palettes, and import and export features to standard file formats. Data layers, object hiding, cutting, pasting, and copying are supported.

By controlling the XYZ axes, NeuroZoom maps structures across the entire extent of the tissue section. Morphometric data tools map locations, rectangles, ovals, contours, and bezier curves. Biological tools map specialized structures, such as somata, nuclei, dendrites, axons, spines, etc. A database dictionary maintains the definitions of all structural objects, characterized by color, line thicknesses, symbols or icons. Specialized functions in NeuroZoom support automatic movement of the stage to adjacent fields of view, automatic splicing of fields, and storing locations of regions of interest when browsing sections. Quenching is minimized by using digital maps instead of live video. Live links to database repositories on the Internet and on local atlas templates are available, as well as 3D expression and manipulation of data objects.

Stereology in NeuroZoom creates collections of stereological disectors of known thickness in a specified volume of tissue, moving the stage to align and focus on each disector, and collecting data in a manner that is constrained by stereology protocols. Statistical analyses are performed and updated as data are entered. An unbiased estimate of the cell densities is then calculated for the area or volume of tissue section. Stereological quantification of length, surface area, and volume will be added. Supported by the Human Brain Project Consortium grant MHDA 52154.

427.10

MAPPING THE SURFACE OF INTACT FROZEN BRAINS USING A STRUCTURED LIGHT BASED 3D SCANNER. J. Nissanov*, C. Ozturk, D. Kozinska. Computer Vision Center for Vertebrate Brain Mapping, Drexel Univ. Philadelphia, PA 19104.

A significant problem in 3D reconstruction of brain tissue from histological material is alignment of the individual sections. Due to the intrinsic curvature of the brain, sequential registration of consecutive sections is inappropriate. Alignment based on blockface imaging, an approach with potentially very high accuracy, is quite expensive to implement. We are developing an alternative approach in which the surface of the tissue is obtained prior to cryosectioning and then utilized to guide registration. Toward that end, we have developed a structured light based technique for imaging frozen rat brains which we describe here.

In structured light imaging, the object is illuminated with a pattern of light while viewed with a camera from a different vantage point. The 3D location of given point within this pattern is computed by comparing its position in the camera image with that on the slide plane of the projector. Our approach relies on a novel coding scheme for the projected light which is based on 2D perfect submaps. Perfect submaps are rxv c-ary arrays in which every nxm c-ary submatrix is unique. In this application, the matrix consists of a 2D pattern of color dots with each uniquely defined by its color and that of its nearest neighbors.

To evaluate performance of this system we imaged 3 frozen rat brains. Each brain was placed on a turntable, rotated and images acquired at 10 degrees intervals to generate 36 views. On average each brain was sampled at over 1000 points. The result matched well the surface of the brain as evaluated by reconstruction from blockface images. (Supported by NIH award P41RR01638, a Biomedical Technology Resource grant).

427.11

SIMULTANEOUS MULTI-SITE RECORDING WITH TWO FLUORESCENT PROBES USING HIGH-SPEED LASER RANDOM-SCANNING MICROSCOPY. S.S. Patel*, A. Bullen and P. Saggau. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

The design and implementation of a high-speed random-scanning laser fluorescence microscope configured for simultaneous recording from dual optical indicators is presented. Previously, we have described a random-scanning system capable of high-speed, high-resolution measurements from small dendritic processes stained with voltage-sensitive dye (Bullen & Saggau, 1994). Here we extend this system to allow simultaneous recording of two fluorescent probes with similar excitation but separate emission spectra. This instrument is comprised of an argon laser (488 nm; Spectra-Physics) and an inverted epifluorescence system equipped with a 100X objective (N.A. 1.3; Zeiss). Two acousto-optical deflectors (1205C-2; Isomet) under computer-control were employed to randomly scan the laser beam at high frequency (>200 kHz). A DIC image was used to interactively select multiple points for optical recording. Emission light was processed by two dichroic mirrors (505 nm & 580 nm) and two emission filters (OG590 & 535/45 bandpass). Two photodiodes were used to capture the resulting fluorescent signals (S_i). A separate photodiode was used to sample the excitation beam reference (R). Each signal was digitized with 16-bit resolution at a frequency up to 133 kHz per detector. At submaximal scanning rates each scanning point was oversampled up to 8 times to improve the signal-to-noise ratio. Cultured hippocampal neurons were stained with two indicators. A voltage-sensitive dye (Di-8-ANEPPS, 100 μM) was applied by bath application. In parallel, an intracellular indicator (CaGreen or NaGreen; 25-100 μM) was introduced through a patch pipette. The fractional changes in fluorescence ($\Delta F_i/F_i$) were obtained by normalizing the ratio of S_i/R by its mean. Typically recordings were made at 6 kHz sampling rate (per scanning spot) and digitially filtered at 1.5 kHz. Spatial resolution was limited by spot size at the level of the preparation (7 μm). Representative dual wavelength recordings from neurons under current-clamp conditions will be presented.

427.12

DERIVATION OF NEURON GEOMETRY FROM CONFOCAL SCANS

Stephen Senft*, Department of Psychology and Center for Theoretical and Applied Neuroscience (CTAN), Yale University, New Haven, CT 06511.

Neuronal geometry is a limiting constraint on our understanding of brain architecture and function. Traditionally, such data has been extracted by tedious *camera-lucida* techniques.

We report an automatic computerized method (based on a thinning approach of Gong and Bertrand, 1990) for deriving the geometries of branched objects from high-resolution 3D confocal scans. Scanned data are partitioned into distinct collections of contiguous volume elements (voxels), and morphometric results are derived from the voxel subsets. The technique is demonstrated using scans of mammalian cerebral cortex.

The advantages of this method include: [1] volumetric data rapidly can be reduced to much more compact skeletal (medial axis) representations; [2] accurate coordinate and diameter information are retained for every voxel that is part of a neurite; [3] skeletons can be traversed automatically to characterize arbor topologies in terms of the number and location of branch and end points; [4] multiple arbors can be analyzed in a single data set; [5] the resulting geometries can be viewed in the context of the original scan and exported in formats useful as input to biophysical simulators.

Several unsolved problems remain that require operator assistance: in practice it can be difficult to automatically identify all voxels of a scanned object in the presence of noise. Loops resulting from inadequate resolution may need to be converted manually to branched structures. Skeletons from distinct scans may need to be annealed into one object. Certain fiducial features can be difficult to identify automatically. *Supported by CTAN.*

427.13

DENDRITIC GEOMETRY IN EPILEPTOGENIC CORTEX FROM THERAPY RESISTANT PARTIAL EPILEPSY (TRPE) AND RETT SYNDROME (RS) PATIENTS, A 3-D CONFOCAL LASER SCANNING MICROSCOPY STUDY P.V. Belichenko and A. B. Dahlström*. Russian Brain Res. Inst., Moscow, Russia; * Dept. of Anatomy & Cell Biology, Göteborg Univ., Medicinareg. 3-5, S-413 90, Göteborg, Sweden

Recently we described a strategy for studying the 3-D dendritic geometry at the microscopic level (1). Using this approach we have investigated biopsy material from the epileptogenic zone of 15 patients with TRPE, undergoing epilepsy surgery and compared these to autopsy brain material from 4 patients with epileptic RS and with 8 normal control cases. Different types of dendritic abnormalities were observed in these diseases (2,3). In the TRPE cases the results show different types of dendritic abnormalities on single pyramid cells in the epileptogenic cortex in layers I, II, III and V, and in the subcortical white matter. Many cells were observed to have two or three dendrites originating from the apical part, rather than, as in normal cases, one single apical dendrite. In RS cases the results demonstrated somewhat different types of dendritic abnormalities as compared to TRPE and control cases. The normally occurring specialization in pyramidal architecture in different cortical areas was absent in RS. Studies on the cytoarchitecture (Nissl staining) revealed neuronal migration disorders (microdysgenesis) in TRPE, but was not observed in RS. Rather, RS appears to be a postnatal developmental, probably genetic, disorder.

Supported by Swedish MRC (2207, 10808), Royal Swed. Acad. Sci., Stockholm, Royal Soc. Arts Sci, Göteborg, and ISF (33000).

References: 1) Belichenko & Dahlström, H.B.M., 1994, **1**:185, 2) Belichenko et al., Epil. Res., 1994, **18**:233; 3) Belichenko et al., NeuroReport, 1994, **5**:1509.

427.14

AUTOMATED COMPUTER TRACING AND MORPHOMETRY OF NEURONS: QUANTITATIVE ANALYSIS OF 3-D CONFOCAL IMAGES

Badrinath Roysam[1], Seetha Kodiyalam[1], Andrew R. Cohen[1], and James N. Turner[2]*. [1]Rensselaer Polytechnic Institute, Troy, NY 12180, USA; [2]Wadsworth Center for Laboratories and Research, Albany, NY 12201, USA.

Current manual and semi-automated methods for tracing and performing morphometry of neurons are tedious and time consuming, and require geometric assumptions that limit their morphometric accuracy. We have developed 3-D image analysis methods for fully automated tracing and analysis of neurons that are labeled with space filling dyes such as Lucifer yellow and HRP. Hippocampal pyramidal cells are impaled, classified electrically, filled with dye, fixed, cleared and imaged using a Bio-Rad MRC-600 confocal attachment and an Olympus microscope. The results of the computer analysis is a labelled laser printout of the tracing, and a computer database representation containing: i) location of all branching and end points, and neuronal spines, ii) spatial adjacency patterns, and voxel-by-voxel path representations, iii) lengths, tortuosities, volume and surface areas of all dendritic and axonal segments, iv) location, volume, and surface area of soma, and v) statistical analysis of all measured features. Comparison of automated results with manual tracing has shown excellent correlation and will be quantitatively compared at the meeting. Support: NSF DIR 9108492 and NIH RR01219.

427.15

MAGNETIC RESONANCE AXONOGRAPHY (MRX) OF THE HUMAN CENTRAL NERVOUS SYSTEM. T. Nakada*[1], H. Matsuzawa[1,2], N. Nakayama[2,3], I. L. Kwee[1], T. Kashiwaba[3], and H. Abe[2]. Dept. of Neurol., Univ. of Calif., Davis, CA 95616[1], Dept. of Neurosurg., Univ. of Hokkaido, Sapporo 060[2], Kashiwaba Neurosurg. Hosp., Sapporo 062[3]

*An*isotropy of apparent diffusivities (D_{app}) of water molecules is known to be an excellent means of elucidating detailed information regarding neuronal fiber direction and density in live subjects (axonography). We have recently developed a simple algorithm which allows for the extraction of the *an*isotropic term vector, D_{app}^{A}, from diffusion tensor, D_{app}^{ξ}, in pictorial form, which in turn provides a novel color-coded contrast for magnetic resonance imaging (MRI). MRI images obtained using this technique, three dimensional anisotropy contrast (3DAC), provided detailed anatomic resolution of the central nervous system (CNS), comparable to *in vitro* histology (NeuroReport 5:2053, 1994). In this study, we extended our investigations to the human CNS utilizing standard MRI systems (1.5T) widely available at clinical institutions. MRX appears to possess immediate applications not only in standard clinical settings but also in various aspects of human neuroscience.

427.16

NEAR-INFRARED SPECTROSCOPY (NIRS) OF THE HUMAN BRAIN WITH IMPROVED SPECTRAL AND TEMPORAL RESOLUTION. A. Villringer*, H. Obrig, Ch. Hirth, T. Wolf, U. Lindauer, U. Dirnagl, Dept. Neurology, Charité Hospital, Berlin

NIRS permits the noninvasive assessment of human brain oxygenation. Most studies so far have employed apparatus using between 2 and 7 different distinct wavelengths of light between 700 and 1000 nm. In order to improve spectral and temporal resolution, in the present study we implemented a whole spectrum CCD approach as previously described by Cope et al. (Adv. Exp. Med. Biol. 247:33). We used a MPP EEV-CCD camera (256x1024), the light source was a halogen lamp. While the spectral resolution was 0.6 nm (at one second sampling time), the best temporal resolution was 37 ms. Using this prototype system, in preliminary human studies, we were able to detect changes in hemoglobin oxygenation occuring during Valsalva maneuver and during oxygen breathing. In the rat, we were able to detect the hemoglobin oxygenation changes associated with spreading depression. When performing a Fourier-analyis of a series of spectra acquired of the human brain with a temporal resolution of 37 ms, we were able to detect frequencies between 60 and 80/min probably corresponding to the heart frequency. In some subjects, we also detected oscillations of approx. 7 Hz and 9-11Hz. We conclude that the CCD-camera approach is a promising technical improvement in NIRS.

427.17

QUANTITATION OF MICROVASCULAR ENZYME ACTIVITY IN RAT BRAIN SECTIONS BY IMAGE CYTOMETRY. L.E. De Bault*. Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Alkaline Phosphatase is one of several enzymes that have a very high expression in brain microvessels. The purpose of these experiments is to demonstrate the feasibility of performing enzyme cytochemistry in real time and quantitate the amount of enzyme by a kinetic method on a DISCOVERY™ *Flu· Or· bance* Image Cytometry System. Formalin-fixed, paraffin-embedded brain sections were incubated at 20°C in Tris buffer containing the sodium salt of 1-naphthyl phosphate as the substrate and fast blue BB as the simultaneous chromogen/coupling agent. Measurements of the same areas were taken at 5 or more time points, and the change in absorbance at 540nm recorded. Units of enzyme activity are calculated by first-order rate reaction method. It can be shown that microvessels of the subfornical organ, microvessels in surrounding brain tissue, as well as vessels in the choroid plexus are high but show varying amounts of enzyme activity. Further work on simultaneous kinetic measurements of 2 or more enzymes is in progress. When properly controlled this enzymecytochemical procedure combined with quantitative Image Cytometry can be used to monitor a variety of cytochemical procedures that use enzymes as indicators. (Supported in part by NIH grant NS-18775 and an OCAST to L.E.D.)

428.1

NITRIC OXIDE INDUCTION IN HUMAN GLIA. M. Ding,* B.A. St.Pierre, K. He, J.E. Merrill. Dept. of Neurology, UCLA School of Medicine, Los Angeles, CA 90024.

Although the importance and a fair understanding of nitric oxide (NO) synthesis in rodent cells has been well established, the induction of NO and regulation of inducible nitric oxide synthase (iNOS) in human cells appears to be substantia different and much less is understood about it. In the present study, we undertook to study the induction of iNOS and NO in human microglia and mixed astrocytes and microglia cell cultures generated from brains taken from fetal, neonatal and adult. By immunohistochemistry and *in situ* hybridization, we identified that both human microglia and astrocytes express iNOS in response to the combination of interleukin-1 (IL-1) and interferon-gamma (IFN-r) stimulation, but the expression was greater when antibody-opsonized myelin was added to the cytokines, especially in neonatal and adult cultures. Northern blot analysis also revealed the expression of 4.1 kb iNOS mRNA in these cultures. Moreover, the studies of the kinetics of NO induction in fetal mixed cultures suggested a delay of NO production after enzyme expression. The expression of mRNA coding for iNOS peaked at 6 hours but was always gone at 24 hours after stimulation, while the enzyme itself appeared at 24 hours, and the maximal level was at day 3, but was still evident at day 7. However, significant amounts of NO were not evident in the supernatant until day 3. NADPH diaphorase staining and nitro-tyrosine as detected by antibody were not observed until day 7. Taking these data together, we suggest that (1)both human microglia and astrocytes are able to express iNOS; (2) the induction of NO in human glia is developmentally controlled; (3) Antigen-antibodies complexes containing myelin induces NO; (4) the delay of production of NO, NADPH diaphorase and nitro-tyrosine suggests a possible post-translational regulation for human iNOS enzyme to be functional.

428.2

EFFECTS OF EICOSANOID & NITRIC OXIDE MEDIATORS ON MICROGLIAL REACTION TO SPREADING DEPRESSION. A.O. Caggiano* and R.P. Kraig. Dept. of Neurology, The University of Chicago, Chicago, IL 60637

To begin examining the mechanisms of gliosis, we studied the effects of inflammatory mediators on microglia made reactive (e.g. microgliosis) by spreading depression (SD), a benign perturbation that lacks cell necrosis.

We elicited SD in 63 halothane anesthetized male Wistar rats by micro-injection of 0.5 M potassium chloride (or 0.5 M sodium chloride, shams) to the parietal cortex for 3 hr at 9 minute intervals, with or without a single treatment with anti-inflammatory agents one hour prior to surgery. SD was confirmed by monitoring DC potentials in frontal neocortices. After a 3 day recovery, brains were removed, sectioned and processed immunohistologically with an antibody to the complement type III receptor, OX-42. Analysis of staining intensity was assessed using a computer-based image analysis system based on a 12-bit cooled CCD camera. The log ratio (lr) of optical density of the experimental cortex divided by the contralateral control cortex was used as a measure of microgliosis. SD induced a significant increase in the log ratio of OX-42 staining intensity (lr= 0.144, $p<.001$). Significant values were not induced, however in animals pre-treated with a single-dose of dexamethasone, a phospholipase A_2 inhibitor, nordihydroguaiaretic acid, a lipoxygenase inhibitor, L-NAM, a NO synthase antagonist (lr's = 0.055, 0.103, 0.092; p's >.05, .05, .05) or sodium nitroprusside, a NO donor, with ephedrine (lr=0.07, statistics incomplete). Pre-treatment with mepacrine or indomethacin, both cyclooxygenase inhibitors, or ephedrine alone did not prevent significant log ratios (p's < .05, .05 and .001, respectively).

We have demonstrated that the induction of microgliosis is sensitive to eicosanoids, particularly those generated by lipoxygenase, and NO. Gliosis, a component of brain inflammation, modulates not only the severity of brain injury but perhaps normal phenomena such as learning. Thus, inflammatory mediators (e.g., eicosanoids & NO) are likely factors which influence glia in their modulation of brain injury and remodeling associated with learning.

428.3

TETRAHYDROBIOPTERIN BIOSYNTHESIS IS REQUIRED FOR LIPOPOLYSACCHARIDE AND CYTOKINE-INDUCED NITRIC OXIDE PRODUCTION IN C6 GLIOMA CELLS. Carrol D'Sa, Anthony West, Maureen Hahn, Kei Hirayama and Gregory Kapatos* Cellular and Clinical Neurobiology Program, Department of Psychiatry and Behavioral Neuroscience, Wayne State University School of Med., Detroit, MI. 48201.

Tetrahydrobiopterin (BH4) is the essential cofactor for nitric oxide synthase and GTP cyclohydrolase I (GTPCH) is the first and rate-limiting enzyme in BH4 biosynthesis. We have examined the requirement of BH4 biosynthesis for the induction of nitric oxide (NO) by LPS and TNF-α in the astrocytic-C6 glioma cell line. Treatment with 10 μg/ml LPS and 50 ng/ml TNF-α caused a time-dependent thirteen-fold increase in the levels of BH4 which preceded NO production. The same stimuli led to a twenty-five-fold increase in GTPCH activity, with a corresponding increase in protein levels as indicated by Western blots. Northern blot analysis showed that levels of GTPCH mRNA were detectable by 3 hrs, reached maximal 25 hrs after activation and were sustained at high levels for at least 50 hrs. 2,4-diamino-6-hydroxypyrimidine, a competitive inhibitor of GTPCH, caused an 83% inhibition of the LPS and TNF-α induced BH4 levels with a corresponding 88% suppression of induced NO production. These results demonstrate that generation of NO from activated C6 cells is dependent on BH4 biosynthesis and suggest that pterin-synthesis inhibitors may be useful for therapy in certain pathophysiological conditions.(Supported by NIH NS26081 and The Joe Young Sr. Research Fund)

428.4

INDUCTION OF GTP CYCLOHYDROLASE I GENE EXPRESSION CONTROLS NITRIC OXIDE SYNTHASE ACTIVITY IN C6 GLIOMA CELLS. Kei Hirayama* and Gregory Kapatos, Cellular and Clinical Neurobiology Program, Department of Psychiatry and Behavioral Neuroscience, Wayne State University School of Med., Detroit, MI. 48201

Tetrahydrobiopterin (BH4) is the essential cofactor for all nitric oxide synthases (NOS), enzymes that produce nitric oxide (NO) from arginine. GTP cyclohydrolase I (GTPCH) is the first and rate-limiting enzyme in BH4 biosynthesis. Treatment of C6 glioma cells with LPS and cytokines such as TNF-α is able to induce NOS activity. We have recently shown that this treatment also induces GTPCH activity and BH4 levels. In order to study the interaction between BH4 and NOS, we have stably transfected C6 cells with rat GTPCH cDNA so that they constitutively express BH4. While BH4 was not detected in non-transfected C6 cells, transfected C6 cells synthesized large amounts of BH4 (0.98 ng/1x10 ^{5} cells). Both transfected and non-transfected cells were treated with 10 μg/ml of LPS and 50 ng/ml of TNF-α for 0-50 hrs and NOS activity in intact cells was measured. In transfected cells, induction of NOS activity was first detected following 2 hrs of treatment. In non-transfected cells NOS activity was not detectable until 7.5 hrs. The level of NOS activity induced by LPS and TNF-α was 3-fold higher in transfected cells than in non-transfected cells. Constitutive expression of BH4 in C6 glioma cells therefore results in a more rapid and robust induction of NOS activity following cytokine treatment, suggesting that in non-transfected C6 cells the level of expression of induced NOS activity is controlled by GTPCH gene expression. (Supported by NIH NS26081)

428.5

CYTOKINE-ACTIVATED ASTROCYTES INDUCE NITRIC OXIDE SYNTHASE TYPE II IN CEREBROVASCULAR ENDOTHELIAL CELLS.
R. Borgerding and S. Murphy*. Dept. of Pharmacology, Univ. of Iowa, Iowa City, IA 52242.

Astrocytes and cells of the cerebrovasculature can be induced by cytokines to express nitric oxide (NO) synthase type II (NOS). However, cerebral vessel-derived endothelial cells exposed to NO from either chemical or cellular sources prior to cytokines are markedly resistant to inducers of NOS. In addition, cytokine-activated astrocytes brought into close proximity with naive endothelium cause a marked induction of NOS in these cells, as revealed by northern blotting for NOS mRNA and their release of nitrite into the medium. Cytokines do not evoke the release of this 'inducing factor' from actinomycin D pretreated astrocytes, suggesting that it is dependent upon transcription. While TNFα is released from astrocytes under these conditions and will transcriptionally activate the type II NOS gene in endothelium, the use of pentoxifylline and neutralizing antibodies indicate that this is not the inducing factor. Using cultures of cytokine-activated cerebrovascular smooth muscle, endothelium and RAW macrophages we can also demonstrate their NOS-inducing activity in naive endothelial cells.

These observations suggest that the expression of NOS type II in cerebral vessels *in vivo* may be regulated by adjacent cells.
Supported by NS24621.

428.6

NITRIC OXIDE LIMITS TRANSCRIPTIONAL INDUCTION OF NITRIC OXIDE SYNTHASE TYPE II IN ASTROCYTES.
S.K. Park*, H.L. Lin and S. Murphy. Dept. of Pharmacology, Univ. of Iowa, Iowa City, IA 52242.

Expression of nitric oxide (NO) synthase type II (NOS) mRNA in astrocytes in response to cytokines is transient but can be amplified by NOS inhibitors or NO-trapping agents (Biochem. Biophys. Res. Commun. 201, 762, 1994) and stabilized by cycloheximide. The regulation by NO appears to be on transcription rather than destabilizing mRNA because: (a) actinomycin D abolished the effect, (b) nuclear run-on experiments reveal increased rate of transcription in the presence of NO-trapping agents, and (c) NO donors did not affect NOS mRNA stabilized by actinomycin D or cycloheximide.

From western blotting, using an antibody against the p65 subunit, we determined that antioxidants such as N-acetylcysteine and DMPO blocked induction of the NOS gene at a step downstream from translocation of the transcription factor NFκB to the nucleus. Exogenous NO (spermine NONOate) alone evoked activation and translocation of NFκB but did not affect translocation of NFκB evoked by cytokines.

These results suggest that NO might regulate NOS gene activation at the level of transcription factor interaction with the promoter, possibly via a redox mechanism.
Supported by NS29226.

428.7

SODIUM CHANNEL DISTRIBUTION IS ALTERED IN EXPERIMENTAL ALLERGIC NEURITIS (EAN). S.D. Novakovic*, S.R. Levinson+, and P. Shrager. Depts. of Physiol., Univ. of Rochester, Rochester, NY 14642 & +Univ. of Colorado, Denver, CO 80262.

Compromised axonal conduction produces well recognized clinical symptoms in EAN. Lewis rats were immunized with bovine root myelin + adjuvant. Using immunofluorescence, we have shown an alteration in Na^+ channel distribution in diseased spinal roots. There is a decrease in Na^+ channel density at the nodes of Ranvier, primarily in large axons, that is observable very early in the disease. The labeling changes from a highly focused fluorescent ring to a more diffuse axonal pattern and, as the disease proceeds, eventually becomes undetectable at the nodes. Clinical symptoms in most rats progress to complete hind limb paralysis, followed by complete recovery within a few weeks. In the second phase of the disease, a few days before clinical recovery starts, new clusters of Na^+ channels are seen. As in our previous study on lysolecithin demyelinated nerve (J.Neurosci. 15:492-502, 1995), these new aggregates form at the edges of newly adhering Schwann cells in regions that previously were internodal. Na^+ channel clusters associated with neighboring Schwann cells then appear to fuse, forming new nodes. In addition, some original nodes of Ranvier seem to regain Na^+ channels, probably due to some influence of associated Schwann cells. Supported by Natl. Multiple Sclerosis Society and NIH.

428.8

GLIAL CELL INTERCELLULAR PERMEABILITY IS INCREASED BY NERVE IMPULSES. R.K. Orkand* and H. Marrero. Institute of Neurobiology, University of Puerto Rico Medical Sciences Campus, 201 Blvd. del Valle, San Juan PR 00901, USA.

In the intact nervous system, glial cells form gap junctions with one another that permit the exchange of ions and small molecules important for their function in regulating the composition of the neuronal microenvironment. We have measured glial intercellular permeability by injecting the fluorescent dye, Lucifer Yellow (LY), into glial cells in the isolated intact optic nerve of the frog, *Rana pipiens,* and monitoring its diffusion into surrounding glial cells with a fluorescence microscope. Normally, 48% of the injections (n = 140) stained only one cell. In 44%, 2 -10 cells were stained and in 8% the dye spread profusely staining more than 20 glial nuclei. Dye coupling increased with electrical stimulation of the axons during the injection (10 Hz for 1 sec/2 sec for 2 min). Raising $[K^+]_o$ to 18-20 mM or adding 0.5 mM Ba^{2+}, to block K channels, which also depolarized glial cells, also increased gap junction permeability. Axonal stimulation in the presence of Ba^{2+} further increased coupling without additional glial depolarization. Thus, it appears that dye coupling of glial cells is increased by nerve impulses not only because of glial depolarization but also by an additional factor released from stimulated axons. The increased coupling helps the role of the glial cells in extracellular homeostasis.

(Supported by NSF (EPSCoR); NIH (PO1, RCMI, MIRDP)

428.9

THE GLUTAMATE TRANSPORTER GLT-1 IS DOWNREGULATED IN HYPERAMMONEMIA AND ACUTE LIVER FAILURE. Z. Huo, J.T. Neary, C.K. Petito*, and M.D. Norenberg. Departments of Pathology and Biochemistry & Molecular Biology, University of Miami School of Medicine and VA Medical Center, Miami, FL 33101.

Recent studies in our laboratory have indicated that ammonia impairs the ability of cultured astrocytes to take up glutamate [J. Neurochem 64 (Suppl):66,1995]. Since ammonia is believed to be a major toxin involved in the pathogenesis of hepatic encephalopathy (HE), we examined whether the glutamate transporter was also affected in animal models of HE as well as in hyperammonemia. Rats were treated with either saline, thioacetamide (TAA) (300 mg/kg; i.p.) or ammonium acetate (5 mmole/kg; i.p.) daily and sacrificed 2 hr after the fourth injection. RNA was extracted from the cerebral cortex and striatum by the guanidinium-thiocyanate-phenol-chloroform method. A 513 bp glutamate transporter (GLT-1) oligonucleotide probe was generated by RT/PCR based the sequence of Pines et al [Nature 360:464,1992]. Northern blot analysis revealed that GLT-1 mRNA was significantly reduced by 22% and 21% in the cortex of TAA and ammonia-treated rats, respectively. GLT-1 mRNA was reduced to an even greater extent in the striatum (TAA, 47%; ammonia, 40%). Our findings indicate that in acute liver failure and in hyperammonemia, there is decreased expression of the glial glutamate transporter. Such a defect could result in elevated extracellular levels of glutamate and possibly contribute to abnormal glutamate neurotransmission and/or excitotoxicity. (Supported by NIH grants DK38153 and NS30291, VA and GRECC)

428.10

INTRACELLULAR GLUTAMINE AS A FACTOR IN AMMONIA-INDUCED ASTROCYTE SWELLING. R.S. Dombro*, A.S. Bender, D.G. Hutson and M.D. Norenberg. VA Med. Ctr., Miami, FL 33125 and Depts. Surg., Pathol., Biochem. & Mol. Biol., Univ. Miami School of Med., Miami, FL 33101

Fulminant hepatic failure is associated with brain edema and astrocytic swelling. The mechanism for this swelling is poorly understood. Takahashi et al. (Am. J. Physiol. 261, H825, 1991) have proposed that ammonia-stimulated production of glutamine (GLN) may play a role in glial swelling by acting as an osmolyte. To test this idea, the effects of ammonia alone, and ammonia plus glutamate (GLU) on GLN levels were determined in cultured astrocytes by HPLC. Experiments were performed using GLN-free DMEM. Cell and medium concentrations of GLN were determined at 10 min, 1, 3, and 24 hr after treatment. Incubation with 1 and 5mM ammonia increased cell GLN at 24 hr by 2.6- and 2.1-fold, respectively. In the presence of 100μM GLU, the corresponding increases were 2.9- and 3-fold. With 1mM GLU, ammonia caused appreciably greater increases in cell GLN content, and these were observed at earlier time points (1 and 3 hr). Medium GLN was markedly decreased at 24 hr by ammonia (55% with 1mM and 76% with 5mM ammonia), suggesting that ammonia may inhibit GLN release. This was confirmed by loading cells with GLN-[^{14}C] and measuring its efflux. Treatment with 5mM ammonia for 1 day caused a 13% decrease, whereas 3 day treatment caused a 46% decrease of GLN release from astrocytes. Our findings indicate that glutamine accumulates in astrocytes following ammonia treatment and that one factor in this accumulation is the diminished efflux of this amino acid. Such accumulation of GLN may contribute to glial swelling and the brain edema associated with acute liver failure. (Supported by VA Medical Research Service, NIH grants DK38153 and NS 30291 and GRECC).

428.11

AMMONIA INHIBITS THE METABOTROPIC GLUTAMATE RECEPTOR IN CULTURED ASTROCYTES. J.H. Bruce*, J.T. Neary, Y. Itzhak and M.D. Norenberg. Departments of Pathology and Biochemistry & Molecular Biology, University of Miami School of Medicine and VA Medical Center, Miami, FL 33101.

Current evidence suggests that ammonia and related abnormalities in glutamate (GLU) neurotransmission play a major role in the pathogenesis of hepatic encephalopathy (HE). While changes in GLU receptors have been described in various models of HE, nothing is known about the properties of astrocytic GLU receptors in HE/hyperammonemia. Since astrocytes are believed to be critically involved in HE, we have initiated studies on the effects of ammonia on the astrocytic metabotropic GLU receptor (mGluR). Cultured astrocytes derived from neonatal rats were treated with 5 mM NH_4Cl for 4 days and properties of the mGluR were determined. GLU-stimulated inositol phosphate formation, which is mediated by mGluRs, was reduced by 60% following ammonia treatment. In fura-2 loaded cells, the transient Ca_i^{2+} peak response to quisqualate, an agonist for the mGluR, was reduced by approximately 50% and the number of cells responding to quisqualate was also reduced. Saturation binding experiments indicated a 35% decrease in the number of AP-3 sensitive [^{3}H]GLU-binding sites in ammonia-treated cells (control Bmax = 1010 ± 20 vs. ammonia Bmax = 658 ± 59 fmol/mg protein). Our findings indicate that ammonia downregulates the astroglial metabotropic glutamate receptor. This may alter glial function, possibly affecting neuronal excitability, and play a role in hepatic encephalopathy. (Supported by NIH grants DK38153, NS30291, VA and GRECC)

428.12

PHARMACOLOGICAL CHARACTERIZATION OF GLUTAMATE-INDUCED ASTROCYTE SWELLING. A.S. Bender*, A. Schousboe+, and M.D. Norenberg. Lab. of Neuropathology, Univ. of Miami Sch. of Med. & VA Med Ctr, Miami, FL 33101 and Dept of Biol. Sci. PharmaBiotec Res. Ctr., Royal Danish School of Pharmacy, DK-2100 Copenhagen, Denmark

L-Glutamate (L-GLU) is well known to induce astrocyte swelling; however, the mechanism is not well understood. In this study, we examined whether such swelling is mediated through GLU receptors, GLU uptake or K^+ channels. Cell volume was measured in cultured astrocytes using 3-0-methyl[^{3}H]-D-glucose method as described by Kletzien et al. (Anal. Biochem. 68:537,1975). L-GLU (1 mM) induced a two-fold increase in astrocyte cell volume, which was not stereospecific, since D-GLU, L-aspartate and D-aspartate at 1 mM induced swelling to about the same degree as L-GLU. K^+-channel inhibitors such as barium, TEA and 4-aminopyridine at 1 mM did not prevent L-GLU swelling, suggesting that K^+ channels are not involved in this process. With the exception of dihydrokainate, L-GLU uptake inhibitors (e.g., β-methylene aspartate, DL-threo-β-hydroxyaspartic acid and L-trans-2,4,PDC) at 1 mM all caused a comparable increase in cell volume when given alone, and did not prevent L-GLU induced swelling. L-GLU receptor agonists such as kainic acid, trans-(1S,3R)-ACPD, AMPA and ibotenic acid at 1 mM did not affect cell volume, whereas swelling was observed with the metabotropic receptor agonists, AP4 and quisqualate. Although involvement of glutamate uptake cannot be completely excluded, our studies indicate that activation of certain metabotropic glutamate receptors may play a role in the mechanism of glutamate-induced astrocyte swelling. (Supported by NIH grants DK38153, NS30291, VA and GRECC)

428.13

THE ROLE OF EXTRACELLULAR IONIC CHANGES IN UPREGULATING mRNA FOR GFAP FOLLOWING SPREADING DEPRESSION. D.J. Bonthius*, E.W. Lothman and O. Steward, Departments of Neurology and Neuroscience, Univ. of Virginia, Charlottesville, VA 22908.

While spreading depression has been shown to upregulate glial fibrillary acidic protein (GFAP) mRNA expression, the specific physiological signal underlying the upregulation is unknown. During spreading depression, extracellular ionic concentrations are altered markedly. This study evaluates the role of these ionic changes as signals influencing GFAP mRNA expression. Gel foam pledgets saturated with artificial cerebrospinal fluid solutions in which [Na+], [Ca++], [K+] and [H+] were altered one at a time to match concentrations seen in spreading depression were applied to exposed parietal cortex for one hour. Dot blot and in situ hybridization techniques were used to evaluate GFAP mRNA levels. Alteration of [K+] was the only ionic change that altered GFAP mRNA expression and led to a many-fold increase in GFAP mRNA levels at the time of maximum expression four days post-exposure. However, the upregulation in GFAP mRNA induced by the potassium exposure was totally blocked by prior administration of MK-801, an NMDA antagonist that blocks spreading depression. This suggests that the upregulation of GFAP mRNA was not due directly to the increased [K+], but to the spreading depression that the elevated [K+] induced. A further finding was that application to the cortex of 60 mM KCl can elicit spreading depression without detectable tissue damage and induces a large transient increase in GFAP mRNA content. This finding demonstrates that an upregulation in GFAP mRNA can occur in the absence of degeneration debris and that the initiating events can be related to physiological changes.

428.14

HETEROGENEITY OF BRAIN PEROXISOMES. A.M. Cimini*, I. Singh+, S.Moreno and M.P. Ceru'. Dept. of Basic and Applied Biology, University of L'Aquilla, L'Aquilla, Italy 67100 +Dept. of Pediatrics, Medical University of South Carolina, Charleston, S.C. 29425

Peroxisomes, a recently recognized subcellular organelle, is known to perform various functions including the metabolism of myelin lipids (plasmalogens and very long chain fatty acid). Morphological studies have previously suggested that peroxisomal functions in brain may be different in different neural cell types (Holtzman et al, 1979). We provide biochemical evidence that brain contains at least two subset populations of peroxisomes based on their density in a gradient and that these two populations of peroxisomes have different enzyme composition. In a Nycodenz density gradient, we observed two different peroxisomal populations characterized by different equilibrium densities and different size. The peroxisomes equilibrated at a density of 1.12gm/ml were of smaller size (0.12μM diameter) and the peroxisomes equilibrated at a higher density of 1.175gm/ml had a large size (0.2μM diameter). The two populations seem to be different also in the enzymatic composition. The lighter peroxisomes (d=1.12gm/ml) had activity for dihydroxyacetone phosphate acyl-transferase (DHAP-AT) and D-amino acid oxidase activities; whereas heavier peroxisomes (d=1.175gm/ml) had high activities for catalase and D-amino acid oxidase. We also examined the latency of these enzymes in these two populations of peroxisomes. Catalase, acyl-CoA oxidase had a latency of 80 to 90 percent; whereas d-amino acid oxidase and DHAP-AT showed no detectable latency. We postulate that peroxisomes with different enzymatic composition for specific functions may be present in different neural cell types. Studies are in progress to characterize peroxisomes in different cell types in brain.

428.15

AGMATINE AND ARGININE DECARBOXYLASE ACTIVITY ARE EXPRESSED IN GLIAL CELLS. S. Regunathan*, D.L. Feinstein, L. Lyandvert, W. Raasch and D.J. Reis. Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

Agmatine, decarboxylated arginine, an endogenous ligand for imidazoline (I-) and α_2-adrenergic receptors has, with its biosynthetic enzyme arginine decarboxylase (ADC), been discovered in mammalian brain (Li et al., *Science* 263:966, 1994). While agmatine is stored in some central neurons, its cellular localization in CNS is not fully known. We investigated whether astrocytes store and synthesize the amine and whether it binds to astrocytic I-receptors (Regunathan et al., *J. Neurosci. Res.* 34:681, 1993). By HPLC measurement, the amount of agmatine was found to be 8.5 $\pm$ 1.4 and 1.8 $\pm$ 0.6 nmol/mg protein in astrocytes and C6 cells, respectively. Agmatine was immunocytochemically localized with a specific polyclonal antibody to cytoplasm of astrocytes cultured from neonatal rat cortex and in rat C6 glioma cells. ADC activity was present in membranes of astrocytes (85.4 $\pm$ 9.2) and C6 cells (18.2 $\pm$ 3.12 nmol/hr/mg protein) indicating that the amine was locally synthesized. Exposure to bacterial lipopolysaccharide (LPS; 1 µg/ml) decreased ADC activity in C6 cells (to 70% of control) but did not affect astrocytic ADC. Interferon-γ (20 Units/ml) significantly ($p<0.05$) increased ADC activity in astrocytes to 130% and C6 cells to 150% of controls. ^{3}H-idazoxan binds with high affinity to I_2 receptors on membranes of cultured astrocytes (K_D: 6.2 nM; B_{max}: 210 fmol/mg protein) but not C6 cells. Astrocytic binding was inhibited by agmatine (K_i:450 nM). We conclude: (a) agmatine is synthesized and stored in astrocytes; (b) astrocytic ADC can be regulated by cytokines; (c) agmatine binds to astrocytic I_2 receptors; (d) agmatine may be an autocrine message in astrocytes.

428.16

CHARACTERIZATION OF AN INWARDLY RECTIFYING CHLORIDE CONDUCTANCE IN DIBUTYRYL-CYCLIC-AMP TREATED CORTICAL ASTROCYTES. S. Ferroni, C. Marchini, M. Nobile° and C. Rapisarda. *Department of Human and General Physiology, University of Bologna, 40127 Bologna, Italy. °Institute of Cybernetic and Biophysics, CNR, 16149 Genova, Italy. (Spon. E.N.A.)*

Prolonged incubation (1-3 weeks) with dibutyryl-cAMP of new-born rat cortical astrocytes in culture induces the expression of inwardly rectifying chloride (Cl^-) and potassium (K^+) conductances. We characterized with the whole-cell patch-clamp technique the gating and pharmacological properties of the Cl^- conductance using intra- and extracellular solutions with symmetrical Cl^- and with monovalent cations substituted by N-methyl-D-glucamine. Hyperpolarizing steps from an holding potential (V_h) of 0 mV activated inward currents which had a slow activation kinetic and did not inactivate. The conductance activation was described by a Bolzman equation. The calculated half conductance was at -70 mV, the slope 18 mV and the gating valence 1.5. Variating V_h of $\pm$ 30 mV did not change the ampitude of the currents. Tail currents analysis performed using different extracellular Cl^- concentrations confirmed the anion selectivity. The pharmacology profile showed that extracellular application of 1 mM DIDS and SITS did not affect the currents. In contrast 1 mM anthracene-9-carboxylate or 1 mM niflumic acid decreased the currents of ~ 50%. Moreover, cadmium and zinc blocked the currents with an half inhibitory concentration of 0.2 and 0.5 mM respectively. These data suggest that the strengthening of the cAMP signalling induces the expression of a Cl^- conductance which can be involved together with the inward rectifying K^+ conductance in the process of the extracellular K^+ buffering via the mechanism of the local accumulation.

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428.17

AUGMENTATION OF EXTRACELLULAR H^+ BUFFERING BY EXOGENOUS CARBONIC ANHYDRASE IN VITRO & IN VIVO. M. Chesler*, W. Huang and S.E. Smith. Depts.Physiol./Neurosurg. NYU Med. Ctr., 550 1st Ave., NY, NY 10016.

In brain, rapid pH buffering is insured by extracellular carbonic anhydrase (CA) [1,2]. We tested whether buffering can be increased by adding CA to Ringer superfused over rat brain slices or cerebral cortex. Artificial alkaline shifts were created by OH^- iontophoresis near a pH microelectrode. Endogenous alkaline shifts were evoked by AMPA ejection. At ~ 0.01 µM, CA had no effect, but at ~ 0.10 µM, CA reversibly decreased the alkaline shifts within 5-10 min. (-61 $\pm$ 4% n=11 slices & -60 $\pm$ 9% , n=6 slices for OH^- and AMPA responses respectively). In HEPES Ringer, CA had no effect on the pH shifts. AMPA-evoked alkaline shifts were also decreased (-53 $\pm$ 6%, n=4 rats) when ~ 0.10 µM CA was superfused over rat cortex *in vivo*. Our data show that CA readily diffuses in brain extracellular space. Thus, endogenous CA activity is unlikely to be an artifact of CA persisting from cell damage. Our results suggest that endogenous [CA] is low, equivalent to exogenous enzyme concentrations of ~ 0.01 - 0.10 µM. These data are consistent with previous PCO_2 and HCO_3^- recordings during alkaline shifts [3], which suggested low extracellular CA activity, insufficient for rapid CO_2-HCO_3^- equilibration. Supported by NIH grants NS32123 & NS10164. [1] Chen & Chesler. *PNAS* 89:7786 [2] Kaila et al. *NeuroReport* 3:105. [3] Chesler & Chen. *Soc. Neurosci. Abstr.* 19:267.

428.18

ENDOGENOUS, PROLIFERATING CELLS IN ADULT RAT WHITE MATTER RESPOND TO INJURY. J.M. Gensert, G.R. Tibbs* and J.E. Goldman. Depts. of Pathology & Physiology, Columbia Univ., NY, NY 10032.

Dividing cells persist in the adult mammalian CNS. Although the antigenic profile of at least a subpopulation of these cells is known, their response to injury has not been described previously. Proliferating cells in adult rat subcortical white matter were examined *in vivo* using stereotactic injections of a replication-deficient retrovirus that expresses ß-galactosidase. These cells were visualized by X-gal histochemistry or by immunocytochemistry with anti-ßgal antibodies. An antigenic profile of the labeled cells was established, and their response to injury was examined. The initially labeled population of proliferating cells exhibited an immature morphology and did not express markers of mature glia 2 dpi. Nestin colocalized with ßgal in approximately half of the labeled cells, and GST-Yp immunoreactivity was detected in approximately half of the ßgal labeled cells; morphologically, these represented destinct populations of proliferating cells. Thirty days after introduction of retrovirus, however, approximately 20% of the labeled cells expressed Rip demonstrating that at least some members of the population of proliferating cells in adult CNS retain the capacity to differentiate and express markers of mature glia *in vivo*. The response of these cells to lesions was examined by disrupting cytoarchitecture with stab wounds and by demyelinating with lysolecithin. These cells responded to injury *in vivo* with an increase in proliferation as assayed by cell counts and BrdU incorporation. At longer time points, many of the ßgal labeled cells differentiated into cells with astrocytic and myelinating oligodendrocytic morphologies. Thus, endogenous cells can develop through oligodendrocyte and astrocyte lineage pathways *in vivo* in response to CNS injury in the adult brain. The signals responsible for proliferation and differentiation of these cells in response to injury remain to be determined.

428.19

TAURINE TRANSPORT KINETICS OF ASTROCYTES EXPOSED TO HYPER-OSMOTIC CONDITIONS. J.W. Beetsch* and J.E. Olson. Departments of Emergency Medicine and Physiology and Biophysics, Wright State University School of Medicine, Dayton, OH 45401.

Hyperosmotic exposure of cultured cerebral astrocytes leads to persistent increases in intracellular taurine content. Taurine uptake rates increase after 24 hr, but then decrease after 2 weeks of hyperosmotic exposure. These results indicate multiple mechanisms contribute to the accumulation and maintenance of high intracellular taurine after hyperosmotic exposure. In this study, we examined changes in taurine regulation in cultured rat astrocytes by comparing rates of uptake and release after growth in isoosmotic and hyperosmotic conditions. Confluent cultures of cerebral rat astrocytes were exposed to control (300 mOsm) medium or hyperosmotic (350, 400, 450 mOsm) medium adjusted with NaCl. After 1, 24, or 48 hr, kinetic parameters of taurine uptake were determined using ^{3}H-taurine together with unlabeled taurine (5-1000 μM). Efflux of preloaded ^{3}H-taurine was measured in parallel studies at these same times. Intracellular taurine content was determined in efflux cultures by HPLC. Compared to control cultures grown in 300 mOsm medium, K_m of taurine uptake was not altered after 48 hr hyperosmotic exposure. V_{max} increased 39% after 24 hr exposure to 450 mOsm medium. After 48 hr exposure to this medium, V_{max} returned to control values. Taurine release from cultures exposed to 450 mOsm medium for 1 and 8 hr increased 2.8 and 3.8 fold, respectively, when compared to control cultures. After 24 and 48 hr, release rates decreased to levels significantly ($p<0.01$) lower than control cultures. Thus, taurine accumulation by cultured cerebral astrocytes exposed to hyperosmotic conditions is due to an alteration in the rates of influx and efflux. Short term hyperosmotic exposure induces an uptake from the extracellular space while prolonged exposure decreases the amount of taurine lost from the cells. Supported by NIH (NS 23218).

428.20

APOLIPOPROTEIN E RECEPTOR PHENOTYPE CHANGES WITH ASTROCYTIC AND OLIGODENDROCYTIC DIFFERENTIATION OF THE 4C8 MOUSE GLIAL CELL LINE. M.C. Irizarry, G.W. Rebeck, D. Strickland, B.T. Hyman, G.W. Van Hoesen*, and C.A. Dyer. Neurology, Mass. Gen. Hosp, Boston, MA 02114; Anatomy, Univ of Iowa, Iowa City, IA 52242; American Red Cross, Bethesda, MD 20855; and Biomedical Sciences, E.K. Shriver Center, Waltham, MA 02254.

The physiologic actions of apolipoprotein E (apoE) in the brain are mediated by apoE receptors--low density lipoprotein receptor (LDL-r), low density lipoprotein receptor-related protein (LRP), very low density lipoprotein receptor (VLDL-r), and GP-330. The 4C8 cell line was subcloned from the glioblastoma cell line MOCH-1 derived from a myelin basic protein/ *c-neu* transgenic mouse. 4C8 cells cultured in low serum conditions resemble O-2A glial progenitor cells morphologically and possess immature myelin markers. LRP and VLDL-r are weakly expressed in these cells. Cells cultured in defined media without serum have the morphologic appearance of mature oligodendrocytes, express all the known major myelin markers, and lack astrocytic markers (GFAP). These oligodendrocytic cells strongly express VLDL-r and weakly express LRP. VLDL-r immunostaining is confined to vesicles in the cell body. 4C8 cells grown in 10% serum resemble reactive astrocytes, expressing GFAP but no myelin markers. These cells strongly express LRP and only weakly express VLDL-r. LRP immunostaining is present in vesicles predominantly located within the cytoplasm and at the tips of processes. Astrocytic 4C8 cells secrete apoE and a soluble form of LRP into the media. All three forms of 4C8 cells express LDL-r and lack GP-330. These results suggest that VLDL-r and LRP serve distinct roles in oligodendrocyte and astrocyte function.

BLOOD-BRAIN BARRIER: PATHOLOGY AND DISEASE

429.1

NEUROCHEMICAL AND CELLULAR MECHANISMS OF RMP-7, A TUMOR-SELECTIVE ADJUVANT TREATMENT FOR NEURO-ONCOLOGY. R.L. Dean[1]*, P.J. Elliott[1], C.M. Oyler[1], S.K. Fisher[2], E.L. McEwen[2] & R.T. Bartus[1]. Alkermes Inc.,[1] 64 Sidney St., Cambridge, MA 02139 and Neuroscience Laboratory,[2] University of Michigan, Ann Arbor, MI 48104.

The blood brain barrier (BBB) severely limits diffusion of chemotherapeutics from the vasculature to brain and brain tumors. To overcome this therapeutic obstacle the bradykinin B2 analog, RMP-7, was designed to have a longer half life and improved selectivity compared to bradykinin. Recent data in a rat glioma (RG2) model (Inamura et al., J. Neurosurg., 1994) not only demonstrated that RMP-7 could permeate the BBB, but that selective permeation of the blood tumor barrier was induced, providing several-fold greater uptake of labeled compounds into the tumor, relative to normal brain. The current work investigates the mechanism(s) of action of RMP-7, as well as gains insight into the nature of its selectivity for permeating brain tumor barriers.

We have demonstrated that in SH-EP cells, a human neuroblastoma x epithelial cell line: (1) RMP-7 induced a B2 receptor-mediated PI response as a key intracellular signal in a dose-dependent manner (EC50 = 30-50 nM); this EC50 is equivalent to that induced by bradykinin. (2) RMP-7 resulted in a marked rise in cytosolic calcium ($[Ca^{2+}]i$) levels (approx. 500nM). The subsequent addition of bradykinin (1 μM) did not elicit any further increase in $[Ca^{2+}]i$. The rise in $[Ca^{2+}]i$ induced by RMP-7 was largely independent of extracellular Ca^{2+} (EC50=40nM). In both series of studies, these responses were abolished by the selective B2 antagonist, HOE-140 (0.1-1.0μM), but not by the selective B1 antagonist, des Arg^{10}-HOE-140 (0.1-1.0μM). These data, thus, provide direct empirical support for biochemical and cellular mechanisms of action of RMP-7

429.2

INTRAVENOUS ADMINISTRATION OF RMP-7 SELECTIVELY INCREASES UPTAKE OF CARBOPLATIN INTO RAT BRAIN TUMORS. P. Elliott*, N. Hayward, M. Huff, T. Nagle, R. Dean, D. Blunt, C. Kim, C. Oyler, A. Schutz, K. Black[1] & R. Bartus. Alkermes Inc., Cambridge, MA 02139 and UCLA Med. Center,[1] Los Angeles, CA 90024.

The brain is isolated from blood-borne-agents by the tight intercellular junctions between the vascular endothelial cells that comprise the blood-brain barrier (BBB). A similar barrier exists around brain tumors, creating a formidable obstacle to effective treatment with chemotherapeutic agents. Intra-arterial administration of the hyper-osmotic agents mannitol and arabinose have been used clinically and are associated with lasting damage to the barrier and increased drug delivery to normal brain, relative to brain tumor. Thus, these methods can increase the neurotoxicity of the CNS.

An alternative approach employs the selective bradykinin B2 agonist RMP-7 which has been shown to selectively open the blood-tumor barrier in a rat glioma (RG2) model (Inamura et al., *J. Neurosurg.*, 1994). This selectively in permeating the blood-tumor barrier offers significant potential advantages for chemotherapy. The present study replicates and extends these earlier observations by: (1) illustrating a significant ($p<0.001$) increase (1.5 fold) in uptake of the chemotherapeutic, ^{14}C-carboplatin, into brain tumor with IV administration of RMP-7 (4.5 ug/kg continuously infused for 15min), (2) demonstrating enhanced carboplatin uptake into the brain area immediately surrounding the tumor with substantially lower amounts in brain tissue distal to the tumor, (3) demonstrating no increase in uptake into peripheral (sciatic) nerve, and (4) characterizing the physiologic state (e.g., blood pressure, body temperature, blood gases, etc.) of brain-tumor-bearing animals treated with RMP-7 versus vehicle, thereby demonstrating that conventional hemodynamic bradykinin responses are not associated with enhanced permeability. These data are discussed as they relate to current attempts to treat gliomas in humans.

429.3

BRADYKININ PERMEATION OF THE BBB: EVIDENCE FOR A SENSITIVE, AUTO-REGULATED, RECEPTOR-MEDIATED SYSTEM. R.T. Bartus[1]*, P. Elliott[1], N. Hayward[1], K. Matsukado[2], & K. Black[2]. Alkermes Inc.,[1] Cambridge, MA and UCLA Med Ctr,[2] Los Angeles, CA

The cytoarchitecture of vessels supplying the brain excludes hydrophilic substances from diffusing into the brain. A primary component of this blood brain barrier (BBB) is the tight junctions between membranes of opposing endothelial cells. The endothelial cells comprising the BBB have a variety of peptide receptors linked to intracellular second messengers (implying complex and sensitive signal transduction systems) and an unusually high concentration of mitochondria (implying high energy demand). Recent data also suggests that one peptide, bradykinin, can cause permeability of the BBB through a B2 receptor-mediated pathway. Using RMP-7, a bradykinin analog that has greater B2 selectivity and plasma stability, we have previously shown that bradykinin permeates the mouse BBB by opening its tight junctions (Sanovich et al., Soc Nsci, '94). Corroborating data for RMP-7's ability to permeabilize vascular barriers in brain and eye has been obtained in rats, guinea pigs, dogs and humans.

In the present studies, we used continuous carotid infusions of RMP-7 (0.1 ug/kg/min), in conjunction with quantitative autoradiography, to study the kinetics of bradykinin's permeation of the BBB to radio-labeled 70kDa dextran. RMP-7 caused a rapid opening of the barrier (e.g., 3-fold increase in Ki within 10min; $p<.0001$) but this permeability was short-lasting (BBB integrity restored within 10 min of cessation of RMP-7; $p>.10$). The response also appeared to be auto-regulated, for permeability decreased rapidly (i.e., to <1/3 Ki within 30min; $p<.001$), even with constant carotid infusion of RMP-7. These studies suggest a very sensitive, but tightly regulated bradykinin system which transiently opens the BBB, for still unknown physiologic purposes.

429.4

RMP-7 SELECTIVELY INCREASES UPTAKE OF CARBOPLATIN INTO RAT BRAIN TUMORS AND SURROUNDING BRAIN. N. Hayward[1]*, P. Elliott[1], M. Huff[1], T. Nagle[1], A. Schutz[1], K. Matsukado[2], K. Black[2] & R.T. Bartus[1]. Alkermes Inc.,[1] Cambridge, MA 02139 and UCLA Medical Center,[2] Los Angeles, CA 90024.

Many agents in the blood are restricted from entering the brain by an anatomical barrier (BBB) comprised of tight intercellular junctions between the endothelial cells of the microvasculature. A similar barrier is found around brain tumors, creating a formidable obstacle to their treatment. The blood-tumor barrier can be selectively permeated by the peptidergic bradykinin agonist RMP-7 in a rodent brain glioma (RG2) model (Inamura et al., *J. Neurosurg.*, 1994).

The present study replicates and extends these observations by demonstrating: (1) enhanced uptake of ^{14}C-carboplatin into brain tumor (1.5-2.0 fold; $p<0.01$) following intracarotid infusion of RMP-7 (0.5-1.5 ug/kg for 5-15min); (2) enhanced uptake of ^{14}C-carboplatin into brain surrounding tumor (up to 2 mm) by RMP-7; although the ki was lower than in tumor, it was significantly higher in RMP-7-treated relative to vehicle-treated animals (2.0 fold; $p<0.01$); (3) enhanced efficacy when RMP-7 was combined with carboplatin (10 mg/kg, IA), reflected in increased survival rates of glioma animals; and (4) that changes in hemodynamic variables often associated with systemic bradykinin (e.g., decreased blood pressure, alterations in blood gases, etc) can be dissociated from the BBB permeability effects and thus are not responsible. These data are discussed as they relate to current attempts to treat recurrent gliomas in humans.

429.5

INTRAVENOUS RMP-7 SELECTIVELY OPENS THE GUINEA PIG BLOOD-RETINAL BARRIER. J. Mackic[1]*, P. Elliott[2], R. Bartus[2] & B. Zlokovic[1]. Dept. Neurol. Surg., USC, Los Angeles, CA 90033 [1], Alkermes Inc, Cambridge, MA 02139 [2]

RMP-7 is a selective B2 agonist with a longer in vivo half life than bradykinin. It has been shown to selectively increase the permeability of the blood-tumor barrier in rats to a number of different molecular weight compounds (Inamura et al., 1994). As such, it is currently in clinical trials as an adjunct to the chemotherapeutic agent carboplatin. RMP-7 is believed to achieve its effects through the opening of tight junctions between endothelial cells via activation of B2 receptors. We have recently shown that intracarotid infusions of RMP-7 can permeate the blood-retinal barrier (BRB) of the guinea pig (Elliott et al., 1995). Currently there are many eye disorders for which treatment is impeded by the BRB, such as cytomegalovirus retinitis (CMV). This study investigated the effects of intravenous (IV) infusion of RMP-7 on uptake of the anti-viral agent, ganciclovir, into the guinea pig eye and brain.

RMP-7 (1μg/kg; IV infusion) was given to guinea pigs as a 5min pretreatment, followed immediately by an IV infusion of 3H-ganciclovir for periods of 15sec to 10min. At the end of the radiolabel administration, the animals were sacrificed and the brain and eyes were rapidly removed and dissected. Levels of radioactivity within various eye segments and the brain were then determined using liquid scintillation counting.

Results illustrate that IV administered RMP-7 significantly increases the uptake of ganciclovir (up to 2 fold) into the guinea pig retina and lens epithelium but did not increase uptake into the brain of the same animals. Significant uptake into the retina and lens was also evident 30 min after cessation of the ganciclovir (5min) infusion. These data highlight the selective nature of RMP-7'S effect and support its potential clinical use as an adjunctive treatment to various eye disorders, including CMV.

429.6

THROMBIN PROTECTS THE BLOOD-BRAIN BARRIER DURING ACUTE HYPERTENSION. J. L. Williams* and C. A. Hathaway. Univ. of South Dakota School of Medicine, Vermillion, SD 57069

Thrombin increases endothelial permeability in cell culture, and pretreatment with L-NAME further increases permeability (*Circ. Res.* 76:199, 1995). Our experiments examined the effects of thrombin alone and combined with L-NAME on blood brain barrier (BBB) permeability in vivo. Evans blue dye was used as a marker for permeability of the BBB in Sprague Dawley rats. Evans blue (3 ml/kg of 2.5% solution) was given intravenously to anesthetized rats and allowed 5 min to circulate. The BBB was then compromised by an abrupt increase in arterial pressure with a bolus of phenylephrine (15 μg IV). Mean arterial pressure (MAP) increased 63.4 ± 21 mmHg (mean ± SD) in control rats and 56.8 ± 11 mmHg in thrombin-treated rats. When MAP started to fall, control rats received a 1 ml bolus of saline, and treated rats received 80 units of thrombin. After the rats were killed, their brains were removed and examined and graded for coloration by a blinded observer. In five pairs of experiments, the brains of the thrombin-treated rats had less staining than sham-treated brains ($p<0.05$). In a similar set of experiments, either 30 mg/kg of L-NAME or saline was given IV to thrombin-treated rats. L-NAME was allowed to circulate for 30 min and MAP rose 18 ± 12 mmHg after treatment. Phenylephrine produced a rise in MAP of 78 ± 11 mmHg in control rats and 83 ± 16 mmHg in L-NAME-treated rats. Both groups were given 80 units of thrombin as soon as MAP started to return towards baseline. The rat brains were evaluated and graded for coloration by a blinded observer. In five groups of experiments, the rats that were treated with L-NAME had brains that were darker than sham-treated rats ($p<0.05$). These findings suggest that thrombin has a protective action on BBB by reducing the increase in permeability caused by an abrupt increase in MAP, possibly by increasing the release of nitric oxide.

429.7

AN AGED RAT STUDY OF ALTERATIONS IN CHOROID PLEXUS EPITHELIUM AFTER ISCHEMIA INDUCED BY CAROTID OCCLUSION. C. Johanson,* D. Palm, M. Primiano, P. Chan, N. Knuckey, and E. Stopa. Dept. Clin. Neurosci., Prog. in Neurosurg., Brown U. & RI Hospital, Providence, RI 02903

In late stages of life, the appearance in choroid plexus (CP) of calcification and hyalinization of connective tissue and blood vessels, undoubtedly alters the secretory and vascular functions of the blood-CSF barrier. Thus, in both elderly humans and *aged* rats, there are substantial reductions in baseline cerebrospinal fluid (CSF) production rate. Given the important role of the choroidal epithelium in CNS extracellular fluid (CSF) homeostasis, it is important to know how the *aging* CP recovers from insults such as interruption of its blood supply.

We used the bilateral carotid occlusion model with hypotension, i.e., 40-45 mmHg, to induce transient forebrain ischemia (TFI). Sprague-Dawley rats, 1.5 to 2 yr of age, were anesthetized with halothane and subjected to carotid flow interruption for 10 min; following reperfusion of the carotids, the animals were then allowed to recover for 30 min or 24 hr. Sham surgical controls were also run.

At 30 min, the lateral ventricle plexus epithelium in aged rats was severely damaged, as evidenced by swollen mitochondria, extensive vacuolization of the cytoplasm, and disappearance of intact apical microvilli. However, by 24 hr of reperfusion following the ischemic insult, the ultrastructure of the choroidal epithelium was restored to a near-normal state. The findings indicate that the blood-CSF barrier in *aged* rats is resilient, and it is apparently as capable of recovering from TFI (10 min) as is the case for young adult rats subjected to a similar degree of blood flow interruption.

Supported by funds from R.I. Hospital, Brown U. Fellowship (D.E.P.), and NIH NS 27601 (C.E.J.)

429.8

BLOOD BRAIN BARRIER PERMEABILITY TO IL-1RA AND BSA AFTER FOCAL CEREBRAL ISCHEMIA IN THE RAT. J.K. Relton* and K. Ash. Dept. Biology, Amgen Inc., 3200 Walnut St., Boulder, CO 80301.

Blood brain barrier (BBB) permeability to the recombinant human protein interleukin-1 receptor antagonist radiolabelled with ^{125}Iodine (I-IL-1ra) and ^{125}I- bovine serum albumin (I-BSA) were compared after focal cerebral ischemia in the rat. The timecourse of cerebral edema formation was also measured. Ischemia was induced by permanent occlusion of the left middle cerebral artery (MCAO) under fluothane anesthesia (2% in O_2). 2, 24, 48 or 72h later rats were anesthetized with sodium pentobarbitol (60mg/kg ip) and the femoral vein and artery cannulated. 13μCi I-BSA or 2μCi I-IL-1ra was injected iv. 1h later arterial blood was collected and the vascular space cleared by cardiac perfusion with 30ml 0.9% saline prior to removal of the brain. 0.5g of cortical and striatal tissue was homogenized in 4.5mL 0.9% saline and radioactivity of plasma and brain samples measured in a Beckman γ-counter. Cerebral tissue H_2O composition was determined from non perfused rat brains by dry and wet weight measurement of right and left hemispheres as an index of edema. BBB permeability to I-BSA and I-IL-1ra was similar for both proteins at 0h in unoperated control rats (I-BSA: $19.7\pm5.5\times10^{-3}$% Total count injected (T_c); I-IL-1ra:$19.55\pm6.02\times10^{-3}$% T_c, n=6). BBB permeability to I-BSA correlated with the timecourse of edema formation. Both were evident 24h after MCAO and peaked at 72h (2.3±0.78% increase in $\%H_2O$, n=4; $49.9\pm10.8\times10^{-3}$% T_c, n=7). In contrast, increased BBB permeability to I-IL-1ra was evident at 24h, peaked 48h after MCAO ($38.5\pm13.2\times10^{-3}$% T_c, n=7) and returned to control levels by 72h. These data indicate that different mechanisms may be responsible for I-BSA and I-IL-1ra entry into the brain after focal cerebral ischemia.

429.9

EFFECT OF ATRIAL NATRIURETIC PEPTIDE AND STEROIDS ON TNF-INDUCED BLOOD-BRAIN BARRIER INJURY. *G. A. Rosenberg*, E. Estrada, and J. E. Dencoff.* Depart. of Neurology, Univ. of New Mexico, and Neurology Service, and VA Medical Center, Albuqueruque, NM 87131.

Delayed proteolytic damage to the blood-brain barrier (BBB) is important in inflammation and ischemia. Atrial natriuretic peptide (ANP) and methylprednisolone decrease BBB damage, possibly by altering proteolytic balance. Therefore, we used intracerebral injection of tumor necrosis factor-alpha to induce BBB damage and studied the effect of ANP and steroids on capillary permeability and MMP production. Adult rats had TNF (100,000U) infused into both hemispheres. BBB permeability was measured with ^{14}C-sucrose 24 hrs later. Two groups of rats had either ANP (0.8mg/kg/24hrs) or methylprednisolone (14mg/kg) given intraperitoneally. Control rats had TNF only. Sucrose uptake into brain was significantly reduced by both agents (3.72±0.30% and 4.27±0.33%, respectively, compared with 5.94±0.35% in controls; $p<0.05$). Production of 92-kD type IV collagense was measured by zymography in TNF-injected rats. Both ANP and steroids significantly reduced MMP production compared with controls. Reduction of proteolytic activity by ANP and steroids may explain their benefical actions in vasogenic brain edema.

429.10

AMINO ACID EFFECTS ON CEREBRAL ENDOTHELIAL CELL (EC) INJURY FOLLOWING OXYGEN-GLUCOSE DEPRIVATION (OGD). J. Xu*, M.P. Goldberg, J.S. Beckman, C.Y. Hsu. Dept Neurology, Washington Univ Sch Med, St. Louis, MO 63110 and Dept Anesthesiology, Univ Alabama, Birmingham, AL 35233.

The endothelium is a primary target of hypoxic or ischemic insult. We studied EC injury following oxygen-glucose deprivation in vitro using cultured bovine cerebral microvascular ECs. The extent of EC injury, based on cell viability (trypan blue exclusion) and LDH release (% of total cell death caused by a calcium ionophore), was dependent upon OGD duration. Cell viability was reduced from 78% to 7% and LDH release increased from 18% to 46% when OGD was extended from 1 to 6 hr. ECs in Dulbecco's modified Eagle's medium (DMEM) sustained greater injury than those in Hank's balanced salt solution. The 15 individual amino acids in DMEM were each tested for its potential deleterious effect on ECs under OGD. The only amino acid showing a substantial effect was L-cyst(e)ine which exerted a concentration-dependent protective effect with an EC50 of approximately 15 uM. ECs after OGD showed nitrotyrosine immunoreactivity suggesting the possible involvement of nitric oxide (NO) derivative in cell injury. However, L-arginine (0.004 - 0.4 mM) was without effect and the addition of L-nitro-arginine, an NO synthase inhibitor, was detrimental. L-arginine at concentrations of 1.2 mM or greater also exaggerated EC injury. Results suggest that amino acids individually and in combination may affect OGD induced EC injury which may be partly related to NO and free radical formation.

429.11

EFFECT OF HYPOXIA ON POTASSIUM TRANSPORT SYSTEMS IN CULTURED BRAIN CAPILLARY ENDOTHELIAL CELLS OF THE RAT.

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Stroke Branch, NINDS, NIH, Bethesda, MD 20892-4128

The specific ion transport systems localized in the brain capillary endothelium have been implicated in the regulation of water-electrolyte homeostasis in the brain. This report describes that hypoxia alters K^+ transport systems in cultured rat brain capillary endothelial cells (RBEC). Uptake of $^{86}Rb^+$ (a tracer for K^+, 2.5 μCi/ml) into RBEC was measured in HEPES-buffered Medium 199 at room temperature for 10 min; ouabain-sensitive and bumetanide-sensitive K^+-uptake was defined as Na^+-K^+-ATPase and Na^+-K^+-Cl^- cotransport activity, respectively. Hypoxia (95% N_2/5% CO_2, 24hr) reduced (61% of control) the Na^+-K^+-ATPase activity, whereas it increased (149% of control) the Na^+-K^+-Cl^- cotransport activity (respective control values = 4.6 and 5.5 nmol/mg protein/min, n = 8). Oligomycin, a metabolic inhibitor (1 μg/ml), similarly affected both ion transport systems in a time-dependent manner, which caused an increased (133% of control) total K^+-uptake. Oligomycin also increased the rate of K^+-efflux up to 129% of control without altering the total intracellular K^+ content (values in μmol/mg protein; control = 0.96, oligomycin = 0.93). The oligomycin-augmented Na^+-K^+-Cl^- cotransport activity was reduced by protein-tyrosine kinase inhibitors (genistein, 50 μM; herbimycin A, 10 μM) without being affected by an inhibitor of protein kinase C (bisindolylmaleimide, 500 nM) or protein kinase A (H8, 20 μM), indicating the involvement of protein-tyrosine phosphorylation. The data indicate that the up-regulated K^+-uptake during hypoxia is due to an increased Na^+-K^+-Cl^- cotransport activity. It is suggested that a similar mechanism may play a role in the disturbance of water-electrolyte homeostasis described in ischemic brain.

429.12

NEUROTOXIC AND IMMUNOTOXIC EFFECTS OF LEAD IN RELATION TO BLOOD-BRAIN BARRIER PERMEABILITY IN A MURINE MODEL OF GENETIC SUSCEPTIBILITY. L. Claudio*, R.V. Rosal, H.A.N. El-Fawal, A. Little, J.G. Wetmur, and D.P. Perl Div. of Environmental Medicine, Mount Sinai Med. Ctr., & Institute of Environmental Medicine, NYU Med. Ctr. New York, NY 10029.

We hypothesize that one mechanism of lead neurotoxicity involves disruption of the blood-brain barrier (BBB), which exposes neural epitopes to the immune system. To explore this hypothesis, we have used two mouse strains which differ in the gene for δ-aminolevulinate dehydratase (ALAD), an enzyme implicated in susceptibility to lead intoxication. We have shown that mice with a duplication of the ALAD gene (strain D) accumulate twice the blood lead levels than mice with a single copy of the gene (strain C) when exposed to the same acute oral doses. This may produce increased accumulation of lead in the brain, kidney and liver as observed by atomic absorption spectrometry. Strain D mice also show increased BBB permeability when exposed to lead. In brain, lead accumulated in astrocytes, as observed by laser microprobe mass analysis (LAMMA).

Exposure to lead increased circulating antibody titers to neural proteins, particularly glial fibrillary acidic protein (GFAP). This effect was higher in strain D mice which showed increased BBB permeability.

These data suggest that lead may induce increased BBB permeability in susceptible animals. Increased BBB permeability may allow exposure of neural epitopes thus raising the production of antibodies against these proteins. Astrocytes may be a primary target for lead toxicity as evidenced by the presence of lead in these cells and increased antibody titers to GFAP. This murine model may be useful for the study of genetic susceptibility to lead-induced toxicity as observed in humans.

429.13

EFFECT OF CAP37 ON BRAIN ENDOTHELIAL CELL PHOSPHATIDYL CHOLINE (PC) HYDROLYSIS. **P. Grammas*, T. Botchlet and H.A. Pereira. Dept. of Pathology, Univ. of Oklahoma HSC, Oklahoma City OK 73104**

CAP37 is a multifunctional protein important in inflammatory and immune responses. We have previously shown, using specific CAP37 antibody, immunochemical staining of brain blood vessels in Alzheimer's brain but not in age-matched non-demented controls. The objective of this study was to elucidate what signalling cascades in brain endothelial cells are regulated by CAP37. Rat cerebral resistance vessels were isolated, collagenase treated, and vessel fragments seeded as explant cultures. Endothelial cells that emerged from the fragments were subsequently cloned to achieve a homogenous population and passages 10-15 were utilized in this study. Confluent monolayers were incubated with serum-free media containing 1 μCi [^{3}H] choline chloride and cells treated with 1 μg/ml CAP37 for 30 minutes. Lipids were extracted using chloroform/methanol/HCl and phospholipids separated by thin layer chromatography. The results demonstrated that CAP37 caused a significant ($P<0.02$) decrease in labelled PC suggesting receptor-mediated hydrolysis of this phospholipid. The data support the notion that CAP37 activates membrane phospholipases that hydrolyze PC and that the mediators released by PC hydrolysis (e.g., diacylglycerol) may mediate CAP37 actions in the cerebral microcirculation. (Supported by NIH NS30457, AI 28018 and OCAST)

429.14

VECTOR-MEDIATED DELIVERY OF [^{125}I]-Aβ^{1-40} THROUGH THE BLOOD-BRAIN BARRIER (BBB) AND BINDING TO ALZHEIMER'S DISEASE AMYLOID OF THE [^{125}I]-Aβ^{1-40}/VECTOR COMPLEX. Y. Saito, J. Buciak, K. Faull*, and W.M. Pardridge. Depts. of Medicine and Psychiatry and Biobehavioral Sciences, UCLA School of Medicine, Los Angeles, CA 90024.

The brain amyloid of Alzheimer's disease (AD) may potentially be imaged in patients with AD using neuroimaging technology and a radiolabeled form of Aβ^{1-40} that is enabled to undergo transport through the brain capillary endothelial wall, which makes up the BBB in vivo. [^{125}I]-Aβ^{1-40} transport through the BBB in the absence of vector-mediated delivery was negligible based on experiments with both an intravenous injection technique and an internal carotid artery perfusion method in rats. Unconjugated [^{125}I]-Aβ^{1-40} was rapidly metabolized following either intravenous injection or internal carotid artery perfusion. To conjugate Aβ^{1-40} to a vector-mediated drug delivery system, [^{125}I]-Aβ^{1-40} was monobiotinylated with NHS-XX-biotin, and bound to a conjugate of streptavidin (SA) and the OX26 monoclonal antibody to the rat transferrin receptor. The OX26 antibody undergoes receptor-mediated transcytosis through the BBB and the OX26/SA conjugate mediates drug delivery of biotinylated peptides through the BBB (Proc. Natl. Acad. Sci., 90, 2618, 1993). Both the rapid systemic clearance and peripheral metabolism of [^{125}I]-Aβ^{1-40} was inhibited by conjugation to the OX26/SA vector. The brain uptake, expressed as % injected dose (ID) per g brain, of the [^{125}I]-bio-Aβ^{1-40}/OX26-SA conjugate was 0.15 ± 0.01 %ID/g, a level that is 2-fold greater than the brain uptake of morphine. The binding of the [^{125}I]-bio-Aβ^{1-40}/OX26-SA conjugate to the amyloid of AD was demonstrated by film and emulsion autoradiography performed on frozen sections of AD brain. Binding of the [^{125}I]-bio-Aβ^{1-40}/OX26-SA conjugate to the amyloid of AD brain was completely inhibited by saturation with high concentrations of unlabeled Aβ^{1-40}. In conclusion, these studies show that BBB transport and access to amyloid within brain may be achieved by conjugation of Aβ^{1-40} to vector-mediated BBB drug delivery systems.

429.15

Blood-brain barrier endothelial cell cultures from post-mortem Alzheimer brains. J. M. Wells[1,2], A. Armaratunga[1], A. McKee*[1,2], C. Abraham[1], D. McKenna[1,2], R. Fine[1,2]. [1]Boston University School of Medicine, Boston, MA and [2]ENRM VA Hospital Bedford, MA 01730.

A number of investigators have suggested that the blood brain barrier is disrupted in Alzheimer's disease. To examine this issue we have developed an in vitro model of the human blood brain barrier. Primary cultures of cerebral endothelial cells were established from microvessels isolated from brains obtained at autopsy from patients with Alzheimer's disease. The cells were able to proliferate and maintain their endothelial characteristics. Doubling time for these cells was between 1.5 and 2 days. Cells stained positive for Factor VIII antigen and formed tight junctions. We have also shown that these cells produce APP and secrete ApoE. We have evidence of proteases that may be involved in APP processing in these cells. We are now examining the role of cerebral endothelial cells in Alzheimer's disease. Supported by the Department of Veterans Affairs.

429.16

LOCALIZATION OF SERUM PROTEINS IN THE BRAINS OF SIV MONKEYS L.A. Raymond*[1], P.D. Cheney[1], S. Joag[2], O. Narayan[2], N.E.J. Berman[3]. Dept. Physiol[1]; Microbiol, Molec Gene & Immunol[2]; Anat & Cell Biol[3], Univ. Kansas Med Ctr, Kansas City, KS 66160 USA.

Symptoms of the human immunodeficiency virus (HIV) include psychomotor slowing and cognitive deficits. For HIV or its animal model, the simian immunodeficiency virus (SIV), to enter the central nervous system, it must traverse the brain-blood barrier (BBB). In human HIV patients, serum proteins have been localized in the brain (Petito & Cash, *Ann. Neurol.* 32:658-666, 1992). The brains of a normal sex and age-matched *Macaca nemestrina* (92424) and another (PHt) infected with neurovirolent SIV-7F,were frozen sectioned and labeled with polyclonal antibodies against serum proteins: fibrinogen, albumin, and IgG. 92424 had positive staining for all three proteins that was confined to the blood vessels of the choroid plexus and microvasculature of the brain with occasional staining surrounding blood vessels causing a "halo" effect. PHt also showed positive staining for all three proteins producing a similar "halo" effect but fibrinogen and albumin localized in many microglia producing large patches of high intensity staining indicating disruption of the BBB. The disruption of the BBB may contribute to the neurological symptoms and cognitive deficits seen in HIV patients and the SIV model will allow for further study. (Supported by HD02528, NS32202, NS32203, RR00166.)

429.17

EFFECT OF LIPOPOLYSACCHARIDE ON 92kD TYPE IV COLLAGENASE IN RAT BRAIN. S. Mun-Bryce* and G.A. Rosenberg. Dept. of Neurology, Univ. of New Mexico School of Medicine, Albuquerque, NM 87131.

Bacterial infection of the brain increases capillary permeability with delayed secondary damage. Matrix metalloproteinases (MMP) mediate proteolysis of the blood-brain barrier, which is comprised of several extracellular matrix components including type IV collagen. We investigated the effect of lipopolysaccharide (LPS) on MMP production in the brain. Adult male rats received an intracerebral injection of either LPS, tumor necrosis factor-α (TNF-α) or sterile saline. Production of MMPs was measured 24 hrs post-injection, using gelatin-substrate polyacrylamide zymography. LPS-injection produced a significant increase in 92-kD type IV collagenase (gelatinase B, MMP-9) as compared to saline injection. TNF-α also stimulated MMP-9 production to a lesser extent than LPS. The time course of LPS-stimulated MMP proteolysis in the brain was characterized by a marked rise in MMP-9 at 6 hrs post-injection in both saline and LPS animals. By 8 hrs, the MMP-9 lysis area in saline animals diminished, as compared to the continued intense MMP-9 lysis seen in LPS animals. Levels of MMP-9 remained elevated 24 hrs after LPS injection. These results show that LPS mediates MMP production, linking LPS with blood-brain barrier damage.

429.18

STIMULATION OF IL-1β, TNFα, IL-6 AND iNOS mRNAs EXPRESSION BY LPS IN THE CULTURED RAT GLIAL AND CEREBRAL MICROVASCULAR ENDOTHELIAL CELLS.

Z. Y. Hu*, J. Xu, Y.Q. Yang and C. Y. Hsu. Dept. Neurology, Washington Univ. Sch. Med., St. Louis, MO 63110

Cytokines (such as IL-1β, TNFα, IL-6) and iNOS are produced by blood-borne and resident brain inflammatory cells. Cytokines mediate the bidirectional communication between cells to facilitate and perpetuate the inflammatory processes. Endotoxin and selected cytokines are know to be the major stimuli to induce iNOS expression in inflammation. Glia and endothelial cells may participate in post-ischemic inflammatory reaction and contribute to the breakdown of blood brain barrier and pathogenesis of vasogenic brain edema. The expression of IL-1β, TNFα and IL-6 mRNAs is studied by quantitative TR-PCR and Western blot in the glial, endothelial cell or mix cultures following incubation with lipopolysaccharide (LPS). Relative change in mRNA content of each gene was calculated after normalization based on endogenous β-actin mRNA. The endogenous IL-1β, TNFα and IL 6 levels were higher in glial cells and mix cultures than in endothelial cells. LPS increased the expression of IL-1β, TNFα, IL-6 and iNOS mRNAs to variable degrees in the glial cell and mix cultured cells. In endothelial culture, only IL-6 mRNA was increased by LPS. No additive effect was noted in cytokine expression in co-cultures. LPS stimulated glial iNOS expression. A synergistic effect of glial-endothelial interaction on iNOS expression was noted following LPS stimulation. IL-1β, TNFα and IL-6 were also detected by Western blot analysis in media of the glial, endothelial and mix cultures stimulated by LPS. Results suggest LPS-stimulated glial-endothelial interaction differentially augments iNOS but not cytokine expression.

429.19

EFFECTS OF TNF-α ON THE PRODUCTION OF VASOACTIVE SUBSTANCES BY CEREBRAL ENDOTHELIAL AND SMOOTH MUSCLE CELLS IN CULTURE. C. Estrada*, C. Gómez, and C. Martín. Department of Physiology, Faculty of Medicine, Universidad Autónoma de Madrid, Spain.

The effects of TNF-α on the production of the vasoactive substances nitric oxide (NO) and endothelin-1(ET-1) were investigated in cerebrovascular cells in culture. Bovine cerebral endothelial cells (BCEC) stained positively for NADPH-diaphorase/NO synthase activity and spontaneously produced nitrite, a stable NO oxidation product, which accumulated in the culture medium in a linear way for 48 hours. Low concentrations of TNF-α (0.5-2 ng/ml) significantly enhanced nitrite production after a 24 hour incubation. Higher concentrations or longer exposure times resulted in a cytotoxic effect which altered cell morphology, released LDH to the culture medium, and reduced the protein content. Dexamethasone, but not the NO synthase inhibitor N-iminoethyl-L-ornithine (L-NIO), prevented the cytotoxic effect of TNF-α in BCEC. TNF-α also significantly enhanced nitrite production in bovine cerebral smooth muscle cells (BCSMC). The enhancement was detected at all times between 8 and 72 hours and at all concentrations tested (2-100 ng/ml). Signs of cytotoxicity were not observed in BCSMC after incubation with TNF-α. ET-1 was constitutively secreted by BCEC. The production of ET-1 was stimulated by thrombin. TNF-α enhanced the release of ET-1 in BCEC, and this enhancement was not modified by the simultaneous addition of IFN-γ. BCSMC did not produce ET-1, either spontaneously, or in the presence of TNF-α, IFN-γ, or of both together. These effects of TNF-α on cerebrovascular cells may explain the microvascular alterations found in some inflammatory or traumatic conditions in which this cytokine is increased in the cerebral tissue.

This work was supported by grants 92/0290 and 94/0388 from FIS, Spain.

429.20

GLYCOSHINGOLIPID ANTIGENS IN CULTURED BOVINE MICROVASCULAR ENDOTHELIAL CELLS OF ENDONEURIAL ORIGIN. T. Kanda*, M. Yamawaki and T. Iwasaki. Department of Neurology, Tokyo Medical and Dental University, Tokyo113, Japan.

Since a number of anti-glycosphingolipid antibody activities have been demonstrated in patients with peripheral neuropathies including Guillain-Barré syndrome, CIDP and macroglobulinemic neuropathy, the presence of common antigens between endoneurial microvascular endothelial cells (ENMEC) and the peripheral nervous tissues presents a potential mechanism for the penetration of macromolecules from the circulation to the peripheral nervous system parenchyma. To investigate the glycosphingolipid antigens in ENMEC, we first established the method to culture bovine ENMECs using a modified method of Kanda *et al.* (1994; originally for brain microvascular endothelial cells). Bovine ENMEC express GM3(NeuGc) and GM3(NeuAc) as the major gangliosides, and GM1, GD1a, GD1b, GT1b, as well as sialyl paragloboside as the minor species. Neutral glycosphingolipids were extremely low and galactosylceramide was not detected. Our study is the first report to describe the successful cell culture method of ENMEC, and revealed that ENMEC and peripheral nervous tissues shared many glycosphingolipids as common antigens. Hence, the presumed cascade of pathological processes - immunological attack of ENMECs, destruction or malfunction of blood-nerve barrier, leakage of immunoglobulins into the endoneurial space and the subsequent destruction of peripheral nerve - can be logically explained in patients with anti-glycolipid antibody.

PRESYNAPTIC MECHANISMS VI

430.1

PRESYNAPTIC MECHANISMS REGULATING CA^{2+} CONCENTRATION TRIGGERING ACETYLCHOLINE RELEASE AT AN IDENTIFIED SYNAPSE OF *APLYSIA*. J.P. Mothet, P. Fossier, J. Stinnakre* and G. Baux. Lab. Neurobiol. cellulaire et moléculaire, CNRS, 91198 Gif-sur-Yvette cedex, France.

The build up of a high concentration of Ca^{2+} in the presynaptic terminal appears as the main trigger of ACh release. We show that both N- and P-type Ca^{2+} channels, sensitive to ω-conotoxin and Funnel Web spider toxin (FTx) respectively, are involved in transmitter release. The Ca^{2+} influx though N-type Ca^{2+} channels and consequently ACh release are modulated by FMRFamide, histamine and buccalin. FMRFamide increases the Ca^{2+} influx by increasing the voltage-sensitivity of N-type Ca^{2+} channels *via* the production of DAG, the activation of PKC and then the phosphorylation of the channels. Histamine has the opposite effect and reduces the voltage-sensitivity of the N-type Ca^{2+} channels, most probably by reducing their rate of phosphorylation. Buccalin decreases the number of available N-type Ca^{2+} channels. The Ca^{2+} influx through P-type Ca^{2+} channels appears unchanged by these neuromodulators. The injection of polyclonal antibodies raised against syntaxin into the presynaptic neuron decreases both ACh release and the Ca^{2+} influx through N- and P-type Ca^{2+} channels. This indicates that some proteins proposed to be involved in transmitter release could interfer with the Ca^{2+} influx, because of their tight association with presynaptic Ca^{2+} channels. Finally the Ca^{2+} concentration which actually triggers ACh release may also depend on the activity of presynaptic Ca^{2+} buffers. A blocker of the reticulum Ca^{2+} pump, 2,5-diterbutyl 1,4-benzohydroquinone (tBuBHQ), facilitates ACh release by increasing the intracellular concentration of Ca^{2+} (detected with aequorin) without affecting the presynaptic Ca^{2+} influx. This indicates that a presynaptic structure sequestrates a part of Ca^{2+} ions flowing into the terminal during the presynaptic depolarization, preventing this fraction to participate in ACh release. Nitric oxide which decreases ACh release without modifying the presynaptic Ca^{2+} influx, could act on the activity of these presynaptic Ca^{2+} buffers.

430.2

ON THE QUANTAL CO-RELEASE OF ATP AND ACETYLCHOLINE FROM FROG MOTOR NERVE ENDINGS AS MEASURED BY THE "PATCH-SNIFF" TECHNIQUE. R.S.Redman*, T.Searl, E.M. Silinsky. Dept. of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, IL 60611.

At the skeletal neuromuscular junction, recent studies have suggested that upon the co-release of the neuromodulator, ATP, with the neurotransmitter, acetylcholine (ACh), ATP is hydrolyzed to adenosine and acts as the primary mediator of neuromuscular depression. While these studies imply that ATP release occurs in a quantal manner, there is little evidence demonstrating that ATP can be released rapidly enough and in temporal harmony with a single nerve impulse under physiological conditions to mediate neuromuscular depression. We used outside-out patches from sympathetic neurons in guinea pig celiac ganglia, which possess ATP-gated ion channels as well as ACh receptors, to test for the co-release of ATP and ACh. Specifically, a coverslip containing acutely dissociated celiac neurons was placed in a chamber in which a cutaneous pectoris nerve-muscle preparation (pretreated with a collagenase-protease digestion protocol) was pinned. Excised patches under voltage clamp were then moved to within 10 μ of the motor nerve ending and single channel currents were recorded both before and after electrical stimulation of the motor nerve. Using this technique, it is possible to demonstrate the rapid (latency <5 msec) release of ATP by discrete nerve impulses in conjunction with ACh. Such release is blocked by the ATP antagonist, suramin (50 μM). Preliminary experiments were made under conditions in which spontaneous ATP release was compared to evoked release; evoked ATP release appeared as integral multiples of the spontaneous events suggesting that ATP is released in a quantal manner from a vesicular store.

430.3

SYNAPTIC STRENGTH OF DEVELOPING GABAERGIC SYNAPSES DEPENDS ON THE ACTIVATION STATUS OF PRESYNAPTIC GLUTAMATE RECEPTORS

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GABAergic synapses between cultured collicular neurons were studied by pair patch clamp recording. Using a local superfusion technique the number of participating terminals was arteficially reduced rendering maximal amplitudes of evoked IPSCs 4-8 times the quantal size. Up to 10 neighbouring boutons were determined in the active window by histological techniques. Using this reduced synapse it was possible to apply unconstrained binomial analysis to estimate the release probability of individual terminals. It was found that the majority of terminals released GABA with low probability ($p<0.2$). We therefore looked for conditions that could transfer GABAergic terminals from a low-efficacy to a high-efficacy release state. Experiments with chronic exposure to the glutamate receptor blockers MK-801 (1 μM), DNQX (10 μM) and CPG (200 μM) increased the average amplitude of evoked IPSCs and reduced the coefficient of variation. Differences between treated and untreated cultures were statistically significant after 9-12 days *in vitro*. Evaluation of amplitude distributions showed that failures were common in untreated pairs, but very rare in treated pairs. These results suggest that the strength of inhibitory coupling may be influenced by heteronymous synaptic input via glutamate receptors.

430.4

A CORRELATION EXISTS BETWEEN REDUCED CATECHOLAMINE RELEASE AND SUPPRESSED DIACYLGLYCEROL GENERATION BY C-TYPE NATRIURETIC PEPTIDE.

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C-type natriuretic peptide (CNP) reduces depolarization induced catecholamine release from rat differentiated pheochromocytoma (PC12) cells. We tested the hypothesis that CNP reduces evoked catecholamine release by inhibiting phospholipase C (PLC) activity. Calcium (10 μM) was used to stimulate PLC. We measured changes in DAG generation as an indicator of PLC activity. CNP (0.1 to 100 nM) suppressed calcium stimulated DAG production concentration dependently. Maximal suppression of DAG production was 34 $\pm$ 5 % at a CNP concentration of 10 nM. CNP (0.1 to 100 nM) also dose dependently reduced calcium evoked dopamine release with a maximal inhibition of 31 $\pm$ 11 % at a concentration of 10 nM. There was a significant correlation between DAG production and dopamine release in response to CNP. DAG is an activator of protein kinase C which potentiates catecholamine release. Since CNP reduced both calcium stimulated DAG generation and dopamine release from PC12 cells, the neuromodulatory effects of CNP are related to a suppression of PLC activity. (Supported by Grant RO1 HL42525 from the PHS)

430.5

PREJUNCTIONAL MODULATION BY ANGIOTENSIN I, II AND III OF NOREPINEPHRINE RELEASE FROM SYMPATHETIC NEURONS.

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The aim of the present work was to examine the modulating effect of angiotensin I, II and III (AI, AII and AIII) on stimulation-evoked norepinephrine release from postganglionic sympathetic neurons in rabbit isolated aorta. Rings of aorta were preloaded with (-)-^{3}H-norepinephrine (10^{-7}M) and subsequently washed with physiological salt solution. The rings were subjected to electrical-field stimulation (S_1-S_8; 200 mA; 300 pulses; 0.5 ms; 1 Hz). The spontaneous ^{3}H-outflow and stimulation-evoked ^{3}H-overflow were collected in fractions (2 ml; 5 min). Cumulative addition of AI (10^{-8}-10^{-6}M), AII (3×10^{-11}-10^{-7}M) and AIII (3×10^{-10}-10^{-6}M) enhanced the stimulation-evoked ^{3}H-overflow up to 154, 169 and 193%, respectively. The order of potency was AII > AIII > AI. Captopril (10^{-6}M) markedly attenuated the facilitating effect of AI (10^{-9}-10^{-7}M) but did not alter the facilitation seen with AII (10^{-10}-10^{-8}M). Captopril (10^{-7}-10^{-4}M) alone did not alter the stimulation-evoked ^{3}H-overflow. Edetate disodium (Na_2EDTA; 3×10^{-5}M) did not alter the enhancing effect of AI (10^{-8}-10^{-7}M). The enhancement seen with AII (3×10^{-11}-10^{-8}M) was independent of stimulation frequency (1-10 Hz). The facilitation induced by single concentrations (10^{-9}-10^{-7}M) of AI, AII and AIII on repeated stimulations waned with time, i.e. development of tachyphylaxis. Cocaine (3×10^{-5}M) plus corticosterone (4×10^{-5}M) did not alter the enhancing effect of AII. We conclude that the AII-induced enhancement of ^{3}H-overflow is due to an increased release of ^{3}H-norepinephrine rather than inhibition of neuronal and extraneuronal uptake and that the enhancing effect of AI is mainly due to its conversion to AII.

430.6

CONTRIBUTION OF PROTEIN KINASE ACTIVATION TO 5-HT$_4$-MEDIATED FACILITATION OF FAST EXCITATORY SYNAPTIC POTENTIALS (fEPSPs) IN ENTERIC NEURONS.

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5-HT$_4$ receptors couple to adenylate cyclase in smooth muscle and in neurons. In the present study we tested the contribution of protein kinase activation to fEPSP facilitation caused by the 5-HT$_4$ receptor agonist, renzapride. Intracellular electrophysiological methods were used to record fEPSPs from neurons in guinea pig myenteric plexus *in vitro*. The stimulus intensity was adjusted to the minimum needed to evoke a stable amplitude fEPSP. Renzapride (10-100 nM) produced an increase in fEPSP amplitude (EC_{50}, 6 nM). The maximum increase was 110 $\pm$ 14% over control (n=14). SDZ 205-557, a 5-HT$_4$ receptor antagonist, caused a rightward shift in the renzapride concentration-response curve (K_B, 8 nM). Forskolin (0.1-1 μM) increased fEPSP amplitude (EC_{50}=126 nM) but the maximum response (55 $\pm$ 10%) was less than that of renzapride. The protein kinase inhibitor, staurosporine, at 1 and 3 μM reduced fEPSP facilitation caused by 100 nM renzapride by 40 and 60% respectively. These data suggest that 5-HT$_4$ receptors may mediate facilitation fEPSPs *via* an increase in cAMP and activation of a staurosporine-sensitive protein kinase. (supported by DK40210 and NS01738)

430.7

PROTEIN KINASE INHIBITORS BLOCK SYNAPTIC TRANSMISSION IN *XENOPUS* EMBRYO SPINAL CORD.

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Glycinergic inhibition plays a key role in the generation of the swimming motor pattern in the *Xenopus* embryo and can be modulated by $GABA_B$ receptors. As well as decreasing the Ca^{2+} currents involved in transmitter release (Wall & Dale, 1994, *J. Neurosci.*, **14**, 6248), $GABA_B$ receptors also reduce the frequency of glycinergic mIPSPs recorded in the presence of TTX and 100μM Cd^{2+} (Wall & Dale, 1993, *J. Physiol.*, **469**, 275).

To examine the possible mechanisms underlying this direct modulation of transmitter release, we applied the protein kinase inhibitors H7 and HA-1004 to the embryo spinal cord while recording both spontaneous glycinergic mIPSPs (with Cd^{2+} and TTX present) and evoked glycinergic IPSPs in motoneurons. Both 1-10μM H7 and 100μM HA-1004 mimicked the actions of $GABA_B$ agonists, by causing a large reversible reduction in the frequency of spontaneous mIPSPs (in some cases abolishing spontaneous mIPSPs completely during the period of drug application). There was no significant change in the size of the spontaneous mIPSPs, suggesting that H7 and HA-1004 act presynaptically. H7 (10μM) also reduced the size of evoked glycinergic IPSPs by 43.1$\pm$4.32% ($\pm$SEM, n=19). However, patch-clamp recordings from acutely isolated *Xenopus* spinal neurons showed that Ca^{2+} currents were not reduced by 10μM H7.

Since H7 and HA-1004 reduce transmitter release without altering the Ca^{2+} currents, we propose that phosphorylation of vesicle proteins by a constitutively active kinase may be necessary to move vesicles into a releasable pool. Presynaptic inhibition by $GABA_B$ receptors may thus involve reduction of the activity of this kinase and hence the number of vesicles in the releasable pool.

430.8

COMPARISON BETWEEN HISTAMINE- AND CAFFEINE-INDUCED SECRETION IN INDIVIDUAL RAT ADRENAL CHROMAFFIN CELLS

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To investigate the effects of intracellular Ca stores on exocytosis, we applied histamine and caffeine, which are considered to stimulate IP_3-induced intracellular Ca release and Ca-induced Ca release, to primarily cultured rat chromaffin cells. The change in membrane capacitance (C_m) was monitored as an assay of the resultant exocytosis along the change in cell membrane conductance (G). Cells with positive exocytosis to membrane potential depolarization (-70 mV, 500 ms) were analyzed.

In histamine (100 μM, 45 sec) treated cells with free external Ca, 24 out of 31 cell showed C_m increases (140.4 $\pm$ 17.8 fF, mean $\pm$ S.E.M.) while majority of responded cells showed no difference in G trace (0.03 $\pm$ 0.10 nS). Neomycin (1 mM, 5 min pretreatment) blocked histamine-induced C_m increase reversibly. On the contrary, caffeine (10 mM, 45 sec) treated cells showed C_m increases (110.6 $\pm$ 20.4 fF) in 33 out of 49 treated cells accompanying changes in G trace (1.78 $\pm$ 0.37 nS). The changes in G trace were blocked by the K channel blockers. The maximum rate of secretion (fF/sec) was also comparable between histamine- (17.1 $\pm$ 1.3 fF/sec) and caffeine-induced secretion (40.7 $\pm$ 5.1 fF/sec).

From the heterogeneity in the responses of chromaffin cells to histamine and caffeine, it is concluded that there are sub-populations of rat adrenal chromaffin cells according to the intracellular Ca stores.

430.9

A NOVEL FAMILY OF PKC SUBSTRATE PROTEINS IN RAT BRAIN AND ITS DEVELOPMENTAL CHANGES. H.H. Miao, C.W. Chak, [1]X.F. Chen, [2]Y.C. Du, F.S. Sheu and Y.F. Han. (SPON: The Hong Kong Society of Neurosciences) Dept. Biochem. Hong Kong Univ. Sci. & Technol., Hong Kong; [1]Shanghai Inst. Physiology; [2]Shanghai Inst. Biochem. Chinese Acad. Sciences, Shanghai, China.

Protein phosphorylation represents a common pathway in the molecular mechanisms through which neurotransmitters, hormones and the nerve impulse produce many of their physiological effects, and elucidation of the precise mechanisms involved in neuronal signalling depends upon the identification and characterization of the *in vivo* substrates for the various protein kinases. Protein kinase C (PKC) and its substrates have received extensive attention since the activation of PKC is coupled to regulations of neuronal development and synaptic plasticity. Here we presented the findings on the identification, distribution and developmental changes of a family of new neuron-specific and membrane-associated PKC substrates with low molecular weights from 12K-16K. Crude membrane of tissues and synaptic plasma membranes (SPM) from male rats of an inbred SD strain were prepared according to Dokas *et al* with minor modifications. PKC was purified from rat brain as previously described by Sheu *et al*. The SPMs were labelled with γ^{32}P-ATP by either endogenous or exogenous PKC, and phosphoproteins were separated by 13 or 15% SDS-PAGE. By means of this approach, it was detected in brain synaptic membranes a family of phosphoproteins, i.e. P12, P15, and P16, with apparent molecular weights of 12K, 15K and 16K, respectively. They have been found to be present in spinal cord, midbrain and cerebellum, but not in peripheral tissues. The phosphorylation of these proteins was selectively inhibited by PKC_{19-31}, a specific PKC inhibitor, and by Ca^{2+}-chelator EDTA and EGTA, or stimulated by PKC activator phorbol 12,13-dibutyrate. Two-dimensional electrophoresis indicated that these small PKC substrates possess the similar neutral property with isoelectric points of approximate to 7. In contrast to the developmental decrease of GAP43 level in rat brain, it was found that the concentrations of P12, P15 and P16 ontogenetically increased in rat cerebral cortex.

430.10

SYNAPTIC AND CELLULAR PROPERTIES OF HIPPOCAMPAL NEURONS IN CALBINDIN-D28K DEFICIENT MICE. S. Vietla*[1], I. Mody[1], M. Meyer[2], M.S. Airaksinen[2], and H. Thoenen[2]. [1]Dept. of Neurology, UCLA School of Medicine, Los Angeles, CA, and [2]Dept. of Neurochemistry, Max-Planck Institut for Psychiatry, Martinsried, F.R. Germany

To date, little is known about the function of high-affinity neuronal Ca^{2+}-binding proteins, but some experimental evidence is consistent with their role as intracellular Ca^{2+}-buffers. We have examined the possible Ca^{2+} sequestering role of the cytosolic Ca^{2+}-binding protein calbindin-D28K (CB). Electrophysiological experiments were carried out in brain slices obtained from female control (CB+/+, n=4) and genetically engineered mice that lacked the gene for CB (CB-/-, n=4). Immunohistochemical labeling of slices not used for physiology confirmed the loss of CB from various neurons of the hippocampal formation.

First we studied the role of CB in Ca^{2+}-sequestration in presynaptic terminals. In the CA3 and CA1 regions, we recorded extracellular field EPSPs evoked by stimulation of the mossy and Schaffer collateral/commissural fibers respectively. The recordings were done in 50 µM bicuculline, 50 µM AP5 and 4 mM $MgCl_2$ to reduce the probability of polysynaptic activation of pyramidal cells. In CB-/- mice, the rates of potentiation of EPSPs during the first 10 pulses of a 33 Hz tetanus were decreased by 66.4% and 36.6% in the CA3 and CA1 regions respectively. Paired-pulse potentiation at inter-stimulus intervals (ISI) between 20-1000 ms was also reduced in CB-/- animals (*e.g.*, at 50 ms ISI, there was 38% and 21% less potentiation in the CA3 and CA1 regions respectively). Second, we examined the effect of a short (3 min) anoxia on synaptic transmission in the CA1 region. No differences were found in the anoxia-induced depression of field EPSPs between CB+/+ and CB-/- mice. Third, we studied the firing properties of dentate granule cells in whole cell recordings. The frequency of action potential firing elicited by depolarizing current pulses (200-400 ms) was significantly lower in CB-/- mice.

Our findings are consistent with an enhanced activation of a Ca^{2+}-dependent K^+ conductance in the absence of CB, and thus indicate an active role for CB in the regulation of neuronal Ca^{2+} homeostasis.

430.11

INHIBITION OF TYROSINE KINASE BLOCKS POTENTIATION IN THE CEREBRAL GANGLION OF *APLYSIA*. S.M. Fredman*. Dept. of Physiology, Meharry Medical College, Nashville, TN 37208.

The A-B neuron synapse in the cerebral ganglion of *Aplysia* exhibits multiple forms of activity-dependent plasticity. Low frequency stimulation of the presynaptic A neurons produces synaptic depression (LFSD) of their EPSPs in B neurons. Brief high frequency A neuron stimulation evokes a long-lasting increase in synaptic transmission, slow developing potentiation (SDP). Work to date indicates that SD is mediated by PKA and can be mimicked by cAMP and its activators. SDP appears due, at least in part to the activation of PKC. Both LFSD and SDP are due to changes in the presynaptic A neurons. Recently evidence has been obtained indicating that a tyrosine kinase (TK) may also contribute to SDP.

Experiments were done on isolated cerebral ganglia in high divalent cation seawater (55 mM Ca^{2+}, 150 mM Mg^{2+}) to suppress polysynaptic connections. Individual A neurons were stimulated with single test pulses every 3 min. SDP was induced after the 3rd test pulse with 4, 500 msec 20 Hz trains. This was followed by 15 additional test pulses. After obtaining a control level of SDP, TK inhibitors were added to the bath. Both 1 µM genistein and 1 µM erbstatin analog gave similar, highly selective, results. In the presence of either inhibitor, there was no significant change in test EPSPs prior to the tetanic stimulation. However, SDP following the high frequency trains was largely eliminated. These inhibitors act at different sites on the kinase. Genistein blocks at the catalytic site, while erbstatin preferentially inhibits the regulatory site of the EGF receptor class of TKs. This suggests that SDP may be in part mediated by a receptor TK. Work is in progress to try and identify potential endogenous ligands and to further characterize the receptor.

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430.12

MECHANISM OF FREQUENCY DEPENDENT FACILITATION OF NICOTINIC TRANSMISSION IN RABBIT PARASYMPATHETIC GANGLIA. T. Nishimura* and T. Akasu. Dept. of Physiol., Kurume Univ. Sch. of Med., Kurume 830, Japan

Fast excitatory postsynaptic current (EPSC) was recorded from neurons in rabbit vesical parasympathetic ganglia (VPG) at 36±1°C *in vitro*, using single electrode voltage clamp techniques. A holding potential ranged between -50 and -60 mV. The fast EPSC was evoked by stimulation of the pelvic nerve at various frequencies (0.03-10 Hz). The amplitude of fast EPSC typically ranged from 0.1 to 0.6 nA at 0.03 Hz. When the frequency of preganglionic nerve stimulation was increased to 3 Hz, the fast EPSC gradually increased in amplitude and then reached 1.5-2 nA at a 10-15th stimulus from the beginning of a 3 Hz train. The facilitation lasted for 1-5 min after the tetanic stimulation (3 Hz for 10 sec). During the frequency facilitation, the amplitude of an inward current evoked by pressure application of acetylcholine (ACh) was not changed in the presence of atropine (1 µM). The amplitude of fast EPSC was depressed and the number of failure was increased in low calcium (0.8 mM) solutions or after the application of BAPTA-AM (200 µM) for 20 min. In these neurons the frequency facilitation still occurred in some extent, however the decay of facilitation was significantly faster than the control. The frequency facilitation occurred even at low temperature (22 °C). Contrary dibutyryl cyclic AMP (1-6 mM) and forskolin (10 µM) presynaptically increased the amplitude of fast EPSC at 36 °C but not at 22 °C. These data suggest that the frequency dependent increase in the ACh-release is responsible for the frequency facilitation of the fast EPSC. The frequency facilitation is explained at least in part by "residual calcium" hypothesis. Also it is independent of cyclic AMP. The frequency facilitation of the fast EPSC may be one of the mechanisms of the high pass filter of nicotinic transmission in the rabbit VPG (Neurosci. Lett., 103: 179-184, 1989).

430.13

FURTHER CHARACTERIZATION OF mGluR MODULATION OF SYNAPTIC TRANSMISSION AT SYNAPSES IN CORTICOSTRIATAL CO-CULTURES. E.C. Tyler*, R. L. Popp, and D. M. Lovinger. Dept. of Molecular Physiology and Biophysics, Vanderbilt Medical School, Nashville, TN 37232.

Previously, we described a corticostriatal co-culture system in which both stimulus evoked and spontaneous, calcium influx independent, synaptic transmission were modulated by mGluR activation. We showed that inhibition of evoked EPSCs (eEPSCs) by application of mGluR agonists exhibited a pharmacology consistent with modulation by class II mGluRs. Application of 50µM *t*-ACPD produced 32.5±4.6% (n=10) inhibition of eEPSC amplitude. In the same cells, application of 2µM DCG-IV and 5µM L-CCG-1 produced 31.7% (n=2) and 34.2±3.7% (n=9) inhibition of the eEPSC, respectively. In 4 of 5 cells examined, 100µM AP4 reduced the eEPSC by less than 10% (4.2±1.3%). We also demonstrated that modulation of evoked synaptic transmission by mGluR activation is mediated via a pertussis toxin (PTX) sensitive G-protein. PTX pre-treatment of culture dishes (500ng/ml, 20-27hr) blocked the ability of 50µM *t*-ACPD to reduce the eEPSC (3.8± 2.7% inhibition, n=5) compared to untreated controls. In controls from the same set of cultures, 50µM *t*-ACPD reduced the eEPSC by 20.3±3.8% (n=5). By comparing manipulations that affect modulation of evoked and spontaneous transmission we hope to determine if modulation of synaptic transmission is mediated via calcium channels or via a mechanism downstream of calcium influx. Previously, we showed that phorbol esters were able to block inhibition of eEPSCs by mGluR activation. We have now shown that phorbol esters also have a similar effect on calcium influx independent miniature EPSCs (mEPSCs). Application of 1µM PDAc significantly decreased the interval between mEPSCs in all neurons examined (n=4, range 20-40% of control) without affecting mEPSC amplitude. Application of 50µM *t*-ACPD in the presence of PDAc had no significant effect in 3 of the 4 neurons examined. In the one neuron showing an effect it was significantly smaller than ACPD effects before PDAc treatment. Our current data suggest that modulation of synaptic transmission at synapses in corticostriatal co-cultures is mediated via a class II mGluR and that this receptor is acting, at least in part, via a pertussis toxin sensitive G-protein. (Supported by NS 30870 and MSTP 5-T32-GM07347-14).

430.14

EFFECTS OF L-ARGININE ON GABA SYNAPTIC CURRENTS IN DOPAMINE NEURONS OF RAT MIDBRAIN SLICE. B.A. Cox*, K.-Z. Shen and S.W. Johnson, Department of Pharmacology, Oregon Health Sciences University, Portland, OR, 97201.

Dopamine neurons were recorded with patch pipettes in the whole-cell configuration from rat midbrain slices, and synaptic currents were evoked by focal electrical stimulation of the slice. The amplitude of the $GABA_B$-mediated inhibitory postsynaptic current (IPSC), recorded in bicuculline (30 uM), APV (50 uM) and CNQX (10 uM), was increased 21 ± 4% by L-arginine (10 mM), whereas the duration of the current at half amplitude was prolonged 50 ± 11%. L-ω-nitro-L-arginine methyl ester (L-NAME; 10 mM), an inhibitor of nitric oxide synthase, reduced the amplitude of the $GABA_B$ IPSC by 58 ± 4%, whereas the addition of L-arginine to L-NAME raised the amplitude of the $GABA_B$ IPSC back to the control level. L-lysine (10 mM), a competitive inhibitor of L-arginine uptake, reduced the $GABA_B$ IPSC by 64 ± 5%. The GABA uptake inhibitor NO-711 (1 uM) mimicked L-arginine by increasing the $GABA_B$ IPSC amplitude by 13 ± 4% and increasing its duration at half-amplitude by 117 ± 22%. Outward current evoked by GABA, applied locally by pressure-ejection with bicuculline (30 uM) in the superfusate, was increased 57 ± 16% by L-arginine. However, L-arginine failed to increase outward current evoked by locally applied baclofen, a $GABA_B$ agonist. These results suggest a presynaptic site of action for the potentiation by L-arginine of $GABA_B$ synaptic transmission. The action of L-arginine may be mediated by release of nitric oxide.

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430.15

INOSITOL 1,4,5-TRISPHOSPHATE MASS MEASUREMENT IN TORPEDO CHOLINERGIC SYNAPTOSOMES UNDER CONDITIONS THAT RELEASE ACETYLCHOLINE. M.A.Carrasco[1], Y.Morot-Gaudry Talarmain, J.Molgo and P.Caviedes[1] * Lab. Neurobiologie Cellulaire et Moléculaire,CNRS,91198 Gif-sur-Yvette Cedex, France; [1]Dept.Fisiol.Biofis.,Fac.Medicina,U.Chile.Casilla 70005,Santiago,Chile.

The cholinergic nerve endings isolated from the electric organ of *Torpedo marmorata* retain most of their metabolic and physiological properties (Israel et al.,Biochem.J.,160,113-115,1976). The intracellular messenger inositol 1,4,5-trisphosphate (IP_3), has not been described in this preparation. We measured both IP_3 mass using a radioreceptor assay and acetylcholine (ACh) release by a choline-oxydase chemiluminiscent method with various agents that trigger ACh release.

IP_3 mass under resting conditions (471.3 ± 25.6 pmol/mg of protein) was independent of external [Ca^{2+}]. IP_3 mass and ACh release increased with [K^+] provided Ca^{2+} was present, as determined in parallel experiments. The threshold for IP_3 mass increase was different from that found for ACh release since 30 mM K^+ which did not modify significantly IP_3 levels with respect to controls, already increased ACh release. The Ca^{2+}-ionophore A23187 and gramicidin-D, induced a significant IP_3 mass increase and a parallel marked increase of ACh release in the presence of Ca^{2+}. Hence, changes in IP_3 mass and ACh release seem to depend on both Ca^{2+} influx and intracellular Ca^{2+} levels.

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430.16

DOES ADENOSINE CONTRIBUTE TO TETANIC FADE AT THE MAMMALIAN NEUROMUSCULAR JUNCTION?. D.F. Wilson*, K.V. Schmit, J.W. Schrock, and P.M. Barr. Zoo Dept., Miami Univ., Oxford, OH 45056.

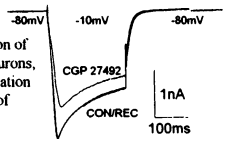
In the amphibian it has been suggested that the major basis for tetanic fade at the neuromuscular junction is due to a feedback action of adenosine that is coupled with neurotransmitter release during repetitive stimulation. Evidence supporting this hypothesis is lacking in the mammalian system. We were interested in determining if a similar mechanism could be operating at the neuromuscular junction of the rat. The presynaptic effect of blocking adenosine A_1-receptors on tetanic fade in the rat diaphragm was examined by testing 100 and 200 pM 8-cyclopentyl-1,3,dipropylxanthine (DPCPX) on evoked transmitter release. Intracellular recording techniques were used to monitor end-plate potentials and miniature end-plate potentials in the isolated cut-muscle rat diaphragm-phrenic nerve preparation (40 stimuli of 1, 25, and 50 Hz were used). Quantal release and tetanic fade in the presence and absence of the antagonist were examined. DPCPX failed to reduce tetanic fade or alter quantal release. These results do not support the hypothesis that adenosine causes tetanic fade by acting on presynaptic adenosine A_1-receptors .

430.17

ACTIVATION OF $GABA_B$ RECEPTORS INHIBITS CALCIUM CHANNELS IN LATERAL ENTORHINAL CORTICAL NEURONS, BUT NOT TRANSMISSION AT THE LATERAL PERFORANT PATH. Nevin A. Lambert* and Wilkie A. Wilson. *Department of Pharmacology, Duke Univ. Med. Ctr. and VAMC, Durham, NC 27705.*

Transmitters and modulators that inhibit transmission at various synapses often inhibit voltage-gated Ca^{2+}channels in the cell bodies of the presynaptic neurons. We asked if Ca^{2+}channels were ever inhibited in neurons that make synapses that are *not* affected by a given transmitter. Lateral perforant path (LPP) fibers arise from neurons in layer II of the lateral enthorhinal cortex (LEC)[1] and terminate in the outer molecular layer (OML) of the dentate gyrus[2]. LPP synaptic responses are unaffected by activation of $GABA_B$ receptors[3].

Field potential recordings in the OML confirmed that LPP responses were not inhibited by the $GABA_B$ receptor agonists baclofen or CGP 27492 in slices from young (PN14-21) rats; LPP responses were reversibly inhibited by adenosine (n=3). Whole-cell voltage-clamp recordings were made from visually-identified neurons in layer II of the LEC. With K^+-based internal solutions, $GABA_B$ and adenosine receptor agonists induced currents which were outward at -60mV, reversed near -90mV and were associated with an increase in conductance (n=6). With Cs^+-based internal solutions and TEA and TTX in the bath, $GABA_B$ and adenosine receptor agonists reversibly inhibited inward currents elicited by depolarizing commands from a holding potential of -80mV (n=25; Figure). These results suggest that activation of $GABA_B$ receptors inhibits Ca^{2+}channels in LEC neurons, but not transmission at their terminals. Thus, regulation of somatic Ca^{2+}channels is not a reliable predictor of regulation of transmission.



Supported by grants from the NIH and the VA.

References: 1. Steward, *J. Comp. Neurol.* 167:285. 2. Steward and Scoville, *ibid.* 169:347. 3. Lanthorn and Cotman, *Brain Res.* 225:171.

430.18

EFFECT OF CAFFEINE ON SYNAPTIC PERFORMANCE AT CRAYFISH NEUROMUSCULAR JUNCTIONS. K. Judd and S.J. Velez*. Dept. of Biological Sciences, Dartmouth College, Hanover, NH 03755.

We had previously reported that caffeine decreased the facilitation of neurotransmitter released during constant 10 Hz stimulation of neuromuscular synapses in the crayfish superficial flexor muscles (Soc. Neurosci. Abstr. 20:1338). A dosage-response curve was now obtained for the largest excitor to this system (axon 6), at a constant 1 Hz or 10 Hz stimulation, while recording junction potential sizes (jp's) from several muscle fibers. Maximum reduction of jp size, within 5 minutes of exposure, was obtained for a .05M solution of caffeine in Ringers; no effect was observed for concentrations lower than .001M. Both jp's generated at 1 Hz and at 10 Hz were affected, as was the facilitation ratio (jp 10Hz/jp 1Hz) of the terminals. Experiments were repeated using .01M caffeine in Ringers that contained 30% or 200% normal calcium concentrations, with no effects observed on caffeine's reduction in jp sizes or time course of action. It appears that caffeine is lowering the intracellular free-calcium ion concentration in this system. Experiments in progress using .01M caffeine in Ringers containing the calcium ionophore A23187 indicate that even after internal calcium stores are compromised by the enhanced calcium permeability induced by the ionophore, caffeine's effects are still observed. This suggest that caffeine is not increasing calcium pumping into intracellular compartments but could be activating molecules that remove free-calcium ions from solution.

430.19

FACILITATION OF ^{3}H-NE RELEASE FROM CEREBRAL CORTICAL SLICES IS MEDIATED PRIMARILY BY β2-ADRENERGIC RECEPTORS. K.D. Murugaiah and J.M. O'Donnell *. Dept. of Pharmacology, L.S.U. Medical Center, Shreveport, LA 71130.

The facilitatory effect of isoproterenol (0.1-10 nM), a nonselective β-adrenergic receptor agonist, on electrical stimulation-evoked release of ^{3}H-NE from cerebral cortical brain slices was antagonized by the nonselective, the β1- and the β2-selective adrenergic antagonists, propranolol, ICI 89,406 and ICI 118,551, respectively. Preliminary data indicated that the β1- and β2-selective adrenergic agonists prenalterol and albuterol (0.1-100 nM) both enhanced electrically-evoked release of tritium. These results were extended to the present study which examined whether the facilitatory effects of prenalterol and albuterol were antagonized by propranolol, ICI 89,406 and ICI 118,551. Rat cerebral cortical slices were preloaded with ^{3}H-NE and superfused with Krebs Ringer buffer for 100 min and electrically stimulated (1Hz, 20 mA, 2 ms, 2 min) at S1 (20 min) and S2 (70 min). Tritium outflow was evaluated as % radioactivity present in each 5 min fraction; results were expressed as S2/S1 ratios. Drugs were added 10 min prior to S2. Prenalterol and albuterol 1) did not alter basal outflow of tritium, 2) enhanced evoked overflow of tritium in a concentration-dependent manner but with differing potency and efficacy (S2/S1 ratios at 10 nM prenalterol = 126 ± 5%; albuterol = 142 ± 6% of control), and 3) antagonism of the effects of both agonists was greater with ICI 118,551 than with propranolol or ICI 89,406. These results suggest that the facilitation of ^{3}H-NE release by β-adrenergic agonists primarily involves β2-adrenergic receptors. (Supported by NIMH Grants MH 40694, MH 51175 & MH 01231).

430.20

MODULATORY EFFECT OF BRADYKININ BY B_1 AND B_2 RECEPTORS ON NORADRENALINE RELEASE IN ISOLATED MOUSE ATRIA. C. Chulak*, R. Couture and S. Foucart Groupe de Recherche sur le Système Nerveux Autonome, Département de physiologie, Université de Montréal, Montreal, Canada, H3C 3J7

The aim of this study was to investigate the modulatory effect of bradykinin (BK) on the release of noradrenaline (NA) from isolated mouse atria. Atria from CD1 mice were isolated and incubated with [^{3}H]-NA and then inserted in a suprafusion system (6 pairs of atria in parallel). After a washing period, the radioactivity was collected at every 5 min for 60 min. During this collecting period, the atria were electrically stimulated (5 Hz, 2 ms, 50 mA, 60 sec) at 10 min (S_1) and at 45 min (S_2). At the end of the experiments, the radioactivity was counted and the results were expressed by the ratio S_2/S_1. The drugs were added 20 min before S_2. The results show that BK (10 nM) alone did not affect significantly [^{3}H]-NA release induced by electrical stimulation. However, in the presence of a B_1 receptor antagonist, [Leu^8]-des-Arg^9-BK (100 nM), BK (10 nM) had a facilitatory effect on [^{3}H]-NA release. This facilitatory action of BK (10 nM) was inhibited by HOE 140 (10 nM), a B_2 receptor antagonist. The B_1 agonist, des-Arg^9-BK (10 nM) had an inhibitory effect on [^{3}H]-NA release when used with the B_2 antagonist (100 nM). Moreover, BK had a facilitatory effect on [^{3}H]-NA release when administered with a cyclooxygenase inhibitor, diclophenac (1 µM). These results suggest that BK had a facilitatory effect through the activation of prejunctional B_2 receptors and also had an inhibitory effect through the activation of B_1 receptors linked to production of prostaglandins.

431.1

TRANSMITTER RELEASE PROBABILITY FUNCTION: A CONTINUOUS DISTRIBUTION. E. P. Huang* and C. F. Stevens. Salk Institute, La Jolla, CA 92037.

Whole cell recording in rat hippocampal CA1 region was performed to examine the distribution of synaptic transmitter release probabilities. Cells in slices were clamped at -40mV, and NMDA receptor mediated EPSCs were isolated by stimulating in the stratum radiatum in the presence of 5 μM DNQX. After application of NMDA receptor open channel blocker MK-801, the amplitude of EPSCs decreases with repeated stimuli as receptor block accumulates; the rate of decrease is determined by the synaptic release probability (Hessler et al., *Nature*, **366**: 569 (1993); Rosenmund et al., *Science*, **262**: 754 (1993)). The blocking rate is also determined by MK-801 concentration; we measured the dose response of NMDA receptor block at 10, 20, and 40 μM MK-801. The fitted K_i is 20 μM. Theoretical analysis shows EPSC amplitude decline in MK-801 is well fitted by a model incorporating a continuous distribution of release probabilities, such as previously reported (Allen et al., *PNAS*, **91**:10380 (1994)). To further characterize this distribution we compared MK-801 block of synapses with release probabilities altered under two conditions: (1) paired pulse facilitation (PPF) and (2) increased or decreased Ca^{2+} concentration. PPF synapses show faster EPSC amplitude decline than control due to increased release probability. The facilitation ratio is measured during the blocking and found to be constant across synapses of all probabilities. Our model of MK-801 block predicts a relationship between the change in decline and the measured PPF ratio; our results are in good agreement with these predictions. The change of release probability in different $[Ca^{2+}]$ is predicted by the Dodge-Rahamimoff model. We measured MK-801 block of synapses in $[Ca^{2+}]$ of 1.0, 1.75, 2.5, and 6.0 mM. We fit the rate of decline versus $[Ca^{2+}]$ to the D-R equation; fitted constants K_m and K_c are 3.4 mM and 0.8 mM, respectively.

431.2

MONTE CARLO SIMULATION OF FAST EXCITATORY SYNAPTIC TRANSMISSION AT A HIPPOCAMPAL SYNAPSE. L. M. Wahl, C. Pouzat, and K. J. Stratford*. Univ. Lab. of Physiology, Parks Rd., Oxford, OX1 3PT, UK.

Fast excitatory synaptic transmission at the hippocampus has been simulated by modelling individual neurotransmitter molecules as they diffuse through the synaptic cleft and interact with postsynaptic receptors. 4000 neurotransmitter molecules are simultaneously released at a point source 15 nm above a rectangular array of 14x14 postsynaptic receptors. The model is able to reproduce published results of patch clamp experiments on CA3 pyramidal cells at room temperature, predicting an excitatory postsynaptic current (EPSC) with a 10-90 rise time (t_r) of 0.28 ms, a peak open probability (P_o) of 0.27, and a decay time constant (τ_d) of 2.33 ms. The coefficient of variation (CV) at the peak of the EPSC is 9.4%. At 37°C, t_r is 0.07 ms, P_o is 0.56, and τ_d is 0.70 ms. The CV at the peak is 6.6%. Slower rates of diffusion of neurotransmitter in the cleft increase the peak response and slow the time course of decay. Random variations in release site position have little effect on the time course of the average EPSC or the peak response, whereas changes to the width of the synaptic cleft, or to the number or spacing of postsynaptic receptors, have marked effects on the peak amplitude of the simulated EPSC. Variations in the number of neurotransmitter molecules released also affect EPSC peak amplitude, but the magnitude of this effect is strongly dependent on the number of postsynaptic receptors and the rate of neurotransmitter diffusion in the cleft.

431.3

MODELING THE CO-LOCALIZATION OF Ca^{2+} CHANNELS AND Ca^{2+} RECEPTORS: CORRECT HANDLING OF Ca^{2+} DOMAINS. R. Bertram* and A. Sherman. Mathematical Research Branch, NIDDK, NIH, Bethesda, MD 20892.

Ca^{2+} channels and Ca^{2+} receptors are often co-localized, so that receptor proteins are influenced almost exclusively by the Ca^{2+} from one or more adjacent channels. Examples include the co-localization of N-type Ca^{2+} channels and vesicle fusion proteins (Stanley, Neuron, 11:1007) and the co-localization of Ca^{2+} channels and calcium-activated K^+ channels (Gola and Crest, Neuron, 10:689). This co-localization makes the modeling of transmitter release from a synaptic terminal or the total K(Ca) conductance of a patch of membrane problematic, due to the stochastic nature of channel openings which gives each binding complex a different history of exposure to Ca^{2+}. We describe a deterministic method which accounts for this stochasticity. Using a model of transmitter release based on single-channel Ca^{2+} domains (Bertram *et al.*, Biophys. J., 68:A396) we show that it is not valid to approximate the domain Ca^{2+} concentration by the average domain Ca^{2+} concentration if one or more of the binding sites comprising the receptor complex has kinetics which are slow compared to the time constant of the channel. Such slow Ca^{2+} binding sites are now thought to be crucial for fast synaptic facilitation (Stanley, J. Neurosci, 6:782). We use this model in conjunction with a model for bursting in dopamine neurons (Li *et al.*, Soci. Neurosci. Abstr., 20:1507) to demonstrate that more transmitter is released when the cell is in bursting mode than when it fires tonically, even though bulk cytosolic Ca^{2+} does not accumulate.

431.4

SPATIALLY RESOLVED MEASUREMENTS OF THE ENDOGENOUS CALCIUM BUFFERING CAPACITY IN ADRENAL CHROMAFFIN CELLS

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In calcium imaging experiments, theoretical considerations predict that the indicator dye, in addition to acting as an exogenous calcium buffer, also contributes to the diffusional equilibration of calcium gradients by virtue of its own mobility. In order to accurately assess its role and that of the endogenous calcium buffers in shaping the spatiotemporal dynamics of intracellular calcium signals, we have measured the diffusional dissipation of calcium gradients introduced by short depolarising voltage clamp pulses in chromaffin cells at different concentrations of the indicator dyes Fura-2 or Fura-2-Dextran or a combination of Calcium-Green-1 and Brilliant-Sulfaflavin.

We use a slow-scan CCD imaging sytem with an acquisition rate of 40-50 Hz. To improve the signal-to-noise-ratio we increase the excitation intensity by means of a pulsed power supply within the period of 200-300 ms during which the calcium gradients disappear. As the intracellular diffusion of calcium and indicator dye is inherently a three-dimensional process, its observation using a two-dimensional detector was achieved by obtaining responses at several planes of focus. The blurring introduced by the optical system was compensated for by using an iterative deconvolution algorithm based on regularisation theory. Using a diffusion model we calculate the endogenous buffering capacities in a spatially resolved manner.

431.5

MICROMOLAR 4-AMINOPYRIDINE ENHANCES INVASION OF A NEUROSECRETORY TERMINAL ARBORIZATION: OPTICAL RECORDING OF ACTION POTENTIAL PROPAGATION USING A PHOTODIODE ARRAY AND AN ULTRAFAST PHOTODIODE-MOSFET CAMERA. A.L. Obaid and B.M. Salzberg*. Department of Neuroscience, UPENN School of Medicine, Phila., PA 19104-6074.

The vertebrate hypothalamo-neurohypophysial system is a useful model for the study of excitation-secretion coupling. Magnocellular neurons located in the hypothalamus project their axons as bundles of fibers through the median eminence and infundibular stalk to arborize and terminate in the neurohypophysis, where the neurohypophysial peptides and proteins are released into the circulation by a Ca-dependent mechanism. Elevating $[Ca^{2+}]_o$ from 2 to 5 mM enhances release of arginine vasopressin from these terminals, as does the addition of 4-aminopyridine (4-AP) in micromolar concentrations. However, preliminary evidence based on the rapid changes in light scattering that accompany release from these terminals (Salzberg, Obaid, & Gainer, J. Gen. Physiol. 86: 395, 1985; Obaid, Flores & Salzberg, J. Gen. Physiol. 90: 32a, 1987), suggested that while Ca increases the amount of peptide released per active terminal, 4-AP acts by increasing the number of active terminals through a mechanism of ***enhanced invasion of the terminal arborization***. Here, we show directly, using the voltage-sensitive dye NK 2761 and a differential photodiode-MOSFET camera (HR Deltaron 1700, Fuji Photo Co.), having 16,384 pixels (128 x 128) and operating at a frame rate of 1.7 kHz, that 10 μM 4-AP dramatically increases the spatio-temporal spread of the action potential in the neurohypophysis of the frog *Xenopus*. We will show moving pictures of the enhanced invasion of the neurosecretory terminal arborization in micromolar 4-AP, together with evidence that elevated Ca has no effect on the pattern of action potential propagation.

We are grateful to Fuji Medical Systems (Stamford, CT) for the loan of the HR Deltaron 1700 and to the NINDS for USPHS grant NS16824.

431.6

ANALOG CIRCUIT OF A PASSIVE BRANCH POINT. M.D. Goldfinger* and K. Moradmand. Dept. Physiology & Biophysics, Wright State U., Dayton, OH.

We solved the cable equation for a 1-dimensional core-conductor consisting of a parent fiber and two daughter branches using a series of connected compartments each of which corresponded to 0.0165λ of a 620.μm-diameter squid axon (λ=4.8216 mm) with R_m=1453.5 Ω·cm^2, C_m=1.0 μF/cm^2, ρ_i=108.5 Ω·cm, and 12 msec-duration constant current pulses. In unbranched configuration, transient and steady-state responses were well-fit by analytic solutions (1) including the shortening of the charging transient when total electrotonic length increased from 1 to 11λ; errors >5% were restricted to the first 0.3 t/τ of the transient. The branch-point circuit (BPC) consisted of a 2.0λ parent and two 2.0λ branches. The second branch attenuated the magnitude of transient and steady-state voltages at any x but did not alter the time course, as reconstructed with a numerical literal compartmental model (LCM) using trapezoidal integration (2) with Δx=0.0165λ and Δt=50 nanosec.

With parent distal-end stimulation, BPC and LCM steady-state potentials declined monotonically with x. Rall's Equivalent Circuit (REC,3) underestimated these voltage changes. BPC and LCM steady-state axial current declined monotonically to the branch point and was equally divided into each branch. REC steady-state axial current increased discontinuously at and because of the diameter expansion. Close to (±0.075λ) the branch point, BPC transients were reconstructed by LCM but not by REC. BPC and LCM axial currents flowing from the parent were twice that flowing into each branch; compared to LCM, REC overestimated the entire axial current waveform at the geometrical transition.

Thus, while LCM accurately reconstructed the passive properties of the BPC, REC was not the electrical equivalent of BPC. Supported by NSF BCS-9315856(MDG).
Ref.s: (1) Jack et al.,1975, *Electric Current Flow in Excitable Cells*; (2) Goldfinger et al.,1992, Biol.Cybern.*66*:399; (3) Goldstein & Rall,1974,Biophys.J.*14*:731.

431.7

FOUR MODES OF GABAERGIC SYNAPTIC TRANSMISSION IN CULTURED EMBRYONIC RAT HIPPOCAMPAL NEURONES.

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Most of the current to clamp at -80mV (whole-cell patch configuration, symmetrical ([Cl^-])) hyppocampal neurones from 18-22 day old rat embryos with CsCl electrodes represents the activity of GABA receptor-coupled Cl^- channels. Analysis of baseline and tranient Cl^- currents reveals that GABA is secreted from these neurones according to four modes as reported for ACh secretion from motor terminals. 1) Tonic secretion is characterized by a baseline noise corresponding to random openings of the Cl^- channels. 2) One or a group of a few poorly synchronized transmitter discharges is characterized by TTX-resistant, small mode Cl^- transient signals with highly variable peak amplitude (PA) and rise-time (RT). Elementary discharges peak at 10-20pA. 3) Well synchronized transmitter discharges (10-30) are characterized by medium mode (100-300pA), temporarily (15min) TTX-resistant, transient signals with moderately variable PA and RT. 4) Presynaptic action potential-evoked release is characterized by large mode (800-1200pA), TTX-sensitive, transient signals with little variability in PA and RT. Reversible, rapid or progressive interconversions beween tonic and pulsatile secretory modes and between poorly and well-synchronized pulsatile modes occur spontaneously or are induced by conditions known to modify $[Ca^{2+}]_i$. Spontaneous or action potential-triggered presynaptic intracellular Ca^{2+} transients are proposed to synchronize transmitter secretion to produce postsynaptic transient currents.

431.8

SYNCHRONOUS AND ASYNCHRONOUS EPSCs EVOKED IN HIPPOCAMPAL SLICES: A TEST OF THE QUANTAL MODEL OF NEUROTRANSMISSION.

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The classical quantal model of synaptic transmission proposes that the amplitudes of evoked excitatory postsynaptic currents (EPSCs) occur as integral multiples of the amplitude of miniature EPSCs (mEPSCs). mEPSCs occur spontaneously in the postsynaptic cell following the asynchronous release of a single vesicle, or 'quantum', of neurotransmitter. Apparent quantization of EPSCs at central synapses has been reported, but mEPSCs generally had too broad an amplitude distribution to underlie the tight quantization of the evoked EPSCs, possibly because the mEPSCs and evoked EPSCs did not originate from the same population of synapses. The goal of this work is to test the quantal model at a central synapse under conditions in which evoked and miniature EPSCs originate at the same release site(s).

Whole-cell patch clamping was used to record EPSCs in dentate granule cells in 400 μm hippocampal slices from juvenile rats while weakly stimulating the perforant path. EPSCs evoked in control external solution (containing 2 mM Ca) showed predominantly synchronous release with short latency (< 8 ms). Exchanging the external Ca for 8 mM strontium (Sr) produced EPSCs with a similar mean amplitude of synchronous release, but in addition a pronounced phase of asynchronous release lasting hundreds of milliseconds. This effect of Sr has been reported for the endplate (*Nature* **212**:1233, 1966) and cultured hippocampal neurons (*PNAS* **91**:12942, 1994) and is presumably due to residual Sr remaining in the stimulated presynaptic terminals. These asynchronous currents resembled mEPSCs in having no obvious inflections in their rising edges. Risetime and amplitude distributions for mEPSCs were similar to those of the synchronous EPSCs. Amplitude distributions for each were skewed (coefficient of variation 0.4-0.5) and had means of 3-6 pA (at -70 mV). These distributions will be subjected to quantal analysis using robust statistical techniques (*Biophys. J.* **67**:532, 1994).

431.9

ONTOGENY OF ADRENERGIC MODULATION OF $GABA_A$ RECEPTOR-MEDIATED INHIBITION IN SOMATOSENSORY CORTEX OF RAT.

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Adrenergic agonists decrease evoked inhibitory postsynaptic currents (eIPSCs) in hippocampal CA1 pyramidal neurons by decreasing the efficacy of excitatory synaptic input to presynaptic GABAergic interneurons (Doze et al., Neuron 6:889). We used whole-cell voltage-clamp techniques in slices of somatosensory cortex to study adrenergic modulation of monosynaptic eIPSCs in layer V pyramidal neurons. Experiments were designed to determine 1) whether adrenergic effects on eIPSCs might differ from those in hippocampus and, 2) to examine the ontogeny of this modulation. Monosynaptic IPSCs were evoked by layer V stimulation in slices bathed with ACSF containing 20 μM DNQX and 50 μM APV. Of 21 neurons studied in slices from animals aged P9-P12, 15 were responsive to 10 μM noradrenaline or adrenaline (bath application). Thirteen displayed a pronounced depression of eIPSCs that was reversible in 5 cells (43%±11%) and irreversible in 8 cells. An enhancement of eIPSCs was seen in 2 neurons and was reversible in 1. By contrast, adrenergic stimulation in 6 neurons from animals aged P15-P18 produced either a reversible enhancement (23%±13%) of eIPSCs (3 of 5 responsive cells) or an irreversible depression of eIPSCs (2 of 5). Adrenergic agonists increased spontaneous IPSCs in almost all neurons of both age groups but did not alter the baseline holding current in any cells. In contrast to findings in the hippocampus, these data indicate that adrenergic agonists directly modulate eIPSCs presumably through actions on presynaptic GABAergic neurons. The effects appear to be age-dependent so that reversible actions change from predominantly a depression of eIPSCs at P9-P12 to an enhancement of $GABA_A$-mediated inhibition in the older age group ($p<0.02$, Mann Whitney U test). These studies were supported by NIH Grant NS12151 from the NINDS and a Pimley fellowship to BDB.

431.10

NICOTINE FACILITATION OF GLUTAMATERGIC SYNAPTIC TRANSMISSION IS MEDIATED BY PRESYNAPTIC CALCIUM INFLUX.

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Both the medial habenula (MHN) and the site of its primary projections, the interpeduncular nucleus (IPN), express nicotinic ACh receptors (nAChRs) and choline acetyltransferase. In recordings from dispersed IPN neurons innervated by explants of MHN, we find that the transmission is glutamatergic but that nAChR activation facilitates both evoked and spontaneous transmission by a presynaptic mechanism. The EC_{50} for nicotine-induced facilitation is 170 nM, and it is blocked by pretreatment with α-bungarotoxin (100 nM). Thus it is likely that the α7 or α8 nAChR subunits are components of the receptors mediating this facilitation. We are utilizing antisense oligonucleotide deletion of the α7 subunit to characterize further the role of this subunit in presynaptic facilitation by nicotine. Since α7 nAChRs expressed in Xenopus oocytes are highly Ca^{2+} permeable, channels whose composition includes this subunit would be ideal regulators of synaptic transmission, particularly if expressed near sites of transmitter release. We have determined that the facilitation induced by nicotine is dependent upon the presence of Ca^{2+} in the external solution. Therefore, we have used fura 2 imaging of intracellular $[Ca^{2+}]$ in the cocultures and in dispersed MHN neurons to examine the subcellular localization of these receptors as well as the intracellular mechanisms involved in this response. In dispersed habenula neurons nicotine induces a concentration-dependent increase in $[Ca^{2+}]_i$ with an EC_{50} of approximately 1 μM. To test whether Ca^{2+} enters the cells through nAChRs *per se* or through voltage gated Ca^{2+} channels (VGCC) we are testing the effects of various VGCC blockers (at concentrations that do not effect nAChR channels). Preliminary data indicate that VGCCs contribute to this response. Experiments are underway to test whether the nAChRs expressed in the periphery of these neurons differ in their Ca^{2+}-permeability or in their concentration-dependence of activation from those found on the somata. Supported by NS22061, Cncl. Tobacco Res. & McKnight Found. grants to LR and NS09395 to DM.

431.11

DOPAMINERGIC INHIBITION OF SYNAPTIC CURRENTS IN CHOLINERIC MAGNOCELLULAR NEURONS IN THE BASAL FOREBRAIN.

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Magnocellular cholinergic neurons in the basal forebrain, which form the main source of cholinergic projection to cortical and subcortical regions of the mammalian brain, receive dopaminergic inputs from both the substantia nigra pars compacta and ventral tegmental area. In the present study whole-cell recording experiments were carried out on thin slice preparations of the rat brain to elucidate the effect of dopamine on postsynaptic currents in the magnocellular basal forebrain neurons. Coronal slices (200 μm) of the horizontal limb of the diagonal band of Broca and substantia innominata/nucleus basalis were cut from 12-14 day old rats. After whole-cell configuration was made from visualised magnocellular basal forebrain neurons (> 20 μm, considered to be cholinergic from morphological studies) a stimulating electrode was placed within the nucleus or on a nearby neuron to evoke synaptic currents. Both glutamatergic EPSCs, which were blocked by CNQX, and GABAergic IPSCs, blocked by bicuculline, were recorded. Dopamine suppressed both EPSCs and IPSCs in a concentration-dependent manner between 10 and 100 μM. SKF 81297, a D_1 agonist (10-30 μM), mimicked the action of dopamine, whereas (-)TNPA, a D_2 agonist, had little effect at concentrations up to 30 μM. Dopamine-induced suppression of EPSCs and IPSCs was antagonized by SCH 23390, a D_1 antagonist, while antagonism by eticlopride, a D_2 antagonist, was less prominent. In addition, the frequency of miniature EPSCs and IPSCs, recorded in the presence of TTX (0.5 μM), was reduced by dopamine (10-30 μM), whereas their mean amplitude was unaffected.

These findings suggest that afferent inputs to magnocellular cholinergic neurons in the basal forebrain express mainly D_1 receptors to modulate excitatory and inhibitory transmission.

431.12

POSSIBLE MECHANISMS UNDERLYING PATTERNS OF ELECTRIC ACTIVITY IN NEUROHYPOPHYSIAL TERMINALS.

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The different bursting patterns of action potentials (APs) in the hypothalamic magnocellular cells are important for neurohypophysial peptide release. Properties of APs in the neurohypophysial terminals, however, are still poorly understood. Recent characterization of the major voltage-activated ion channels allows us to gain insights into the mechanisms underlying AP patterns in the isolated terminals. Current-clamping with conventional and perforated 'whole-terminal' configurations shows that APs can be elicited from resting potentials (Vm) of -70 to -85 mV. The duration (12.7 ms) of AP (APD) was increased in a frequency- and Vm-dependent manner. The depolarizing phase of the AP was dependent only on Na^+ channel activity, but the repolarizing phase could be divided into an early, fast and a late, slow phase. The first phase was affected by the K^+(A) channel blocker 4AP, producing a substantial prolongation of the APD. Using the perforated configuration or with low (0-50 nM) intraterminal Ca^{2+} concentrations ($[Ca^{2+}]i$), the second phase was even slower. Acceleration of this slow phase by high (10 μM) $[Ca^{2+}]i$ could be strongly antagonized by the K^+(Ca) channel blockers Ba^{2+}, tetrandrine and TEA. In addition, the K^+(A) and K^+(Ca) channel blockers had opposite effects on the Vm: the former increased it whereas the latter decreased it. These data suggest that: (1) the fast and slow repolarizing phases of the terminal's AP are dependent on K^+(A) and K^+(Ca) channel activities, respectively; (2) the genesis of bursting patterns might be due to an initially low $[Ca^{2+}]i$ which would result in a slower repolarization of the second phase and make the next AP trigger easier, while gradually accumulating high $[Ca^{2+}]i$ would eventually terminate the burst. (Supported by NIH NS29240)

431.13

KAPPA-OPIOID RECEPTOR ACTIVATION MODULATES Ca^{2+} CURRENTS AND SECRETION IN ISOLATED NEUROENDOCRINE NERVE TERMINALS. K.I.Rusin*, D.R.Giovannucci, H.C.Moises and E.L.Stuenkel. Department of Physiology, University of Michigan, Ann Arbor, MI 48109-0622.

Whole cell patch clamp recordings were obtained from nerve terminals acutely dissociated from neurohypophysis of adult rats to investigate modulation of calcium currents and secretion by activation of opioid receptors. External and internal solutions were chosen to suppress sodium and potassium currents and calcium (10 mM) or barium (10 mM) was used as the charge carrier through calcium channels. Observed currents were characterized as high-threshold calcium currents. Bath administration of the κ-opioid agonists U-50488 (1-10 μM) and U-69593 (1 μM) suppressed peak amplitude of the calcium currents by 32.8±12.9% (n=4) and 43.1±24.7% (n=3), respectively. Measurements of cell capacitance showed that κ-opioids reduce depolarization-induced exocytosis in nerve terminals of the neurohypophysis. In contrast, administration of the μ-opioid agonist H-Tyr-D-Ala-Gly-Phe(N-Me)-Gly-ol (DAGO, 3 μM) did not effect calcium currents (n=6). These data suggest that κ- but not μ-opioid receptors are negatively coupled to calcium channels in nerve endings of the rat neurohypophysis and may regulate release of oxytocin and vasopressin from these terminals. (Supported by NSF 9410834 to ELS and DA 03365 to HCM).

431.14

PRESYNAPTIC MUSCARINIC INHIBITION OF NICOTINIC TRANSMISSION IN BULLFROG SYMPATHETIC GANGLIA W-X Shen* and JP Horn
Department of Neurobiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

Presynaptic muscarinic modulation was analyzed by recording nicotinic EPSCs from bullfrog sympathetic neurons under 2-electrode voltage-clamp in normal Ringer. Muscarine was bath-applied to cells in which EPSCs were evoked at 0.10 - 0.17 Hz. In B cells, EPSC amplitude was reduced by 60% (EC_{50} = 105 nM, n = 8). Muscarine (0.3 to 3 μM) had no effect on EPSCs in 4 C cells. Results from two types of experiment suggest that muscarine acts presynaptically to inhibit evoked acetylcholine (ACh) release. First, in 4 B cells, muscarine did not change nicotinic currents produced by iontophoresis of ACh. Second, when the coefficient of variation (CV) of EPSC amplitude was measured in the presence and absence of muscarine (10 B cells), the CV increased in proportion to the mean level of EPSC inhibition.

Presynaptic muscarinic receptors are activated by ACh that is released during repetitive nerve stimulation. Trains of 40 EPSCs were recorded in control Ringer and 300 nM atropine. Their difference was used to estimate the time course and magnitude of muscarinic inhibition. At 1 Hz, EPSC amplitude progressively declined by 10 - 15% with a latency of 4 - 5 s. At 5 Hz, the latency decreased to 2 s and EPSC inhibition increased to 40%. However, at 20 Hz, EPSC amplitude remained unaltered.

These results show that presynaptic muscarinic receptors in a subclass of preganglionic sympathetic nerve terminals inhibit ACh release during low-frequency stimulation. (Supported by NIH NS21065 and a Grant-in-Aid from the AHA, PA Affiliate).

431.15

EXCITATORY SYNAPTIC INPUT TO AXONS IN THE SPINAL CORD. Amanda J. Holt* and Simon Alford. Dept. of Physiology, Northwestern University Medical School, Chicago IL 60611.

In axons, release of transmitter follows transmission of action potentials from the soma to presynaptic terminals. The traffic of signals in axons is considered unidirectional, originating in the soma and ending at the axon termination. Release is subject to modification by autoreceptors and axo-axonic presynaptic inhibition, however there is evidence that vertebrate axons receive excitatory inputs. Recordings from cat primary afferents (Gossard etal 1989, J. Neurophysiol. 62:1177) indicate that the spinal cord is capable of causing antidromic spike activity. Additionally, synaptosomes release transmitter following application of excitatory amino acids (Barnes etal 1994, Br. J. Pharmacol. 113:339). We made whole cell patch recordings from reticulospinal axons in the spinal cord of the lamprey in which the cell body was removed. Axons ranged in input impedance from 350 to 12 MΩ and in diameter from 20 to 4 μm, as identified by dye filling. Upon achieving whole-cell access, spontaneous transient inward currents are readily apparent which show variable rise times (range: 2 to 20 ms). Fictive locomotion was activated by bath application of 200 μM NMDA. This caused a marked increase in the frequency of these events. Pressure ejection of AMPA over the spinal cord in the presence of TTX caused an immediate inward current in these axons whilst, in contrast, kainate and NMDA pressure ejection had no effect. In 32 recordings, a stimulation electrode was placed over the spinal ventromedial tracts. Stimulation evoked transient inward currents in axons whose rise times ranged from 1.1 to 23 ms. The responses showed a reversal potential markedly positive to resting membrane potential. Inward currents were abolished by the application of the AMPA receptor antagonist CNQX (10 μM) in all cells tested (n=14). In 7 cells the recording method was switched to whole-cell current clamp. Spinal cord stimulation, subthreshold to direct stimulation of the axon, was then capable of evoking a spike in 6 of the 7 recorded axons. In summary, large descending excitatory axons of the spinal cord receive excitatory synaptic input mediated by AMPA/kainate receptors. This input is capable of depolarizing the axon to initiate an action potential. Supported by NINDS grant NS31713.

431.16

ω-CONOTOXIN GVIA-INSENSITIVE NEUROTRANSMITTER RELEASE IN THE ISOLATED RAT ANOCOCCYGEUS MUSCLE: AN ELECTRO-PHYSIOLOGICAL STUDY. A. B. Smith, R. S. G. Jones*, and T. C. Cunnane. Univ. Dept. of Pharmacology, Mansfield Rd, Oxford, OX1 3QT, U.K.

It is generally accepted that N-type calcium channels control action potential-evoked neurotransmitter release in sympathetic nerve terminals. Low concentrations of the N-type calcium channel blocker ω - conotoxin GV1A (ω - CTX) irreversibly abolished excitatory junction potentials (EJPs) evoked by nerve stimulation at ≤ 10 Hz (≤ 5 stimuli). In the present study we have investigated the effects of ω - CTX on EJPs evoked by higher frequencies of nerve stimulation.

Conventional intracellular microelectrode techniques were used and changes in membrane potential of individual smooth muscle cells were recorded to monitor neurotransmitter release. Trains of stimuli at 5-50 Hz were delivered through Ag/AgCl electrodes positioned close to the point of insertion of the pelvic nerves supplying the anococcygeus muscle.

Low concentrations of ω - CTX (10nM) greatly reduced all EJPs in trains of 3 to 5 stimuli at 10 Hz. When the frequency of stimulation was increased (10-50 Hz) trains of stimuli evoked EJPs even in the presence of 1 μM ω-CTX. We have termed this ω-CTX-resistant release 'residual release'. EJP amplitude depended on both the frequency and number of stimuli in a train.

'Residual release' was inhibited to some extent by the P-type calcium channel blocker ω - Agatoxin IVA (100 nM). However, even in the presence of both toxins, longer trains of stimulation could still evoke neurotransmitter release.

Therefore, it would appear that a heterogeneous population of calcium channels is involved in mediating norepinephrine release from these sympathetic nerve terminals.

431.17

PRESYNAPTIC METABOTROPIC INHIBITORY EFFECT INDUCED BY BACLOFEN AT THE CRAYFISH EXCITATORY SYNAPSES. B. Alkahe, H.Golan and Y. Grossman*.
Department of Physiology, Faculty of Health sciences, Ben - Gurion University of the Negev, Beer - Sheva 84105, Israel.

Mechanisms of presynaptic inhibition were studied in the crayfish neuromuscular synapses. Excitatory post synaptic currents (EPSCs) were recorded by using a macropatch clamp technique. Inhibition was induced by either stimulating the inhibitory axon a few ms before the evoked EPSC or, by application of 5-50μM GABA to the bath solution for a period of 1-2 min. Short train of stimuli to the inhibitor usually decreased the potentiated EPSC by 43%. Application of 10, 30 and 50μM GABA caused 30%, 50% or 100% reduction in EPSC amplitude, respectively. Application of 100μM baclofen, a known $GABA_B$ agonist, did not affect the single EPSC amplitude. However it increased the efficacy of the evoked inhibition by 22% (n=4). Moreover, baclofen induced full recovery of evoked inhibition in synapses treated (for 60 - 90 min) with aminooxy acetic acid, a known glutamate decarboxylase blocker, which depressed inhibition efficacy by 38% (n=4). Baclofen effects were observed after 60 - 90 min incubation, and blocked by application of 50 μM picrotoxin to the bath solution. These results suggest a modulatory effect by a presynaptic metabotropic GABA receptor whose activation increases the efficacy of a picrotoxin-sensitive GABA-dependent chloride inhibition.

431.18

NMDA-INDUCED CHLORIDE CURRENT IN PC12 CELLS A. Yoshida*, H. Takagi[1], Y. Kudo[1] and T. Yoshioka. Department of Molecular Neurobiology, Waseda University, Tokorozawa, Saitama 359, JAPAN. [1]Tokyo University of Pharmacy and Life Science, Hachiooji, Tokyo 192-03 JAPAN.

NMDA receptors have been shown to participate in synaptic plasticity and neuronal cell death. This type of glutamate receptor has a high permeability to Ca^{2+}. In this study, we found a novel NMDA-evoked inward current which was a slowly activated after NMDA application to whole-cell voltage-clamped PC12 cells, and these responses appeared in the presence of up to 10μM NMDA. The inward current induced by 100μM NMDA was blocked by the competitive NMDA antagonists AP7 and APV (100μM). However, no NMDA-dependent increase in $[Ca^{2+}]_i$ was detected by fura-2-Ca^{2+} measurement, and unusually the reversal potential of the NMDA-dependent current was nearly -40mV. To determine whether Cl^- could cause the NMDA-evoked current, the reversal potential was measured at various $[Cl^-]_i$. The reversal potentials were shifted to the positive side by increasing $[Cl^-]_i$ and fitted the calculated value. It was previously reported that syntaxin 1B forms an NMDA receptor channel in a *Xenopus* oocyte expression system. The properties of the syntaxin channel were similar to the NMDA-dependent Cl^- channel in PC12 cells detected in this study. To examine whether syntaxin forms this Cl^- channel, we treated the PC12 cells with botulinum neurotoxin C1 (BoTx C1) which specifically cleavages syntaxin and blocks transmitter release. The treatment of toxin prevented the NMDA-induced inward current. However, the treatment of BoTx A and C, which don't cleave syntaxin but block transmitter release, also blocked this current. These results suggest that the NMDA stimulation opened Cl^- channels in relation to exocytotic events in PC12 cells.

431.19

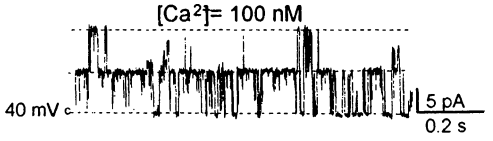
SEROTONIN PROLONGS ACTIVITY-DEPENDENT SYNAPTIC ENHANCEMENT AT CRAYFISH NEUROMUSCULAR JUNCTION S.-M. Qian* and K.R. Delaney Dept. of Biosciences, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6

Recovery of activity-dependent synaptic enhancement (ADSE) after moderate trains of action potentials (30 secs, 15 Hz) exhibits characteristic rapid (τ <1sec; facilitation) and slow (τ ≈10 secs at 15.5°C; augmentation) phases. Serotonin (5-HT) increases the *amplitude of excitatory junctional potentials (ejps) at crayfish* neuromuscular junction (nmj) by 2-5 fold through presynaptic mechanisms (e.g. Dixon and Atwood 1985; 1989). To explore possible neuromodulation of ADSE, we have examined the effect of 5-HT on ejps after trains of action potentials. 5-HT (1μM) slows the recovery of the slow phase of ADSE up to 4.5 fold (2.92±1.20, mean±sd; n=16) without changing the initial magnitude of slow ADSE measured immediately after the train. Reducing extracellular [Ca^{2+}] by 50% reduces transmitter release but has little or no effect on the prolongation of ADSE by 5-HT, suggesting the prolongation is not an artifact of saturation of transmitter release. The prolongation of ADSE and enhancement of ejp amplitudes reverse with a similar time course during several hours of washing (linear correlation, r=0.84). Preliminary data indicate that the effects of 5HT on the slow phase of ADSE may result from increased intracellular Ca^{2+} buffering, either the amount or the affinity of endogenous Ca^{2+} buffer. However, it is not consistent with a slowing of Ca^{2+} removal by pumps. Such neuromodulation of the time course of decay of ADSE may be imporant for the implementation of time-integrative properties of neural networks particularly for storage of short-term memory "traces". Support: NSERC, Canada OGP0121698

431.20

DIRECT RECORDING OF A HIGHLY CALCIUM-SENSITIVE POTASSIUM CHANNEL ON THE PRESYNAPTIC NERVE TERMINAL OF THE CHICK CILIARY GANGLION. Sun, X.P.* and Stanley, E.F. SMS, NINDS, NIH, Bethesda, MD 20892.

Ca^{2+}-activated potassium channels have been localized to presynaptic nerve terminals (Robitaille, et al.: Neuron, 11:645) where they are believed to be important in the termination of action potential gated *transmitter release. We have examined these channels by patch* clamping on the calyx presynaptic nerve terminal of chick ciliary ganglion. A Ca^{2+} and voltage-sensitive potassium channel was recorded with a single channel conductance of 219 ± 15.1 pS (n=8). The open probability of the channel was half maximal in 100 nM Ca^{2+} at +40 mV and exhibited an e-fold change over 33.5 ± 6.5 mV (n=3). The channel was blocked by charybdotoxin (10 nM) but not by apamin. The high calcium sensitivity of the presynaptic terminal maxi-K' channel suggests that it plays an important role in defining the time course of transmitter release.



PRESYNAPTIC MECHANISMS VIII

432.1

EFFECTS OF THE PESTICIDE ALDICARB ON CHOLINERGIC TRANSMISSION M.F.M. Braga, O.V. Sousa, M.D. Santos, L.E.F. Almeida, W.S. Cortes, W.M. Cintra, Y. Aracava*, and E.X. Albuquerque Lab. Mol. Pharmacol., IBCCF, UFRJ, Rio de Janeiro, RJ 21944, Brazil; Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201, USA.

The carbamate aldicarb (ALD), like other cholinesterase (ChE) inhibitors, has been reported to interact directly with the muscle nicotinic receptor (nAChR, *Soc Neurosci. Abs.* 20: 1138, 1994). In the present study, we investigated the effects of ALD on the neuromuscular transmission in various preparations. In Mg^{2+}-paralyzed sciatic nerve-sartorius muscle preparation from frog (*L. occelatus*), ALD (0.1-300 μM) increased the amplitude and prolonged the decay time constant of evoked and spontaneous end-plate potentials (EPP) in a concentration-dependent manner. These effects could not be fully explained by the anti-ChE activity of ALD, because maximal ChE inhibition could be obtained with 10 μM ALD. To investigate whether the effects of ALD (30-300 μM) could be attributed to a direct interaction of this compound with the muscle nAChR, we applied the whole-cell and the cell-attached modes of the patch-clamp technique to cultured rat myoballs and to frog single muscle fibers, respectively. In rat myoballs, ALD (1-50 μM) increased the peak amplitude of ACh (1 μM)- or anatoxin-*a* (1 μM)-evoked whole-cell currents, although ALD alone was unable to elicit macroscopic currents. Thus, the potentiating effect of ALD could be the result of its interaction with an allosteric site on the nAChRs. In frog muscle fibers, ALD (1-100 μM) decreased the mean open time of ACh-activated single channels and increased the number of brief closures within a burst, indicating that ALD can act as an open-channel blocker at muscle nAChRs. Our findings indicate that in addition to acting as a ChE inhibitor, ALD could facilitate the neuromuscular transmission by acting on an allosteric site on the muscle nAChRs, and that such a facilitation may be counteracted by its open-channel blocking activity. *Support:* FINEP/UMAB, FINEP, and CNPq.

432.2

DOES (+)-AMPHETAMINE FACILITATE THE TRANSMITTER RELEASE OF MOTOR NERVE ENDING? M.C. Tsai*, Y.H. Chen, and C.C. Ho. Department of Pharmacology College of Medicine, National Taiwan University, Taipei, Taiwan R.O.C.

(+)-Amphetamine has powerful CNS stimulant action and causing dopamine release in the brain. However, it decreased the amplitude of end plate potential (EPP). The effect of (+)-amphetamine on synaptic transmission and motor nerve terminal activity were assessed on mouse *triangularis sterni* nerve-muscle preparations. The perineural waveforms were recorded with extracellular electrodes placed in the perineural sheath of motor nerves. (+)-Amphetamine (13.6 μM) decreased the components of waveforms associated with potassium current and the slow potassium current of nerve terminal while it had no effect on that associated with the sodium, calcium and the calcium activated potassium currents of the nerve terminal. (+)-Amphetamine also increased the frequency of spontaneously generated MEPP and the quantal content of EPP. However, (+)-amphetamine decreased the amplitude of EPP. The MEPP was diminished in amplitude to the extent that the EPP was reduced despite an increase in quantal content. It is concluded that amphetamine decreased the potassium current in the nerve terminal. The effect may contribute to the pharmacological actions of amphetamine on synaptic transmission (Supported by NSC-82-0420-B002-M10 and NSC-84-2331-B002-107).

432.3

EFFECTS OF PARAOXON ON HIPPOCAMPAL GABAERGIC-SYNAPTIC TRANSMISSION. Y. Aracava[1,2], E.S. Rocha[1*], K.L. Swanson[1] and E.X. Albuquerque[1,2]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., USA; [2]Lab. Mol. Pharmacol. II, IBCCF, UFRJ, Rio de Janeiro, Brazil.

Several studies have suggested that the toxicity of paraoxon, mainly its convulsant properties, are not solely related to the irreversible inhibition of acetylcholinesterase. In the present study, we used the GABA-mediated transmission system in the mammalian central nervous system as a model to study the effects of paraoxon that are not correlated with cholinesterase inhibition. The whole-cell patch-clamp technique was applied to cultured rat hippocampal neurons, and spontaneous miniature postsynaptic currents (SMPSCs) mediated by GABA were pharmacologically identified and recorded. Paraoxon at several concentrations was applied for 5 to 10 min to the neurons. Each concentration was delivered through one of six parallel barrels placed 100 μm away from the soma. The frequency, peak amplitude, and decay time constant of the SMPSCs were determined. Paraoxon (300 nM) irreversibly increased the frequency of GABA-mediated SMPSCs to 180 ± 15% (mean ± SE, n=5) of control by a local presynaptic mechanism that was independent of either cholinesterase inhibition or action potential activity. In addition, exposure of neurons to paraoxon (3 μM to 1 mM) caused a 30 - 50% reduction of the peak amplitude and decay time constant of the SMPSCs (n=9). These blocking properties of paraoxon were immediately reversed upon washing. These data suggest that the marked potentiation of transmitter release and the blockade of $GABA_A$ postsynaptic receptors may contribute to the toxicity of paraoxon in the mammalian central nervous system. (USPHS Grants NS 25296, ES05730 and T32-ES07263).

432.4

N-ETHYLMALEIMIDE (NEM) BLOCKS DEPOLARIZATION-INDUCED SUPPRESSION OF INHIBITION IN RAT HIPPOOCAMPAL PYRAMIDAL CELLS. B.E. Alger*, T.A. Pitler, and J.J. Wagner Univ. Maryland Sch. Med., Baltimore, MD 21201.

Depolarization-induced suppression of inhibition (DSI) is the transient decrease in $GABA_A$ IPSCs that follows the activation of CA1 pyramidal cells or Purkinje cells with brief trains of action potentials or a voltage step (Pitler and Alger, 1994, Neuron, 13:1447-55). DSI lasts 60-120 s and is mediated by a retrograde signalling process (Pitler, et al., Soc. Neurosci. Abstr., 1995). DSI is blocked in slices from pertussis-toxin-treated hippocampi, implying the existence of a G-protein-linked step somewhere in the DSI process. It will be useful to apply the criterion of G protein sensitivity in future studies of DSI, but treatment with pertussis toxin itself is laborious, and the technique does not permit confirmation of the capability for DSI in any given pertussis-toxin-exposed cell.

N-ethylmaleimide is a sulfhydryl alkylating agent that inhibits certain G-protein-mediated actions (e.g., Shapiro et al., 1994, J. Neurosci., 14:7109-16). We now report that bath-applied NEM blocks DSI of spontaneous inhibitory postsynaptic currents (sIPSCs) recorded under whole-cell voltage clamp in neurons recorded from rat hippocampal slices. A high-[Cl^-]-containing intracellular solution was used, and DSI was induced by 1-s, 60-mV depolarizing steps from -70 mV. NEM did not alter IPSCs or passive cell properties, although it did block G-protein-mediated baclofen responses. The NEM method is easy to apply and permits study of DSI in a cell prior to and after the G proteins are blocked. These results support the hypothesis of G protein involvement in DSI. Supported by NS30219 and NS22010 to B.E.A.

432.5

ZINC MODULATES INTRACELLULAR CALCIUM IN SALAMANDER ROD PHOTORECEPTORS. I.U. Willcockson[1], P. Saggau[#] and S.M. Wu.[#*] Dept. of Molecular Physiology & Biophysics[1], Division of Neuroscience[#] Cullen Eye Institute, Baylor College of Medicine, Houston, Tx 77030.

Dim background light increases the gain between rod photoreceptors and second-order neurons in the salamander retina. One possible mechanism involves modulation of the presynaptic calcium current, via neurotransmitters/modulators. Using retinal whole mounts, Zn^{+2} (5-50μM) blocked synaptic transmission from photoreceptors to second-order neurons. Patch-clamping rods in retinal slices showed that Zn^{+2} reversibly and dose-dependently inhibits the Ba^{+2} current with an IC_{50} of 20 - 50 μM.

To measure presynaptic calcium, rods were isolated enzymatically from the retina of larval tiger salamanders (*Ambystoma tigrinum*), and plated onto dishes coated with Sal-1 antibody (courtesy of P. MacLeish). Cells were bath-loaded for 10-20 min with the calcium indicator Fura-2 AM (5 mM) and examined on an inverted microscope. Fluorescence, excited at 380 nm, 360 nm and 340 nm, was recorded from the synaptic terminal of individual rods using a single photodiode. Changes in intracellular calcium were measured using fluorescence ratios.

Perfusing the cells for 2 - 4 min with saline containing 15 mM K^+ and 1.8 mM Ca^{+2} (high K^+ saline) resulted in an increase in intracellular calcium in the terminal which could be reversed upon washing the cells for several minutes with normal saline. Addition of 100 μM Zn^{+2} to high K^+ saline resulted in reduced intracellular calcium increase. Present studies are examining the dose-response for zinc as it affects changes in intracellular calcium levels in the synaptic terminal as well as other potential sites of Zn^{+2} action.

432.6

INHIBITION BY Zn^{2+} OF URIDINE 5'-TRIPHOSPHATE-INDUCED Ca^{2+}-INFLUX BUT NOT Ca^{2+}-MOBILIZATION IN PC12 CELLS. S. Koizumi, K. Nakazawa and K. Inoue*. Div. Pharmacol., Natl. Inst. Health Sci., Kamiyoga, Setagaya, Tokyo 158, Japan

We previously demonstrated that Zn^{2+} potentiated the responses mediated by P_{2X}-purinoceptors forming non-selective cation channels using rat pheochromocytoma PC12 cells. In the present study, we show that the effects of Zn^{2+} on the responses mediated by G-protein coupled P_2-purinoceptors in the cells. Uridine 5'-triphosphate (UTP; 1-100 μM) stimulated a rise in intracellular Ca^{2+} concentration ([Ca]i). This response was decreased to about 30 % by external Ca^{2+}-depletion, but not abolished. This [Ca]i rise was mimicked by 100 μM ATP but not by 2-methyl-thio-ATP or α,β-methylene-ATP in the absence of external Ca^{2+}, suggesting that the response was mediated by P_{2U}-purinoceptors, a subclass of P_2-purinoceptors. The UTP-evoked [Ca]i rise consisted of two components; a transient and a sustained one. When external Ca^{2+} was removed, the sustained component was abolished, on the other hand, the transient component was decreased by about 70 % but did not disappear. These results suggest that UTP induces Ca^{2+}-mobilization and, subsequently, Ca^{2+}-influx. The UTP-evoked increase in [Ca]i was not affected by Cd^{2+} (100 & 300 μM) or nicardipine (30 μM), inhibitors of voltage-gated Ca^{2+} channels, but was significantly inhibited by Zn^{2+} (10-300 μM) in the presence of external Ca^{2+}. Zn^{2+}, however, did not affect the Ca^{2+} response to UTP in the absence of external Ca^{2+}. UTP evoked the release of dopamine from the cells. This dopamine release was abolished by Ca^{2+}-depletion or Zn^{2+}, but not by Cd^{2+} or nicardipine. Taken together, the data demonstrate that UTP stimulates P_{2U}-purinoceptors and induces a rise in [Ca]i both by Ca^{2+}-mobilization and Ca^{2+}-influx in PC12 cells. The UTP-evoked dopamine release requires external Ca^{2+} which may enter the cells through pathway sensitive to Zn^{2+}, but insensitive to Cd^{2+} or nicardipine.

432.7

INTRACELLULAR CADMIUM REDUCES PAIRED-PULSE FACILITATION AT A SYNAPSE BETWEEN IDENTIFIED LEECH NEURONS IN CULTURE. Y. Liu* and E. F. Stanley. Synaptic Mechanisms Section, NINDS, NIH, Bethesda, MD 20892.

Previous studies have indicated that cadmium affects neurotransmitter release but examining the mechanism of this effect is severely limited by the low permeability of Cd^{2+} through calcium channels. We have used the cultured leech Retzius cell-pair preparation to inject Cd^{2+} directly into a presynaptic neuron. Neurons were impaled by intracellular electrodes filled with either 3M KCl (control) or 3M KCl plus 0.1M $CdCl_2$. One neuron was stimulated with brief duration single or paired-pulses while postsynaptic potentials (PP) were recorded from the other. Facilitation (PP_2/PP_1) was significantly lower with Cd^{2+} in the electrode, 1.97±0.32 (SD; n=3), than in controls, 3.32±0.49 (n=3; $p<0.02$).

Since facilitation is believed to reflect a step subsequent to calcium entry, this result suggests that cadmium affects synaptic transmission by competing for a calcium binding site.

432.8

NH_4^+ DECREASES FREQUENCY BUT NOT AMPLITUDE OF MINI-EPSCS IN CEREBELLAR NEURONS IN TISSUE CULTURE. W. Raabe*. Depts. Neurology and Physiology, VA Medical Center and University of Minnesota, Minneapolis, MN.

NH_4^+ plays a role in the pathogenesis of hepatic encephalopathy. To study effects of NH_4^+ on excitatory synaptic transmission, effects on action potential-independent, spontaneous EPSCs (miniEPSCs) were investigated in cerebellar neurons in tissue culture. Whole cell patch clamp recordings were obtained from large neurons (> 15 μm ∅) presumed to be Purkinje cells. Extracellular solutions contained TTX (1 μM) and bicuculline (10 μM) to block action potential generation and IPSCs. CNQX (10 μM) completely abolished miniEPSCs indicating that these were generated by glutamate acting on AMPA receptors. NH_4^+ (2.5-5 mM) did not affect the amplitude, however, decreased the frequency of miniEPSCs. Intracellular alkalinization with trimethylamine (5 mM) had no effect on amplitude or frequency of miniEPSCs.

NH_4^+ ≥2.5 mM decreases spontaneous transmitter release from cerebellar granule cells, the only excitatory neuron in the experimental preparation, independent of a change in intracellular proton concentrations. The mechanisms by which NH_4^+ decreases transmitter release are currently further investigated. The lack of an effect of NH_4^+ on the amplitude of miniEPSCs indicates that NH_4^+ has no effect on the sensitivity to glutamate of cerebellar postsynaptic AMPA-receptors. This result contrasts with a report that NH_4^+ decreases excitatory synaptic transmission in hippocampus by affecting AMPA-receptors (Brain Res. 632: 225, 1993).

432.9

ZINC INCREASES INTRINSIC EXCITABILITY OF CA3 INTERNEURONS IN ORGANOTYPIC SLICE CULTURES OF RAT HIPPOCAMPUS. Ricciardi, T.N.* and Malouf, A.T. Neurological Surgery RI-20, University of Washington, Seattle, WA 98195.

Zn^{++} is a modulator of GABAergic synaptic transmission in the hippocampus. Application of exogenous Zn^{++} (300 μM) results in net excitation and GABAergic giant depolarizing potentials (GDPs) in hippocampal neurons. GABA can cause depolarizations in pyramidal cells when it is focally applied to the dendrites. Thus, GDPs may arise from activation of GABAergic neurotransmission to dendrites of pyramidal neurons. In this study, whole-cell patch and sharp intracellular recordings were used to study the actions of Zn^{++} on CA3 interneurons in slice cultures of hippocampus. Interneurons were recorded from stratum pyramidale (SP), stratum oriens (SO), and stratum radiatum (SR). Interneurons were physiologically identified by their characteristic fast spikes, large spike afterhyperpolarization, and lack of spike frequency adaptation. Interneurons were filled with biocytin for subsequent morphological identification. All interneurons showed an increase in intrinsic excitability in the presence of Zn^{++}, as determined by an increase in the slope of the input-output response to intracellular current injection. Zn^{++} dramatically reduced the spike afterhyperpolarization, which could contribute to the enhanced intrinsic excitability. Zn^{++} reduced anomalous rectification (sag), prolonged the membrane time constant, and increased the input resistance. In the majority of interneurons, Zn^{++} caused rhythmic bursting and giant depolarizing potentials (GDPs) similar to those previously seen in pyramidal neurons. These data suggest that Zn^{++} can alter interneuron activity by increasing their intrinsic excitability and synaptic drive. Supported by NIH-NS28650 (A.T.M.) and NIH-T32NS-07144-15 (T.N.R.).

432.10

MECHANISMS OF MOSSY FIBER ASYNCHRONY IN THE RAT HIPPOCAMPUS. D.A. Henze* and G. Barrionuevo. *Dept of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.*

Previous studies have reported that mossy fiber (MF) excitatory postsynaptic currents (EPSCs) recorded from CA3 pyramidal cells may show inflections during the rising phase suggesting an inherent asynchrony of neurotransmission at this synapse. Two different mechanisms have been proposed to underlie the observed asynchrony: Asynchronous release of quanta (Jonas et al., 1993), and asynchronous MF bouton spiking (Langdon et al , 1993). Asynchronous spiking could reflect heterogeneous conduction velocities, heterogeneous conduction distances, or differential bouton invasion delays. We have studied the MF fiber volley to determine if the MFs exhibit heterogeneous conduction velocities and conduction distances in the hippocampal slice. MF fiber volley recordings were made at 22 °C in the presence of Kynurenate (10 mM) or CNQX (10 μM) and MK801 (10 μM) to block synaptic transmission. Stimulation of the *s. granulosum* in the suprapyramidal blade of the dentate gyrus (DG) caused asynchronous MF fiber volleys in area CA3. Following the placement of extensive cuts which removed the hilus, the *s. oriens* and the *s. radiatum*, a synchronous MF fiber volley was observed by direct stimulation and recording in the *s. lucidum* of area CA3. The synchronous MF fiber volley had a conduction velocity of 0.3 m/sec for both anti- and orthodromic conduction. It was also possible to obtain synchronous MF fiber volleys in the intact slice by stimulating the *s. moleculare* in the DG adjacent to the hippocampal fissure. Stimulating the DG at locations progressively closer to the hilus lead to an increasing asynchrony in the MF fiber volley recorded in CA3. We conclude that asynchrony observed in the MF fiber volley is solely due to heterogeneous conduction distances arising from the anti/orthodromic activation of hilar MF collaterals and is not due to different MF fiber conduction velocities. We also conclude that the mechanism underlying the asynchronous unitary MF EPSCs as observed by Jonas et al. (1993) must occur at the level of the bouton and remains to be determined. Supported by NIMH Predoctoral Fellowship MH10474 and NS 24288.

432.11

REGULATION OF HIPPOCAMPAL CA3-CA1 PRESYNAPTIC FUNCTION ACROSS DEVELOPMENT T.C Dumas* and T.C. Foster, Dept. of Psychology, Univ. of Virginia, Charlottesville, VA. 22903.

Excitatory synaptic transmission increases postnatally in area CA1 of the developing rat hippocampus due, in part, to an increase in presynaptic function. Decreased transmitter release in younger animals might be due to differences in presynaptic receptors. We have measured changes in paired-pulse facilitation of CA3-CA1 synaptic responses during application of putative presynaptic receptor agonists and antagonists to investigate the development of presynaptic inhibition. Evoked field EPSPs (~1mV, 0.1Hz) were recorded in hippocampal slices taken from 3 and 5 week old rats. Paired-pulse stimulation (50ms ISI) was applied in order to measure changes in facilitation before and after bath application (5min) of adenosine (50uM), 1S,3R-ACPD (50uM), or baclofen (15uM). Recording was performed for 10min before and continued for at least 30min after drug application. All three agonists increased facilitation over baseline in the older animals suggesting functional presynaptic modulatory systems at week 5. In contrast to 1S,3R-ACPD which increased facilitation in the younger animals, adenosine and baclofen showed little to no effect at week 3. Decreased effects on facilitation could be due to lack of presynaptic receptors, saturation by endogenous ligands, or basement levels of release probability. After increasing probability with increased Ca2+, adenosine and baclofen significantly increased facilitation in week 3 slices, indicating the presence of functional presynaptic receptors that are not saturated. Receptor antagonists, (+)-MCPG (250uM), phaclofen (800uM), or DPCPX (1uM), were without effect suggesting no tonic receptor activity. However, theophylline (25uM) decreased facilitation at both ages and the effect was larger in the younger animals. Theophylline could be acting at adenosine receptors or on second messenger systems. Current work addresses these possibilities. Supported by NS31830 to TCF.

432.12

MUSCARINIC ENHANCEMENT OF DEPOLARIZATION-INDUCED SUPPRESSION OF INHIBITION IN RAT HIPPOCAMPAL PYRAMIDAL CELLS. L.A. Martin, T.A. Pitler, R.A. Lenz, J.J. Wagner*, and B.E. Alger, Dept. Physiol., Univ. Maryland, Sch. Med., Baltimore, MD. 21201.

Depolarization-induced suppression of inhibition (DSI) is the transient decrease in $GABA_A$ IPSCs that follows the activation of a hippocampal CA1 pyramidal cell with brief trains of action potentials or voltage steps (Pitler and Alger, 1994, Neuron, 13:1447-55). DSI of spontaneous IPSCs is often absent or weak under normal conditions. However, activation of muscarinic receptors with carbachol can enhance or induce DSI. To investigate the mechanism of this effect we examined the dose-response relationship between carbachol (0.1-30 μM) and DSI in pyramidal cells in hippocampal slices. We used whole-cell recording pipettes filled with a high-[Cl^-]-containing solution and studied the ability of a 1-s depolarizing voltage pulse (V_H= -70 mV) to cause DSI of sIPSCs.

At concentrations $\leq$ 1 μM, carbachol inhibited the slow outward AHP current elicited by the voltage step and increased the frequency of small to medium sIPSCs (50-200 pA). DSI was not induced by these low levels of carbachol. At 1-3 μM, carbachol typically induced the onset of large sIPSCs (>300 pA) and DSI. Further increases in carbachol concentration had little additional effect. In general, however, muscarinic receptor activation is neither necessary nor sufficient for DSI since in some cells carbachol did not induce large sIPSCs or DSI at any dose, and, in other cells, atropine did not block DSI when it occurred in the absence of carbachol. We conclude that muscarinic agonists primarily activate a set of interneurons whose output is particularly susceptible to DSI. Supported by NS 30219 and 22010 to B.E.A.

432.13

SHORT TERM SYNAPTIC POTENTIATION AND DEPRESSION IN THE PERFORANT PATH - DENTATE GYRUS SYNAPSE.

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Synaptic response depends on the amount of neurotransmitter released, and on the availability of postsynaptic receptors. Both factors are affected by previous use. Short term synaptic activity was studied in rat cortico-hippocampal slices. The perforant path was stimulated in the subiculum with series of 5 stimuli at frequencies from 0.1 to 100 Hz, and extracellular field potentials were recorded at the molecular layer of the dentate gyrus. Under these conditions, the responses of the perforant path axons and the presynaptic volley were stable, whereas the population excitatory post synaptic potentials (pEPSP) displayed frequency and time dependent depression. The slope of the fifth pEPSP declined by 5, 14, 32, 61, and 82 % (normalized to the first pEPSP), at 1, 10, 25, 50, and 100 Hz respectively. The synaptic decline was enhanced at 4-8 mM $[Ca^{2+}]_o$, and attenuated either at 1mM $[Ca^{2+}]_o$, or when $[Mg^{2+}]_o$ was raised in normal solution containing 2 mM $[Ca^{2+}]_o$. At 0.5 mM $[Ca^{2+}]_o$, pEPSPs were reduced but were frequency - potentiated. 3.4 μM ω-conotoxin reduced the synaptic potentials by 50 %. but failed to prevent the frequency-dependent decline. Nifedipine (10-45 μM) did not affect synaptic activity. Blockers of calcium dependent potassium channels, apamin (100 nM), charybdotoxin (10-30 nM), and quinidine (75-200 μM) were ineffective in reducing the synaptic decline. The modulators of AMPA receptors desensitization, aniracetam (0.7 mM), and cyclothiazide (0.1 mM) prolonged the time course of the single pEPSP, but paradoxically increased its paired pulse depression. Synaptic decline was not affected by the $GABA_A$ blocker bicuculline methiodide (10μM), nor by the $GABA_B$ blocker saclofen (0.5 mM). In contrast, the $GABA_B$ agonist baclofen (1-25μM) and the non- NMDA-R blocker CNQX (0.5 μM) diminished the decline. A calcium dependent, glutamate and $GABA_B$-modulated mechanism, possibly a specific presynaptic potassium channel, may be responsible for this synaptic depression.

432.14

PATHWAY-SELECTIVE *l* - ISOPROTERENOL POTENTIATION OF PYRAMIDAL CELL ACTIVATION IN FIELD CA1 OF THE RAT HIPPOCAMPAL SLICE. D. Cooper, D. Dahl, G. Epling, V. Nejtek, and G. Moushegian *. School of Human Development, The University of Texas-Dallas, Richardson, TX 75083.

Norepinephrine or *l* - isoproterenol (a β-adrenergic agonist) produces potentiation of pyramidal cell activation by the Schaffer Collateral pathway (SC) in hippocampal field CA1. Whether this potentiation is due to direct effects of adrenergic agonists on pyramidal cells, or indirectly via modulation of SC is not known.

The locus of adrenergic potentiation was investigated in the rat hippocampal slice. Independent pathways activating field CA1 pyramidal cells were stimulated by monopolar electrodes placed in mid-*stratum radiatum* (to activate SC) and in the heavily myelinated perforant pathway (PP) proximal to the hippocampal fissure. After Colbert and Levy, field potential profiles and baclofen were employed to verify isolation of the SC and PP. Intracellular recordings of excitatory postsynaptic potentials (EPSPs) were taken to alternating SC and PP stimulation. Activation of pyramidal cells by the weak PP to field CA1 was enhanced by application of bicuculline methiodide (a γ-amino butyric $acid_A$ antagonist [1.0 μM]).

Concentrations of *l* - isoproterenol in the range 500 nM - 1.0 μM produced significant potentiation of SC-evoked EPSPs, but were without effect on PP-evoked EPSPs: *In no experiment did PP-evoked EPSPs undergo potentiation in parallel to potentiation of the SC.* Initial EPSPs of only a few mV frequently potentiated to well beyond action potential threshold.

These results are consistent with the hypothesis that adrenergic potentiation of pyramidal cell activation in field CA1 probably involves a pre-synaptic locus.

Supported by grants to DD from the Whitehall Foundation and NSF.

432.15

HETEROGENEITY BETWEEN NON-NMDA SYNAPTIC SITES IN PAIRED-PULSE SYNAPTIC RESPONSES IN CA1 HIPPOCAMPAL NEURONS. D.A. Turner*, Y. Chen, J. Isaac, M. West and H.V. Wheal. Neurosurg., Neurobiol. and ISDS, Duke Univ., Durham VAMC, Durham, NC, 27710. Physiol. and Pharmacol., Univ. Southampton, Southampton, SO16 7PX, UK.

Dynamic changes in synaptic release probability and synaptic amplitude are critical aspects of neural plasticity. We have developed a Bayesian statistical method which predicts both the likely number of activated synapses (k) and synaptic amplitude and probability without assuming a statistical model (SITE analysis). We analyzed single synaptic site parameters during paired-pulse changes using this approach.

Non-NMDA excitatory synapses onto CA1 hippocampal neurons were activated using microstimulation, block of inhibition, a paired-pulse stimulus protocol and whole-cell recording (n=16 ensembles exhibiting stationarity and <1.5 pA σ_{noise}). EPSC ensembles (#=375±130) showed 1st pulse=4.61±3.67 pA and 2nd pulse=5.03±3.61 pA, with an average of k_1=3.0 and k_2=3.4 synaptic sites, respectively [to a marginal P(k|D)>0.5]. These synapses exhibited significant heterogeneity of both site probability (range 0.03-0.99; mean 1st=0.43±0.31, mean 2nd=0.46±0.32) and site amplitude (range 1.09-19.4; mean 1st=5.20±2.82, mean 2nd=4.75±2.68 pA). There was greater inter-site amplitude variability (CV=77%) than that found at the same site over time (6.5%). Paired-pulse plasticity (depression, potentiation) primarily included alterations in the number of activated synapses and their release probabilities.

Heterogeneity between non-NMDA synapses onto CA1 neurons may preclude use of Poisson or compound binomial statistical models. These data suggest that a wide array of mechanisms may contribute to synaptic plasticity in the CNS, including alterations in the number of synaptic sites as well as parameter values (amplitude, probability) at each site. The Bayesian SITE analysis may also be extended to a time series approach which may give clues to dynamic synaptic plasticity. Supported by NSF (DAT, MW), VAMC (DAT), Wellcome Trust (HVW) and MRC (HVW).

432.16

DIFFERENTIAL MODULATION OF NEUROTRANSMITTER LEVELS IN RAT HIPPOCAMPUS BY ARACHIDONIC ACID. A.I.M. Breukel, E. Besselsen, V.M. Wiegant., C.M.A. Pennartz*, F. H. Lopes da Silva and W.E.J.M. Ghijsen, Graduate School for the Neurosciences, Institute of Neurobiology, University of Amsterdam, Kruislaan 320, 1098 SM, Amsterdam, The Netherlands.

Glutamate and GABA play an important role in signal transduction in the hippocampus. Release and clearance of these neurotransmitters are regulated by specific messengers. We investigated the modulatory role of arachidonic acid (AA), which may act as a retrograde messenger in synaptic plasticity. Purified nerve terminals (synaptosomes) isolated from rat hippocampus were used to study AA effects on basal release of glutamate, aspartate and GABA and also of the neuropeptide cholecystokinin (CCK).

AA (25 μM) increased the extracellular levels of glutamate, aspartate, GABA, and also of CCK under basal conditions. This effect was not due to nonspecific disturbances in the synaptosomal energetic balance (ATP/ADP ratio) or membrane integrity (LDH leakage). The AA-effect on the amino acid levels was completely Ca-independent, suggesting that the fatty acid acts on the cytoplasmic amino acid pool. Although AA is a potent presynaptic PKC activator, no effects of the inhibitors H-7 and sphingosine on Ca-independent amino acid release could be observed, excluding PKC involvement on this process. In contrast, AA-mediated enhancement of CCK release was blocked by these drugs. Possible effects of AA on re-uptake of spontaneously released amino acids were investigated by measuring accumulation of [^{3}H]-GABA or [^{3}H]-D-aspartate in synaptosomes. Under these conditions, AA inhibited the net Na-dependent uptake of [^{3}H]-GABA by 20% and of [^{3}H]-D-aspartate by 30%. In addition to its reported effects on stimulated release, especially of glutamate, we conclude that AA enhances basal, extracellular levels of amino acids by inhibition of their Na-dependent uptake, whereas it enhances those of the neuropeptide CCK via presynaptic PKC activation.

432.17

CORRELATED PHYSIOLOGY AND ANATOMY OF PYRAMID TO BURST FIRING INTERNEURONE CONNECTIONS IN RAT NEOCORTEX IN VITRO. Jim Deuchars and Alex Thomson, SPON:Brain Research Association, Dept. of Physiology, Royal Free Hospital School of Medicine, London, NW3 2PF, U.K.

Paired intracellular recordings were made using conventional sharp microelectrodes (2M $KMeSO_4$/2% biocytin) in rat neocortex in vitro. Synaptic connections from pyramidal cells to spiny, burst firing interneurones were recorded and the cells filled with biocytin for histological analysis. In one pair the average EPSP amplitude recorded in the burst firing interneurone at a postsynaptic membrane potential of -79mV in response to single action potentials was 1.12±0.66mV. Between 30 and 40% of presynaptic spikes failed to elicit discernable EPSPs in the interneurone. Light microscopic examination revealed 12 close appositions between pyramid axon and interneurone dendrites. Electron microscopic examination of 6 of these 12 close appositions revealed that they were synaptic contacts. In another pyramid to burst firing interneurone pair the average EPSP amplitude of 0.12±0.21mV was associated with 80% apparent failures of transmission to single presynaptic spikes. Electron microscopic examination revealed that 3 of 6 close appositions were synaptic contacts. In response to pairs of presynaptic spikes profound paired pulse facilitation was evident in both pairs. These correlated data indicate that pyramidal terminals onto spiny, burst firing interneurones exhibit a low probability of release.

432.18

SINGLE AXON PYRAMID-PYRAMID AND PYRAMID-INTERNEURONE EPSPS COMPARED IN AREA CA1 OF RAT HIPPOCAMPAL SLICES. A. Barclay, J. Deuchars and A.M. Thomson* Department of Physiology, Royal Free Hospital School of Medicine, London NW3 2PF, UK.

Paired intracellular recordings were made in 450μm thick slices of adult hippocampus from CA1 pyramidal cells and from interneurones at the stratum oriens/alveus border whose axons ramified in stratum lacunosum-moleculare. Of 12 pyramid-interneurone pairs tested, four were monosynaptically connected. EPSPs elicited in the interneurones by single pyramidal action potentials had mean amplitudes of 0.2-1.1mV. The proportion of apparent failures of transmission at these connections could be high (16-50%) and paired pulse facilitation was profound (>100%). In contrast, in the one CA1 pyramid-pyramid connection studied to date, EPSPs with a mean amplitude of 0.8mV displayed no apparent failures of transmission under similar conditions and paired pulse depression was apparent. This pyramid-pyramid connection appeared to be partially mediated by NMDA receptors.

After recording, cells were filled with biocytin which was visualized using Avidin-HRP and recorded cells identified. Morphological analysis is in progress, but one presynaptic CA1 pyramidal axon appears to contact several basal dendrites of the postsynaptic CA1 pyramidal cell.

432.19

PRESYNAPTIC CALCIUM INFLUX IS MODULATED DURING MUSCARINIC RECEPTOR-INDUCED PRESYNAPTIC INHIBITION AND LTP AT CA3-CA1 SYNAPSES IN THE GUINEA PIG HIPPOCAMPUS. J. Qian, L.G. Wu*, and P. Saggau* Div. Neuroscience, Baylor College of Medicine, Houston, TX 77030; *Dept. Physiology, University of Colorado HSC, Denver, CO 80262.

Cholinergic receptors are involved in the regulation of synaptic transmission. The muscarinic receptor agonist carbachol (CCh) was previously shown to inhibit synaptic transmission and to modulate stimulation-induced LTP. Recently, CCh was found to induce by itself a novel form of LTP (muscarinic LTP, LTP_m, Auerbach and Segal, *J. Neurophysiol.*, 1994). Ca^{2+} influx through voltage-dependent calcium channels (VDCCs) plays a key role in transmitter release. In this study, presynaptic Ca^{2+} influx at CA3-CA1 synapses was measured, using a previously developed optical technique (Wu and Saggau, *J. Neurosci.*, 1994), to address the role of presynaptic VDCCs in these modulations of synaptic transmission.

Bath application of 5 µM CCh for 20 min inhibited synaptic transmission to less than 10% of baseline (slope of the extracellular field EPSP, low frequency stimulation), during which the presynaptic Ca^{2+} influx was reduced to 40-50% of baseline. About 30 min after such CCh application, a long-lasting potentiation developed. This LTP_m was 150 - 200% of baseline and lasted for more than 2 hours, during which the presynaptic Ca^{2+} influx was enhanced by 20 - 40% of baseline. Administration of 10 µM CNQX and 50 µM APV did block glutamatergic transmission, however, this did not prevent the expression of LTP_m, which developed even without any stimulation. Both CCh effects could be completely blocked by application of the muscarinic receptor antagonist atropine (10 µM). Bath application of 1 µM CCh produced the same LTP but showed less inhibitory effect.

These results suggest that presynaptic inhibition caused by activation of muscarinic receptors and LTP_m are both at least partially due to a modulation of presynaptic Ca^{2+} influx. Presynaptic inhibition and LTP_m of synaptic transmission may be differentially regulated by activation of different subtypes of muscarinic receptors.

432.20

NOREPINEPHRINE DIFFERENTIALLY REGULATES SYNAPTIC INHIBITION IN THE RAT HIPPOCAMPUS. V. A. Doze*, D. E. Bergles, S. J Smith, and D. V. Madison. *Department of Molecular and Cellular Physiology, Beckman Center, Stanford University School of Medicine, Stanford, CA 94305-5426.*

Inhibition in the hippocampus is generated by a diverse group of local circuit interneurons which release GABA onto the principal cells. Numerous anatomical studies have suggested that these inhibitory interneurons are a primary target of the dense noradrenergic projection to this area. We have previously shown that norepinephrine (NE) reduced evoked polysynaptic IPSPs in hippocampal slices; however, monosynaptic IPSPs elicited in stratum radiatum were not affected.

In this study, we investigated the actions of NE on evoked monosynaptic IPSPs elicited in the various strata of area CA1 of the rat hippocampus, and compared these to the responses of identified interneurons to NE found in these same strata. Monosynaptic IPSPs elicited in stratum oriens consisted of a fast early IPSP only, whereas IPSPs evoked in stratum lacunosum-moleculare (L-M) near the hippocampal fissure consisted of a $GABA_A$-mediated IPSP with a slow time-course (slow early IPSP) followed by a late $GABA_B$-mediated IPSP. NE (10 µM) caused a small increase (~10%) in the size of monosynaptic fast early IPSPs elicited in stratum oriens. In contrast, both the slow early and late IPSPs produced by L-M stimulation were decreased by >35% in NE (10 µM). These results correlate with recordings from identified interneurons showing that those in stratum oriens depolarized in response to NE, while those located in stratum L-M were hyperpolarized by NE.

These results point to an important role for NE in regulating the efficacy of synaptic inputs in both spatial and temporal domains.

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LONG-TERM POTENTIATION: PHYSIOLOGY III

433.1

ANALYSIS OF Sr^{2+}-INDUCED ASYNCHRONOUS RELEASE IN CA1 HIPPOCAMPAL NEURONS REVEALS CHANGES IN QUANTAL SIZE ASSOCIATED WITH BOTH LTP AND LTD. Stéphane H.R. Oliet*, Robert C. Malenka and Roger A. Nicoll. *Depts. of Cellular & Molecular Pharmacology, Physiology and Psychiatry; University of California, San Francisco CA 94143-0450.*

At central synapses extracellular Sr^{2+} has recently been shown to reduce the "synchronous" component of excitatory postsynaptic currents and facilitate the "asynchronous" phase of the synaptic response (Goda & Stevens, *PNAS 91:12942-12946*, 1994). The latter is characterized by a tail of quantal events following the decreased synchronous response which originate from the stimulated synapses. We took advantage of this observation to measure the changes in quantal size associated with homosynaptic LTP and LTD of synaptic transmission at Schaffer/collateral-CA1 synapses. When compared to the unitary events associated with the asynchronous component of the evoked response in an independant naive pathway, those originating from potentiated synapses (n=8) showed a significant increase in amplitude (+29.1± 2.3%) and frequency (+62.0±26.2%). At depressed synapses the amplitude and frequency of these events were reduced by 18.4±2.3% and 12.4±7.2% respectively (n=7). In 6 cells where the stimulus strength of one pathway was increased, the frequency of unitary events associated with asynchronous release was increased significantly (+165.3±39.4%) whereas their size was unaffected (+3.0±1.2%). The changes in quantal size reported here are consistent with a post-synaptic site for expression of both homosynaptic LTP and LTD while the variations in frequency could reflect either pre- or post-synaptic modifications. *Supported by the International Human Frontier Science Program and the NIMH.*

433.2

HETEROSYNAPTIC LONG TERM DEPRESSION IN THE HIPPOCAMPUS. M. Scanziani*, R.C. Malenka and R.A. Nicoll. *Depts. of Cellular & Molecular Pharmacology, Physiology & Psychiatry; UCSF, San Francisco, CA 94143-0450.*

Long term heterosynaptic depression (LTD_H) of excitatory postsynaptic potentials was studied in the CA1 region of hippocampal slices of 2-4 week old rats using both field and whole cell recordings. Two independent afferent pathways were stimulated by either placing two bipolar electrodes in the radiatum or by placing one in the radiatum and one in the oriens. After blockade of GABAergic transmission, four tetani of 100Hz for 1s delivered to one of the pathways induced LTD_H of the other synaptic input. LTD_H could be followed up to 40 min and averaged 85% of base line. Saturation of homosynaptic LTD (1Hz stim; 10 min; 4x) prevented subsequent generation of LTD_H. D-APV (25 µM) blocked LTD_H but MCPG (500 µM) and nimodipine (50 µM) did not.

The NMDA receptors responsible for the induction of LTD_H could be located either at the synapses of the tetanized pathway or at those synapses which express LTD_H. The latter case would occur if ambient glutamate levels are high enough to activate NMDA receptors while the cell is being depolarized by the tetanus. We distinguished between these two possibilities by selectively blocking the NMDA receptors of one input with the use-dependent NMDA receptor channel-blocker MK801. LTD_H could be expressed at synapses functionally devoid of NMDA receptors indicating that NMDA receptor activation at the tetanized synapse is sufficient to induce LTD_H. *Swiss National Science Fund and NIMH.*

433.3

INDUCTION OF LTD IN HIPPOCAMPUS *IN VIVO* BY PAIRED-PULSE STIMULATION: ROLE OF PAIRED-PULSE INHIBITION. E. Thiels*[1], G. Barrionuevo[1], T.W. Berger[2]. [1]*Dept. of Neuroscience, U. Pittsburgh, Pittsburgh PA 15260, and* [2]*Dept. of Biomedical Engineering and Programs in Neurobiology and Neuroscience, U. Southern California, Los Angeles CA 90089.*

We demonstrated that repeated paired-pulse stimulation (PPS; 0.5Hz) of commissural afferents to area CA1 in hippocampus of anesthetized rats produces NMDA receptor-dependent long-term depression (LTD) at the commissural-CA1 pyramidal cell synapse (Thiels et al., 1994). The stimulation paradigm effective for producing LTD included 200 paired pulses with a 25-ms interstimulus interval (ISI). At this ISI, CA1 pyramidal cell firing to the second pulse of a pair was inhibited completely during most of the train. When the train was delivered in the presence of the $GABA_A$ receptor antagonist, bicuculline, paired-pulse inhibition during the train was largely abolished and LTD failed to develop. When the ISI was lengthened to 1000 ms, paired-pulse inhibition was absent and, again, LTD failed to develop. To characterize the relation between paired-pulse inhibition and the ability of PPS to induce LTD in intact hippocampus, we assessed the effect of various ISI durations on pyramidal cell firing to the second pulse of a pair and on the induction of LTD.

During a train with either a 75-ms or a 125-ms ISI, paired-pulse inhibition was present during only the first half of the train and significantly reduced compared to that seen with a 25-ms ISI. The amplitude of the CA1 population spike (PS) evoked by the second pulse of a pair for the first 100 pairs was 70±22 % of pre-train baseline level for the 75-ms ISI (n=8) and 67±20% for the 125-ms ISI (n=8). In parallel with the attenuated amount of inhibition, the train with a 75-ms ISI caused a smaller reduction in the amplitude of the PS evoked 30 min after the train (-46±8%, compared to -76±6% for the 25-ms ISI). However, the train with a 125-ms ISI failed to produce a lasting depression in PS amplitude (-10±9%). During a train with a 200-ms ISI, paired-pulse inhibition was absent (the PS amplitude evoked by the second pulse for the first 100 pairs was 141±24% of baseline; n=6), and this train produced no change in PS amplitude (-3±7%). These findings indicate that temporal overlap of postsynaptic inhibition and excitatory synaptic activation of CA1 pyramidal cells alone is not sufficient to induce LTD *in vivo*. (Supported by ONR, NIMH, NIH, and BMSR).

433.4

MAINTENANCE OF LTD IN HIPPOCAMPUS *IN VIVO* IS ACCOMPANIED BY INCREASED PROTEIN PHOSPHATASE ACTIVITY. E.D. Norman*, G. Barrionuevo, E. Klann, E. Thiels. *Department of Neuroscience, University of Pittsburgh, Pittsburgh PA 15260.*

Repeated paired-pulse stimulation (PPS; 0.5 Hz) of the commissural fibers has been shown to induce NMDA receptor-dependent long-term depression (LTD) of the commissural input to CA1 pyramidal neurons in the hippocampus of anesthetized rats (Thiels et al., 1994). It has been suggested that protein phosphatase (PPase) activity participates in NMDA receptor-dependent LTD induced by low-frequency stimulation in hippocampal slices (e.g., Mulkey et al., 1993). To determine whether an increase of PPase activity accompanies LTD induced by PPS in hippocampus *in vivo*, we measured PPase activity in homogenates of dorsal area CA1 (recording site) 30 min after the final train of 200 paired pulses (25-ms interpulse interval) delivered to the contralateral dorsal area CA3.

Tissue from dorsal and ventral (control) area CA1 was homogenized in buffer and added to reaction mixtures that included phosphorylase kinase (PK), a general PPase substrate. Incorporation of ^{32}P into the α and β subunits of PK was quantified using autoradiography and densitometry. Initial experiments indicated no difference in PPase activity between dorsal and control tissue using this assay. Three trains of PPS produced a significant reduction in the amplitude of the evoked CA1 population spike (PS: 22±8% of baseline; *n*=8). This effect was accompanied by a significant decrease in the phosphorylation of the α subunit of PK (^{32}P remaining = 79±7% for dorsal and 94±7% for ventral) but not of the corresponding β subunit (90±3% and 98±3%, respectively). These findings indicate that LTD induced by PPS in intact hippocampus is associated with an increase in PPase activity. Injection of the NMDA receptor antagonist, D-APV (100 μM), near the recording site blocked the induction of LTD (evoked PS amplitude: 88.5±11.2% of baseline; *n*=3) and attenuated the increase in PPase activity (^{32}P remaining on the α subunit of PK = 93±7% for dorsal and 92±4% for ventral). These findings indicate that NMDA receptor activation is necessary for the enhancement of PPase activity associated with LTD. Taken together, our findings suggest that PPase activity plays a role in the maintenance of NMDA receptor-dependent LTD in hippocampus *in vivo*. (Supported by NIMH and NINDS).

433.5

QUANTAL ANALYSIS OF LONG TERM DEPRESSION AT EXCITATORY SYNAPSES ON CA1 PYRAMIDAL NEURONES. S.P. Perrett*, C. Stricker and S.J. Redman. Division of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra, Australia.

Long term depression (LTD) of transmission at CA1 synapses is thought to be the complementary process to long term potentiation (LTP) that can allow synaptic weights to remain flexible in response to different patterns of afferent activity. However, the precise mechanisms through which such changes in synaptic strength occur are unknown. The changes that occur in EPSCs of pyramidal cells following the induction of LTD were investigated using quantal analysis. Hippocampal slices obtained from young rats (17-24 days) were prepared using conventional techniques and maintained at 30°C. Small EPSCs (average peak amplitude <10pA) were evoked by extracellular stimulation of stratum radiatum and recorded using whole cell techniques. Control records were obtained at 0.33Hz, and homosynaptic LTD was then induced by a prolonged period of 1Hz stimulation with the membrane potential maintained at -70mV. When a noticeable reduction of the EPSC had occurred, 0.33Hz stimulation was resumed, and conditioned responses were recorded for at least 30 mins. Probability density functions of the peak amplitudes of the control and conditioned EPSCs were constructed and quantal analysis followed the procedures described in Stricker *et al.*[1]. Four EPSCs have been analysed for which the control responses were clearly quantised, and for which a significant and stable reduction of the EPSC was maintained for >30 min. For three EPSCs, the quantal current decreased following conditioning (21%, 21%, and 51%), while no change occurred in the fourth. The probability of response failures increased and the probability of multiquantal responses decreased for all EPSCs following conditioning. These results are very similar, though opposite in direction, to changes recently observed for quantal analysis of LTP, and suggest that the mechanisms responsible for LTP and LTD may be complementary.

[1] Stricker, C., D. Daley & S.J. Redman (1994) *Biophys. J.*, **67**, 532-547

433.6

PROPERTIES OF LTP AND LTD DURING THE CHOLINERGIC THETA STATE. P.T. Huerta* and J.E. Lisman. Center for Complex Systems, Brandeis University, Waltham, MA 02254-9110.

Cholinergic modulation (50 μM) of the rat hippocampal slice activates a theta (θ) network oscillation and makes CA1 synapses highly plastic. In this state, a single burst of only 4 pulses (100 Hz) to the Schaffer inputs induces LTP if given at the peak of θ. In potentiated pathways, two types of LTD can be elicited by a burst. Homosynaptic LTD is produced by a burst at the trough of θ; heterosynaptic LTD is obtained in inactive synapses when other synapses are stimulated at the peak of θ. The scheme below summarizes the synaptic changes during θ. Numbers indicate % of maximal LTP, or % of total reversal in stimulated (s) and unstimulated (ns) pathways (n=10, each).

Burst at θ Peak		Burst at θ Trough	
s ↙	↘ ns	s ↙	↘ ns
LTP (81.7%)	Heterosynaptic LTD (34.0%)	Homosynaptic LTD (101.4%)	No change

These synaptic modifications can be saturated and require muscarinic receptors since they are blocked by scopolamine (1-5 μM). Cholinergic LTP occludes with standard LTP and like standard LTP, exhibits a cooperativity requirement (threshold for LTP was ~20% maximal stimulation). We conclude that the similar bursts observed during θ *in vivo* may be a natural stimulus for synaptic modifications, and that it is the timing rather than the frequency of stimulation that determines the sign of the change.

433.7

HOMOSYNAPTIC LONG TERM DEPRESSION IN THE DEVELOPING RAT DENTATE GYRUS. L. Kojic*, R.M. Douglas, M.S. Cynader. Department of Ophthalmology, University of British Columbia, 2550 Willow St., Vancouver, B.C. Canada V5Z 3N9.

Long-term depression (LTD), an activity-dependent decrease in the strength of synaptic transmission, has been described in several brain regions. Heterosynaptic LTD has been frequently reported in dentate gyrus (DG) granule cells, indicating that synaptic efficacy could be decreased in nonactive synapses. However, homosynaptic LTD induced by prolonged low frequency stimulation of perforanth path-granule cell synapses has been proven difficult to obtain, either *in vivo* or *in vitro*. The aim of this study was to examine the age as a factor in the induction of homosynaptic LTD in the DG *in vitro*. Recordings of field potentials were made from the molecular and granule cell layers of hippocampal slices from Wistar rats 4-93 days of age. Dentate granule cells were synaptically activated by electrical stimulation of the molecular layer (0.067 Hz), while LTD was induced by using prolonged low frequency (LFS) afferent stimulation (1Hz, 900 pulses). This form of LTD was input specific, was saturable upon repeated stimulation and was reversible by induction of LTP. Homosynaptic LTD was most frequently induced in young animals at up to 3 weeks of age (21/29=72%). In the first two weeks LTD was clearly seen in the granule cell layer, expressed mostly as a long-lasting depression of the population spike. At the same age, LFS could induce either LTD or LTP of the molecular layer EPSP, simultaneously with the LTD observed in the granule cell layer. Later in DG development (3-8 weeks), homosynaptic LTD was produced in both molecular and granule cell layers, but at a lower frequency (12/23=52%) than it was in younger animals. In mature animals (8-12 weeks) this LTD was not inducible in either layer of the dentate gyrus (0/4=0%). Modulation of GABAergic transmission by bicuculline (0.1-10μM) and muscimol (1-10μM), during the first five weeks of age, did not significantly change the occurrence of homosynaptic LTD. The results suggest that this form of synaptic plasticity is developmentally regulated in the DG, in a manner complementary to the developmental pattern of dentate gyrus LTP.

433.8

THE INVOLVEMENT OF ADENOSINE A1 RECEPTOR ACTIVITY IN THE INDUCTION OF LONG-TERM DEPRESSION Kunio Kato*, Anne Williamson. *Dept. of Surgery, Yale University School of Medicine, New Haven, CT 06520, U.S.A.*

Long-term potentiation (LTP) is a widespread phenomenon in the central nervous system and is proposed as a mechanism for memory formation. Long-term depression (LTD) is another form of synaptic plasticity, which decreases the synaptic response. LTD is regarded as a 'forgetting' mechanism, since LTP and LTD are induced alternately. We investigated the role of adenosine receptor activation in LTD induction. 500 μm hippocampal slices were prepared from 20-25 days old rats, and field EPSPs and EPSCs were monitored using field and whole cell recordings. 2 Hz stimulation was applied for 15 min (low frequency stimulation; LFS) to induce LTD (-19.8 ± 0.9%; N=20). 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX) 0.1 μM and 2 μM 8-Cyclopentyl-1,3-dimethylxanthine (CPT), adenosine A1 receptor antagonists, blocked LTD induction (+3.5 ± 0.3 % ; N=8). Adenosine deaminase 2 IU/ml also blocked the induction of LTD (N=3). 0.2 μM DPCPX and 5 μM CPT which is applied after LTD induction reversed LTD. This reversal effect was more obvious when these drugs were applied closer to the time of LTD induction. These data suggest that adenosine A1 receptor activity is essential for the induction of LTD. The site of LTD expression was investigated using a failure study under whole cell voltage clamp. These data indicated that the site of LTD expression is presynaptic. Application of 0.1 μM DPCPX with LFS prevented the induction of LTD and decreased the number of synaptic failures, suggesting enhancement of transmitter release. APV 50 μM, an NMDA receptor antagonist, also blocked the induction of LTD. Application of 50 nM N6-cyclohexyladenosine (CHA), an A1 adenosine receptor agonist, reversed the blocking effect of APV and induced LTD (-15.2 ± 2.8 % ; N=5). These data suggest that A1 receptor activity which is enhanced by NMDA activation, contributes to the induction of LTD.

433.9

REVERSAL OF LONG-TERM POTENTIATION IS DIFFERENT FROM LONG-TERM DEPRESSION. U. Staubli*, D. Chun, F. Xu & X. Li. Center for Neural Science, New York University, New York 10003.

Previous studies (Staubli & Lynch, 1987, 1990) have shown that theta burst stimulation produces an extremely stable form of hippocampal LTP in area CA1 of awake rats that can be reversed with single pulse stimulation patterned at the frequency of the theta rhythm (5 Hz or theta pulse stimulation). The present set of *in vitro* studies extended these results by demonstrating that reversal of LTP is time dependent and does not require NMDA receptor activation. Specifically, a 1-min episode of theta pulse stimulation delivered 30 sec after LTP induction consistently produced nearly complete reversal. The same stimulation was progressively less effective at causing depotentiation at longer delays with virtually no impact at 15 min. Theta pulse stimulation never produced any depression when delivered to naive (non-potentiated) pathways, consistent with the idea that depotentiation is an active process that reverses increments in synaptic strength of recently potentiated synapses and is effective up to the time at which LTP stabilization is complete. In contrast, we found that a 15-min episode of low-frequency stimulation at 1 Hz produced a 15-20% stable homosynaptic depression (LTD) in adult slices, independent of whether the stimulated pathway was naive or potentiated. Unlike LTP reversal, this LTD effect was not time dependent and was blocked by APV. Moreover, while reversal of LTP by theta pulse stimulation (1 min) is readily obtained both *in vitro* and *in vivo*, we so far have been unable to produce LTD in the awake chronic CA1 preparation, despite the use of extended trains (up to 15 min) of low-frequency stimulation at 1 Hz, 5 Hz or 10 Hz. In sum, the above results do not support the notion that reversal of LTP represents a weak version of LTD, but suggest that the mechanisms underlying LTP reversal and LTD are qualitatively different.

433.10

SEX DIFFERENCES IN THE EXPRESSION OF HIPPOCAMPAL LTP IN CHRONICALLY PREPARED RATS. T.L. Ivanco*, C. Trepel, and R.J. Racine. Department of Psychology, McMaster University, Hamilton, ON, Canada, L8S-4K1.

Recent evidence in anaesthetized animals indicates that there are sex differences in hippocampal LTP, possibly modulated by hormonal fluctuations during the estrus cycle. We investigated LTP in awake rats to determine the long-term effect of sex on plasticity in the hippocampus. Female rats were chronically implanted with stimulating and recording electrodes in the perforant path and ipsilateral dentate gyrus under somnotol anaesthsia. Baseline input/outpus evoked response tests were run, and vaginal smears were taken, daily for ten days. Only those females with regular cycles were included in the experiment. Animals were divided into one of three groups: proestrus, estrus, and diestrus. Baseline measures continued until stable I/Os had been collected and smears indicated that the animal was in the appropriate phase of the estrus cycle. High frequency trains at 1259 uA (pairs of 400 Hz bursts with burst durations of 35 ms, separated by 200ms) were then delivered to the perforant path to induce LTP. Baseline responses were retested 20 minutes, 24 hrs, and 48 hrs following trains. A group of males underwent the same electrophysiological manipulations and served as controls. There were no significant differences in baseline measures between the four groups. All groups showed similar short term potentiation effects in response to the trains. No potentiation was seen in females given trains during the estrus or diestrus phase, whereas both the females stimulated during the proestrus phase and the males showed significant LTP up to 48 hrs after the trains. The stimulation effects in females were not due to the shift from one phase of the estrus cycle to another following trains. We conclude that the induction of LTP here is consistent with the literature based on anaesthetized preparations. The difference in LTP induction between males and females is a result of the phase of the estrus cycle the females are in when given trains to induce LTP.

433.11

IMPAIRED MAINTENANCE OF HIPPOCAMPAL LONG-TERM POTENTIATION (LTP) IN MAP 1B-DEFICIENT MICE. M. Zervas[1], W. Edelmann[2], R. Kucherlapati[2], Bruce Wainer[1]*, and P.K. Stanton[3]. Departments of [1]Pathology, [2]Molecular Genetics, and [3]Neuroscience and Neurosurgery, Albert Einstein Coll. Med., Bronx, NY, 10461.

Mutant MAP 1B/- mice are deficient in the expression of microtubule associated protein 1B (MAP 1B) in the hippocampus, cerebellum, and olfactory cortex, and display a range of clinical phenotypes including tremor, ataxia, and slow growth rates. Electrophysiological recordings of Schaffer collateral-evoked excitatory post synaptic potentials (epsps) from field CA1 of hippocampal slices reveals that MAP 1B/- mice (n = 16) display normal short-term potentiation, but show a decremental loss of long-term potentiation (LTP) when compared to slices from wildtype littermates (n = 8). The largest decrement in LTP was observed between 60 and 90 minutes post-tetanus. A separate set of littermates were examined for an extended time of four hours post-tetanus in response to saturating LTP stimulation (100 Hz/2Sec x 3, 5 min apart). Slices from MAP 1B/-mice (n = 6) showed smaller LTP that decayed more rapidly than age-matched wildtype littermates (n = 6). These data indicate that the dendritic expression of the cytoskeletal protein, MAP 1B, is important in the long term maintenance of LTP in hippocampal field CA1.

433.12

RE-DISTRIBUTION OF DENDRITIC SPINES FOLLOWING LONG-TERM POTENTIATION (LTP) IN THE HIPPOCAMPUS OF THE AWAKE RAT. M.G. Stewart, D.A. Rusakov, G. Richter-Levin, and T.V.P. Bliss. Open University, Milton Keynes, MK7 6AA, and NIMR, Mill Hill, London NW7 1AA, U.K. (SPON: BRA)

The fine composition of dendritic spine populations in hippocampal dentate gyrus was studied 24h after LTP was induced and maintained unilaterally in 7 awake rats. Hippocampi were dissected, and Golgi preparations of granule cells from both potentiated and control hemispheres were analyzed via computerized microscopy. A multi-factorial statistical design included paired blocks (potentiated vs control hemispheres), 7-19 neurons per hemisphere, 3 dendritic branch orders per neuron, and 1-5 dendritic fragments (normal to the viewer's sight) per branch order. Identification of spines and quantification of branch-spine geometry was performed using an image recognition algorithm and standard image analysis methods[1].

Statistical analysis of data obtained from light microscopic images revealed the existence of dense clusters of spines (spine collars) in potentiated and unpotentiated preparations. The frequency of spine collars decreased in 2nd and 3rd branch orders. Comparing potentiated and unpotentiated tissue with unbiased stereological estimators (the tilting disector[1]), we have found: (i) no significant changes in the mean spine density along dendrites; and (ii) a significant change in the variability of dendritic-spine geometry towards less variable spine densities, and more variable spine lengths after LTP, as revealed by principal component analysis.

These results suggest that long-term potentiation in rat hippocampus is accompanied by specific re-arrangement of synaptic connections. [1] Rusakov DA, Stewart MG (1995) *J Neurosci. Meth (in press)*

433.13

THE BASOLATERAL AMYGDALA MODULATES THE FORMATION OF HIPPOCAMPAL LONG-TERM POTENTIATION IN VIVO. Y. Ikegaya , K. Abe, H. Saito* and N. Nishiyama. Dept. of Chem. Pharmacol., Fac. of Pharmaceut. Sci., The Univ. of Tokyo, Bunkyo-ku, Tokyo 113.

Long-term potentiation (LTP) of evoked potentials in the hippocampus is a form of activity-dependent synaptic plasticity which may underlie learning and memory. The amygdala is involved in emotional and motivational aspects of behavior. Recent behavioral studies have provided evidence that the amygdala modulates hippocampal-dependent memory. Therefore, in the present study, we investigated whether the amygdala contributes to hippocampal long-term potentiation. Male Wistar rats 8 - 9 weeks old were anesthetized with urethane and α-chloralose. A bipolar electrode was placed in the entorhinal cortex to stimulate the medial perforant path, and the evoked potential was extracellularly recorded from the granule cell layer of the dentate gyrus. We first examined the effect of surgical lesions (80 °C for 10 sec) of the basolateral amygdala (BLA) on LTP. Synaptic potential evoked by low-frequency test stimulation did not change following lesion of the ipsilateral BLA. However, when tetanic stimulation (30 pulses at 60 Hz) of the perforant path was applied 60 min after BLA lesion, the magnitude of LTP was significantly attenuated. This LTP-attenuating effect of BLA lesion was an ipsilateral and unilateral phenomenon. Lesion of the BLA 20 min after tetanic stimulation did not affect the established LTP. Next, we tested the effect of high-frequency stimulation of BLA on LTP. Although a conditioning stimulation (100 pulses at 100 Hz) of the ipsilateral BLA had no influence on basal transmissions in the perforant path-dentate gyrus synapses, the magnitude of LTP was significantly facilitated, when the BLA conditioning stimulation was applied simultaneously with tetanic stimulation of the perforant path. The BLA conditioning stimulation did not enhance contralateral LTP. The BLA stimulation 1 sec - 10 min prior to tetanic stimulation did not affect LTP. These results suggests that the ipsilateral BLA neurons modulate the induction of LTP in the hippocampal dentate gyrus in vivo. Activity-dependent facilitation of hippocampal LTP by the amygdala may underlie associative memory processing with emotion.

433.14

SELECTIVE BLOCKADE OF THE LATE PHASE OF LTP IN TRANSGENIC MICE EXPRESSING AN INHIBITORY FORM OF THE REGULATORY SUBUNIT OF PROTEIN KINASE A. P. V. Nguyen*, T. Abel, M. Barad, and E.R. Kandel. Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, New York, New York 10032.

LTP in the CA1 region of the hippocampus can persist for up to 8 hr in slices and for days in intact animals. The late phase of LTP (L-LTP) in CA1 differs from short-term LTP in requiring protein kinase A activity, protein synthesis, and transcription. Indeed, pharmacological inhibitors of PKA selectively block L-LTP. To genetically reduce PKA activity in the hippocampus, we generated transgenic mice that express an inhibitory form of the regulatory subunit of PKA. Expression of this subunit in the hippocampus, cortex, striatum and thalamus was driven by the promoter from the Ca^{2+}/calmodulin protein kinase II gene. We found that L-LTP was significantly decreased in area CA1 of transgenic mice: the mean field EPSP slope measured 30 min and 3 hrs post-induction in transgenic mice were 173 ± 14% and 105 ± 6% of pre-tetanus baseline, respectively (n = 6 mice, 22 slices), while wild-type values were 226 ± 24% and 171 ± 13%, respectively (n = 5 mice, 13 slices, $p < 0.05$). These results show that targeted suppression of PKA activity eliminates a late phase of LTP in CA1. These transgenic mice provide us with a model system with which to probe the behavioral roles of cAMP, PKA, and L-LTP in the mammalian hippocampus. Biochemical experiments are in progress to determine the effects of this inhibitory subunit on PKA activity, and behavioral studies are underway to investigate effects on spatial learning. [Supported by MRC Canada, Runyon-Winchell Fdn., NIH, and HHMI.]

433.15

TETRACYCLINE REGULATED EXPRESSION OF AN ACTIVATED FORM OF CaM KINASEII IN THE FOREBRAIN OF TRANSGENIC MICE M. Mayford*, L. Wang, K. Podsypanina, and E. R. Kandel. Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, NY, NY 10032.

One of the drawbacks of using genetically modified mice in the study of learning and memory is the lack of temporal control over the expression of the genetic change (Mayford et al., 1995). Recently, Furth et al. (1994) have described a binary system for the regulation of transgene expression in peripheral tissue using tetracycline. We have used this system to drive expression of an activated form of CaM kinase II, that is Ca^{2+}-independent due to the introduction of an aspartate at amino acid 286. Previous studies have shown that expression of this mutant form of the kinase shifts the response of hippocampal synapses to low-frequency trains in the 5-10 Hz range, so that LTD is enhanced and LTP is reduced (Mayford et al., *Cell*, in press). Using the tetracycline system combined with the CaMKIIα promoter, we generated mice that show strong transgene expression, which is limited to specific regions of the forebrain. Preliminary results indicate that transgene expression can be blocked by the administration of tetracycline analogs to the mice. The use of this system should allow the developmental consequences of transgene expression to be distinguished from the consequences of acute expression at both the electrophysiological and behavioral level.

433.16

RESCUING IMPAIRMENT OF LONG-TERM POTENTIATION IN FYN DEFICIENT MICE BY INTRODUCING THE FYN TRANSGENE. N. Kojima[1], J. Mansuy[2], J. Wang[2], M. Pragnell[2*], and E. R. Kandel[2]. [1]Lab. Neurochem., Natl. Inst. Physiol. Sci., Okazaki 444, Japan; [2]HHMI, Ctr. Neurobiol. & Behav., Columbia Univ., NY, NY 10032.

To understand the physiological roles of the tyrosine kinase Fyn in the brain, we generated transgenic mice expressing fynB cDNA under the control of calmodulin kinase IIα promoter. Regional distribution and time course of transgene expression were very similar to those of calmodulin kinase IIα mRNA. Strong expression of the transgene was observed in the adult forebrain, but hardly detected in the neonatal brain. To determine whether the impairment of long-term potentiation (LTP) in Fyn deficient mice is caused by the lack of Fyn in the adult hippocampus or by the developmental abnormality observed in neuronal connections, we generated Fyn deficient mice carrying the Fyn transgene. We were able to rescue LTP, at least partially, in the hippocampal slices from these mice, even though we did not rescue the characteristic morphological abnormalities in the hippocampus. These results suggest that Fyn contributes, in part, to the molecular mechanisms of LTP induction.

433.17

AGED RATS SHOW INCREASED SENSITIVITY TO LTD-INDUCING STIMULATION C.M. Norris#* and T. C. Foster#+. #Neuroscience Program and +Dept. of Psychology, Univ. of Virginia, Charlottesville, VA, 22903

We tested the hypothesis that the rapid decay of long term potentiation (LTP) in aged animals is due to enhanced long term depression (LTD). Low frequency stimulation (LFS) (900 pulses at 1 Hz) depressed CA3-CA1 EPSPs more in slices from aged (21-24 mos) vs young (6-8 mos) Fischer 344 rats ($p < .05$). No age-related differences were found in the magnitude of LTP and LFS delivered 15-20 min post LTP induction significantly reversed LTP in both age groups. However, only aged slices completely reversed to pre-LTP levels. LFS delivered 1 hr after LTP tended to reduce responses more in aged animals. To determine potential changes in the sensitivity to LTP-reversal, multiple short-duration episodes of LFS (6 sets, 30 pulses, separated by10 min baselines) were employed. Aged rats showed significant reversal after the 1st set of LFS and responses were completely reversed by the 5th set of LFS. Young rats did not show significant reversal until the 5th set of LFS and at no time were responses reversed to pre-LTP levels (see Figure). The results indicate (1) LTD/LTP-reversal can be observed in CA1 of aged rats and (2) aged rats are more sensitive to LTD-inducing stimulation. Together, the findings suggest that LTD mechanisms could play a role in the rapid rate of LTP decay and the rapid rate of forgetting observed in aged animals.

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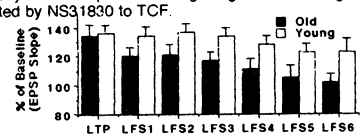

433.18

REPEATED CO-ACTIVATION OF THE CORPUS CALLOSUM AND HYPOTHALAMUS INDUCES A LONG-TERM POTENTIATION OF THE NEOCORTEX IN AWAKE BEHAVING RATS. P.A. Valentine* and G.C. Teskey. Behav. Neurosci. Res. Grp., Dept. of Psychology, Univ. of Calgary, Calgary, AB, Canada T2N 1N4.

The neocortex of the awake, behaving, adult rat has been found to be highly resistant to the induction of long-term potentiation (LTP). However, LTP of the neocortical evoked response has been observed when several sessions of high frequency stimulation (HFS) were applied to the corpus callosum over several days. It has also been shown that modulatory systems, which project to the cerebral cortex, play a role in LTP. This study was undertaken to examine the effects of co-activation of the ascending endogenous modulatory systems and corpus callosum on LTP of the neocortex. Male, Long-Evans hooded rats were chronically implanted with bipolar stimulating electrodes in the corpus callosum and the region of the dorsomedial-posterior hypothalamus (DMPH), and recording electrodes in the neocortex. Following a two-week recovery, baseline input/output (I/O) response curves were generated. These were based on the application of rectangular 200 μsec pulses at 11 intensities through the callosal electrodes. The potentials evoked in the neocortex were analyzed for changes in the size (area) of the negative component. This was calculated by subtracting baseline field potentials from post-treatment field potentials. Experimental animals received HFS trains to the callosum concurrent with DMPH stimulation once a day for a total of five days. Post-treatment I/O's were recorded for several weeks. We report potentiation of a late component of the neocortical evoked response to concurrent application of HFS to the corpus callosum and stimulation of the DMPH. Animals that received either HFS to the corpus callosum alone or DMPH stimulation alone showed no change in the evoked response. Supported by NSERC.

433.19

LONG TERM POTENTIATION (LTP) IS IMPAIRED IN BDNF-DEFECTIVE MICE. ***M. Korte, P. Carroll, H. Thoenen, and T. Bonhoeffer*, Max Planck Institute for Psychiatry, D-82152 Munich-Martinsried, Germany.***

There is increasing evidence that neurotrophins might be involved in neuronal plasticity. Application of BDNF (brain-derived neurotrophic factor) can induce synaptic strengthening, synthesis of BDNF and of NGF (nerve-growth factor) can rapidly be regulated by physiological stimuli, and secretion can occur from axonal and dendritic processes in an activity-dependent manner. To determine whether BDNF has a function in processes like long-term potentiation (LTP), we produced a strain of mice with a deletion in the coding sequence of the BDNF-gene. We then used hippocampal slices from these mice to investigate whether LTP is affected by this mutation. Homo- and heterozygous mutant mice show significantly reduced LTP in terms of the magnitude of the potentiation as well as the percentage of cases in which LTP could be induced successfully. This was true intra- and extracellularly. In a number of electrophysiological control experiments we measured responses to the application of GABA, AMPA, and NMDA. Furthermore we measured important anatomical and morphological parameters, like cell shape, cell size, dendritic branching, and spine density. No differences could be detected between wildtype and mutant animals. Paired pulse facilitation as an important parameter for normal synaptic function was also not affected in mutant animals. Since no obvious developmental deficits seem to be responsible for the failure to induce LTP in BDNF-deficient mice we suggest that BDNF is causally involved in the expression of LTP. In an attempt to obtain further support for this notion we are currently testing whether LTP can be "rescued" by re-introducing the BDNF-gene with an adenovirus construct in slices from BDNF-deficient animals.

434.1

CHANGES IN EARLY AND LATE IPSPs IN CA1 PYRAMIDAL CELLS AFTER HIGH FREQUENCY STIMULATION OF SCHAFFER COLLATERALS.

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Stimulation paradigms inducing long-term potentiation in hippocampal pyramidal cells will also affect local inhibitory circuits. To investigate plasticity in these circuits, the effect of high frequency stimulation of Schaffer collaterals (100Hz, 1s) was examined on early and late IPSPs using intracellular recordings from CA1 pyramidal cells in conventional rat hippocampal slices. In a first series of experiments with K-acetate containing microelectrodes, the mean amplitude of early IPSPs 30 min after tetanization was increased to 129±8.4% of control in 37.5% of cells ($p \leq .005$), decreased to 67±4.0% of control in 37.5% of cells ($p \leq .001$) and unchanged in the other 25% of cells. The mean amplitude of late IPSPs was increased to 142±4.3% of control in 50% of cells ($p \leq .04$), decreased to 64±9.5% of control in 25% of cells ($p \leq .0001$) and unchanged in the remaining 25%. For the pooled data (n=8 cells), mean amplitude of early and late IPSPs were unchanged. In another series of experiments, microelectrodes containing 200mM BAPTA were used to chelate intracellular Ca^{2+}. In these conditions, Ca^{2+}-dependent afterhyperpolarizations and accommodation were eliminated, and the mean amplitude of early IPSPs was increased 25-30 min after tetanization to 141±3.5% of control in all cells tested (n=5; $p < .0001$). The amplitude of late IPSPs was increased to 172±28.2% of control in 40% of cells ($p \leq .0001$) and unchanged in the other 60%. For pooled cells, the late IPSPs was increased to 138±7.2% of control ($p \leq .0001$). These preliminary results suggest that tetanization may result in either potentiation or depression of inhibition in pyramidal cells. Preventing Ca^{2+} rises in pyramidal cells blocked the depression and unmasked a reliable potentiation of both early and late IPSPs. Thus, tetanization may produce persistent changes within inhibitory circuits resulting in potentiation of inhibition in pyramidal cells.

(Supported by the MRC, FCAR, and FRSQ)

434.2

SATURATED HIPPOCAMPAL LTP IN RATS DOES NOT PRECLUDE ADDITIONAL POTENTIATION AT LATER PHASES.

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Hippocampal long-term potentiation may serve as an elementary process underlying certain forms of learning and memory in vertebrates. As is the case with behavioural memory, hippocampal long-term potentiation in the CA1 region and in the dentate gyrus exhibits distinct phases. These comprise a short-term early potentiation which lasts 1 to 3 hours and is independent of protein synthesis and is characterized in general by the activation of NMDA-receptors and protein kinases; and a later, longer lasting phase, which can be separated by inhibitors of protein synthesis.

Here, we report that the prior induction of long-term potentiation, both in the dentate gyrus in vivo and in the CA1-region in vitro, precludes further long-term but not short-term potentiation by means of a newly delivered conditioning stimulus to a subset of the same activated synapse population during the early stage (~1-3 hours post tetanus). In contrast, a subsequent, long-lasting potentiation can be induced after the establishment of the late phase of potentiation (>4 hours). Thus, the system preserves the capacity for short-term LTP immediately after potentiation, but the capacity for the induction of longer lasting plastic changes is recovered only after about 4 hours. Our results demonstrate that, once long-term potentiation has been established, hippocampal neurons do not lose their capacity for functional plasticity during certain phases of the maintenance of long-term potentiation.

This work was supported by the German BMBF, FKZ: 0310258A.

434.3

PLASTICITY AT 'SILENT' SYNAPSES EXPRESSING FUNCTIONAL NMDA RECEPTORS ONLY.

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NMDA receptor (NMDAR) activation is required for the induction of homosynaptic LTP and LTD in the hippocampal CA1 region. A recent study on CA1 using the analysis of coefficient of variation suggested that synapses may exist which express functional NMDARs only (Kullmann, *Neuron* 1994. 12,1111-1120). To examine this possibility, whole-cell recordings were made from CA1 pyramidal neurons in rat hippocampal slices (12-20 day-old) in the presence of 100 µM picrotoxin, and synaptic responses were elicited by minimal stimulation in the stratum radiatum. In 27/73 cells, synaptic events were detected at holding potentials between +30 and +60 mV, at a stimulus intensity which elicited no detectable synaptic response at -60 mV. The responses at positive potentials were completely blocked by D-APV. A comparison of spontaneous activity at -60 mV and +60 mV, using Fourier analysis, also revealed a much higher number of events at +60 mV. These additional events had the same frequency spectrum as evoked NMDAR-mediated EPSCs, and were completely blocked by D-APV. In 10/14 cells at a stimulus intensity which initially elicited no synaptic events at -60mV, AMPA receptor- (AMPAR) mediated responses appeared following a pairing protocol (100 stimuli, 1Hz, -10mV). In the 4 cells in which pairing did not cause the appearance of events at -60mV, the existence of NMDAR-mediated responses was confirmed at the end of the experiment. This form of potentiation was stable for the duration of recordings, and in a series of interleaved experiments, was blocked by D-APV. These data demonstrate the existence of synapses that express functional NMDARs but no detectable AMPARs, and suggest that these synapses can undergo a form of LTP in which functional AMPARs are uncovered or inserted into the postsynaptic membrane. *Supported by the Wellcome Trust and NIH*

434.4

INDEPENDENT MECHANISMS FOR LTD OF AMPA AND NMDA RECEPTOR MEDIATED SYNAPTIC TRANSMISSION IN AREA CA1 OF RAT HIPPOCAMPUS.

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Long-term depression (LTD) of excitatory synaptic transmission is a long lasting, activity dependent decrease in synaptic strength thought to be important for learning, memory, and neural development. Although LTD of AMPA responses (AMPA LTD) has been well characterized, relatively little is known about LTD of NMDA responses (NMDA LTD). We therefore decided to characterize NMDA LTD and compare its mechanism to that of AMPA LTD.

Using standard electrophysiological techniques to make field and whole cell recordings from area CA1 of the hippocampus, we produced input-specific NMDA LTD (-23±4%) by pharmacologically isolating the NMDA responses (0.1 Mg^{++}, 10 µM CNQX, 100 µM picrotoxin) and by applying low frequency stimulation (LFS; 1 Hz, 5-10 min). To determine whether NMDA LTD occurs under more physiological conditions, we first obtained AMPA LTD using LFS (-28±4%), and then pharmacologically isolated the NMDA responses. We found that the NMDA response in the test pathway was reduced relative to the control pathway (-41±5%). This NMDA LTD was blocked when D-APV (50 µM) was present during LFS.

To assess the mechanism responsible for NMDA LTD, we measured the coefficient of variation (CV) during whole cell voltage clamp recordings in which LTD was induced on pharmacologically isolated NMDA responses. We found that $(1/CV)^2$ was unchanged following NMDA LTD, implying a decrease in q, the uniquantal NMDA response. Repeating this experiment under normal recording conditions, we obtained simultaneous AMPA and NMDA LTD and found that while AMPA LTD resulted in a proportional decrease in $(1/CV)^2$, implying a decrease in n or p, $(1/CV)^2$ of the NMDA response was again unchanged.

These results suggest that NMDA responses undergo LTD under physiological conditions, and that this LTD has a mechanism different from that of AMPA LTD.

434.5

THE SPATIAL DISTRIBUTION OF LONG-TERM POTENTIATION IN THE HIPPOCAMPAL CA1 AREA DEPENDED ON THE TEMPORAL INFORMATION

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Long-term potentiation (LTP) in the CA1 area of hippocampus are highly sensitive to the higher order statistical characteristics of the stimulus (correlation between successive pairs of inter-stimulus intervals) (Tsukada et al. 1994). We observed the change of the spatial distribution of LTP depended on the number of stimulus in a slice preparation from the hippocampus of a guinea-pig by using the optical-imaging method. And we compared the spatial distribution of LTP induced by the temporal-pattern stimuli which have the same mean frequebcy but different time structure, different correlation, in inter-stimulus intervals.

As the results that the number of the stimulus increase, consequently, the area of LTP came more wide and shifted far from the stimulation site. And the spatial pattern of LTP in CA1 area was made in the area corresponding to the number of stimulus and the temporal-pattern sensitivity in LTP was identified in the area. And they differed depending on the temporal pattern of stimulus; positively correlated sequences were much more effective (large area) in producing LTP, while negatively correlated sequences were ineffective (small area), and randomly correlated sequence were medium. These results suggest that there is a transformation from the temporal pattern into the spatial pattern in the coding process of the hippocampal learning system.

434.6

THE LONG-TERM SUPPRESSIVE EFFECTS OF LOW-FREQUENCY STIMULATION ON THE INDUCTION OF LONG-TERM POTENTIATION IN CA1 NEURONS OF GUINEA PIG HIPPOCAMPAL SLICES.

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Low-frequency afferent stimuli (LFS) erases LTP (depotentiation; Fujii, et al. Brain Research,555, 112, 1991) but also suppresses the induction of LTP in CA1 neurons of guinea pig hippocampal slices. The parameters of the LFS (frequencies of 1 and 5 Hz; number of pulses of 80, 200 and 1000; and time-lag prior to tetanus of 20, 60 and 100 min) were altered systematically and their effects on the induction of LTP were evaluated. When the LFS of 1000 pulses at 1 Hz was delivered, the slope of the field EPSP (S-EPSP) and the amplitude of population spike (A-PS) 50-60 min after the LFS were reduced by 3.0 % and 7.5 %, respectively (n=6). Tetanus (100 Hz, 1 s) given 60 min after the LFS failed to induce LTP leaving a short-term potentiation which declined gradually close to the pre-tetanic level within at most 40-50 min. Significantly less effectiveness was observed for the LFS at 5 Hz and the LFS at 1 Hz with either a shorter (20 min) or a longer (100 min) time-lag before tetanus. When the LFS of 1000 pulses at 1 Hz was delivered in the presence of 50 µM APV (D,L-4-amino-5-phosphonovalerate), the S-EPSP and the A-PS 50-60 min after the LFS were considerably potentiated by 24.8 % and 31.8 %, respectively (n=6) and tetanus given 60 min after the LFS resulted in the induction of LTP. Therefore, the mechanisms of this particular form of synaptic plasticity may involve in the activation of NMDA receptor / Ca^{2+} channels by low-frequency synaptic inputs.

434.7

INTRACELLULAR CORRELATES OF EPSP-TO-SPIKE POTENTIATION. L.W. Campbell* and T.J. Sejnowski. Howard Hughes Medical Institute, The Salk Institute for Biological Studies, La Jolla, CA 92037

EPSP-to-Spike (E-S) potentiation has been observed in the rat hippocampus following stimulation trains which lead to LTP in the CA1 cell layer. Most commonly, E-S potentiation is noted as a potentiation in the population spike amplitude in excess of that predicted by the potentiation of the field EPSP slope. Jester, *et al.* (J Physiol, 1995) reported an associative stimulation paradigm which directly produces an E-S potentiation with no change to the EPSP slope. Further work with field potentials recordings and $GABA_B$ antagonists suggest that $GABA_B$ receptors are necessary for the expression of this associative E-S potentiation. What are the intracellular correlates of associative E-S potentiation and in what way are $GABA_B$ receptors involved in its expression?

Intracellular recordings were made from 75 g male Sprague-Dawley rats using sharp electrodes filled with 2M potassium acetate. In order to measure Na spike excitability and accommodation, a series of intracellular depolarizing 150 ms D-C current injections were given at approximately 5 minute intervals. The population spike amplitude and EPSP slope were monitored every 20 seconds with a single shock to the Schaffer-Collateral pathway . The associative tetanization consisted of 50 bursts of 5 antidromic pulses at 100 Hz with an interburst interval of 200 ms, paired with one shock to the Schaffer-Collaterals per antidromic burst.

Tetanization resulted in a slowly developing depolarization of the RMP and an increase in spikes generated in the intracellular current injection series. Counteracting the depolarization with holding current did not fully counteract the increase in spikes generated in the series. Changes in the $GABA_a$-mediated IPSP do not appear to contribute to the expression of E-S potentiation.

434.8

LONG-TERM POTENTIATION INITIATED BY EPILEPTIFORM ACTIVITY. V. Valenzuela & L.S. Benardo*. Departments of Neurology and Pharmacology, State University of New York Health Science Center, Brooklyn, NY 11203.

We have developed an *in vitro* model of epileptogenesis in neocortex in which paroxysmal activity persists for several hours after a limited exposure to magnesium-free solution. We have investigated whether similar activity is observed in hippocampal slices following such treatment.

Rat hippocampal slices (P20-P36) were exposed to magnesium-free solution for 30 min and then returned to normal saline. Interictal spiking in CA1 led by activity in CA3 occurred during exposure to magnesium-free media, and for 2-6 hrs after slices were returned to normal media. In addition, evoked extracellular field potentials recorded in CA1 and CA3 in response to single electrical stimuli applied to stratum radiatum remained potentiated for the duration of the experiment (up to 6 hrs) by 75-100%.

Exposure to the NMDA receptor antagonist CPP (10 μM) did not alter the potentiated field potentials, nor the spontaneous activity. However, the non-NMDA receptor antagonist CNQX (10 μM) abolished both of them. These results show that long-term potentiation mediated through non-NMDA receptors occurs after a brief period of epileptic activity. This has implications for the role of synaptic plasticity in the process of epileptogenesis.

434.9

CA2 REGION MODULATES ACTIVITY PROPAGATION FROM CA3 TO CA1 IN RAT HIPPOCAMPAL SLICES. Y. Sekino[1] and K. Obata[2*]. [1]PRESTO Res. Develp. Co. of Japan, c/o Mitsubishi-kasei Inst. of Life Sci., Machida, Tokyo 194, [2]Lab. Neurochem., National Inst. for Physiol. Science, Okazaki 444, Japan.

Many electrophysiological studies on hippocampal slices and theoretical analysis of hippocampal function have been performed on the basis of a classical trisynaptic circuit from the perforant path to CA1 in the transverse section. We previously reported the existence of a delayed synaptic transmission between CA3 and CA1 in rat hippocampal slices cut rather obliquely along alveus fibers and suggested the involvement of the CA2 region (Soc. Neursci. abst. 907,19,1993). In the present study, we investigated the pharmacological properties of this local circuit around the CA2 region with an optical measurement system (Fuji SD1001). Slices were stained with a potential sensitive dye, RH-482, and mossy fibers were electrically stimulated to produce synaptic excitation of CA3 and CA1 neurons. In some slices the delayed CA1 activity was not induced by the CA3 activation. When those tissues were treated with disinhibitory agents, the activity around the CA2 region was induced and the CA1 region was activated. These data suggest that the region including CA2 usually has a high threshold of excitation that is controlled by GABAergic and/or purinergic inhibitory systems. We propose that the CA2 region acts as a gate for the information processing along the longitudinal propagation in the hippocampal formation.

434.10

LONG-TERM TRANSFORMATION OF HIPPOCAMPAL GABAergic SYNAPSES SHARES MECHANISMS WITH PAIRING-INDUCED LTP, BUT NOT TETANUS-INDUCED LTP. D.Dahl*, C.Collin, W. Devane, J. Axelrod, and D.L. Alkon. School of Human Development, The University of Texas @ Dallas, Richardson, TX 75080 and the Laboratory of Adaptive Systems, NINDS, Bethesda, MD 20814.

Long-term potentiation (LTP) is the enhancement of excitatory synaptic transmission following afferent tetanization. Afferent tetanization may be paired with intracellular injection of depolarizing current (pairing-induced LTP) or not (tetanus-induced LTP). Here we present new evidence for the long-term transformation (LTT) of GABAergic inhibitory synaptic transmission into synaptic excitation by a pairing-specific paradigm. The mechanisms underlying LTT, pairing-induced LTP, and tetanus-induced LTP have not previously been distinguished.

Tetanus of the GABAergic afferents on to CA1 somata paired with injection of depolarizing current induces LTT in hippocampal field CA1 pyramidal neurons. The transformation response is not blocked by the glutamatergic antagonists CNQX and AP5. However, furosemide (600 μM), a chloride-pump blocker, decreases or eliminates LTT and pairing-induced LTP. This result is in contrast to furosemide's enhancement of tetanus-induced LTP. Similarly, anandamide (1 μM), an endogenous cannabinoid ligand, prevents LTT and pairing-induced LTP, but variably affected tetanus-induced LTP.

The results of the furosemide experiments suggest that LTT significantly contributes to pairing-induced LTP, but not to tetanus-induced LTP. Anandamide/cannabinoid receptor activation inhibits adenylate cyclase and N-type Ca^{2+} channels and thus provides a possible link between LTT and pairing-induced LTP.

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434.11

STIMULATION-DEPENDENT DIFFERENTIAL EXPRESSION OF LTP IN CA1 INTERNEURONS AND PYRAMIDAL CELLS. A.Stelzer* & S.Karnup, Department of Pharmacology, SUNY Brooklyn, Brooklyn, NY 11203.

Double intracellular recordings were performed in basket or axo-axonic cells (identified by biocytin labelling) and apical dendrites of CA1 pyramidal cells in hippocampal slices to compare tetanization effects. In recording pairs, elicitation of orthodromic postsynaptic potentials (PSPs) in interneurons was implemented with lower stimulation intensities (ranging from 7-160 μA for exclusive PSPs in interneurons, denoted I(i)). PSP elicitation in pyramidal cell dendrites required intensities from 80 - 430 μA, denoted I(p). When I(i) stimulation intensities were used during tetanization (n=10 pairs), 8/10 interneurons exhibited LTP of orthodromic EPSPs, whereas no PSPs could be evoked in pyramidal cells at any point of time following tetanization. In contrast, when I(p) stimulation intensities were used during tetanization (n=7 pairs), pyramidal cells exhibited LTP of EPSPs in 6/7 recordings, whereas interneuron excitability was depresssed in all 7 cells. These data show (1) stimulation-dependent differential LTP in pyramidal cells and interneurons, (2) a presynaptic strengthening of synaptic inhibition via interneuron LTP at I(i) tetanization stimulation intensities and (3) a presynaptic mechanism of disinhibition via interneuron depresssion at I(p) tetanization stimulation intensities at which pyramidal cell LTP was generated.

434.12

LTP INDUCED BY A PAIRING PROTOCOL REVEALS CELLULAR PLASTICITY IN ONLY ONE OF THREE CELL TYPES LOCATED OUTSIDE St. PYRAMIDALE. C. J. McBain* and G. Maccaferri NICHD-LCMN, NIH. Bethesda, MD, 20892.

We have studied the effect of pairing low frequency stimulation (1 Hz, 1 min.) of st. radiatum afferent fibers with cell depolarization (-20 mV) in three different populations of cells of the rat hippocampal slice preparation. Excitatory post-synaptic potentials (EPSPs) were recorded from single cells of CA1 under whole-cell current clamp conditions and field population spikes (fPSs) were simultaneously monitored in st. pyramidale. After establishing a stable EPSP baseline, the pairing procedure was applied to the cell under study. Long-term potentiation (LTP) could be induced in a subpopulation of st. radiatum cells, yielding a ~50% increase of the EPSP slope, while the fPS amplitude was decreased of ~40%. A second population of morphologically distinct st. Radiatum cells was totally insensitive to this protocol, and no change of the EPSP slope could be observed, while the fPS amplitude was again decreased. Finally, the effect of the pairing protocol on st. oriens-alveus cells elicited a depression of the EPSP slope, similar in magnitude to the decrease of the fPS amplitude. However, in this latter kind of cells LTP could be subsequently obtained by repeated tetanic stimulation (4X100 Hz, 1s) concomitant with an increase in the fPS amplitude. These results suggest the occurrence of: i) a "conventional" form of LTP in one cell type in st. Radiatum, independent of pyramidal cell activity, ii) the absence of synaptic plasticity in a second distinct st. Radiatum population, and iii) a "passive propagation" of synaptic plasticity from pyramidal to st. oriens-alveus cells (Maccaferri & McBain, Neuron 1995).

434.13

TETANUS-INDUCED LTP IN HIPPOCAMPAL PYRAMIDAL CELLS IS ACCOMPANIED BY SYNAPTIC DEPRESSION IN NEIGHBORING INTERNEURONS. L.L. McMahon* and J.A. Kauer. Dept. of Neurobiology, Duke University Medical Center, Durham, NC 27710

Previous work in hippocampal slices suggests that after tetanic stimulation in s. radiatum (s.r.) that induces LTP, GABAergic transmission is significantly impaired (Abraham, et al., J. Physiol. 394:367,'87; Miles and Wong, Nature 329:724,'87). We directly examined the effects of tetanus on excitatory synaptic transmission in inhibitory interneurons in s.r. Whole-cell recordings were made from CA1 s.r. interneurons in slices from 16-22 day old rats. Simultaneous field recordings in CA1 monitored synaptic transmission onto pyramidal cells. Tetani (100 Hz, 1 second at 1.5X test intensity) were delivered twice 20 seconds apart through electrodes in s.r. Tetanic stimulation triggered LTP in pyramidal cells, but depressed excitatory transmission onto the interneurons. Interneuron EPSCs were depressed by -34+/-7% (n=8) at 1 min., -33+/-7% (n=8) at 10 min., and -40+/-10% (n=3) at 40 min. post-tetanus. Depression was maximal just after tetanus, and usually lasted throughout the experimcnt, as long as 69 minutes post-tetanus. Similar depression was observed in current-clamp, showing that changes in series resistance do not account for the reduced EPSC size (EPSP at 1 min. post-tetanus was -27+/-9%, n=7; at 10 min., -21+/-9%, n=7). Neighboring synapses on pyramidal cells were simultaneously potentiated (EPSP at 10 minutes was +140+/-9%, n=5; LTP is persistent under our recording conditions (30-40 minutes post-tetanus, +130+/-6%, n=6)). A second afferent pathway onto the interneuron (stim. electrodes averaged 870 um apart), unstimulated during the tetanus, nonetheless was also depressed, suggesting a postsynaptic locus of depression (unstimulated pathway, -25+/-7% at 10 min. post-tetanus , n=7). These data suggest a mechanism to decrease feedforward inhibition of pyramidal cells that reinforces the effects of LTP and may contribute to epileptiform activity. Supported by NIH grant NS30500-03.

434.14

ROLE OF $GABA_A$ MEDIATED INHIBITION IN THE INITIATION AND MAINTENANCE OF LTD AND LTP IN RAT HIPPOCAMPAL PYRAMIDAL CELLS. S.V.Tisi, R.J.Holland, H.V.Wheal & J.E.Chad.* Department of Physiology and Pharmacology, University of Southampton, Hants., UK. SO16 7PX.

Long Term Depression and Long Term Potentiation were investigated in the hippocampi of adult Wistar rats (200g). A simple LTD/LTP protocol was carried out on slices, 400μM thick, which had had the CA3 pyramidal cell layer removed. The Schaffer collateral pathway was routinely stimulated every 30 seconds with a bipolar stimulating electrode. Stimulus strength was set to the level required to elicit half-maximal population spike response under control conditions. Population responses were recorded from CA1 and radiatum. Under control conditions, population spike depolarisations in CA1 were 2.42±0.28mV (mean±SD) while in radiatum initial population epsp slopes were 0.12±0.02mV/ms. LTD was generated by delivering 500 pulses at 1Hz producing a reduction in the slope of 38%± 0.03 and a reduction in the spike of 48%±0.26. Paired pulse inhibition at 20ms IPI was abolished. Following LTD, LTP could be produced by five 1 second bursts of 100Hz stimulation causing an increase in the measured slope of 34%±0.03 and an increase in the spike of 43%±0.33. We are investigating the role of inhibition in controlling synaptic plasticity. Bicuculline (10μM) was used to block $GABA_A$ mediated effects, increasing population spike amplitude (55%±0.18) and causing multiple spiking. From this baseline, both LTD and LTP could be initiated. In the majority of slices, the level of depression reached a maximum immediately after the LTD stimulus and there was a recovery to a stable LTD level. The LTP stimulus increased population spike amplitude (55%±0.171) and epsp slope (20%±0.019). Intracellular recordings of spontaneous and evoked ipscs are being used to investigate modulation of synaptic inhibition and its effects on synaptic plasticity. *Supported by the MRC and the Wellcome Trust.*

434.15

LONG-LASTING FACILITATION OF EXCITATORY TRANSMISSION INDUCED BY STIMULATION OF A MONOSYNAPTIC INHIBITORY PATHWAY IN THE RAT HIPPOCAMPAL SLICE. T. Taira[1,2]*, K. Lämsä[1] and K. Kaila[1] [1]Department of Biosciences, Division of Animal Physiology, P.O. Box 17 and [2]Institute of Biomedicine, Department of Physiology, 00014 University of Helsinki, Finland.

Depolarizing inhibitory postsynaptic potentials (dIPSPs) can be evoked in CA1 pyramidal cells of the rat hippocampal slice upon monosynaptic high-frequency activation of GABAergic interneurons in the absence of excitatory transmission. We have previously shown that large dIPSPs trigger spikes accompanied by activation of voltage-gated calcium channels (VGCCs). We now show that pharmacologically-isolated dIPSPs exert a long-term modulatory influence on glutamatergic transmission. Tetanic (100 Hz/1 s) stimulation applied at s. radiatum close to the recording site in the presence of the glutamate antagonists AP5 (80 μM) and CNQX (40 μM) facilitated the recovery of field excitatory postsynaptic potentials (EPSPs) following washout (>70 min) of the antagonists. In the tetanized pathway the recovery of the EPSPs was consistently faster and the synaptic responses larger (by up to 50%) than those recorded simultaneously in a separate control pathway which received the test stimuli only (given at 0.05 Hz). If the tetanization was given in the simultaneous presence of the glutamate antagonists and the $GABA_A$-receptor antagonist PiTX (100 μM), no difference in the recovery of the EPSPs between the two pathways was seen. This indicates that activation of $GABA_A$ receptors during tetanization is required for the facilitated recovery of the EPSPs. Application of Ni^{2+} (50 μM) during the high-frequency stimulation also prevented the facilitation of the EPSPs thus indicating an involvement of VGCCs. The results provide the first evidence for the idea that excitatory synaptic transmission can be potentiated upon activation of VGCCs by GABA-mediated depolarizing responses.

434.16

EXPRESSION OF SYNAPTIC POTENTIATION IN THE VISUAL CORTEX IS DUE TO ENHANCEMENT OF EXCITATORY SYNAPTIC TRANSMISSION AND NOT DUE TO A REDUCTION IN CHLORIDE-DEPENDENT INHIBITORY SYNAPTIC TRANSMISSION K. Harsanyi* and M.J. Friedlander, Neurobiology Research Center, University of Alabama at Birmingham, AL, 35294, USA.

Our laboratory has previously shown that potentiation of compound postsynaptic potentials (PSPs) in adult visual cortical slices can be induced by low frequency pairings of presynaptic afferent-stimulation and coincident postsynaptic depolarization (Harsanyi and Friedlander, Soc. Neurosci Abstr. 20: 1471, 1994). Stimulation at the white matter/layer VI border at 0.1 Hz was paired with postsynaptic depolarizing pulses (2.1 ±0.1nA; 80 ms; 60 pairings). Fifty eight percent (n=35/60) of the pairing protocols evoked potentiation of the PSP peak amplitude (26.5 ±3.1 percent change) in visual cortical slices from adult guinea pigs (31-90 days). In the present study, we evaluated whether the expression of this type of potentiation is due to a reduction in the IPSP, or a pure enhancement of the EPSP. Conventional intracellular recordings were made from cortical slices of adult animals (35-45 days). To selectively inhibit IPSPs only onto the neuron that participates in the pairing, a combination of cesium to block potassium channels, and the disulfonic stilbene, DNDS (4,4'-dinitro-stilbene-2,2' disulfonic acid; 500μM) to block chloride channels, was added to the micro-pipette filling solution (Dudek, et al. Soc. Neurosci. Abstr. 20: 1471, 1994). DNDS is an organic acid compound that acts as an open-channel flicker-type blocker of chloride channels and decreases open probability in a concentration dependent manner (Singh et al. Am. J. Physiol. 260, C51-C63, 1991). Diffusion of DNDS from the micropipette into the cell greatly reduced or eliminated early IPSPs, that are known to be mediated by movement of chloride ions through the $GABA_A$ channels. The probability of potentiation is similar with (n=6/9) or without (n=35/60) the intracellular block of chloride channels. Our data suggest that our pairing protocol induces an enhancement of compound PSPs by potentiating excitatory synaptic transmission. Supported by NIH grant EY 05116 and HFSP RG-69/93

434.17

USE OF TS-TM-CALIX[4]ARENE FOR INTRACELLULAR BLOCKADE OF $GABA_A$-MEDIATED INHIBITION IN VISUAL CORTICAL LAYER IV NEURONS. S. M. Dudek* and M. J. Friedlander. Neurobiology Research Center, Univ. of Alabama at Birmingham, AL 35294.

Since many forms of synaptic potentiation and depression are dependent on activation of NMDA receptors and/or voltage-gated calcium channels for their induction, postsynaptic inhibition has been extensively studied as a possible modulator of these types of plasticity. Previously, we have shown that the presence of inhibitory postsynaptic potentials (IPSPs) that are evoked with white-matter stimulation is negatively correlated with the ability to induce long-term depression of postsynaptic potentials recorded from visual cortical layer IV neurons maintained *in vitro* (Dudek & Friedlander, *Soc. for Neurosci. Abstr.* 19:894, 1993). Previous studies investigating the role of synaptic inhibition in the induction and maintenance of synaptic plasticity primarily have used extracellular application of various GABA receptor blockers by bath application or iontophoresis in order to reduce synaptic inhibition. These procedures have the disadvantage that entire networks of neurons are influenced, often leading to hyperexcitable states. Thus, introducing an agent intracellularly offers the advantage that only the neuron impaled is influenced by the blockade of synaptic inhibition directly onto that cell. We and others have determined that such studies are feasible for determining the effects of blocking GABA conductances both *in vitro* (DNDS: Dudek, *et. al., Soc. for Neurosci. Abstr.* 20:1471, 1994) and *in vivo* (DIDS: Nelson, *et. al., Science*, 265:774-777, 1994). Here we present the use of a novel compound for the intracellular blockade of $GABA_A$-mediated IPSPs. Although first described for the blockade of colonic chloride channels (Singh, *et. al., Biophys. J.*, 64 ,2(2):A17, 1993), 5,11,17,23-tetrasulfonato-25,26,27,28-tetramethoxy-calix[4]arene was found to potently inhibit the early IPSPs evoked by stimulation of white matter in slices of visual cortex (n=8). This study suggests that use of TS-TM-calix[4]arene may facilitate our understanding of the role of $GABA_A$ channels in modulating the induction and maintenance of synaptic potentiation and depression.

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434.18

BLOCKADE OF $GABA_A$ RECEPTORS FACILITATES INDUCTION OF NMDA-RECEPTOR INDEPENDENT LTP. Lawrence M. Grover*. Dept. Physiology, Marshall Univ. Sch. Med., Huntington, WV 25755.

High frequency (200 Hz) tetanization can induce a slow onset, NMDA-receptor independent LTP in hippocampal area CA1. This LTP requires Ca^{2+} influx through postsynaptic voltage-dependent Ca^{2+} channels (VDCCs). During tetanization, GABAergic inhibition limits the degree of postsynaptic depolarization, and thus the magnitude of Ca^{2+} influx through VDCCs. Relief from inhibition might therefore enhance NMDA-receptor independent LTP. To test this possibility, extracellular field potentials were recorded from CA1 stratum radiatum of rat hippocampal slices. Slices were tetanized with a single 25 Hz, 1 sec stimulus train, or four 200 Hz, 0.5 sec stimulus trains. APV (50 μM) was applied to all slices to prevent induction of NMDA-receptor dependent LTP. In some slices the $GABA_A$ antagonist, bicuculline (10 μM), was applied with APV. 200 Hz tetanization in APV produced a mean EPSP potentiation of 28.2 ± 2.5 % (n=13). The same 200 Hz stimulation delivered in APV and bicuculline induced significantly greater potentiation (mean EPSP increase of 50.5 ± 11.0 %, n=11). Bicuculline reduced the time required for maximal expression of LTP from 25 min (in APV alone) to 15 min (in APV and bicuculline). 25 Hz tetanization in APV did not induce LTP (-3.1 ± 6.3 % change in EPSP, n=7), but 25 Hz tetanization in APV and bicuculline induced a slow onset LTP in 5 of 11 slices (mean EPSP increase in these 5 slices was 31.0 ± 4.6 %; mean increase for all 11 slices was 10.7 ± 6.5 %). These data show that $GABA_A$ inhibition can regulate the magnitude, kinetics, and probability of induction of NMDA-receptor independent LTP. Neuromodulatory control of inhibitory circuitry might therefore be important in regulating NMDA-receptor independent LTP *in situ*.

434.19

DOES THE LATE MAINTENANCE OF CA1-LTP DEPEND ON GABA-ERGIC INHIBITION ?

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LTP serves as a model for the investigation of distinct forms of learning and memory formation. LTP consists at least of three phases: a potentiation lasting up to one hour precedes an early stage lasting three to four hours and a third stage which maintains the potentiation for at least eight hours and which depends on protein synthesis.

We examined hippocampal CA1-LTP *in vitro* of male wistar rats as previously described (Frey et al., *Brain Res:* 452. 57 - 65. 1988).

We have investigated, whether the GABAergic system plays a role in mechanisms responsible for the prolonged maintenance of hippocampal CA1-LTP. When $GABA_A$- and $GABA_B$-antagonists were applied simultaneously, the induction of LTP was facilitated for the two recorded parameters, the population spike and the field-EPSP. The late phase of LTP was not negatively influenced. Interestingly, a slight additional potentiation was observed 3 - 4 hours after LTP induction. Our recent studies (Frey et al., Neurosci. in press) demonstrated that persistent LTP does not preclude further potentiation 4 hours after LTP-induction. We have now investigated, whether spontaneous, intrinsic bursts, which occur after application of GABA-receptor antagonists, are able to induce a potentiation by themselves. Therefore we used a low-frequency stimulation protocol. In these experiments we observed a delayed, developing potentiation after about 3 - 6 hours. This delayed potentiation induced via spontaneous, intrinsic bursts in the presence of GABA-receptor inhibitors may mask a possible blockade of a late phase of hippocampal LTP, when the inhibitory system is blocked during LTP-induction.

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434.20

MODULATION OF PAIRED PULSE DEPRESSION ASSOCIATED WITH LONG-TERM POTENTIATION OF INHIBITORY SYNAPTIC TRANSMISSION IN RAT VISUAL CORTEX.

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To study the expression mechanism for long-term potentiation (LTP) of GABAergic inhibitory synaptic transmission in rat visual cortex, paired pulse depression (PPD) was compared before and after the induction of LTP. Inhibitory postsynaptic currents (IPSCs) evoked by layer IV stimulation were recorded from layer V cells while excitatory synaptic transmission was pharmacologically blocked. LTP of IPSCs induced by high-frequency stimulation accompanied no change in the mean value of PPD (ratio of the 2nd to the 1st IPSC amplitude), but did a significant reduction in the variation of PPD in all tested cells. The same change was observed when transmitter release was facilitated by bath application of a K^+ channel blocker 4-aminopyridine. In contrast, the changes of IPSC amplitude produced by changing the holding potential of postsynaptic cells accompanied no change in PPD. These results suggest that the expression of LTP involves an increase of transmitter release.

EXCITATORY AMINO ACIDS: PHARMACOLOGY—SECONDARY MESSENGER

435.1

DUAL ACTION OF CYCLOTHIAZIDE ON QUISQUALATE-STIMULATED PHOSPHOINOSITIDE HYDROLYSIS IN CEREBELLAR NEURONS.

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In primary cultures of cerebellar granule cells quisqualate stimulates phosphoinositide (PI) hydrolysis by acting at metabotropic glutamate receptors (mGluRs). Quisqualate is also known to be the most potent agonist at mGluR1 and mGluR5 receptors, both coupled to PI hydrolysis in CHO cells transfected with these receptors. In addition, quisqualate acts also as an agonist of ionotropic AMPA receptors. We have previously observed that in cerebellar neurons the action of quisqualate on PI hydrolysis can be enhanced by the presence of concanavalin A, a lectin known to decrease the desensitization of AMPA receptors. Hence, the stimulation of PI hydrolysis by quisqualate may include both a metabotropic and ionotropic component. In order to study further the possible contribution of AMPA receptors to the stimulation of PI hydrolysis we used cyclothiazide (CTZ) which decreases the desensitization of AMPA receptors. In addition, we used cerebellar neurons cultured in presence of 10 mM (K10)and 25 mM K^+ (K25) which express a different pharmacology of PI hydrolysis stimulation. Quisqualate enhances PI hydrolysis in both cultures, while *trans*-ACPD acts potently only in K10 cells. In K10 cells CTZ produced a biphasic effect on the basal PI hydrolysis, causing an increase at concentrations up to 10 μM, followed by an inhibition at higher concentations. CTZ, at concentrations above 10 μM also inhibited the stimulation of PI hydrolysis induced by both quisqualate and ACPD. In-K-25 cells, CTZ had little effect on basal PI hydrolysis, however, at low concentrations (10 μM) it potently enhanced PI hydrolysis stimulated by quisqualate. This effect of CTZ was inhibited by NBQX, a selective antagonist of AMPA receptors. These results suggest that in K25 granule cells the stimulation of PI hydrolysis by quisqualate is mediated by both mGluRs and by ionotropic AMPA receptors. In contrast, in K10 the contribution of the ionotropic AMPA receptors to PI hydrolysis appears negligible. Instead, in these cells CTZ inhibits quisqualate stimulated PI hydrolysis, possibly through a direct action at mGluRs. In fact, the application of CTZ to CHO cells transfected with mGluR1 caused an inhibition of both quisqualate and ACPD-induced PI hydrolysis, similar to that observed in K10 cerebellar granule cells.

435.2

MODULATION OF THE RETINAL ON-BIPOLAR CELL mGluR6 CASCADE BY PHOSPHORYLATION.

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The ON-bipolar cell metabotropic glutamate receptor (mGluR6) is thought to be coupled to a cGMP-phosphodiesterase (PDE) via a G-protein. Receptor binding leads to the closure of a cGMP-regulated cation channel. Intracellular dialysis of the tiger salamander on-bipolar cell with 1mM cGMP and 2mM ATP causes the development of an inward cation current that is reversibly suppressed by the glutamate analog L-AP4. The inward current and L-AP4 responses run-down with time (n=12) despite the continued presence of cGMP, suggesting that cGMP is not sufficient for maintaining the channel in the open state. We find that the rate of run-down increased when ATP was excluded from the pipette (n=14) or when ATP was replaced by ATP-γS (n=6), indicating a requirement for ATP, and by extension phosphorylation. The PDE inhibitor IBMX (100μM) failed to enhance the size of the inward current in the presence of 1mM ATP-γS, indicating that ATP-γS did not merely enhance PDE activity. This concentration of IBMX restored the inward current in the presence of GTP-γS which should maximally activate PDE, indicating that IBMX was capable of PDE inhibition. These results provide evidence that at least one site of phosphorylation is at the level of the channel. The specific cyclic-nucleotide dependent-protein kinase inhibitor HA1077 (50μM, n=6) decreased not only the inward current ($P<0.05$) and L-AP4 response ($P<0.01$) but also the fraction of current suppressed by L-AP4. The current evidence is consistent with the presence of phosphorylation sites associated with both the channel and the mGluR6-PDE pathway that may be important in adaptation state. Supported by NIH grant EY 10254

435.3

STRUCTURE FUNCTION ANALYSIS OF THE COUPLING BETWEEN METABOTROPIC GLUTAMATE RECEPTORS AND SECOND MESSENGER PATHWAYS.

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The function of metabotropic glutamate receptors (mGluRs) is dependent on the specific second messenger pathway(s) activated and thus on the specificity of coupling to different G proteins. To determine which regions of the receptor are involved in this interaction, we have introduced specific amino acid substitutions in the second and third intracellular domains of mGluR1a by site-directed mutagenesis. Messenger RNA, encoding wild-type and mutant receptors was synthesized *in vitro* and injected into *Xenopus* oocytes. The injected oocytes were challenged with 1 mM L-glutamate (Glu) for 15 sec to activate the receptors. The ability of the wild-type and mutant receptors to couple to G proteins was tested by recording the Ca^{2+}-gated chloride current resulting from the activation of a second messenger pathway involving phospholipase C, IP_3 turnover, and intracellular Ca^{2+} release. Since mGluR1a activity is partially sensitive to Pertussis toxin (PTX), we also tested the mutant receptors for their PTX sensitivity by pretreating the injected oocytes with PTX (2 μg/ml) for 24 hours before the recording. Two of the receptors mutated in the third intracellular loop showed no response following Glu stimulation, indicating that this domain is probably essential for the coupling to G proteins. One of the receptors mutated in the second intracellular loop showed a different response profile and PTX sensitivity compared to the wild type mGluR1a, suggesting that this domain is important in determining the specificity of the coupling to G proteins. (Supported by EY09534, RPB and New York Academy of Medicine).

435.4

ACTIVATION OF PHOSPHOLIPASE D MEDIATED BY A NOVEL METABOTROPIC GLUTAMATE RECEPTOR DOES NOT DEPEND ON PHOSPHOINOSITIDE HYDROLYSIS.

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We (*Soc. Neurosci. Abstr.* **20**: 487, 1994) and others (Holler et al., *J. Neurochem.* **61**: 1569, 1993; Boss et al., *Mol. Pharmacol.* **45**: 1177, 1994) have shown that metabotropic glutamate receptor (mGluR) agonists stimulate phospholipase D (PLD): their rank order of potencies in adult rat hippocampal slices (quisqualate > ibotenate > L-CCG-I > 1S,3R-ACPD > L-cystein sulfinate = L-AP3 = L-glutamate) indicates that the response is not mediated by a II or III group mGluR. In order to investigate the relationship between PLD activity and the stimulation of phospholipase C (PLC) induced by mGluRs of the I group (mGluR1 and mGluR5), we examined the effects of a series of agents on *i)* the formation of inositol phosphates and *ii)* the PLD-specific transphosphatidylation reaction resulting in the formation of [^{3}H]phosphatidyl-ethanol ([^{3}H]Pet). In rat hippocampal slices, the selective mGluR1 and mGluR5 agonist (RS)-DHPG (100 μM) stimulated inositol phosphate formation but had no effect on PLD activity. Conversely, L-AP3 (1mM) and the phorbol ester TPA (100 nM) antagonized mGluRs linked to PLC but stimulated the mGluR linked to PLD. (RS)-MCPG (0.5-1 mM), an antagonist of mGluRs linked to PLC, stimulated the formation of [^{3}H]Pet and decreased the PLD response induced by 1S,3R-ACPD, acting as a partial agonist on this receptor. The aminoindane derivative UPF-523, a selective mGluR1 antagonist (see Lombardi et al., these proceedings), had no effect on PLD activity. Finally, in cultured granule cells and in cerebellar slices, 1S,3R-ACPD promoted inositol phosphate but not [^{3}H]Pet formation, which was instead stimulated by the Ca^{2+} ionophore A23187. Our results indicate that the mGluR coupled to PLD does not belong to any of the three mGluR groups identified so far and that mGluRs may activate PLD and PLC via separate transduction pathways. Independent activation of PLD may be an important and specific mGluR mechanism to increase diacylglycerol formation in brain tissue.

435.5

Characterization of metabotropic glutamate receptors linked to IP3/Ca^{2+} cascade in transfected cells.

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Among metabotropic glutamate receptors, mGluR1 and R5 are coupled to IP3/Ca^{2+} cascade. These receptors share more than 60% of amino acid identity, and show similar pharmacological properties when expressed in CHO cells. We transfected two types of cells, HEK293 and NIH3T3 cells with cDNA encoding either rat mGluR1α or mGluR5a and studied the activation of PI hydrolysis in response to glutamate and its agonists. In accord with the results in CHO cells, inositol phosphates formation increased 3- to 6-fold over basal levels in these cells. The coupling system of these receptors therefore are consistent among different cell types. Both rank order and potencies of agonists in inducing PI hydrolysis were closely similar in both receptors. We also studied the increases in $[Ca^{2+}]_i$ triggered by glutamate exposure. The patterns of increase in $[Ca^{2+}]_i$ were carefully examined by the single cell calcium recording technique. We will present these results in the meeting.

435.6

NITRIC OXIDE SYNTHASE INHIBITORS DECREASE NMDA-INDUCED ELEVATIONS OF EXTRACELLULAR GLUTAMATE AND INTRA CELLULAR CALCIUM ($[Ca^{2+}]_i$) VIA A cGMP INDEPENDENT IN CULTURED CELLS.

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These studies were designed to examine the effect of nitric oxide (NO) on glutamate neurotransmission. In primary cultures of cerebellar granule cells the glutamate receptor agonist, N-methyl-D-aspartate (NMDA), stimulates an elevation of intracellular calcium ($[Ca^{2+}]_i$), the release of glutamate, the synthesis of nitric oxide (NO) and an increase in cGMP. Although it is clear that NMDA-induced NO stimulates guanylyl cyclase, it is unclear whether NO augments the NMDA-induced elevations of extracellular glutamate or $[Ca^{2+}]_i$ levels. We show that the NO synthase inhibitor, N^G-monomethyl-L-arginine (MMA), partially prevents the NMDA-induced extracellular glutamate and $[Ca^{2+}]_i$ and with complete blocking of cGMP elevations. These effects of NO on glutamate release and $[Ca^{2+}]_i$ elevation are unlikely to be secondary to cGMP elevations as evidenced the cGMP analogue, dibutyryl cGMP (dBcGMP), does not augments NMDA-induced elevations of extracellular glutamate or $[Ca^{2+}]_i$ levels. MMA was unable to suppress the kainate- or KCl-induced elevations of extracellular glutamate. Furthermore, MMA did not affect glutamate uptake. This results suggest that physiological concentrations of NO has a role in maintaining the NMDA receptor activation in a cGMP independent manner.

435.7

CHANGES IN $[Ca^{2+}]_i$ INDUCED BY ACTIVATION OF GLUTAMATE RECEPTORS IN CULTURED RAT HIPPOCAMPAL NEURONES.

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Confocal laser scanning microscopy was used to study changes in $[Ca^{2+}]_i$ produced by pressure ejection of glutamate to rat cultured hippocampal neurones. Cells were pre-loaded at 37°C with the fluorescent calcium-sensitive dye fluo-3 (2μM for 45-60 min). TTX (0.5 μM) was routinely used to block fast sodium currents. Glutamate (0.1mM) induced a transient increase in $[Ca^{2+}]_i$ as shown by a 3-5 fold rise in fluorescence which reached a peak in 2-5 s and exponentially declined in 10-20 s. The response was abolished in Ca^{2+}-free solution. The Ca^{2+} increase signal was strongly and reversibly depressed (72 ± 6%, *n=17*) by CPP (20μM). The residual response was completely (6/9 cells) and reversibly blocked by CNQX (20μM). However, CNQX alone caused a full inhibition (5/8 cells) of the glutamate-induced response. When 100μM glycine was added to CNQX the block was only 30%, suggesting that CNQX acted also on glycine binding sites of the NMDA receptors. In addition, the metabotropic glutamate receptor antagonist MCPG (1mM), significantly and reversibly decreased the background level of $[Ca^{2+}]_i$ by 17 ± 1% *(n=8)* and reduced the effect of glutamate by 25 ± 6% *(n=8)*. The latter effect was probably indirectly mediated since a complete suppression of calcium signalling was obtained when ionotropic glutamate receptor antagonists were applied after MCPG was washed out.

These data suggest that the rise in intracellular calcium by glutamate was dependent on extracellular calcium and involved mainly NMDA receptor activation.

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435.8

EXTRACELLULAR MG^{2+} CAN CARRY CURRENT THROUGH THE NMDA-ACTIVATED CHANNEL.

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Glutamate increases the free intracellular $[Mg^{2+}]$ in cultured rat cortical neurons in the absence of extracellular Ca^{2+} and Na^+. The characteristics of this increase suggest that Mg^{2+} can enter neurons through NMDA channels (see Stout *et al.*, this volume). In this study we directly measured NMDA-activated whole-cell currents in extracellular solutions in which Mg^{2+} and NMDG were the only cations.

When 130 mM Cs^+ was included in the pipette solution, application of 100 μM NMDA plus 10 μM glycine activated an inward current in an extracellular solution containing 70 mM Mg^{2+} and 31 mM NMDG (n = 13) at potentials from -40 to -120 mV. At potentials more positive than -75 mV, the NMDA solution activated an outward current in a solution containing 2 mM Mg^{2+} and 140 mM NMDG (n = 6). Addition of 200 μM DL-APV to the NMDA solution inhibited the inward current by 95 ± 2.7% (n = 5). When the internal Cs^+ was entirely replaced by 130 mM NMDG, the NMDA solution activated an inward current in the 70 mM Mg^{2+} solution at potentials more negative than +20 to +30 mV. The amplitude of the inward current ranged from -20 pA to -280 pA. In individual neurons, the amplitude of the Mg^{2+} current was proportional to that of the control current (recorded in 140 mM extracellular Na^+). The correlation between the two currents was highly significant (r^2 = 0.98, $P < 0.001$, n = 9), indicating that Mg^{2+} and Na^+ permeate the same population of NMDA channels. Further analysis shows that the current recorded near -55 mV in 70, 2, or 0.9 mM Mg^{2+} solution was 7.3 ± 0.5 (n = 9), 2.5 ± 1.4 (n = 5), and 0.9 ± 0.4% (n = 3) of the control current. The influx of Mg^{2+} through NMDA channels may explain the glutamate-stimulated increase in the intracellular $[Mg^{2+}]$ in the absence of extracellular Ca^{2+} and Na^+. Supported by NIH grants MH00944, MH45817, MH18273, DA07409 and NS34138 and the American Heart Association.

435.9

GLUTAMATE-STIMULATED MAGNESIUM INFLUX AND SUBSEQUENT MAGNESIUM EFFLUX IN NEURONS.

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Previous magfura-2 fluorescence microscopy studies using cultured rat forebrain neurons have demonstrated that glutamate exposures which trigger excitotoxicity (100 μM, 5 min) can also produce large increases in intracellular free magnesium concentrations ($[Mg]_i$). A large component of the glutamate-induced increase in $[Mg]_i$ is probably due to displacement of bound intracellular Mg by influx of Ca ions through the NMDA receptor channel (Brocard *et al.* 1993). In this study we have investigated a smaller glutamate-stimulated increase in $[Mg]_i$ which remains in the absence of external Ca ($[Ca]_e$) and Na ($[Na]_e$). This glutamate-stimulated increase in $[Mg]_i$ was not significantly reduced by prior depletion of intracellular Na (induced by a fifteen-minute pretreatment of neurons with Na-free buffer), suggesting that the increase in $[Mg]_i$ does not occur via reverse Na-Mg exchange. The increase in $[Mg]_i$ was blocked by the NMDA channel blocker MK-801 (Brocard *et al.* 1993) and was enhanced by increasing external Mg ($[Mg]_e$). Furthermore, the increase in $[Mg]_i$ was reduced when NMDA channel-permeant Cs or K ions were used as a Na substitute instead of the presumably NMDA channel-impermeant ion N-methyl-D-glucamine (NMDG). These results suggest that the glutamate-stimulated increases in $[Mg]_i$ that occur in the absence of $[Ca]_e$ and $[Na]_e$ result from Mg entry through NMDA-gated ion channels. Consistent with this hypothesis, application of NMDA + glycine in an extracellular solution containing Mg (70 mM) and NMDG (31 mM) as the only cations induced inward currents at negative membrane potentials in whole-cell patch-clamp experiments (see Li-Smerin *et al.*, this volume). The initial rate of recovery of $[Mg]_i$ after glutamate stimulation was significantly inhibited either by complete removal of $[Na]_e$ (31% of control) or by elevation of $[Mg]_e$ to 30 mM (36% of control), suggesting that Na-Mg exchange plays a role in Mg efflux. If the alterations of Mg homeostasis that occur upon glutamate stimulation contribute to excitotoxicity, then modulation of these Mg fluxes may provide a possible therapy for ischemic brain injury. Supported by the American Heart Association and NIH grants MH18273, MH45817, MH00944, DA07409, and NS34138.

435.10

DEVELOPMENT OF GLUTAMATE-INDUCED $[Ca^{2+}]_i$ AND $[Mg^{2+}]_i$ CHANGES IN CULTURED FOREBRAIN NEURONS.

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Glutamate acting through the NMDA receptor produces a characteristic excitotoxic profile: a transient increase in $[Ca^{2+}]_i$ during the toxic stimulus and cell death can be detected 24 hrs later. It has been suggested that both $[Ca^{2+}]_i$ and $[Mg^{2+}]_i$ may be important in this type of excitotoxic neuronal injury. The aim of this study was to look at the development of agonist-induced changes in $[Ca^{2+}]_i$ and $[Mg^{2+}]_i$ (using fura-2 and magfura-2, respectively) in cultured rat forebrain neurons at 5, 10, 14, 18, 21, 28 days in culture. A non-toxic stimulation with NMDA, AMPA, kainate, glutamate, or depolarization produces a change in $[Ca^{2+}]_i$ at day 5 that was lower than the other developmental days. A toxic stimulation with glutamate appears to induce a change in $[Ca^{2+}]_i$ that is lower at day 5 than at day 28, whereas glutamate seems to produce an increasing change in $[Mg^{2+}]_i$ up to day 14. Our preliminary results indicate that after 20 days in culture, it took longer for the cells to recover to basal $[Ca^{2+}]_i$ and $[Mg^{2+}]_i$ after an excitotoxic stimulus compared to less mature cells. We are also currently investigating the relationship between the expression of various NMDA receptor subunits and sensitivity of these cells to excitotoxicity over this developmental time course. These results indicate that the neurons in our culture are functional and responsive to the various agonists tested, even at the earliest day studied. Although there does not appear to be a good correlation between glutamate-induced changes in $[Ca^{2+}]_i$ or $[Mg^{2+}]_i$ and sensitivity to excitotoxicity during the maturation of these cells, the difference in recovery times suggests a change in homeostatic mechanisms of the more mature neurons in buffering these ions upon glutamate stimulation.

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436.1

IBOGAINE BLOCKS NMDA RECEPTOR CURRENT BY AN OPEN CHANNEL MECHANISM. K. Chen, T.G. Kokate, S. Yamaguchi*, F.I. Carroll† and M.A. Rogawski. Neuronal Excitability Section, Epilepsy Research Branch, NINDS, NIH, Bethesda, MD 20892, and †Research Triangle Institute, Research Triangle Park, NC 27709.

Ibogaine is a hallucinatory indole alkaloid with antiaddictive properties that is under investigation as a treatment for chemical dependency. Recently, ibogaine was reported to be a competitive inhibitor of [^{3}H]dizocilpine binding (Popik et al., *Psychopharmacology* **114**: 672–674, 1994), suggesting that it interacts with the channel blocking site of NMDA receptors. We further investigated the effects of ibogaine on NMDA receptors in whole cell voltage-clamp recordings from cultured rat hippocampal neurons. At a holding potential of –60 mV, ibogaine caused a concentration-dependent block of NMDA-evoked currents (IC_{50}, 3.1 μM). In contrast, ibogaine failed to affect either kainate- or GABA-evoked currents. The blockade of NMDA currents by ibogaine was use-dependent, long lasting and highly voltage-dependent, being significantly relieved at positive holding potentials. The block could be substantially reduced by co-application of Mg^{2+}, indicating that ibogaine is an open channel blocker. In confirmation of previous studies, ibogaine produced a concentration-dependent inhibition of [^{3}H]dizocilpine binding to rat brain membranes (IC_{50}, 2.5 ± 0.9 μM). Finally, in mice, ibogaine had a dose-dependent protective effect against NMDA-induced lethality. These data indicate that ibogaine is a channel blocking antagonist of the NMDA receptor complex. Whether this contributes to its acute behavioral effects or reported ability to modify drug-seeking behavior remains to be determined.

436.2

BIII 277 CL AND BIII 281 CL, TWO ENANTIOMERIC NMDA OPEN-CHANNEL BLOCKERS: ELECTROPHYSIOLOGICAL CHARACTERIZATION. M. Grauert, J.M. Rho, M.A. Rogawski*. Epilepsy Research Branch, NINDS, NIH, Bethesda, MD 20892.

BIII 277 CL {2R-[2α,3(R*),6α-1,2,3,4,5,6-hexahydro-3-(2-methoxypropyl)-6,11,11-trimethyl-2,6-methano-3-bezazocin-9-ol hydrochloride} is a potent antagonist of the NMDA receptor-channel complex which is currently under preclinical evaluation as a treatment for acute thrombotic stroke. We investigated the mechanism by which BIII 277 CL and its enantiomer BIII 281 CL block NMDA receptors using whole-cell and single channel recording techniques in cultured hippocampal neurons; for comparison we also examined the effect of the drugs on AMPA and $GABA_A$ receptor currents. In whole-cell recordings, both compounds produced a long lasting, use-dependent block of NMDA-evoked currents that could be substantially reduced by coapplication with Mg^{2+}. In contrast, the compounds had no effect on currents evoked by kainate, AMPA or GABA. The block of NMDA-activated current by BIII 281 CL occurred in a concentration-dependent fashion with IC_{50} of 63.5 nM (Hill coefficient, 1.1). The data obtained with BIII 277 CL was fit better to a two binding site model, with IC_{50}'s of 1.96 and 58.4 nM. In single-channel recording from outside-out patches, BIII 281 CL caused rapid flickering of NMDA-evoked unitary currents, compatible with a channel blocking mechanism. In contrast, BIII 277 CL caused flickering early in the drug application, but then virtually eliminated all channel currents, possibly because of slow binding to a high affinity blocking site. We conclude that both enantiomers selectively block NMDA receptors by an open-channel mechanism. BIII 281 CL likely interacts with the NMDA receptor at one site, whereas BIII 277 CL seems to interact with two sites, one of which may correspond to the BIII 281 CL blocking site.

436.3

IN VIVO EFFECTS OF THE SELECTIVE KYNURENINE 3-HYDROXYLASE INHIBITOR FCE 28833 IN THE RAT. [1]A. Molinari, [1]M. Cini, [1]M. Marconi, [2]H.-O. Wu, [2]R. Schwarcz, [1]A. Bonsignori, [1]R.A. McArthur*, [1]M. Yarasi and [1]C. Speciale. [1]Pharmacia R&D CNS, Nerviano, Italy and [2]Maryland Psychiatric Research Center, Baltimore, MD 21228.

A shift in the equilbrium between the neuroprotectant kynurenate (KYNA) and the neurotoxin quinolinate (QUIN) towards the latter has been proposed to increase excitotoxic vulnerability in the CNS. The inhibition of kynurenine 3-hydroxylase (KYN 3-OHase), an enzyme of the kynurenine pathway, could conceivably attenuate kynurenine degradation to QUIN and at the same time increase the synthesis of KYNA. FCE 28833 [(R,S)-2-amino-4-oxo-4-(3'-4'-dichlorophenyl)butanoic acid] was identified *in vitro* as a specific and potent inhibitor of KYN 3-OHase (IC_{50} = 0.2 μM). Drug effects were tested *in vivo* using male Wistar rats (200-250 g). After a single oral dose of 50-400 mg/kg, FCE 28833 caused increases in KYNA content in all tissues investigated (plasma, liver, kidney, brain). Brain KYNA increased approximately 14-fold by 8 hr after the administration of 400 mg/kg FCE 28833, p.o. The time course of this effect was further examined by intrahippocampal microdialysis in freely moving rats. A 10-fold increase in extracellular KYNA was thus obtained between 3 and 10 hr after 400 mg/kg FCE 28833, p.o. Data returned to baseline values by 20 hr after drug administration. FCE 28833 (400 mg/kg, p.o.) also reduced the duration of maximal electroshock-induced tonic-clonic convulsions by approximately 30%, without affecting motor coordination on the rotarod. FCE 28833 is a novel KYN 3-OHase inhibitor which constitutes a useful tool to investigate the neurobiological relevance of modulating kynurenine pathway function in the brain.

436.4

DERIVATIVES OF KYNURENINE AS INHIBITORS OF RAT BRAIN KYNURENINE AMINOTRANSFERASE. [1]M. Yarasi*, [1]P. Pevarello, [1]C. Speciale, [2]P. Guidetti, [2]D.R. Wells and [2]R. Schwarcz. [1]Pharmacia R&D CNS, Nerviano, Italy and [2]Maryland Psychiatric Research Center, Baltimore, MD 21228.

Previous studies have indicated that a single enzyme, kynurenine aminotransferase (KAT), is responsible for the synthesis of the neuroinhibitory metabolite kynurenic acid (KYNA) in the rat brain. The structural requirements of the enzyme's catalytic site were now examined using analogs and derivatives of L-kynurenine (KYN), the natural substrate of KAT. KYNA production from KYN was routinely monitored in rat brain homogenates and brain tissue slices in the presence or absence of the novel compounds. Any modification of KYN's alanine side chain or its ring amino group resulted in compounds which (up to 1 mM) did not affect KYNA synthesis from KYN. In contrast, ring chlorination in positions 3, 4, 6 and 5 yielded KYN analogs which interfered with KYNA synthesis with increasing potency. L-5-Cl-KYN was found to be the most active of several 5-substituted kynurenines. Using partially purified rat KAT, L-5-Cl-KYN was shown to be an excellent substrate of KAT, yielding 6-Cl-KYNA. In kinetic studies using partially purified KAT, L-5-Cl-KYN was found to have an approximately 5 times higher affinity to the enzyme than KYN (K_i for L-5-Cl-KYN: 5.4 μM vs. K_m for KYN: 28.3 μM). Ongoing studies are designed to examine the usefulness of L-5-Cl-KYN as a tool for the study of brain kynurenines.

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436.5

MICROSPECTROFLUORIMETRIC STUDIES OF UNCOMPETITIVE NMDA ANTAGONISTS. D. Sawyer, J.G. McLarnon, and J. Church*[1] Departments of Pharmacology & Therapeutics and [1]Anatomy, The University of British Columbia, Vancouver, BC, Canada V6T 1Z3.

Cultured rat cortical neurons loaded with the calcium-sensitive fluorescence indicator Fura-2 responded to 20 s applications of NMDA, AMPA, kainate or 50 mM potassium with transient increases in intracellular free calcium ($[Ca^{2+}]_i$). Superfusion of the cultures with either the non-opioid antitussive dextromethorphan or the benzomorphan opiate (-)-β-cyclazocine resulted in suppression of responses to NMDA but not to AMPA or kainate. Responses to high K^+ were also unaffected by (-)-β-cyclazocine, whereas a slight decrease was observed with dextromethorphan, consistent with its known blockade of high-voltage activated Ca^{2+} channels . Concentration-effect curves showed dextromethorphan and (-)-β-cyclazocine to inhibit NMDA-evoked reponses with IC_{50}'s near 5 μM and 0.2 μM, respectively. The actions of β-cyclazocine on NMDA-evoked $[Ca^{2+}]_i$ increases were stereoselective, as the (+)-enantiomer at 5 μM decreased NMDA responses by only 16±2%. The actions of (-)-β-cyclazocine exhibited use-dependence in that several applications of agonist were required in order to achieve a steady-state block. On the other hand, with dextromethorphan an equilibrium level of blockade was apparent with the initial application of agonist. The results show that while both compounds are inhibitors of NMDA-evoked responses in cultured rat cortical neurons, there may be important differences in their mechanism of block.

436.6

CEREBRAL PRODUCTION OF 7-CHLOROKYNURENIC ACID AFTER SYSTEMIC ADMINISTRATION OF ITS BIOPRECURSOR 4-CHLOROKYNURENINE IN MICE. H.-O. Wu* and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD 21228.

We have demonstrated previously that 4-chlorokynurenine (4-Cl-KYN) can be enzymatically converted into the specific NMDA/glycine antagonist 7-chlorokynurenic acid (7-Cl-KYNA) in the rat brain (J. Med. Chem., 37: 334, 1994). Through conversion to 7-Cl-KYNA, 4-Cl-KYN exhibits potent neuroprotective properties *in vivo* (Soc. Neurosci. Abstr. 19: 554.7, 1993). We now explored the effects of systemically administered 4-Cl-KYN (300 mg/kg, i.p.) on cerebral 7-Cl-KYNA synthesis using hippocampal microdialysis in unanesthetized CD-1 mice. Extracellular 7-Cl-KYNA was found to rise rapidly, reaching a plateau of 600 nM at 3 h. 7-Cl-KYNA levels declined slowly over the next 20 h. Infusion of 1 mM aminooxyacetic acid through the dialysis probe reduced the levels of 7-Cl-KYNA by 90%, indicating that enzymatic production of 7-Cl-KYNA occurs in the brain. Systemic administration of 4-Cl-KYN also increased endogenous extracellular kynurenic acid levels two-fold. Tissue levels of 7-Cl-KYNA were examined in separate animals which were killed 4 h after the i.p. administration of 4-Cl-KYN (100-1000 mg/kg). 7-Cl-KYNA was measured in the hippocampus and striatum of these saline-perfused mice and was found to increase linearly with the dose of 4-Cl-KYN. Unexpectedly, the 7-Cl-KYNA content was almost twice as high in the hippocampus as in the striatum (1.3±0.2 vs. 0.7±0.1 pmol/mg prot. after 300 mg/kg 4-Cl-KYN; $P < 0.05$). These data indicate that systemically administered 4-Cl-KYN may provide region-selective NMDA/glycine receptor antagonism in the brain.

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436.7

ACEA 1021: AN ANTAGONIST AT THE STRYCHNINE-INSENSITIVE GLYCINE RECEPTOR - ELECTRO-PHYSIOLOGICAL CHARACTERIZATION *IN VIVO*. K. Lingenhöhl*, T. Ferrat, and R. Hiltscher. Ciba, 4002 Basel, Switzerland.

ACEA 1021 is an antagonist at the strychnine-insensitive glycine receptor site of the NMDA receptor and has been shown to exhibit neuroprotective, anticonvulsant and analgesic properties *in vivo* (Woodward et al. 93, Soc. Neurosci. Abstr., 19: p. 718).

In the present study the effects of ACEA 1021 were examined on NMDA induced as well as acoustically induced excitation in the rat *in vivo*. Single and multibarrel electrodes were used for extracellular recordings and the application of NMDA. ACEA 1021 was either iontophoretically or intravenously applied. In the CA1 region iontophoretically applied ACEA 1021 abolished NMDA induced excitation. In the same area of the brain systemically applied ACEA 1021 dose-dependently reduced NMDA induced excitation. At 30 mg/kg the NMDA induced excitation was reduced to 5.4 %. In the inferior colliculus acoustically induced excitation, which is NMDA and non-NMDA mediated, was maximally reduced by iontophoretically applied ACEA 1021 to 32.1 %. Intravenously applied ACEA 1021 reduced acoustically induced excitation at 30 mg/kg to 35.0 %. In summary, these data show that following systemic application ACEA 1021 is centrally active on exogenously evoked NMDA responses as well as endogenously mediated NMDA responses.

436.8

FREE L-ASPARTATE, L-GLUTAMATE, D-SERINE AND GLYCINE CONCENTRATIONS IN THE DISCRETE BRAIN AREAS OF MK-801(DIZOCILPINE)-TREATED RAT.

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Concentrations of free L-aspartate, L-glutamate, D-serine and glycine have been measured in the rat cerebral cortex, hippocampus and striatum using high-performance liquid chromatography with fluorometric detection after the blockade of the N-methyl-D-aspartate (NMDA) receptor. Systemic administration of a selective and non-competitive antagonist of the NMDA receptor, MK-801 (0.05-0.15mg/kg, S.C.), decreased and increased the levels of L-aspartate and L-glutamate, respectively, in the three areas at 90 min post-injection. The contents of D-serine were found to be increased in the striatum. There was no change in glycine concentration in the three areas. These results indicate that contents of endogenous amino acids acting at the excitatory amino acid site (L-aspartate and L-glutamate) and the glycine site (D-serine) of the NMDA receptor might depend upon the NMDA receptor-mediated neurotransmission.

436.9

PHARMACOLOGICAL CHARACTERIZATION OF MDL 105,519, A SYSTEMICALLY ACTIVE ANTAGONIST OF THE NMDA RECEPTOR-ASSOCIATED GLYCINE RECOGNITION SITE B.M. Baron*, B.W. Siegel, B.L. Harrison, J.H. Kehne, T.C. McCloskey, C.J. Schmidt, V.L. Taylor, G.M. Fadayel, M.K. Murawsky, P.L.M. van Giersbergen, H.S. White[1], and F.G. Salituro Marion Merrell Dow Research Institute, Cincinnati, OH, 45215 and Strasbourg, France; University of Utah, Salt Lake City, UT 84108

MDL 105,519 ((Z)-2-(phenyl)-3-(4,6-dichloroindol-3-yl-2-carboxylic acid)propenoic acid) is a potent inhibitor of [^{3}H]glycine binding to the NMDA receptor (Ki = 11 nM) exhibiting > 1,000-fold binding selectivity vs. a panel of 16 ionotropic and G-protein coupled receptors. Antagonism was evident in that MDL 105,519 prevented both NMDA-dependent increases in [^{3}H]TCP binding and NMDA-elicited biochemical responses. The IC_{50} value vs NMDA-stimulated cGMP accumulation in cerebellar slices was 282 nM. Inhibition was non-competitive and could be nullified with D-serine. MDL 105,519 (1 μM) reversibly inhibited by ~90% NMDA-induced elevations in cytosolic calcium and whole cell currents.

Inhibition of harmaline-stimulated increases in cerebellar cGMP content (CD-1 mouse, ED_{50} 56 mg/kg i.v.) provided evidence of NMDA antagonism in vivo. Anticonvulsant activity was quantitated vs audiogenic seizures (DBA/2J mouse, ED_{50} 12 mg/kg ip), icv quinolinic acid (CD-1 mouse, ED_{50} = 37 mg/kg iv), and maximal electroshock (CDF rat, ED_{50} 29 mg/kg iv). Duration ($t_{1/2}$) was 1-3 h in the various models. MDL 105,519 showed anxiolytic activity in the rat separation-induced vocalization model (ED_{50} 40 mg/kg ip) but muscle relaxant activity was evident at lower doses (ED_{50} 28 mg/kg ip). Side effect liability was also evaluated using the mouse rotorod test (ED_{50} 73 mg/kg iv), rat mesolimbic dopamine turnover or release (no effect at ≤100 mg/kg ip), and prepulse inhibition of the rat startle reflex (inhibited baseline response but did not abolish prepulse inhibition even at doses up to 200 mg/kg).

436.10

[^{3}H]MDL 105,519, A HIGH AFFINITY RADIOLIGAND FOR THE NMDA RECEPTOR-ASSOCIATED GLYCINE RECOGNITION SITE B.W. Siegel*, B.M. Baron, B.L. Harrison, R.S. Gross, C. Hawes[1], and P. Towers[1] Marion Merrell Dow Research Institute, Cincinnati, OH, 45215; [1]Amersham International plc, Cardiff Laboratories, Whitchurch, Cardiff CF4 7YT, Wales UK.

MDL 105,519 ((Z)-2-(phenyl)-3-(4,6-dichloroindol-3-yl-2-carboxylic acid)propenoic acid) is a potent ligand at the NMDA receptor-associated glycine recognition site. For purposes of characterizing its action at the glycine binding site, a halogenated analog was reduced with tritium to form radiolabeled MDL 105,519. [^{3}H]MDL 105,519 bound to a rat brain membrane P2 fraction with high affinity (K_d = 3.77 nM) and capacity (B_{max} = 12.1 pmol / mg protein). Specific binding was ~90% of total binding. Centrifugation and filtration gave identical levels of specific binding.

The kinetics of the binding reaction were studied. Association was monophasic with k_{on} equal to 7.0 x 10^7 $M^{-1}min^{-1}$. Dissociation was also monophasic with the k_{off} value calculated from association experiments (0.257 min^{-1}) being similar to that measured directly in dissociation experiments (0.232 min^{-1}). A kinetically-derived value for the equilibrium dissociation constant was calculated using the two values for k_{off} and the association rate constant. The values (K_d = 3.67 and 3.31nM, respectively) agreed well with those obtained from the saturation experiments.

Comparing the pK_i values for a series of glycine site agonists, partial agonists, and antagonists, the pharmacology of the site labeled by [^{3}H]MDL 105,519 matched that of the recognition site labeled by [^{3}H]glycine (r = 0.90). With the exception of HA-966, Hill slopes were not significantly different from unity. No effect on specific binding was observed with ligands interacting with other sites on the NMDA receptor complex or with non-NMDA glutamate recognition sites.

436.11

PHARMACOLOGICAL STUDIES OF AMINO ACID NEUROTRANSMITTERS USING SILICON SUBSTRATE DRUG DELIVERY PROBES. J.F. Hetke, S.C. Bledsoe, Jr.*, J. Chen, J.A. Wiler, K.D. Wise and D.J. Anderson. Dept. Elec. Engin. & Comp. Sci. and Kresge Hearing Res. Inst., Univ. of Michigan, Ann Arbor, MI 48109

Traditionally, in vivo pharmacological studies of the nervous system have largely relied on microiontophoretic and micropressure techniques. With this approach multibarrel pipettes are used to apply drugs in the vicinity of a single neuron. An improvement would be the ability to record simultaneously multiple neurons at different locations while applying the drug at a given site.

Toward this end, we report on the development and utilization of silicon substrate drug delivery "puffer" probes. These probes, fabricated using integrated-circuit processing techniques, bury microchannels within the substrate. The process is compatible with the inclusion of multiple recording sites on the same substrate, permitting multipoint drug delivery and multichannel recording with minimal tissue disruption. Separations between the electrode sites and outlet ports can be as small as 2.5μm.

Utilization of puffer probes reveals that kainic acid, a neural excitant, produces excitation in the superior colliculus but not the inferior colliculus (IC). We also have preliminary results that the application of γ-aminobutyric acid (GABA), a known inhibitory transmitter in the (IC), produces both inhibition and excitation of IC neurons. This new technology should prove useful for connectivity studies in neural networks and for pharmacological manipulation of tissue reactions in chronic electrophysiological studies.

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436.12

BIOCHEMICAL, PHARMACOLOGICAL AND STRUCTURAL CHARACTERIZATION OF KAINATE RECEPTORS. Z.G. Wo, M.J. Sutcliffe§ and R.E. Oswald*. Dept. Pharmacology, Cornell Univ., Ithaca, NY 14853 USA; §Dept. Chemistry, Univ. Leicester, UK.

Based on the studies of two goldfish kainate receptor subunits (GFKARα and GFKARβ), we have previously proposed a model with three transmembrane segments (3-TMs) for the topology of ionotropic glutamate receptor family (Wo, Z.G. and Oswald, R.E. TiNS 18, 161-8, 1995). In the present study, GFKARα and GFKARβ were transiently expressed in transfected HEK293 cells either alone or in combination. [^{3}H]kainate saturation binding analysis showed that GFKARα and GFKARβ have K_D's for kainate of 20 nM and 35 nM, respectively. The affinity of GFKARα and GFKARβ for various glutamatergic ligands (L-glutamate, quisqualate. domoate, AMPA and CNQX) was measured by the inhibition of [^{3}H]kainate binding. While GFKARα and GFKARβ have similar affinity for quisqualate, domoate, AMPA and CNQX, these two subunits showed marked difference (> 20-fold) in the affinity for L-glutamate. When co-expressed, GFKARα and GFKARβ assemble into hetero-oligomers as indicated by the changes in ligand binding profiles. Thus, these two kainate receptor subunits (sharing 55% identity) provide an interesting system for the identification of the residues determining ligand binding specificity. The affinity for [^{3}H]kainate was increased upon treatment with the reducing agent dithiothreiotol (DTT). Detailed analysis indicated the presence of S-S bond in GFKARα and GFKARβ. Given the 3-TM model for the transmembrane topology and a body of structural information available, we will present a three dimensional structural model of non-NMDA glutamate receptors.

436.13

PHENYLGLYCINE ANALOGUES CAN EVOKE THE AP6 "PRIMING" RESPONSE IN RAT CINGULATE CORTEX.
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The priming or AP6 response following quisqualate application to slices of rat cingulate cortex can be evoked in a dose dependent manner by L-AP4 and L-AP6. A series of phenylglycine analogues, which have no intrinsic depolarizing activity, can evoke large depolarizations of rat cingulate cortex slices, when administered 20 min following a 2 min application of 30 μM quisqualate. These depolarizations were inhibited by NBQX (0.3-1.0 μM). The most potent compound was (S)-4C3HPG (MEC 3 μM). The order of potency was (5)-4C3HPG ≥ (S)-3C4HPG > (S)-4CPG > (S)-3HPG > (RS)-α-M4CPG. None of these comounds showed any significant affinity for the AMPA or CPP binding sites, and their pharmacology at mGluR subtypes was heterogeneous. All of the compounds showed affinity for a low affinity [^{3}H] AP4 binding site. The potency at this site was correlated to (P = 0.001) the MEC in the AP6 response in the cortex. None of the compounds have affinity for mGluR4 receptors.

436.14

EFFECTS OF CYCLOTHIAZIDE AND GYKI 52466 ON BINDING PROPERTIES OF AMPA RECEPTORS. M. Kessler*, A. Arai, A. Quan, and G. Lynch. Ctr. for the Neurobiology of Learning and Memory, Univ. of California, Irvine CA 92717.

The binding affinity of the AMPA receptor for [^{3}H]AMPA was shown in an earlier report to be reduced about five-fold by cyclothiazide (Hall *et al.*, Brain Res. 628 (1993) 345-348). Binding in that study had been measured in the presence of the chaotropic ion thiocyanate. When similar tests were carried out without thiocyanate, cyclothiazide was found to have minimal effects on [^{3}H]AMPA binding or on the displacement of [^{3}H]CNQX binding by AMPA and glutamate. The interaction of cyclothiazide with the receptor itself seemed not to be affected by thiocyanate. The results, when interpreted in a five-state receptor model, suggest that cyclothiazide does not reduce receptor desensitization by making the desensitized state inaccessible but rather by increasing the affinity of the active (non-desensitized) state for agonists to such an extent that the active state becomes energetically more stable than the desensitized state.

The inhibition of AMPA receptor currents by GYKI 52466 has been reported to be reversed by cyclothiazide in an apparently competitive manner. The interaction between these two compounds was examined further by testing whether the effects on binding that cyclothiazide produces in the presence of thiocyanate are influenced by GYKI. This was not the case with GYKI concentrations up to 400 μM, i.e., concentrations far exceeding those effective in physiological experiments. The lack of effect of GYKI is not likely to be due to a negative interaction with thiocyanate since AMPA-induced currents in patches excised from hippocampal pyramidal cells were inhibited to a similar extent in the absence and presence of the latter. The results suggest that GYKI and cyclothiazide do not bind to the same site on the AMPA receptor. (Supported by grants from AFOSR and Cortex Pharmaceuticals).

436.15

KINETIC ANALYSIS OF AMPA RECEPTOR AND EPSC MODULATION BY NON-SATURATING DOSES OF CYCLOTHIAZIDE. R. Baehring, D. K. Patneau#, L. Vyklicky Jr and M. L. Mayer*. LCMN, NICHD, National Institutes of Health, Bethesda, MD 20892; and #Department of Pharmacological & Physiological Sciences, University of Chicago, Chicago, IL 60637.

In whole cell experiments on cultured hippocampal neurons AMPA receptors show 90-95% desensitization (τ 8-15 ms) in response to fast superfusion of 1 mM glutamate. The amount of desensitization was reduced by cyclothiazide in a concentration-dependent manner (IC_{50}=1μM). For equilibrium responses to non-saturating concentrations of cyclothiazide the desensitization time constant of the initial component of the glutamate response was indistinguishable from control values. Similar results were obtained during both wash-in and wash-out of cyclothiazide. Thus, at low concentrations of cyclothiazide two distinct receptor populations could be distinguished, one with cyclothiazide bound, showing block of desensitization, and a second receptor population which desensitized normally.

We have also studied effects of cyclothiazide on evoked excitatory postsynaptic currents (EPSCs). The decay of EPSCs was prolonged in a dose-dependent manner, but with the most pronounced effect at concentrations of cyclothiazide greater than those required to fully block desensitization. Assuming that receptor deactivation is the main factor for determining the decay kinetics of EPSCs we expected cyclothiazide to exhibit comparable effects on responses to brief glutamate applications (1 ms) recorded in outside-out patches. Indeed, we found that deactivation was prolonged by cyclothiazide, however, at 100 μM the slowing of deactivation did not match the effects on EPSC decay kinetics: In the presence of cyclothiazide a fast component of EPSC decay was followed by a pronounced slow component (τ 50 ms; relative weight 40 % of the total current decay). In contrast, the slow component of deactivation (τ 12 ms) was considerably more rapid than for EPSC decay and contributed only 25% to the total amplitude. Together, these results suggest more than one mechanism for modulation of EPSCs by cyclothiazide. The examination of lower concentrations of cyclothiazide on deactivation kinetics are in progress and should shed further light on this issue.

436.16

THE POLYAMINE SITE ANTAGONIST IFENPRODIL BLOCKS THE EXCITATORY EFFECTS OF DYNORPHIN ON NMDA RECEPTOR-MEDIATED SYNAPTIC CURRENTS IN THE CA3 REGION OF THE GUINEA PIG HIPPOCAMPUS. R.M. Caudle* and R. Dubner, Neurobiology and Anesthesiology Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20892.

In a previous study we demonstrated that dynorphin had a biphasic concentration response relationship on N-methyl-D-aspartate (NMDA) receptor-mediated synaptic currents in guinea pig CA3 pyramidal cells (J. Neurosci. 14:5580, 1994). Concentrations of dynorphin < 1 μM enhanced the synaptic current whereas concentrations > 1 μM inhibited the current. In our previous study, we demonstrated that the inhibitory effects of dynorphin were blocked by naloxone while the excitatory effects of dynorphin were non-opioid. The inhibitory effects were demonstrated to be mediated by $kappa_2$ opioid receptors. The present study focused on the excitatory actions of dynorphin on NMDA receptor-mediated synaptic currents. Synaptic currents were evoked by stimulating the Schaffer collateral pathway while voltage clamping the CA3 pyramidal cell. Non-NMDA excitatory amino acid receptors were blocked by CNQX (10 μM) and GABA receptors were blocked by bicuculline (20 μM). The patch electrode contained cesium and tetraethylammonium chloride to block potassium currents. In the absence of any antagonists dynorphin 1-17 had a biphasic concentration response relationship as previously described. When the NMDA receptor polyamine site antagonist ifenprodil (10 μM) was included in the superfusion buffer, dynorphin's concentration response relationship was monotonic and inhibitory. The IC50 for dynorphin was shifted from approximately 1.1 μM to 28 nM by the addition of ifenprodil. Ifenprodil alone had no effect on NMDA mediated synaptic currents. The inhibitory effect of dynorphin in the presence of ifenprodil was blocked by naloxone. These findings indicate that dynorphin can act as an agonist at the polyamine site of the NMDA receptor in the guinea pig hippocampus.

436.17

IN VITRO ACTIVITY OF THE NOVEL NMDA RECEPTOR GLYCINE SITE ANTAGONIST ACEA 1021. J.E. Hawkinson*, C.L. Kimbrough, Z.O. Egbuwoku, and R.M. Woodward. CoCensys, Inc., 213 Technology Dr., Irvine, CA 92718.

ACEA 1021 is a systemically active quinoxalinedione currently under clinical evaluation as a neuroprotectant. Electrophysiological data indicates that ACEA 1021 is a selective antagonist of the NMDA receptor glycine site. In this report, we compare the electrophysiological, neurochemical, and binding properties of ACEA 1021 with R(+)-HA-966, a systemically active glycine site partial agonist/antagonist. ACEA 1021 and R(+)-HA-966 inhibit NMDA/glycine currents in *Xenopus* oocytes expressing rat brain NMDA receptors with K_b values of ~ 5 nM and ~ 15 μM, respectively, and inhibit the binding of the glycine site antagonist ligand [^{3}H]5,7-dichlorokynurenic acid ([^{3}H]DCKA) to rat cortical membranes with IC_{50} values of 3 nM and 8 μM, respectively. In neonatal rat cerebellar slices *in vitro*, ACEA 1021 and R(+)-HA-966 block NMDA-stimulated cGMP accumulation with IC_{50} values of 2 μM and 1 mM, respectively. Thus, ACEA 1021 inhibits NMDA/glycine currents and [^{3}H]DCKA binding with high affinity and is > 2000-fold more potent than R(+)-HA-966. Although less active in cerebellar slices, ACEA 1021 retains 500-fold greater potency than R(+)-HA-966 in blocking cGMP accumulation. These electrophysiological, neurochemical, and binding data provide further evidence for ACEA 1021 antagonism of glycine sites associated with the NMDA receptor.

437.1

EFFECT OF THIOCYANATE ON AMPA RECEPTOR MEDIATED RESPONSES IN EXCISED PATCHES AND HIPPOCAMPAL SLICES

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The binding affinity of AMPA receptors for [^{3}H] AMPA is increased 10-30-fold by the chaotropic anion thiocyanate. The present experiments tested if thiocyanate alters AMPA receptor mediated currents and if any such effects are reflected in the waveform of synaptic responses. Currents induced by glutamate and AMPA were measured in patches excised from pyramidal cells of hippocampal slice cultures. Thiocyanate accelerated the decay of AMPA responses two-fold and reduced the peak current by 30-50% with an EC_{50} of 3.2 mM which is comparable to its EC_{50} for enhancing binding. Effects on the desensitization of glutamate induced responses were much smaller and only evident at the highest thiocyanate concentration. Binding and physiological effects can be adequately explained by assuming that thiocyanate enhances conversion from the sensitive to the desensitized state of the receptor and reduces ligand dissociation from the desensitized state. Synaptic responses were measured in disinhibited hippocampal slices. Thiocyanate increased the slope of the field excitatory postsynaptic potential by 45 ± 4% and reduced its decay time by 10 ± 4 %. The former effect appears to result at least in part from an increase in transmitter release. The decrease in the decay time constant points to an effect of thiocyanate on AMPA receptors in situ which is similar to that seen in excised patches. These results demonstrate that an increase in binding affinity may be indicative of reduced rather than enhanced current flow through AMPA receptors. In addition, the results provide further evidence that the kinetics of the AMPA receptor channel contribute significantly to at least the decay phase of fast excitatory synaptic responses. (Supported by AFOSR).

437.2

HALOTHANE INDUCED SYNAPTIC DEPRESSION AND METABOTROPIC GLUTAMATE RECEPTORS.

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We examined the effect of metabotropic glutamate receptor (mGluR) activation by trans-(±)-1-amino-1,3-cyclopentanedicarboxylic acid (ACPD) on control and halothane depressed population spikes (PS's) in the rat hippocampal slice. Hippocampal slices 400μM thick were cut and placed in a submersion type bath. The stratum radiatum was stimulated at the CA1-CA2 boundary with PS recorded from the CA1 stratum pyramidale and the field EPSP from the stratum radiatum. PS's were analyzed for amplitude and latency EPSP for slope of onset. In some studies staurosporine was added to examine the possible interaction of protein kinase C. ACPD 100μM produced a biphasic effect on the PS consisting of a transient increase in amplitude followed by a decline to about 4.3% of the control value. Prior application of halothane prevented the initial increase in PS amplitude produced by ACPD but did not alter the later decrease. The slope of the EPSP was only reduced without a preceding enhancement. The prior application of staurosporine did not alter the effect of ACPD.

The initial increase in amplitude caused by ACPD is reported to be related to enhanced excitability of the CA1 pyramidal cell. The secondary decrease in amplitude is reported to be related to decreased glutamate release. Halothane prevented the primary increase in amplitude but did not alter the secondary decrease. Halothane action was not altered by the prior application of staurosporine. Part of the action of ACPD may be related to a reduction of inhibitory mechanisms.

437.3

MODULATION OF DOPAMINE (DA), NORADRENALINE (NAD) AND SEROTONIN (5-HT) RELEASE IN FRONTAL CORTEX, ACCUMBENS AND STRIATUM OF FREELY-MOVING RATS BY PSYCHOSTIMULANT DRUGS.

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A perturbation of mesolimbic and mesocortical monoaminergic transmission is implicated in the etiology of schizophrenia. Here, we compared the influence of several psychostimulants upon the release of DA, NAD and 5-HT in freely-moving male Wistar rats implanted with a single cannula in the frontal cortex, or with a cannula in the accumbens and the contralateral striatum. Rats were dialysed and monoamines measured by HPLC with coulometric detection. Samples were taken every 20 min. Three basal values were taken, then amphetamine (2.5 mg/kg, i.p.), phencyclidine (PCP) (20.0 mg/kg, s.c.) or dizocilpine (0.16 mg/kg, s.c.) injected and samples taken for a further 180 min.

	Frontal Cortex			Nucleus Accumbens			Striatum	
	DA (1.2)	NAD (1.8)	5-HT (0.8)	DA (4.9)	NAD (0.6)	5-HT (0.8)	DA (9.2)	5-HT (0.8)
PCP	1085	436	447	383	1037	246	268	372
DIZO	163	263	896	108	217	138	105	129
AMPH	817	1560	293	922	4391	220	915	954

Absolute, basal levels are given (pg/20 min) in parentheses. Maximum mean % change is expressed relative to basal levels (= 100%).

Vehicle (water) did not significantly modify release (not shown). For PCP and dizocilpine, but not amphetamine, the increase in DA and 5-HT release was more marked in cortex than elsewhere. These findings reveal distinctive patterns of modulation of monoaminergic transmission by several psychostimulant drugs.

437.4

GLUTAMATE MODULATES SPINAL CORD NOREPINEPHRINE RELEASE.

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Previous studies have demonstrated that glutamate (GLU), via N-methyl-D-aspartate (NMDA) receptors modulates the release of various neurotransmitters including dopamine, acetylcholine and norepinephrine (NE) from brain regions. In the spinal cord, the presence of GLU terminals in anatomically similar areas where NE and serotonin (5-HT) terminals are located suggests a possible interaction between these neurotransmitter systems. The present studies, therefore were undertaken to determine if GLU modulates the release of spinal neurotransmitters such as 5-HT and NE.

Spinal cords from male Sprague-Dawley rats (250-300g) were harvested and chopped into 300μm slices. The slices were incubated with 0.025 μM [^{3}H]NE or [^{3}H]5-HT and tissue aliquots placed in 1 ml chambers and then perfused with Mg^{++} free Krebs-bicarbonate buffer. GLU agonists were added to the perfusion buffer and fractions of perfusate collected. When included, drugs were added 15 min prior to stimulation with glutamate agonists. [^{3}H]NE or [^{3}H]5-HT release was expressed as a percentage of the total tissue radioactivity remaining at the beginning of that fraction.

In the absence of Mg^{++}, NMDA induced a concentration dependent increase in the release of [^{3}H]NE but not of [^{3}H]5-HT. [^{3}H]NE release was Ca^{++}-dependent and blocked by Mg^{++} and the NMDA antagonists MK-801, APV and ketamine. NMDA was more efficient at inducing [^{3}H]NE release than was kainate or glutamate. NMDA-stimulated [^{3}H]NE release from dorsal spinal cord was 2-fold greater than from ventral cord. These findings suggest an interaction between GLU and NE that may be involved in spinal sensory processing.

437.5

PROLINE-INDUCED POTENTIATION OF SYNAPTIC TRANSMISSION IS LONG-LASTING AND NMDA RECEPTOR-DEPENDENT.

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We previously reported that concentrations of proline known to be present in CSF enhance synaptic transmission at Schaffer collateral-commissural synapses in rat hippocampal slices, when proline is continuously present in the medium. At high concentrations, proline can activate NMDA receptors. We are therefore investigating the relationship between proline-induced potentiation and NMDA-dependent LTP. Two concentrations of proline were used: a concentration typical of normal CSF (3 μM) and a concentration present in the CSF of persons with hyperprolinemia type II (30 μM). When proline was added to the superfusion medium for 20 min, the initial slope of the field EPSP began to increase after about 10 min in 85% of rat hippocampal slices and continued to increase after proline was removed. Plateau values, reached in 50-60 min, were 36% and 46% greater than baseline for 3 and 30 μM proline, respectively. In the absence of proline, field EPSP slope declined slightly during the same time period. When applied concurrently with proline, 50 μM D-AP5 blocked proline-induced potentiation. When 25 nM NMDA was added to the superfusion medium for 20 min, it increased the field EPSP slope to a comparable degree. However, the effect of NMDA was immediate and, unlike proline, it increased the amplitude of the fiber volley. In addition, proline-induced potentiation did not reduce the amplitude of subsequent electrically-evoked LTP.

These results suggest that proline-induced potentiation is long-lasting, requires the activation of NMDA receptors and is separable from electrically-evoked LTP.

437.6

MODULATION OF GLUTAMATE AND ASPARTATE RELEASE FROM HIPPOCAMPAL SYNAPTOSOMES BY ARACHIDONIC ACID AND THROMBOXANE A_2.

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Previous studies on slices of hippocampal area CA1 that utilized inhibitors of eicosanoid metabolism suggested that arachidonic acid and/or platelet-activating factor (PAF) enhances the K^+-evoked, Ca^{2+}-dependent release of glutamate and aspartate and that a prostaglandin or thromboxane inhibits the release of glutamate. To identify the specific eicosanoids involved in regulating glutamate/aspartate release, we tested the effects of these compounds on release from rat hippocampal synaptosomes. Arachidonic acid (60 μM), but not linoleic acid (300 μM), enhanced the K^+-evoked, Ca^{2+}-dependent release of glutamate and aspartate. The PLA_2 inhibitor aristolochic acid (50 μM) reduced the release of these amino acids. c-PAF (3-10 μM), in contrast, enhanced the release of aspartate only. Flurbiprofen (100 μM), an inhibitor of both isoforms of cyclooxygenase, selectively enhanced glutamate release from both CA1 slices and hippocampal synaptosomes. In synaptosomes, prostaglandins D_2 (1 and 10 μM), E_2 (1 and 10 μM), $F_{2\alpha}$ (1 μM) and c-I_2 (10 μM) did not alter the release of aspartate or glutamate. However, two metabolically-stable forms of thromboxane A_2 (1 and 20 μM c-TXA_2 and 20 μM p-TXA_2) inhibited the release of both amino acids. SQ 29,548 (5 μM), an antagonist of TXA_2 in other systems, partially reversed the depression of aspartate release and fully reversed the depression of glutamate release.

These results suggest that arachidonic acid and TXA_2 act as modulators of excitatory transmitter release in the rat hippocampus. Arachidonic acid enhances and TXA_2 depresses release. These effects may be especially important during seizures and cerebral ischemia, when these lipids are released in large quantities.

437.7

PHENCYCLIDINE AND MK801 PRODUCE TIME-DEPENDENT REGIONAL ALTERATION IN GAD mRNA IN RAT BRAIN. T. Hashimoto*, X.-M. Gao and C.A. Tamminga. Maryland Psychiatric Research Center, University of Maryland School of Medicine, Baltimore MD 21228

Interactions between glutamatergic and GABAergic transmission in CNS have been previously demonstrated. We have studied this interaction in rat brain pharmacologically by measuring GAD mRNA after blockade of glutamate transmission at the NMDA receptor with PCP or MK801. These non-competitive antagonists at the NMDA receptor have extended, time-dependent actions in rat brain on regional cerebral glucose utilization, hippocampal glutamate receptor density and expression of multiple immediate early genes. In this study, we report the extended actions of PCP (8.6 mg/kg) and MK801 (1 mg/kg) on the expression of GAD_{67} mRNA at 1, 3, 6, 24, and 48 hours after drug administration. We employed *in situ* hybridization with a specific GAD cRNA probe and quantified the film densities autoradiographically. Expression of GAD_{67} mRNA was reduced by 15%-25% in neocortex including anterior cingulate, medial prefrontal (MPC), dorsolateral frontal (DFC) and sensorimotor cortices ($P<0.05$) 24 hours after PCP. The caudate also showed a decrease in the expression of GAD_{67} mRNA ($P<0.05$) 24 hours after PCP. After MK801 GAD_{67} mRNA was reduced in MPC and DFC 24 hours after dosing. Data from additional times and drug doses will be reported. At higher PCP doses, this reduction in inhibitory transmission might be the basis of the PCP-induced signs of neurotoxicity, like intracellular vacuolization (Olney et.al. 1989) and HSP elevation (Sharp et.al. 1991, 1992). This action of PCP and MK801 in inhibiting GAD mRNA production, suggests that glutamatergic stimulation at the NMDA receptor activates GAD activity regionally and maintains inhibitory transmission.

437.8

POTENTIATION OF SPONTANEOUS EXCITATORY POSTSYNAPTIC CURRENTS BY PREGNENOLONE SULFATE IN CULTURED NEURONS. M. Park-Chung*, T.T. Gibbs and D.H. Farb. Laboratory of Molecular Neurobiology, Department of Pharmacology, Boston University School of Medicine, Boston, MA 02118.

We have shown recently that the naturally occurring sulfated neurosteroids pregnenolone sulfate (PS) and 3α-hydroxy-5β-pregnane 20-one sulfate (5β3αS) are glutamate receptor modulators. PS potentiates and 5β3αS inhibits the response to exogenously applied N-methyl-D-aspartate (NMDA), and both PS and 5β3αS inhibit responses to kainate and a-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA). However, the effects of steroids on glutamate-mediated synaptic responses have not been studied. Here we report that PS potentiates spontaneous excitatory post synaptic currents (EPSCs) in cultures of rat hippocampal neurons. Using whole-cell recording methods, cells were voltage-clamped at -70 mV. Drug solutions were applied to single neurons by pressure ejection from 7-barrel pipets. EPSC potentiation by PS is concentration-dependent, with an EC_{50} of 5.6 μM and maximum potentiation of 198.2 %. An analog of PS, 11-keto PS, has no effect on EPSCs, suggesting the effect of PS on EPSCs is specific. When EPSCs mediated by NMDA receptors are blocked with the specific NMDA receptor antagonist APV (40 μM), the potentiation of EPSCs by 100 μM PS is reduced. Conversely, when EPSCs mediated by non-NMDA glutamate receptors are blocked with the specific non-NMDA receptor antagonist DNQX (10 μM), 100 μM PS produces a greater potentiation of EPSCs (453 %). These results indicate that PS primarily potentiates EPSCs mediated by NMDA receptors. The effects of PS on EPSCs agree with those of PS on the response induced by exogenously applied NMDA. These observations provide further evidence that neurosteroids such as PS can exert direct neuromodulatory effects on excitatory synaptic transmission in the CNS.

437.9

EVALUATION OF NEUROSTEROID MODULATION OF KAINATE RECEPTORS USING AN AUTOMATED SYSTEM FOR OOCYTE ELECTROPHYSIOLOGY. N. Yaghoubi*, T.T. Gibbs, and D.H. Farb. Laboratory of Molecular Neurobiology, Department of Pharmacology, Boston University School of Medicine, Boston, MA 02118.

Recent studies from our laboratory have shown that neurosteroids can directly modulate glutamate-gated ion channels. Although modulation of NMDA receptors has been well documented, modulation of kainate receptors has not been studied in detail. To characterize neurosteroid modulation of the kainate response, kainate receptors expressed in *Xenopus* oocytes from rat brain mRNA were evaluated using a novel automated system for oocyte electrophysiology. To provide precise control over voltage and drug application protocols during data acquisition, an automated system was developed that integrates drug application, instrument control, data acquisition, and waveform analysis through a computer-based "virtual instrument." The apparatus consists of a custom recording chamber that is coupled through a multi-barrel manifold to a programmable perfusion system that is controlled by a Mac-based data acquisition and instrument control platform running SuperScope II software (GW Instruments). Automated protocols have been implemented to generate dose-response curves, reversal potential determinations, and modulation studies while automatically measuring waveform parameters and generating session transcripts. The neurosteroids pregnenolone sulfate and 3β-hydroxy-5β-pregnan-20-one sulfate were found to act as negative allosteric modulators at the kainate receptor, with respective IC_{50} values of approximately 142 μM and 270 μM. Various other steroids, including progesterone, corticosterone, and 17β-estradiol, were evaluated and found to be without effect on the kainate receptor. These studies may provide a basis for using neurosteroids in the clinical management of disorders involving glutamatergic neurotransmission. The automation techniques that have been developed significantly improve the efficiency of oocyte electrophysiology while enabling the development of more complex protocols and are amenable to implementation with patch-clamp recording rigs.

437.10

CHRONIC EXPOSURE OF MOUSE CORTICAL NEURONS TO NMDAR ANTAGONISTS DIFFERENTIALLY REGULATES NMDAR SUBUNITS mRNA AND POLYPEPTIDE EXPRESSION. P. Follesa* and M.K. Ticku. Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284-7764.

Chronic exposure of neurons to N-methyl-D-aspartate receptor (NMDAR) antagonists produced upregulation as measured by radioligand binding. In this study we examined the possibility of changes in mRNA and polypeptide expression encoding for the R1, R2A and R2B subunits of the NMDAR after chronic antagonist D(-)-2-Amino-5-phosphonopentanoic acid (AP-5) exposure in cultured mouse cortical neurons. Exposure of neurons to AP-5 (100 uM, 5 days) produced a 45% increase in the levels of mRNA encoding for the R2B subunit. There was no significant change in the mRNA levels encoding for the R1 and R2A subunits. Using different antibodies against NMDAR subunits, we observed 40-60% increase in R1 polypeptide despite no apparent change in the levels of mRNA encoding for the R1 subunit. More pronounced increase was observed for the R2A-B polypeptite (2-3 fold). The effect of chronic antagonist treatment was time and concentration dependent and reversible after 48 h removal of the antagonist. Concomitant exposure of neurons to AP-5 and NMDA (1mM) was able to block the increase in gene expression of the NMDAR subunits. Noncompetitive antagonist of the NMDAR, MK-801 (1 uM), was also able to upregulate the NMDAR. In contrast non-NMDAR antagonists such as CNQX did not have any effect on NMDAR expression. These data suggest that the upregulation of the NMDAR induced by AP-5 is due to a selective increase in NMDAR mRNA and polypeptide expression by a translational or postranslational regulation for the R1 subunit and a transcriptional regulation for the R2 subunits. Supported by NIH-NIAAA grant # AA10552.

437.11

CNQX-AND APV-RESISTANT, G PROTEIN-UNCOUPLED SLOW EPSCS IN SUBSTANTIA GELATINOSA NEURONS OF THE ADULT RAT SPINAL CORD SLICES Y. Yajiri*, M. Yoshimura, H. Baba and H. Higashi. Dept. Physiol., Kurume Univ. Sch. Med., Kurume 830, Japan and Dept. Orthop. Surg, Niigata Univ. Sch. Med., Niigata 951, Japan

To characterize the slow EPSCs evoked by Aδ afferent fibers, blind patch clamp recordings were made from SG neurons in the adult rat spinal cord slices in which an attached dorsal root was retained. The slow EPSC evoked by Aδ afferent fibers reversed polarity at holding potential of +15mV which was similar to that of fast EPSC. The slow EPSC evoked by primary afferents was abolished by CNQX (10-20 μM), while the slow EPSC evoked by focal stimulation was depressed but not abolished by CNQX. The slow EPSC could be elicited for up to two hours after establishing whole cell configuration and was insensitive to injection of GDP-β-S. These observations suggest that the slow EPSC is mediated by a ligand-gated channel. However, none of the antagonists for the known ligand gated channels had significant effect on the slow EPSC. Currents produced by glutamate or aspartate revealed similar properties to those of slow EPSC and were depressed but not abolished by CNQX and APV (CNQX, 40 μM; APV, 100 μM). Furthermore, the slow EPSC was occluded during the aspartate induced current but not during the glutamate induced current. These observations suggest that the slow EPSC is mediated by a transmitter released from interneurones through CNQX and APV-resistant receptors and that this slow synaptic response may contribute to processing nociceptive information in the spinal dorsal horn.

437.12

DESCENDING AND SEGMENTAL EXCITATORY AMINO ACID-MEDIATED SYNAPTIC TRANSMISSION TO RAT SYMPATHETIC PREGANGLIONIC NEURONS (SPN). D. Spanswick, S.D. Logan and L.P Renaud*. Neuroscience Unit, Loeb Research Institute, Ottawa Civic Hospital, Ottawa, Canada K1Y 4E9 *and* Univ. of Aberdeen, Dept Biomedical Science, Marischal College, Aberdeen, AB91AS, U.K.

Thoracolumbar SPN receive both segmental and supraspinal afferents. To investigate their nature and source we utilized whole-cell recording techniques from SPN in neonatal rat spinal cord slices maintained in-vitro. Electrical stimulation of ipsilateral dorsal horn (DH), ipsi- and contralateral lateral funiculus (iLF, cLF) evoked fast EPSPs in all SPN tested. EPSPs displayed constant latencies, rise-times and no failures at 10Hz, suggesting a monosynaptic origin. Bath application of the non-NMDA receptor antagonist CNQX (5-12μM) or NBQX (1-2μM) reduced all EPSPs. An NMDA receptor-mediated component was evident by sensitivity of EPSPs to D-CPP (5-12μM) and extracellular magnesium. D-CPP reduced EPSPs in some SPN during low frequency (0.03Hz) stimulation of DH, iLF and cLF. However, a D-CPP-sensitive component was observed during repetitive 10 Hz stimulation, or uncovered during low frequency stimulation following removal of magnesium from the media. These observations demonstrate a role for excitatory amino acids, acting through both NMDA and non-NMDA receptors, in synaptic transmission in descending inputs from both sides of the spinal cord, and from ipsilateral segmental inputs. Supported by MRC, Heart and Stroke Foundation of Canada, British Heart Foundation and Wellcome Trust.

437.13

KINETIC PROPERTIES OF RECOMBINANT NMDA RECEPTORS IN OUTSIDE-OUT PATCHES

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The NMDA component of excitatory synaptic currents mediated by glutamate is long-lasting (~100-300 ms). This slow decay can be mimicked by brief (1 ms) applications of glutamate to NMDA receptors in outside-out patches from hippocampal granule cells (13 day-old rats), suggesting that the slow decay arises from the fact that glutamate remains bound to the receptors for a prolonged period after the 1 ms exposure. To investigate the single-channel basis for the slow decay, we have attempted to obtain ensemble currents with only a single channel in the patch, and the results from one such patch suggest that a long latency to first opening (after agonist binding) is a major contributor to the slow decay. Obtaining single-channel patches from granule cells has, however, proved extremely difficult, making it difficult to substantiate our observations. Therefore, we are now pursuing this problem by performing concentration jump experiments on recombinant NMDA receptors (NR-1 plus NR-2A, -2B, -2C or -2D) expressed in *Xenopus* oocytes. Preliminary data suggests that it is possible to reduce the density of receptor expression by altering the amount of mRNA injected into the oocyte. Thus, we hope to increase the probability of obtaining single-channel patches using this strategy. Moreover, since NMDA receptors with different subunit compositions are known to have different macroscopic decay kinetics, the results of jump experiments on a variety of recombinant receptors expressed at low density will allow us to compare the single-channel mechanisms underlying the decay timecourses.

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437.14

THE ROLE OF SYNAPTIC GEOMETRY IN NEURAL TRANSMISSION: A MODELING STUDY. J.-S. Liaw*, M. Baudry, G.A. Chauvet, and T.W. Berger. Depts. of Biomedical Eng. and Biological Sciences, Program in Neuroscience, University of Southern California, Los Angeles, CA 90089-2520

We have developed a computational model of a glutamatergic synapse to study the structural correlates of synaptic transmission. In particular, we examine factors that contribute to the differential expression of short-term potentiation (STP) in AMPA and NMDA-mediated conductances. It has been demonstrated that during the expression of STP, the evoked responses of both AMPA and NMDA components are greatly increased. Furthermore, sensitivity to microejection of exogenous agonist is selectively enhanced for NMDA; responses to microejection of AMPA are unchanged (Xie & Berger Soc. Neurosci. Abstr., 1994). We investigated the role of the relative positions of presynaptic release site and postsynaptic receptor-channels in determining the magnitude and time course of the EPSCs. Our simulations show that AMPA-mediated EPSC is 42% higher while NMDA-mediated EPSC is 17% higher when the receptors are right underneath the release site than when they are 40 nm away. Furthermore, the rising and decaying phases of AMPA-mediated EPSC becomes faster, consistent with experimental data. NMDA receptor has a higher affinity and is thus less sensitive to the relative position. If glutamate is delivered to the synapse by means of perfusion, then its distribution becomes more uniform and the influence of receptor location should be reduced for both AMPA and NMDA-mediated EPSCs. Surprisingly, AMPA-mediated EPSC is 7% *lower* when the receptor and the release site are aligned. The simulations strongly support the hypothesis that the potentiation of AMPA-mediated EPSC is due to the relocation of the receptor on the postsynaptic membrane while changes in the kinetic parameters underlies the potentiation of NMDA-mediated EPSC. NRSA fellowship, ONR, NIMH (MH51772, MH00343, MH52194), NIH Simulations Resource, and the Human Frontiers Science Organization.

EXCITATORY AMINO ACID RECEPTORS X

438.1

CHARACTERIZATION OF NATIVE NMDA RECEPTOR CHANNELS IN ADULT RAT HIPPOCAMPUS. P. Miu*, L. Zhang, & P.L. Carlen
Playfair Neuroscience unit, Bloorview Epilepsy Program, Toronto Hospital, Rm 12-413, 399 Bathurst St., Toronto, Ontario, Canada M5T 2S8.

Earlier studies using outside-out patch clamp configuration have shown that the selective agonist NMDA activates multiple single channel conductances, which can be categorized into small (5-15 pS), medium (20-35 pS), and large (40-50 pS) opening events. Since the outside-out configuration may alter the functional integrity of NMDA receptors, we compared the NMDA-evoked single channel events recorded in both outside-out and cell attached patch clamp configurations of hippocampal CA1 neurons of mature rat brain slices.

In the outside-out configuration, 5 μM NMDA evoked predominately one main conductance level of 42.9 ± 3.6 pS at -80 mV. The slope conductance of the main conductance state, determined by fitting a first order regression line through the data in the current-voltage plot, was 48.3 ± 1.7 pS, which is consistent with previous findings for the large opening events. However in the cell attached configuration, the single channel main conductance level was consistently higher (66.7 ± 1.4 pS, at -70 mV) than that of outside-out configuration. The slope conductance of the main conductance state obtained from four cells was 61.7 ± 7.1 pS. We also found that when the recording temperature was increased from room temperature (22° C) to 33° C, the NMDA main conductance level was further increased to 97.9 ± 9.3 pS.

We hypothesize that the larger NMDA-evoked single channel events observed in the cell attached mode, particularly in mature rat brain slices, reflect the preservation of intracellular modulation of the NMDA receptor channel activity.

Supported by the MRC of Canada and Bloorview Epilepsy Program.

438.2

DEVELOPMENTAL AND REGIONAL EXPRESSION PATTERN OF A NOVEL NMDA RECEPTOR-LIKE SUBUNIT (NMDAR-L) IN THE RODENT BRAIN. Nikolaus J. Sucher*, Schahram Akbarian, Carlin L. Chi, Cynthia L. Leclerc, Marc Awobuluyi, David L. Deitcher, Michele K. Wu, Joseph P. Yuan, Edward G. Jones, and Stuart A. Lipton. Dept. of Neurology, Children's Hospital and Harvard Medical School, Boston, MA 02115; Dept. of Anatomy and Neurobiology, Univ. of California, Irvine, CA 92717.

NMDAR-L shares ~27% identity with NMDA receptor subunits. *In situ* hybridization experiments indicate that NMDAR-L mRNA is expressed in the developing rodent CNS. On postnatal day 1 (P1), NMDAR-L mRNA expression was pronounced in the entorhinal cortex (Ec), the subiculum (Su) and the thalamus (Th), in layer V of the developing neocortex (Nc), in the superior and inferior colliculi, and the hindbrain excluding the cerebellum. On P5, NMDAR-L mRNA was expressed in layer V of the Nc, in the Ec, in the Su, and in the Th. On P14, NMDAR-L mRNA was expressed in layers II-VI of the Nc, in the Ec and piriform cortex, in the Su and CA1 field, and in the nucleus of the lateral olfactory tract (NLOT). In the adult brain, NMDAR-L mRNA was detected exclusively in the NLOT. Injection of NMDAR-L cRNA into *Xenopus* oocytes did not lead to the expression of homomeric glutamate-activated channels. However, co-injection of the triple combination of NMDAR-L with NMDAR1 and NMDAR2B cRNAs led to a striking decrease in the current magnitude compared to currents obtained after co-expression of the double combination of NMDAR1 with NMDAR2B. While the function of NMDAR-L remains to be established, its developmental and regional expression pattern suggests that NMDAR-L may play an important role in brain development.

438.3

CLONING OF χ-2: A PUTATIVE MEMBER OF THE IONOTROPIC GLUTAMATE RECEPTOR SUPERFAMILY.

M.S. Forcina, A.M. Ciabarra, and K.A. Sevarino*. Div. of Molecular Psychiatry, Dept. of Psychiatry, Yale School of Medicine, New Haven, CT 06508

Synaptic transmission is mediated by ligand-gated ion channels which transduce ligand binding into electrical stimuli; glutamate plays the major role in excitatory signalling in the mammalian brain. Molecular cloning techniques have been employed to identify 16 rat glutamate receptor subunits. As reported elsewhere in these abstracts, Ciabarra et al. and Sucher et al. used degenerate PCR to isolate and characterize χ-1, the first member of a new class of ionotropic glutamate receptors.

For this study, degenerate primers were designed based on the sequences of χ-1 and the NMDA receptors. Degenerate PCR was used in concert with homology screening to identify 8 novel PCR fragments, 7 of which possess 40-71% identity to residues 575 to 688 in χ-1. These data strongly suggest that the novel clones are members of the χ class of glutamate receptors. The fragment possessing 71% identity has been identified as χ-2.

Further sequencing is necessary to establish whether χ-2 and other clones represent one or several different χ relatives. Northern blot analysis, *in situ* hybridization, ligand binding and electrophysiological studies with χ-2 and other χ-1 relatives will elucidate the function of χ receptors in neuronal development and may allow pharmacological characterization of this new class.

438.4

CHARACTERIZATION OF A MEMBER OF A NOVEL CLASS OF THE GLUTAMATE RECEPTOR FAMILY. A. M. Ciabarra*[1], J. M. Sullivan[2], L. G. Gahn[1], G. Pecht[2], S. Heinemann[2], and K. A. Sevarino[1]
[1]Division of Molecular Psychiatry, Departments of Pharmacology and Psychiatry, Yale University School of Medicine, 34 Park Street, New Haven, CT 06508; [2]Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92037.

Ionotropic glutamate receptors mediate the majority of rapid excitatory transmission in the CNS. We have cloned a novel member of the rat glutamate receptor family that has an average amino acid identity of 27% to NMDA receptor subunits and 23% to non-NMDA receptor subunits. *In situ* hybridization experiments reveal that the regional transcript levels of this subunit are elevated just prior to and during the first postnatal week, with the highest levels present in the spinal cord, brainstem, hypothalamus, thalamus, and CA1 field of the hippocampus. Transcript levels decline after postnatal day 7 (P7) and are very low in the adult brain. This subunit does not form functional homomeric channels when expressed in *Xenopus* oocytes. However, co-expression studies have demonstrated that this subunit diminishes the current responses of NMDA channels, but not non-NMDA channels, suggesting a functional interaction with NMDA subunits. Ligand binding experiments conducted in HEK-293 cells did not demonstrate detectable binding of this subunit to [^{3}H]CGP 39653 or [^{3}H]DCKA. A C-terminal antisera has been developed which recognizes a 135 kDa protein in membranes from transfected HEK-293 cells and from P7 brain. Immunoprecipitation experiments to determine if this subunit assembles with NMDA subunits are in progress. This receptor is also being studied by Sucher et al. Further characterization will be required to establish the precise role of this subunit in neuronal signaling.

438.5

REGIONAL EXPRESSION AND CLONING OF THE HUMAN NMDA NR2C SUBUNIT. Y.J. Lin*, J. Carver, and T. Giordano. Symphony Pharmaceuticals, Inc., Malvern, PA 19355.

The NMDA glutamate receptor family is composed of at least five subunits, NR1 (present in various splice variants), NR2A, NR2B, NR2C and NR2D as determined by cloning of cDNAs from rat and mouse. We have cloned cDNAs from human hippocampal and cortical libraries representing the complete coding sequence of the human NR2C. Sequence analysis of approximately 1/3 of the > 4 kb nucleic acid contig in the coding region has shown 90% and 97% identity to the rat NR2C at the nucleic acid and amino acid levels, respectively. When compared to the human NR2A subunit, this homology is decreased to 70% and 75%. Northern analysis has demonstrated highest levels of expression in the cerebellum, moderate levels of expression in the amygdala, caudate nucleus, corpus callosum, hippocampus, subthalamic nuclei and thalamus, and low abundance levels in the hypothalamus and substantia nigra. This is in contrast to previously published reports suggesting the lack of expression of NR2C in the rat hippocampus. The NR1 subunit is also detected in all nine brain regions analyzed, with the highest levels observed in the hippocampus and hypothalamus. In the human periphery, NR2C was detected in the heart, skeletal muscle and pancreas by Northern analysis. In contrast, NR1 was not detected in any peripheral tissues. Further work is required to elucidate the function of NR2C in the periphery.

438.6

ISOLATION AND ANALYSIS OF TWO SPLICE VARIANTS OF A HUMAN METABOTROPIC GLUTAMATE RECEPTOR. P.J. Flor, P. Grandes, D. Rüegg, S. Lukic, T. Leonhardt, T. Knöpfel and R. Kuhn*. CNS Research, Pharmaceuticals Division R & D, Ciba, CH-4002 Basel, Switzerland and Departamento de Neurociencias, Universidad del Pais Vasco, Apdo. 699-48080 Bilbao, Spain.

The metabotropic glutamate receptor subtype 7 has previously been isolated from rat brain (rmGluR7) and is a member of a family of at least eight G-protein coupled receptors (Okamoto et al., 1994; Saugstad et al., 1994).

Here we report the isolation of two full-length cDNA clones encoding two splice variants of human mGluR7 (hmGluR7a and -b). hmGluR7b differs by 23 amino acids from hmGluR7a and is generated by alternative splicing at the C-terminus. hmGluR7b stably expressed in Chinese hamster ovary cells mediates depression of forskolin-stimulated cAMP accumulation. The rank order of potency of five agonists is comparable to that described for rmGluR7a (L-SOP $\geq$ L-AP4 > L-glutamate >> 1S,3R-ACPD, quisqualate). Northern blot analysis shows a strong expression of hmGluR7 in human fetal brain and a wide distribution in adult human brain. Immunohistochemical studies with polyclonal antibodies raised against the different C-termini of hmGluR7a and -b demonstrate distinct and differential distribution of the two splice variants in rat brain.

438.7

NOVEL HUMAN NMDA RECEPTOR SUBUNITS: CLONING AND IMMUNOLOGICAL CHARACTERIZATION OF EXON 5 CONTAINING ISOFORMS. N.R. Nash*, C.J. Heilman, H.D. Rees, A.I. Levey. Dept. of Neurology, Emory Univ. Sch. of Med. Atlanta, GA 30322.

The NMDA subtype of the glutamate receptor family is important in neuronal plasticity and neurotoxicity. Nine isoforms of the rat NMDAR1 receptor subunit have been previously distinguished, based on alternatively spliced exons. In four of the isoforms, exon 5 (encoding 21 amino acids) is spliced into the N-terminus. This insert has a high charge density (9 of 21 amino acids), is predicted to be located at the surface of the receptor, and has been implicated in both H^+ sensitivity and regulation by polyamines.

The cloning of the human homologue to NMDAR1-3b and a second isoform NMDAR1-4b are reported here; both of these contain the N-terminal insert, but differ in their C-termini as a result of differential splicing. An E. coli expression construct was made splicing amino acids 22-268 behind glutathione S-transferase in pGEX2T. The resulting fusion protein was used to generate rabbit antisera, which recognize at least two distinct protein bands (~115 kD) by Western blot analysis of human and rat brain. A rat monoclonal antibody has also been obtained using this antigen. Epitope mapping demonstrated the recognition site is within the 21 amino acid N-terminal insert. Immunocytochemistry and immunoblotting show insert containing NMDA receptor subunits are heterogeneously distributed in brain.

438.8

MOLECULAR CLONING AND CHARACTERISATION OF THE HUMAN $mGlu_3$, $mGlu_5$ AND $mGlu_6$ RECEPTORS B. Sommer*, H. Boddeke, P. Schoeffter, K. Stöckli, K-H Wiederholt, T. Tölle†, D.J. Laurie. Sandoz Pharma AG, Basle, Switzerland and †Max-Planck Institute for Psychiatry, Munich, Germany.

Glutamate receptors in the mammalian CNS are categorised as ionotropic or metabotropic receptors. Seven rat metabotropic receptor sequences have been published and allocated to three main groups, based on pharmacological preferences and sequence homologies (Nakanishi, 1994, Neuron 13: 1031). In CHO cells, group I receptors ($mGlu_1$ and $-_5$) positively couple to phospholipase C, while receptors of groups II ($mGlu_2$ and $-_3$) and III ($mGlu_4$, $-_6$ and $-_7$) negatively couple to adenylate cyclase. Sequences for the human $mGlu_3$ and $-_5$ receptors have recently been described (Flor et al., 1994, Eur. J. Neurosci. (Suppl. 7), Abst. 47.01; Minakima et al., 1994, Biochem. Biophys. Res. Commun. 199: 1136). We here report the cloning of cDNAs encoding three human metabotropic receptors, $hmGlu_3$, $-_5$ and $-_6$, one from each group. These were obtained from human brain and retina cDNA libraries by homology screening using radiolabelled fragments of the rat $mGlu_1$ and $-_4$ receptors. The deduced amino acid sequences are 846, 1180 and 866 residues in length, respectively, and display ~95% homology to the published rat sequences. *In situ* hybridisation on human brain sections revealed that $hmGlu_3$ mRNA is widely expressed and $hmGlu_5$ mRNA is strongly expressed in the hippocampus, but no $hmGlu_6$ mRNA is expressed. PCR analysis showed that, as in the rat, $hmGlu_6$ mRNA is virtually restricted to the retina. In CHO cells stably expressing $hmGlu_5$ receptors, glutamate raised intracellular $[Ca^{2+}]$ (detected by æquorin luminescence), while in cells expressing $hmGlu_3$ and $-_6$ receptors glutamate lowered the forskolin-elevated intracellular cAMP concentration. L-CCG-1, quisqualate and L-AP4 were, respectively, the most potent agonists at the $mGlu_3$, $-_5$ and $-_6$ receptors, in agreement with published studies on the corresponding rat receptors. The pharmacological preferences and coupling mechanisms of these receptors are currently under study.

438.9

BIOPHYSICAL PROPERTIES OF HUMAN NMDA RECEPTORS STABLY EXPRESSED IN MAMMALIAN CELLS. S.D. Hess*, C. Deal, M. Urcan, R. Skvoretz, E.C. Johnson and G. Veliçelebi. SIBIA Inc., La Jolla, CA 92037.

Mouse Ltk$^-$ cells were stably transfected using cDNAs encoding human NMDAR1A/2A or NMDAR1A/2B receptors. Subclones that reliably displayed an increase in $[Ca^{2+}]_i$ following application of NMDA and glycine (see Varney *et al.*, these abstracts) were examined using the whole-cell mode of the patch-clamp technique. Cells were typically held at -60 mV in mammalian Ringer with 0 external Mg^{2+} and tested with saturating concentrations of NMDA (1 mM) and glycine (100 µM) delivered *via* a pressure pipette positioned within 20-30 µm of the cell. The recording pipette contained (in mM): 135 $CsCl_2$, 10 EGTA, 1 $MgCl_2$, 10 HEPES and 4 ATP. For NMDAR1A/2A-expressing cells, currents ranged from 10 to 408 pA, and the mean (± S.D.) current density was 2.6 ± 2.7 pA/pF (n = 27 cells). Analysis of currents elicited by voltage ramps showed a reversal potential of approximately 0 mV, and the maximal inward current in the presence of 1 mM external Mg^{2+} occurred at -23.6 ± 1.4 mV (mean ± S.D., n = 3 cells). Currents ranged from 9 to 1390 pA for NMDAR1A/2B-expressing cells, and the current density was 5.9 ± 6.8 pA/pF (mean ± S.D., n = 14 cells). The reversal potential in Ringer was approximately 0 mV, and the maximal inward current with external Mg^{2+} present occurred at -24.3 ± 7.7 mV (mean ± S.D., n = 3 cells). These current densities were observed over 6 and 13 cell passages for the hNMDAR1A/2A and hNMDAR1A/2B lines, respectively. The NMDA-site competitive antagonist CGS19755 (30 µM) reversibly blocked 90-100% of the current elicited with 100 µM NMDA and 30 µM glycine for either the hNMDAR1A/2A or hNMDAR1A/2B-expressing cells. Based on these results, these cell lines represent useful experimental systems to study the functional and pharmacological properties of human NMDA receptor subtypes.

438.10

STABLE EXPRESSION AND CHARACTERIZATION OF RECOMBINANT HUMAN DIMERIC NMDA RECEPTOR SUBTYPES 1A/2A AND 1A/2B IN MAMMALIAN CELLS. M.A. Varney*, M. Urcan, C. Jachec, R. Skvoretz, L.P. Daggett, F.F. Lin, T. Moran†, J. Morrison†, E.C. Johnson, G. Veliçelebi. SIBIA, La Jolla, CA 92037, and †Mount Sinai School of Medicine, New York, NY 10029

Human hippocampal and fetal brain cDNA libraries were screened with rat or human NMDA receptor sequences, and overlapping cDNAs obtained corresponding to the human NMDAR1A, 2A and 2B subunits. These cDNAs were subcloned into plasmids containing the MMTV inducible promoter, and stable co-transfections initiated in mouse fibroblast Ltk$^-$ cells. After G418 selection, transfected cells were cloned by limiting dilution and cells screened for NMDA/glycine-stimulated $[Ca^{2+}]_i$ responses in Mg^{2+}-free buffer. Clones expressing hNMDAR1A/2B displayed peak $[Ca^{2+}]_i$ responses of approximately 3 to 4-fold above basal levels. Functional expression was also confirmed by measurement of NMDA-induced currents in whole-cell patch-clamp recordings (Hess *et al*, this meeting). The presence of both subunits in the receptor complex was confirmed by non-denaturing detergent-solubilization of membranes and immunoprecipitation of receptor complexes using an antibody to the 1A subunit, followed by Western blotting and detection of the 2B subunit.

The pharmacology of recombinant hNMDAR1A/2B in Ltk$^-$ cells, as measured by the Ca^{2+} response in Mg^{2+}-free buffer, is consistent with that reported from *Xenopus* oocyte expression. NMDA increased $[Ca^{2+}]_i$ with an EC_{50} value of 10.4 ± 2.3µM, and responses to submaximal concentrations of NMDA were inhibited by the competitive NMDA antagonist CGS19755, and by the noncompetitive channel blockers MK801 and ketamine. The glycine-site antagonist 5,7-DCKA also inhibited $[Ca^{2+}]_i$ responses to NMDA. The same strategy has been used for the stable expression of hNMDA1A/2A receptor subunits, and functional clones are currently undergoing stability tests and pharmacological characterization.

438.11

CLONING AND FUNCTIONAL EXPRESSION OF THE HUMAN GluR3-FLIP IONOTROPIC GLUTAMATE RECEPTOR. L.P. Daggett, F.-F. Lin, C. Jachec, C.R. Deal, S. Rao, C.-C. Lu, E. Santori*, M.A. Varney, S.D. Hess, E.C. Johnson and G. Velicelebi. SIBIA, Inc., La Jolla, CA 92037.

A full-length human GluR3-flip cDNA (hGluR3$_f$) was constructed using two overlapping cDNA fragments isolated from human hippocampal and human fetal brain cDNA libraries. The deduced amino acid sequence of the hGluR3$_f$ receptor is 894 amino acid residues which is 6 amino acids longer than rat GluR3.

Human embryonic kidney (HEK293) cells were transiently transfected with hGluR3$_f$ and tested for functional expression of the receptor in several assays. A 110-kDa immunoreactive species was identified in membranes prepared from hGluR3$_f$-transfected cells when probed with anti-rat GluR2/3 antibody in Western blots. This is the expected size for a full-length GluR3 polypeptide. Treatment of the hGluR3$_f$-transfected cells with 100 μM AMPA in the presence of 100 μM cyclothiazide elicited inward currents of 285 ±116 pA (mean ± S.D., n=5 cells) that were reversibly blocked by 10 μM CNQX. Treatment of cells with 100 μM AMPA or kainate stimulated intracellular calcium levels ($[Ca^{2+}]_i$) 3- to 4-fold above basal levels in the presence of cyclothiazide. Both the AMPA- and kainate-induced $[Ca^{2+}]_i$ responses were inhibited by CNQX. No increase in $[Ca^{2+}]_i$ was detected in the absence of cyclothiazide. Stable transfections of cells with hGluR3$_f$ have been initiated with the aim of using these cells as targets in drug screening.

438.12

CHARACTERIZATION AND TIME-COURSE OF AGONIST-INDUCED DESENSITIZATION OF HUMAN mGLUR1α. M.A. Desai*, J.P. Burnett, N.G. Mayne and D.D. Schoepp. Central Nervous System Research, Eli Lilly & Co., Indianapolis, IN 46285.

We have studied the properties of a human mGluR1α (HmGluR1α) receptor in a novel cell line (AV12-664; ATCC CRL 9595) and examined its pharmacological profile when co-expressed with a rat glutamate/aspartate transporter (GLAST). Using RT-PCR to compare mRNA levels, we found that HmGluR1α was similarly expressed in AV12 cells with or without co-expression of GLAST. However, HmGluR1α-mediated phosphoinositide (PI) hydrolysis was appreciably elicited only in cells co-expressing the rat GLAST transporter (RGT cells). Pre-treatment and subsequent washout of RGT cells with the glutamate uptake blocker, L-*trans*-pyrrolidine-2,4-dicarboxylic acid (*t*-PDC), had no effect on agonist-stimulated PI hydrolysis at 6 or 12 hours; whereas similar pre-treatment with 3,5-dihydroxyphenylglycine (DHPG), an mGluR1 agonist, inhibited glutamate-stimulated PI hydrolysis by 15% and 47%, respectively. Upon 24 hour pre-treatment with *t*-PDC, glutamate-stimulated PI hydrolysis was inhibited by 40%. These effects were reversed by (+)-α-methyl-4-carboxyphenylglycine (MCPG), an mGluR1 antagonist. These studies demonstrate that activation of HmGluR1α, either through inhibition of transport and resulting accumulation of glutamate in the extracellular medium or through direct activation of the receptor, elicits a time-dependent, receptor-mediated inhibition of agonist-stimulated PI hydrolysis.

438.13

CLONING AND EXPRESSION A HUMAN CELL LINE cDNA WHICH ENCODES A GLUTAMATE-GENERATING NEUROPEPTIDASE ACTIVITY. R.E. Carter*, A. Feldman, and J.T. Coyle. Laboratory of Molecular and Developmental Neuroscience, Massachusetts General Hospital and Consolidated Department of Psychiatry, Harvard Medical School, Boston, MA.

The N-acetylated alpha-linked acidic dipeptidase (NAALADase) is a novel membrane hydrolase which has been characterized in the mammalian nervous system on the basis of its catabolism of the neuropeptide N-acetylaspartylglutamate (NAAG) to N-acetylaspartate (NAA) and glutamate. The isolation of a similar rat brain cDNA has led us to discover that the cDNA for an investigational prostate cancer marker known as the prostate-specific membrane antigen (PSM) encodes a hydrolase with the substrate and pharmacologic properties of NAALADase (Carter et al. [1994] Soc. Neurosci. Abstr. 308.13 , Israeli et al. [1993] *Cancer Res.* **53**, 227-230.). We have recently cloned a cDNA containing the 2250-base PSM open reading frame by RT-PCR from the human prostatic carcinoma cell line LNCaP. Transfection assays of this cDNA demonstrate NAAG-hydrolyzing activity which is inhibited by the specific NAALADase inhibitors quisqualic acid and β-NAAG. Thus, as defined by activity and pharmacology, PSM is a NAALADase enzyme, and its corresponding cDNA is the first full-length NAALADase species to be identified. Northern analyses detect at least six transcripts which hybridize to both the rat brain and human cell line cDNA clones. These RNAs are variably expressed in rat brain and other NAALADase-positive but not in NAALADase-negative rat tissues and human cell lines. Our findings indicate that PSM and/or species which share molecular similarities to PSM account for the NAAG-hydrolyzing activity characterized previously in the nervous system.

438.14

CLONING OF A 3.8 kb cDNA FOR A BRAIN SYNAPTIC MEMBRANE PROTEIN THAT BINDS NMDA RECEPTOR ANTAGONISTS. K.N. Kumar*, X. Y. Chen, P.S. Johnson, M. Ahmad and E.K. Michaelis. Dept. of Pharmacol. & Toxicol. and the Center for Neurobiol. & Immunol. Res., Univ. of Kansas, Lawrence, KS 66045.

We have previously reported on the purification and the immunological and pharmacological characterization of a complex of four proteins having the characteristics of an NMDA receptor (Kumar et. al. *J. Biol. Chem.* 269, 27384, 1994). This complex consists of an ~70 kDa glutamate-binding protein, an ~80 kDa CPP-binding protein, and an ~60 kDa glycine/TCP-binding protein. In the present studies, we have used the polyclonal antibodies raised against the ~80 kDa antagonist-binding protein to screen hippocampul cDNA libraries for clones expressing the antigenic protein. The largest cDNA clone identified, pNRA2, had a size equal to 3.8 kb. This clone was sequenced and found to contain a unique sequence. An open reading frame for a protein of similar size to the synaptic membrane CPP-binding protein was identified. In Northern blot analyses, an ~3.9 kb mRNA hybridized with the cDNA for this protein. Based on the Northern blot analyses, the mRNA levels for this protein were approximately equal in hippocampus, cerebral cortex, and cerebellum. The properties of ligand binding to the protein expressed in *E. coli* following transformation with the pNRA2 clone were distinguishable from those of the NMDA receptor protein NMDAR1 but were similar to those of the CPP-binding protein purified from rat brain synaptic membranes (Cunningham and Michaelis, *J. Biol. Chem.* 265, 7768, 1990). These results indicate the presence of proteins that differ from the NMDAR1/R2 families yet have the ligand recognition sites associated with brain NMDA receptors. [Supported by grants AA 04732, DAAL 031-91-G-0167, and an unrestricted grant from PARKE-DAVIS]

438.15

EXPRESSION OF THE GLUTAMATE-BINDING PROTEIN IN CEREBELLAR GRANULE CELLS: A RECEPTOR PROTEIN MEDIATING NMDA-INDUCED CELL DEATH AND NMDA-DEPENDENT SYNAPTOGENESIS. Y. Xia*, R. Ragan, L. Shen, M. L. Michaelis and E. K. Michaelis. Dept. of Pharmacol. & Toxicol. and the Higuchi Biosciences Center, Univ. of Kansas, Lawrence, KS 66045

The glutamate-binding protein (GBP) is a subunit of an NMDA receptor-like complex isolated from rat brain synaptic membranes (Kumar et al., *J. Biol. Chem.* **269,** 1994, 27384-27393). Cerebellar (CB) granule cell cultures were previously used as a model system to study the role of the GBP in NMDA-induced neuronal development and neurodegeneration (Xia et al., *Neurochem. Res.* **20,** 1995, 545-557). At 14 days *in vitro*, NMDA caused significant cell damage. Anti-GBP and anti-NMDAR1 antisense oligonucleotides were used to inhibit the expression of GBP and NMDAR1. Following a 20 hr incubation with the antisense oligos, the expression of both proteins was inhibited by about 30% as detected by immunoblot analyses for the GBP and NMDAR1. However, only the anti-GBP antisense oligos produced significant inhibition of the NMDA-induced cell death ($P<0.01$), whereas the anti-NMDAR1 antisense and the missense oligos for both proteins had no effect. NMDA also has a trophic effect on CB granule cells in culture. These cells survive under non-depolarizing conditions when NMDA is present. The expression level of GBP was regulated by the presence of NMDA in the culture medium and this effect was sensitive to the presence of the antagonist 2-AP-5. Confocal images of cells labeled with fluorescently-tagged antibodies showed clear localization of the GBP on the growth cones of CB granule cells at 4.5 and 18 hr in culture. NMDA also increased the levels of intracellular calcium at the growth cones of CB granule cells. These data indicate that the GBP may play an important role in both neuronal survival and synaptogenesis (Supported by an MMD fellowship and grant and by grant AA04732 from NIAAA).

439.1

THE FAILURE OF NO DONORS TO MODULATE AMPA BINDING IN THE ABSENCE OF THE CHAOTROPIC AGENT, POTASSIUM THIOCYANATE. L.M. Hawkins and P.J. Roberts. (SPON: Brain Research Association)*. Department of Pharmacology, University of Bristol, Bristol, BS8 1TD U.K.

In several regions of the rat brain α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor mediated synaptic transmission has been shown to undergo both long-term potentiation (LTP) and long-term depression (LTD). The mechanisms underlying these changes in the functional responsiveness of AMPA receptors are not yet understood. However, the ability of phosphorylation and dephosphorylation to alter the responsiveness of glutamate receptor gated ion channels in opposing ways suggests a role for protein kinases.

Both nitric oxide (NO) donors and cyclic GMP analogues have been shown to increase the affinity of [^{3}H]AMPA binding in rat cerebellum, suggesting that a cyclic GMP-dependent protein kinase can modulate the AMPA receptor. In those studies [^{3}H]AMPA binding was performed in the presence of the chaotropic agent potassium thiocyanate (KSCN), which enhances AMPA binding by an unknown mechanism. We have recently described a new radioligand, (S)-[^{3}H]5-fluorowillardiine, which, in contrast to [^{3}H]AMPA, displays high levels of specific binding in the absence of KSCN.

In this study we assessed the ability of NO donors to modulate the binding of 20nM (S)-[^{3}H]5-fluorowillardiine to sections (10μm) of rat brain. The specific binding in the forebrain represented 97.0 ± 4.5 % and 97.5 ± 4.9 % of control binding in the presence of the NO donors S-nitroso-N-acetylpenicillamine (SNAP) (200μM) and sodium nitroprusside (SNP) (1mM) respectively. Similarly in the cerebellum, binding in the presence of 200μM SNAP and 1mM SNP represented 98.7 ± 7.2 % and 93.4 ± 4.7 % of control binding. Therefore we conclude that the binding of (S)-[^{3}H]5-fluorowillardiine to rat brain, in the absence of KSCN, cannot be modulated by compounds which release NO in physiological tissues.

439.2

REGIONAL HETEROGENEITY IN AGONIST ENHANCED [^{3}H]MK-801 BINDING FOLLOWING VARIABLE PREWASH CONDITIONS IN RAT BRAIN. L.McCoy*, and E.K. Richfield. Departments of Psychiatry, Neurology and Pharmacology, University of Rochester School of Medicine, Rochester, NY 14642

NMDA receptor function is modulated through distinct sites that regulate channel opening. The objectives of the present study in Sprague Dawley rat were threefold: (i) determine the contribution of prewash variables on agonist stimulation of the NMDA receptor, (ii) compare regional differences in functional glycine, spermidine and NMDA binding sites under optimized prewash conditions, and (iii) attempt to define the effects of endogenous factors at the different NMDA modulatory sites. The effect of prewash on [^{3}H]MK-801 binding in rat tissue sections was volume, temperature, and time dependent and regionally variable. Variations in prewash time and temperature at a constant volume and agonist concentration produced a wide range (0-700% increase) of agonist enhanced binding values. Prolonged prewash caused a regionally specific *decrease* in unenhanced [^{3}H]MK-801 binding, which was restored to unwashed binding levels with exogenously added glycine, NMDA or spermidine. Short prewash times caused a regionally specific biphasic effect on enhanced [^{3}H]MK-801 binding supporting the presence of both endogenous agonists and antagonists at the various NMDA modulatory sites. To detect regional differences in NMDA binding, a 4°C prewash of at least 120 min using a minimum volume of 25 ml/section is suggested. The regional heterogeneity both in [^{3}H]MK-801 binding following washout of endogenous factors and in agonist modulation of binding following a prolonged prewash supports the hypothesis that NMDA receptor function is dependent on the interaction of the specific receptor subtype expressed and the concentrations of one or more endogenous factors. *Supported by MH18911; MH40381 and the Tourette Syndrome Association, Inc.*

439.3

WATER DEPRIVATION INCREASES THE NUMBER OF NON SYNAPTIC AMPA/GLUR RECEPTORS IN RAT TELENCEPHALON S. Standley*, S. Maren, K. Aquino, R. F. Thompson, and M.Baudry., University of Southern California, HEDCO Neurosciences Bldg., Los Angeles, CA 90089-2520

Water deprivation increases the degree of long-term potentiation (LTP) of synaptic transmission in hippocampus and performance in cognitive tasks in rats. This study examined the effects of water deprivation on the properties and subcellular distribution of AMPA receptors. Long Evans rats were water deprived for 3 days and sacrificed. Crude synaptic (P2) and microsomal (P3) fractions from telencephalon were prepared by differential centrifugation. Binding properties were determined by saturation kinetics with ^{3}H-AMPA, and relative amounts of receptor subunits were obtained with Western blots using antibodies against GluR1, and GluR2/3.

As previoulsy reported, greater numbers of high affinity binding sites were found in P3 fractions than in P2 fractions. Water deprivation resulted in a significant increase in the number of high affinity binding sites in both P3 and P2 fractions. P3 fractions also showed an increased amount of GluR2/3 subunits compared to controls. Neither number of low affinity binding sites nor amount of receptor subunits in P2 fractions were altered. Our results indicate that water deprivation alters the number of non-synaptic AMPA receptors in rat telencephalon. The increased number of receptors might provide the basis for the increased degree of synaptic plasticity and learning.

This work was supported by a grant from Sankyo Pharmaceuticals, NSF IBN 9215069, and NIA AFO 5142 for R.F.T., and Sankyo Pharmaceuticals for M.B.

439.4

KINDLING-INDUCED LONG-LASTING PROLONGATION OF SINGLE NMDA CHANNEL OPENINGS OCCLUDES THE EFFECT OF PHOSPHATASE INHIBITION. D.N. Lieberman* and I. Mody. Dept. of Neurology, UCLA School of Medicine, Los Angeles, CA

We previously reported that the kinetics of single NMDA receptor-channel openings are profoundly altered by kindling-induced epilepsy, and that the openings are similarly prolonged following inhibition of the Ca^{2+}-dependent phosphatase calcineurin by okadaic acid (OA). We have now investigated the persistence of these kindling-induced modifications in the NMDA receptor-channel complex.

Adult rats were implanted with stimulating electrodes in the hippocampal commissures and were either stimulated daily or were handled but not stimulated. Cell-attached recordings in acutely dissociated control and kindled dentate gyrus granule cells were obtained in Mg^{2+}-free medium using L-aspartic acid (250-500 nM) and glycine (3-8 μM) to elicit openings primarily to the 50 pS level. We previously showed that in kindled neurons 2 days after the last seizure, channel openings were significantly prolonged compared to controls. We now examined the longevity of the plastic changes induced by kindling. NMDA channel openings recorded in granule cells 28 days after the last seizure showed a 67% increase in mean open time, a 127% increase in burst duration, a 78% increase in cluster duration, and a 76% increase in supercluster duration. These effects were comparable to those seen 2 days after the last seizure. Thus, the molecular mechanisms responsible for the alteration in NMDA channel function must persist for at least four weeks *in vivo*.

In control granule cells, serine/threonine phosphatase inhibitors produce an enhancement of NMDA channel openings comparable to that induced by kindling. When 10 μM OA was perfused onto control neurons, channel activity was enhanced as has been reported previously. In contrast, granule cells obtained from rats 28 days after the last seizure did not respond to OA. The occlusion of the OA effect on NMDA channel openings in epileptic neurons is consistent with a profound and long-lasting alteration in the phosphorylation/dephosphorylation machinery.

Supported by NINDS grants NS-27528 and NS-12151 (I.M.), the Sid W. Richardson Foundation, and a Howard Hughes Predoctoral Fellowship (D.N.L.).

439.5

PRESYNAPTIC NMDA AND AMPA BUT NOT KAINIC ACID RECEPTORS EVOKE RELEASE OF PRELOADED NORADRENALINE FROM RAT SPINAL CORD SLICES. E. Sundström*, L. Holmberg, L.-L. Mo and F. Souverbie. Dept. of Clin. Neurosci., Karolinska Institutet, Huddinge Univ. Hosp., S-141 86 Huddinge, Sweden.

Presynaptic excitatory amino acid (EAA) receptors have been shown to induce release of noradrenaline (NA) in several supraspinal regions. To determine if EAA receptors exists on spinal NA terminals we studied release of [^{3}H]NA from preloaded slices in a superfusion system. We found that NMDA at concentrations similar to those that stimulated binding of [^{3}H]MK-801 to spinal cord synaptic membranes gave a potentiation of NA release induced by 10 mM K^+. NMDA-induced release was inhibited by μM concentrations of MK-801, whereas [^{3}H]MK-801 showed a nM affinity for binding to spinal NMDA receptors. AMPA gave an evoked release of NA only when 300 μM cyclothiazide was used to prevent receptor desensitization. AMPA-mediated release was apparently mediated by the low affinity AMPA receptor since high μM concentrations were necessary to evoke release. AMPA-evoked NA was inhibited by NBQX indicating that similar to the NMDA-stimulated release these processes are receptor mediated. Kainic acid at concentrations of up to 100 μM failed to induce release of NA even when the lectin Concanavalin A was used to prevent receptor desensitization. It has previously been shown that presynaptic α_2 receptors inhibit release of glutamate in the spinal cord. Considering the present data it is possible that glutamate released from primary afferents, interneurons or descending axonterminals increase extracellular levels of NA which in turn inhibits release of glutamate thereby creating a negative feed-back loop in the spinal cord.

439.6

BINDING OF [^{3}H]CGP-39653 AT THE NMDA RECEPTOR IN PRESENCE OF GLYCINE AND ANTAGONISTS IN THREE BRAIN REGIONS OF THE RAT BRAIN. R. Robichon*, P.K. Randall and S.W. Leslie, Div. of Pharmacol. and Toxicol., The Univ. of Texas at Austin, Austin, TX 78712.

Previous studies have shown that glycine agonists and antagonists differentially affect the binding of antagonists and agonists at the glutamate site of the NMDA receptor. To further elucidate the relationship between the glutamate and the glycine sites, we studied the effect of glycine and its antagonists on the binding of [^{3}H]CGP-39653 in cortex, hippocampus and cerebellum of adult male rats.

Cortex, hippocampus and cerebellum membrane preparations were prepared according to the method of Sills et al. (Eur. J. Pharmacol. 192:19-24, 1991). The tissue (0.10 - 0.15 mg protein) was incubated with 10 nM [^{3}H]CGP-39653 and 10 concentrations of glycine or DCKA (10^{-4} - 10^{-8} M) for 2 hrs at 4 °C. For saturation experiments, 11 concentrations of [^{3}H]CGP-39653 (0.5 - 60 nM) and 10^{-5} M glycine or 10^{-6} M DCKA were used. Non-specific binding was determined with 10 uM glutamate.

Glycine decreased the binding of [^{3}H]CGP-39653 with an IC50 of 261 and 290 nM in cortex and hippocampus, respectively,. In the cerebellum, only 3 x 10^{-5} and 10^{-4} M of DCKA decreased [^{3}H]CGP-39653 binding. The decrease in binding was due to a lower Bmax and affinity of [^{3}H]CGP-39653 at the NMDA site in presence of glycine. The glycine antagonist DCKA decreased [^{3}H]CGP-39653 binding with an IC50 of 41 and 36 nM in cortex and hippocampus, respectively, with no effect on the cerebellum. The decrease in [^{3}H]CGP-39653 binding was due to a lower affinity of [^{3}H]CGP-39653 for the NMDA receptor in cortex and hippocampus without any change in the Bmax. These results suggest that cerebellum behaves differently from cortex and hippocampus regarding the effect of glycine and its antagonists on the binding of [^{3}H]CGP-39653. It appears that at low concentrations glycine increases the binding of [^{3}H]CGP-39653 but that at higher concentrations it decreases [^{3}H]CGP-39653 binding. Thus, glycine may modulate the antagonist binding. (Supported by NIAAA grants RO1 AA05809 and RO1 AA09337).

439.7

CP-101,606, A POTENT AND SELECTIVE ANTAGONIST OF FOREBRAIN NMDA RECEPTORS: BINDING TO A NOVEL RECOGNITION SITE. W. F. White*, M.F. Ducat, B.L. Chenard, T.W. Butler, R.T. Ronau. Pfizer Inc., Central Research Division, Groton, CT, 06340.

CP-101,606, the dextrorotatory (+) enantiomer of (1S,2S)-1-(4-hydroxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol, is a potent and selective antagonist of NMDA-mediated responses *in vitro* and *in vivo*. However, binding studies indicate that CP-101,606 does not interact with the glutamate, glycine, or TCP modulatory sites on the NMDA receptor. Thus, the NMDA antagonist activity of CP-101,606 is not mediated through interaction with these sites. CP-101,606 is also essentially inactive at a variety of other neurotransmitter receptors, enzymes, and uptake systems. Instead, CP-101,606 interacts specifically at a novel regulatory site on the NMDA receptor. The affinity of racemic CP-101,606 for its binding site in membranes from rat cortex is 12±2 nM and the B_{max} is 1.7±0.2 pmols/mg protein. In membranes from human frontal cortex these values are 18±4 and 0.7±0.1. The potency of CP-101,606 analogs to displace racemic ^{3}H-CP-101,606 binding correlates with potency in protecting hippocampal neurons from glutamate toxicity. This strongly suggests that the mech-anism of neuroprotective activity is mediated through interactions at this binding site. *In vitro* autoradiographic studies on sections from rat brain reveal that the racemic ^{3}H-CP-101,606 binding site has a much more restricted distribution than do ligands acting at the glutamate, glycine, or TCP modulatory sites on the NMDA receptor. The binding site is most dense in hippocampus and cortex with low to very low densities seen in more posterior regions of the brain. Thus, these data are consistent with CP-101,606 acting at NMDA receptors containing the NR2B subunit through a novel mechanism.

439.8

CP-101,606, A POTENT AND SELECTIVE FOREBRAIN NMDA RECEPTOR ANTAGONIST: EFFECTS ON NMDA RECEPTOR-MEDIATED ION FLUX. A.H. Ganong*, J.T. Lazzaro, K.E.G. Richter, L.-H. Tang[1], E. Aizenman[1], F.S. Menniti, W.F. White. Pfizer Inc, Groton, CT 06340, [1]Dept. Neurobiology, University of Pittsburgh, PA 15261.

The effects of CP-101,606 on NMDA receptor-mediated responses were studied using patch clamp recording and single cell calcium imaging. Whole cell recordings from rat cortical cells 12-14 days in culture showed dose-dependent inhibition of 100 µM NMDA-induced responses by 32 nM - 10 µM CP-101,606. NMDA responses were inhibited maximally by 76% by CP-101,606 with an IC50 value of 196 nM. Inhibition by CP-101,606 was slow to develop and did not reverse with 5-10 min of washout. The inhibition of NMDA responses by CP-101,606 appeared to be noncompetitive since increasing concentrations of NMDA did not overcome the block. 1 µM CP-101,606 blocked kainate-induced currents by 8±2% (n=3). CP-101,606 had heterogenous effects on single NMDA receptor channels studied in patches taken from cultured cortical neurons. CP-101,606 (0.3-3 µM) decreased the frequency of channel opening in 14/17 patches and decreased single channel conductance in 9/17 patches. There were no consistent effects on single channel open time. Single cell calcium imaging in cultured cortical cells revealed that CP-101,606 decreased NMDA-induced calcium influx in about 80% of the cortical neurons (100 nM, 57% inhibition; 1 µM, 72%; 114 cells on 5 coverslips). There was large variability in the inhibition by CP-101,606 in the population of cells sensitive to the drug. NMDA receptor-mediated synaptic field potentials recorded in the Schaffer-CA1 pathway in rat hippocampal slices were also inhibited by CP-101,606 with maximal inhibition of 84% and an IC50 value of 212 nM. The results of these studies are consistent with the hypothesis that CP-101,606 differentially affects NMDA receptor subtypes.

439.9

CP-101,606, A POTENT AND SELECTIVE ANTAGONIST OF FOREBRAIN NMDA RECEPTORS: *IN VIVO* ACTIVITY. M.J. Pagnozzi*, L.K. Chambers, F.S. Menniti, B.L. Chenard, W. F. White. Pfizer Inc., Central Research Division, Groton, CT, 06340.

NMDA antagonists have been shown to have neuroprotective effects in animal models of disease. However, the adverse behavioral effects of the available agents may limit their therapeutic usefulness. CP-101,606 acts to selectively inhibit forebrain NMDA receptor function through a novel mechanism. This agent retains the neuroprotective effects but appears to be very well tolerated. The immediate early gene *c-fos* is induced in rats following a stab wound to the brain and the fos gene product is elevated in mice following subconvulsant doses of NMDA. Both of these responses are blocked by CP-101,606 (ED_{50} = 4 mg/kg iv and 1 mg/kg ip in rat and mouse model, respectively). The ED_{50} for MK-801 in both models is approximately 1 mg/kg; SL-82.0715 was inactive in the mouse model at doses up to 56 mg/kg. CP-101,606 also blocks haloperidol induced catalepsy with an ED_{50} of 0.3 mg/kg SC; MK-801 is approximately 10x more potent whereas neither SL-82,0715 or ifenprodil were active at doses up to 32 mg/kg. The noncompetitive NMDA antagonist MK-801 at doses greater than 0.1 mg/kg SC caused marked stimulation of locomotor activity in rats. It also disrupted memory consolidation in a mouse step-through passive avoidance paradigm at doses greater than 1 mg/kg IP. CP-101,606 had neither effect at the highest doses tested, 56 mg/kg SC and 100 mg/kg IP. Unlike both competitive and noncompetitive NMDA antagonists, CP-101,606 does not produce neuronal vacuolization in the cingulate or retrosplenial cortex. These data support the NMDA antagonist activity of CP-101,606 in a number of animal models and suggest that it may be better tolerated and/or more efficacious than other available NMDA antagonists.

439.10

PUTATIVE COGNITION ENHANCERS REVERSE INHIBITION BY KYNURENATE OF [3H]MK-801 BINDING. John C. Lehmann* and Servane Hamelin, Dept. Neurosurgery MS 407, Medical College of Pennsylvania and Hahnemann University, Philadelphia PA 19102-1192.

Pittaluga and co-workers have reported that nootropics such as aniracetam reverse the inhibition by kynurenate (100 uM) of release of [3H]norepinephrine stimulated by NMDA (100 uM) from hippocampal slices (Eur. J. Pharm. 272:203, 1995). To further elucidate this effect, we attempted to replicate the findings in a simpler system, namely, [3H]MK-801 binding in rat brain membranes.

Kynurenate (100 uM) inhibited this binding 75 %. The addition of D-cycloserine, piracetam, or aniracetam reversed this inhibition in a concentration dependent manner. Neither D-cycloserine nor aniracetam increased [3H]MK-801 binding in the absence of kynurenate, although piracetam represented an exception and stimulated [3H]MK-801 binding in the absence of kynurenate. The maximal reversal obtained by the nootropics was less than that observed by Pittaluga et al., possibly due to a different population of NMDA-type receptors residing on noradrenergic terminals as opposed to those found in total brain, which may have different pharmacological responses to nootropics. These findings have relevance for determining potentially efficacious cognition-enhancing compounds in a quantitative, mechanistic manner.

439.11

POLYAMINE INTERACTIONS WITH IFENPRODIL'S HIGH AFFINITY INHIBITION OF [3H]TCP BINDING. L.L. Coughenour, J.J. Cordon and P.A. Boxer*. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor MI, 48106.

Ifenprodil's high affinity inhibition of the binding of channel blockers such as thienylcyclohexylpiperdine (TCP) and dizolcipine to the NMDA complex have been reported to reflect its selectivity to the NR2B subtype of the receptor. Ifenprodil also has been characterized as a polyamine antagonist. Using [3H]TCP binding under nonequilibrium conditions we found that the addition of spermidine at a concentration that enhances the binding (10 uM) or the addition of arcaine at a concentration (10 uM) which inhibits 50% of the binding did not significantly shift the IC50 of the high or low affinity inhibition of ifenprodil. Spermidine significantly increased the percentage of high affinity binding by 15%. High affinity binding disappeared as [3H]TCP was allowed to come to equilibrium
with only 7% of the sites detectable at 3 h. Increasing the concentration of spermidine to 100 uM also eliminated the high affinity binding. Under both these conditions we could again detect high affinity inhibition by ifenprodil by adjusting the equilibrium time of the [3H]TCP. This suggests that spermidine does not interact at ifenprodil's high affinity (NR2B selective) site but alters the binding kinetics of [3H]TCP. Assay parameters that increase the access or binding within the channel may mask the detection of this high affinity site without direct interaction.

439.12

CHARACTERIZATION OF PD 158473, AN ORALLY ACTIVE COMPETITIVE ANTAGONIST OF THE NMDA RECEPTOR, AS MEASURED BY [^{3}H] TCP BINDING.

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The competitive antagonist, -Amino-3-(3-naphthalen-1-yl-5-phosphonomethyl-phenyl)-propionic acid (PD 158473), inhibits the binding of [^{3}H] CGP 39653 to the NMDA recognition site and the release of lactate dehydrogenase, as a measure of cell death, in cell cultures exposed to application of NMDA in vitro. In vivo it is orally active in preventing seizures caused by maximal electroshock.. This study investigated the mechanism of action of PD 158473 using [^{3}H] thienylcyclohexylpiperdine (TCP), a high potency blocker of the NMDA channel, as a probe for measuring NMDA receptor activation. PD 158473 inhibited the binding of [^{3}H]TCP in well-washed, frozen/thawed, rat forebrain membranes; IC_{50} = 0.021µM. In comparison, the IC_{50} for reference NMDA antagonists was SDZ-515 (S), 0.29µM; CGS 19755, 0.508 µM; and CPP 1.55 µM. In the presence of PD 158473 (0.01 µM to 1 µM) and glycine 0.03 µM the EC_{50} for the enhancement of [^{3}H]TCP binding by glutamate was increased from 0.256 µM to 11.5 µM. The enhancement of [^{3}H]TCP binding by glycine in the presence of glutamate (0.1 µM) was inhibited in a concentration dependent manner by PD 158473 (0.01 to 1 µM) without altering the EC_{50} of glycine.

439.13

RPR 104632: A POTENT AND SPECIFIC GLYCINE-SITE NMDA RECEPTOR ANTAGONIST AND NEUROPROTECTIVE AGENT. S. Mignani, G.A. Böhme*, A. Boireau, G. Birraux, M.C. Burgevin, A. Doble, P. Jimonet, C. Malgouris, J.C.R. Randle and J.C. Blanchard, Rhône-Poulenc Rorer Central Research, CNS Pharmaceuticals Research Program, 94400 Vitry-sur-Seine, France.

RPR 104632 (2*H*-1,2,4-benzothiadiazine-1-dioxide-3-carboxylic acid) is a novel ligand of the glycine-site of NMDA receptors that displaces [^{3}H]-dichlorokynurenic acid binding to rat cortical membranes with a K_i of 4.9 nM. In cerebellar slices from immature rats, RPR 104632 blocked the NMDA-induced increase in cGMP levels (IC_{50} = 0.9 µM) and [^{3}H]-thienylcyclo-hexylpiperidine (TCP) binding to the NMDA receptor/channel (IC_{50} = 55 nM) in a glycine-sensitive manner. In rat hippocampal slices, long-term potentiation (LTP) of the Schaffer collateral-CA1 pathway EPSP was reduced from 163 ± 8% (n=6) to 105 ± 7% when 10 µM RPR 104632 was applied 30 min before tetanic stimulation, an effect that was not seen in the presence of 1 mM glycine (LTP = 147 ± 4%). In anesthetized rats, RPR 104632 (1-30 mg/kg *iv.*) inhibited (up to 70% and for 30-60 mn) the excitatory responses of thalamic neurons to repetitive iontophoretic NMDA ejections. In hippocampal slices from immature rats, RPR 104632 (1 and 10 µM) reduced pyramidal cell necrosis induced by NMDA (100 µM) by 50 and 100%, respectively, an effect that was reversed by 1 mM glycine. In 8 day-old primary cultures of rat cortical neurons, RPR 104632 concentration-dependently (EC_{50} = 0.5 µM) protected against cell death caused by NMDA (1 mM) as measured by trypan blue exclusion and MTT staining, an effect that was also sensitive to 1 mM glycine. Therefore, RPR 104632 is a potent antagonist at the glycine-site of the NMDA receptor/channel and an effective neuroprotective agent.

Supported by Rhône-Poulenc with the participation of the French Ministries of Research and Industry (Bio-Avenir Program).

439.14

BEHAVIORAL AND NEUROCHEMICAL ASSESSMENT OF THE PSYCHOTOMIMETIC POTENTIAL OF THE COMPETITIVE NMDA ANTAGONIST MDL 100,453. T.C. McCloskey*, R.A. Padich, V.L. Taylor, C.J. Schmidt and J.H. Kehne. Marion Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, Ohio 45215.

MDL 100,453, a competitive NMDA antagonist being developed for the treatment of stroke, was evaluated for duration of action using baseline startle depression, psychotomimetic potential using disruption of prepulse inhibition and stimulation of rat forebrain dopamine synthesis. Time course studies showed that MDL 100,453 has a considerably shorter duration of action than either D-CPPene or CGS 19755, suggesting the potential for improved control over possible side effects. Dose-response studies showed that the ion channel blockers phencyclidine, MK-801, and CNS 1102 consistently disrupted auditory and visual prepulse inhibition, and stimulated dopamine synthesis, indicative of high psychotomimetic potential. MDL 100,453, CGS 19755 and D-CPPene all depressed baseline startle but did not produce a channel blocker-like disruption of prepulse inhibition or stimulation of dopamine synthesis. There was a trend in both behavioral and biochemical studies for an improved psychotomimetic profile of MDL 100,453 relative to CGS 19755 and D-CPPene. Glycine antagonists resembled competitive NMDA antagonists in having minimal effects on prepulse inhibition and on dopamine synthesis. These data suggest that MDL 100,453 has a desirable pharmacokinetic and psychotomimetic side-effect profile relative to other competitive and uncompetitive NMDA antagonists.

NEUROPEPTIDE LOCALIZATION: SENSORY PEPTIDES

440.1

IMMUNOHISTOCHEMICAL DETERMINATION OF SUBSTANCE P RECEPTORS IN RAT CENTRAL NERVOUS SYSTEM. Y. Takeda[1], H. Kawano[2], K. Kawamura[2], H. Asou[1]* and K. Uyemura[1]. Departments of Physiology[1] and Anatomy[2], Keio University School of Medicine, Shinjuku-ku, Tokyo 160, Japan.

Localization of substance P receptor (SPR) in mammals are extensively studied by radiolabeled binding assays. In this study, we immunohistochemically characterized a distribution of SPR in rat central nervous system. An antiserum against rat SPR was obtained by immunizing rabbits with C-terminal peptide (SPR398-407) of SPR and was characterized by using CHO cells transfected with rat SPR cDNA. A strong immunorectivity was detected on the membranes of CHO cells stably expressing rat SPR. The immunoreaction on the membranes of cell bodies and processes in distinct populations of neurons were found throughout the central nervous system, such as olfactory bulb, septum, hypothalamus, cerebral cortex, hippocampus, superior colliculus, locus coelureus, dorsal parabrachial nucleus and dorsal horn of the spinal cord in adult rat. In the primary culture of astrocytes isolated from neonatal rat cerebral cortex, SPR immunorectivity was detected in O-2A progenitor cells and type-2 astrocytes, but not in type-1 astrocytes. Immunostaining with the anti-SPR antiserum preabsorbed with antigen peptide (10µM) completely abolished all of the positive reactions.

The distribution of the SPR-like immunoreactivity detected in neuronal and glial cells coincides well with that of SP binding sites previously reported, indicating that the antibodies generated in this study recognize the physiologically active SPRs in rat central nervous system.

440.2

NEUROKININ-3 RECEPTOR (NK-3R) DISTRIBUTION IN RAT AND HUMAN BRAIN: AN IMMUNOHISTOCHEMICAL STUDY. D. Mileusnic, D. J. Magnuson, M. J. Hejna, J. B. Lorens, S. A. Lorens and J. M. Lee. Depts. Pharmacology and Pathology, Loyola Univ. Medical Center (Bldg. 135), Maywood IL 60153; Department of Molecular Biology, University of Bergen, Norway.

Autoradiographic studies have shown that the NK-3R is widely distributed in the rodent CNS. Expression of the NK-3R in human brain, however, has been debated, mainly due to low levels of the receptor mRNA. We examined ^{3}H-senktide binding in homologous cortical areas of the human and rat brain. Autoradiography in rat showed dense binding in the middle cortical layers and the ventral CA3 hippocampal region. The autoradiographic analysis of the human frontal cortex revealed a distinct superficial cortical binding, limited to only the glia limitans. The conflicting findings between the two species as well as the inadequate autoradiographic image resolution prompted us to develop a primary polyclonal antibody against both rat and human NK-3R. We used an oligopeptide derived from the carboxy-terminus (CASTTSSFISSPYTSVDEYS; amino acids 434-452 of the rat receptor; the underlined Cys was added). The C-terminus was considered the best candidate for generation of the antibody since it is highly conserved between species, while divergent between different neurokinin receptors. NK-3R autoradiography and immunohistochemistry in young rat brain (4 mo) were highly correlated, with the highest concentrations of the receptor in cortical layers II-IV and the hippocampal CA2 and CA3 regions. In contrast, NK-3R immunoreactivity in normal humans (mean age 80 years) was found in the superficial cortical layers, mainly in cells that appear to be astrocytes. The Western blot analysis of the human frontal cortex and hippocampus as well as rat hippocampus revealed a major band in the molecular weight range of 60,000 to 70,000 D which corresponds to the proposed molecular weight of the NK-3R, based on the amino acid sequence. These findings suggest that there may be interspecies differences with regard to NK-3R expression and distribution. However, because there were major age differences between the human and rodent subjects in the present study, we are investigating the effects of aging on NK-3R expression.

440.3

SUBSTANCE P AND ITS RECEPTOR NK-1 IN RAT DURA MATER ENCEPHALI - AN IMMUNOCYTOCHEMICAL STUDY. U. Hanesch, S. Moussaoui[†], R.F. Schmidt*. Physiologisches Institut, Röntgenring 9, D-97070 Würzburg, Germany. [†]Dept. of Biology, Rhône-Poulenc Rorer, 13, quai Jules Guesde, 94403 Vitry-sur-Seine, France.

Neurogenic inflammation of the meninges that is characterized by vasodilatation and plasma extravasation has been proposed as a pathogenic mechanism in headache and migraine pain. It is mainly mediated by the release of the sensory neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP) from afferent nerve fibers. While the vasodilatation seems to be mediated by CGRP, the plasma extravasation is very likely caused by SP, since it can be markedly attenuated by application of SP-antagonists that act at the NK-1 receptor.

The aim of the present study was to characterize the types of the target cells bearing the NK-1 receptor and to investigate the relationship between the site of release of SP (the primary afferents) and its site of action in the dura mater encephali. Therefore, single and double immunostaining with antibodies directed against SP and the NK-1 receptor was performed on whole mount preparations of the rat dura mater by using the ABC-method and the immunogold-silver staining.

NK-1 receptor immunoreaction product was found in the inner layer of venous vessels, on cells that resemble immune cells, mast cells and fibroblasts, and on Schwann cells of small nerves and nerve bundles. SP-immunopositive nerve fibers terminated as free nerve endings not only in close association to blood vessels, but also within the connective tissue between vessels. To now, no clear correlation could be found between terminals of SP-containing fibers and the localization of the NK-1 receptor.

We conclude that SP released from afferent fibers in the dura mater may have multiple effects when activating its receptor NK-1 found associated with different cell types. SP may mediate plasma extravasation by acting on the endothelial layer of venules, stimulate inflammatory mediators (e.g. prostaglandins) by activating fibroblasts and regulate the metabolism and excitability of nerve fibers by binding to Schwann cells.

440.4

LOCALISATION OF NK1 RECEPTOR IMMUNOREACTIVITY IN NEURONS AND INTERSTITIAL CELLS OF THE GUINEA-PIG GASTROINTESTINAL TRACT. A.L. Portbury*, J.B.Furness, H.M. Young and S.R. Vigna[▲] Department of Anatomy and Cell Biology, University of Melbourne, Parkville, Victoria, Australia, 3052 and [▲]Department of Cell Biology, Duke University Medical Centre, Durham, North Carolina 27710, USA.

The localisation and distribution of the NK1 receptor was studied throughout the gastrointestinal tract of the guinea-pig using a polyclonal antiserum raised against the C-terminal 15 amino acids of the NK1 receptor. NK1-immunoreactive nerve cell bodies were found in the myenteric and submucous plexuses as well as in the interstitial cells of Cajal, at the level of the deep muscular plexus. No immunoreactivity was seen in either the circular or longitudinal muscle layers in any of the regions examined. Immunoreactivity was largely confined to the cell surface. The majority of NK1-immunoreactive nerve cells had Dogiel type 1 morphology. About two-thirds of NK1 reactive nerve cells were also reactive for nitric oxide synthase. NK1 was located in neuropeptide Y-containing secretomotor neurons of the myenteric and submucous plexuses. A population of substance P-containing neurons in submucous ganglia also exhibited NK1 receptors.

440.5

RECEPTOR ENDOCYTOSIS OF THE SUBSTANCE P RECEPTOR AND DENDRITE RESHAPING IN SPINAL NEURONS AFTER SOMATOSENSORY STIMULATION

SD Rogers, JR Ghilardi, E DeMaster, H Liu, AI Basbaum, CR Mantyh, SR Vigna, GR Strichartz*, JE Maggio, A Malhotra, DA Simone, PW Mantyh
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Neurons in the mammalian brain express postsynaptic receptors that mediate responses to extracellular signals. The largest class of transmitters in the brain is the G protein-coupled receptor family, which includes adrenergic and muscarinic receptors and nearly all neuropeptide receptors, including the substance P (SP) receptor. Here we show that in vivo somatosensory stimuli, which evoke release of SP from primary afferent neurons terminating in the dorsal spinal cord, stimulate rapid and massive endocytosis of SP receptors in dorsal spinal neurons. In addition, thin distal dendrites which show SP receptor internalization undergo a morphological reorganization, from a roughly tubular structure to one of large swollen varicosities connected by thin segments. This internalization and dendritic structural rearrangement provides a pharmacologically specific image of neurons activated by SP. These data suggest that receptor internalization can drive reversible structural changes in neurons of the central nervous system in vivo. Both of these processes may be involved in neuronal plasticity. Supported by the NIH and the VA.

440.6

ORGANIZATION OF TACHYKININ PEPTIDE mRNAs WITH OPIOID AND DOPAMINE SYSTEMS IN THE NUCLEUS ACCUMBENS OF THE RAT.

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There are two genes that encode the tachykinins; preprotachykinin A encodes the precursors for both substance P (SP) and neurokinin A (NKA), and preprotachykinin B encodes the precursor for neurokinin B (NKB). The mRNAs for both of these tachykinin genes are expressed in the nucleus accumbens (NAcc). The distribution pattern for SP/NKA mRNA within the NAcc is rather homogeneous although there are regional variances in the magnitude of expression. In contrast, the distribution pattern for NKB mRNA is more distinct. NKB-positive cells are primarily observed in the rostral pole and shell where they aggregate together to form a patch-like pattern.

The interaction of the tachykinins with dopamine and opioid systems within the NAcc is thought to play a role in the motivational and motor properties of dopaminergic and opiate drugs. In a series of double in situ hybridization studies, we analyzed the colocalization patterns of SP and NKB mRNAs with opioid peptide, opioid receptor, and dopamine receptor mRNAs in the NAcc of Sprague-Dawley rats. Complex and distinguishable patterns of colocalization were observed for SP and NKB. The distribution pattern of NKB mRNA overlaps extensively with mu opioid receptor mRNA. Previously described substructures within the rostral pole and shell of the NAcc that uniquely coexpress proenkephalin and prodynorphin also overlap with NKB cell groups. Ongoing studies combining tract-tracing with in situ hybridization will determine how these different cell types are related to specific efferents of the NAcc. [This work was supported by DA 02265 and DA 08920]

440.7

CHOLINE ACETYLTRANSFERASE AND NEUROKININ B mRNAs ARE COLOCALIZED IN NEURONS OF THE HUMAN MAGNOCELLULAR BASAL FOREBRAIN COMPLEX.

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Our previous mapping study of the location of substance P and neurokinin B (NKB) mRNAs in the human hypothalamus and basal forebrain identified numerous NKB neurons in the magnocellular basal forebrain complex (Soc. Neurosci. Abstr., 20:516,1994). In the present study, we determined if there is colocalization of choline acetyltransferase (ChAT) and NKB mRNAs in this region. Basal forebrains were collected from 3 adults without histories of neurological disease. Coronal blocks were snap frozen and serially sectioned at 10 µm thickness. *In situ* hybridization was performed on every 10th(ChAT) and 11th(NKB) section using ^{35}S-labeled 48-base synthetic cDNA probes. In the nucleus basalis of Meynert, 216 labeled (> 5X background) magnocellular neurons were identified in adjacent sections hybridized for ChAT and NKB. Of these neurons, 41% contained both ChAT and NKB mRNAs, 50% contained only ChAT mRNA, 3% contained only NKB mRNA, and 6% were unlabeled. In the diagonal band of Broca, of 189 magnocellular neurons, 34% were double-labeled , 56% contained only ChAT mRNA, 4% contained only NKB mRNA, and 6% were unlabeled. Because the basal forebrain cholinergic system degenerates in Alzheimer's disease, it will of interest to determine if the cholinergic NKB neurons are also affected (Supported by NIH AG-09214).

440.8

THE DISTRIBUTION OF NEUROKININ B RECEPTOR (NK-3) mRNA IN THE FEMALE RAT CNS.

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The tachykinins, substance P, neurokinin A and neurokinin B, are a family of neuroactive peptides that appear to have three distinct receptors termed NK-1, NK-2 and NK-3. While the distribution of the NK-1 receptor has been elucidated, little is known about the topography of NK-2 and NK-3 in the rodent brain. Autoradiographic binding studies with tachykinin agonists have provided some insight about the distribution of NK-3 receptors in the rat brain, however the results of these studies varied with different agonists. With the cloning of the rat NK-3 cDNA, it is now possible to evaluate the distribution of NK-3 mRNA in the rat brain. The brains of female rats were frozen and 20µm cryostat sections collected on microscope slides, hybridized with a ^{35}S-labeled antisense riboprobe complimentary to NK-3 mRNA, stringently washed and appossed to emulsion. The results of these studies revealed an extensive distribution of NK-3 mRNA throughout the rostral-caudal extent of the brain. In agreement with previous binding studies, we observed NK-3 mRNA in the cerebral cortex; amygdala; hippocampus; medial habenula; zona incerta; the paraventricular and supraoptic nuclei of the hypothalamus; substantia nigra; ventral tegmental area; interpeduncular nuclei; raphe nuclei, dorsal tegmental nucleus and the nucleus of the solitary tract. In contrast with binding data, only a few NK-3 mRNA cells were detected in the striatum. In addition, the results of the present studies have demonstrated the presence of NK-3 mRNA in the olfactory bulb; dentate gyrus and subiculum of the hippocampus; medial septum; diagonal band of Broca; ventral pallidum; globus pallidus; bed nucleus of the stria terminalis; the arcuate, premammillary and mammillary nuclei and the dorsal and lateral regions of the posterior hypothalamus; central gray; cerebellar cortex; parabrachial nuclei; pontin gray; nucleus of the spinal trigeminal tract and the dorsal horn of the spinal cord. The results of these *in situ* hybridization histochemical studies have provided detailed and novel information about the distribution of NK-3 mRNA in the rat central nervous system. This morphological data also elucidates the putative sites of neurokinin B action in the central nervous system which is an important step towards understanding the neuronal systems modulated by this neuropeptide.

440.9

QUANTITATIVE THREE-DIMENSIONAL MAPPING OF HUMAN BRAINSTEM SEROTONERGIC SYSTEMS.

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Investigation of the serotonergic systems of the human brainstem is necessary not *only from a basic scientific point of view, but also from a clinical standpoint.* 'Atypical' antipsychotics such as clozapine and risperidone have clear actions on 5-HT receptors, which may be the key to their usefulness as therapeutic agents in certain cases of schizophrenia. One cannot hope to determine their site(s) of action and develop even more useful therapies without a greater knowledge of the serotonergic circuits of the brain. To this end, we have developed a method for the generation of a three-dimensional (3-D) map of expression of key genes in the serotonergic systems of the human brainstem.

Brainstem blocks were obtained *post mortem* from seven normal controls, frozen rapidly on dry ice, and stored at -80ºC until use. Tissues were then mounted on individual chucks and four fiducial holes were drilled in the blocks prior to sectioning, using a #55 wire drill bit (0.052"). These fiducial holes allow tissue autoradiograms to be placed in register, using specially-developed macros incorporated into the latest version of *NIH IMAGE*. This generates a 3-D map of gene expression in specific subregions of this area of the brain. Quantitative, digitized data are left intact and easily accessible. *In situ* hybridization protocols utilizing cRNA probes for tryptophan hydroxylase, the serotonin transporter and 5-HT$_{1A}$ 'autoreceptor' have been developed to allow quantitation of the levels of mRNA encoding these molecules, the three main components determining the concentration of any given monoamine transmitter in the synapse: the biosynthetic enzyme, transporter and autoreceptor.

It is hoped that this normative 3-D map of gene expression in the human brainstem will become the basis for future studies of the serotonin system, including studies into schizophrenia and depression.

440.10

COMPARATIVE DISTRIBUTION OF AMYLIN AND CALCITONIN GENE RELATED PEPTIDE (CGRP) IMMUNOREACTIVITIES IN THE ADULT RAT BRAIN

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Calcitonin gene related peptides (CGRP) and amylin share almost 50% sequence identity and can interact at receptors within the rat brain. While CGRP-like immunoreactivity (CGRP-IR) has been extensively studied, comparatively little is known about the distribution of amylin-like immunoreactivity (amylin-IR). Therefore, we investigated the distribution of amylin-IR within the rat brain using monoclonal antibodies, F024-4.4 and F055-24, raised against human amylin, and compared this to CGRP-IR. Serial 40 micron sections of perfusion fixed rat brains were prepared. Sections were exposed to a 1:5000 dilution of guinea pig anti-CGRP serum (Accurate Chemical Co., Westbury, NY) and/or a monoclonal antibody concentration of 1 µg/ml. Primary antibodies were detected using biotinylated donkey anti-guinea pig IgG and/or anti-mouse IgG (Jackson Immunoresearch Laboratories Inc., West Grove, PA) coupled with either streptavidin-peroxidase, DAB reaction product intensified with cobalt and imidazole, or with fluorescence utilizing strepavidin-Cy3 or FITC. Amylin-IR was most notable within the spinal trigeminal tract and sparsely distributed within the area postrema and solitary tract nucleus. No amylin-IR was observed within the superior olivary nucleus where CGRP-IR fibers, as well as cell bodies, were easily discerned. Amylin-IR was also observed within the central amygdaloid nucleus. Thus, it appears that amylin-IR represents a highly localized subset of CGRP-IR fibers and cell bodies within the rat brain.

440.11

ENKEPHALINS IN THE DEVELOPING CENTRAL NERVOUS SYSTEM OF THE AXOLOTL, *Ambystoma mexicanum*. Sánchez-Islas E., Sánchez-Alvarez M., Pellicer F.* and León-Olea M. Div. de Investigación en Neurociencias, Instituto Mexicano de Psiquiatría. Tlalpan México, D.F. C.P. 14370.

Endogenous opioid systems have been shown to play an important role in the regulation of neural development. This system may serve as a trophic factor that regulates cell proliferation and influences cell differentiation and neurobehavioral ontogeny through inhibitory mechanisms (Hess and Zagon. Brain Res. Bull. 20:473-478. 1978). In this study we determined the existence and anatomical localization of the immunoreactive neurons (IRN) and fibres (IRF) to enkephalins (LE, ME) and the octapeptide (MERGL) in the developing central nervous system of the axolotl until it reaches two months old. We carried out experiments using the indirect immunofluorescence method with antibodies directed against LE, ME and MERGL. The results shown cover the days 10, 21, 26, 33 and 49 after the first cleavage. Our main results show no immunoreactivity (IR) present in the brain at day 10. At day 21, we found IRF in the motor zone (MZ) of the ventral part of rombencephalon and a low density of IRF in the reticular formation. At day 33, IRN were clustered in groups covering all brain regions, and there was an appreciable increase in the density of IRF compared to previous stages. A high density of IRF to ME were found in the thalamic paraventricular nucleus (TPv) and hypothalamus, whereas in amygdala, preoptic nuclei, ventral thalamus and ventral tegmentum a moderate density was present. A moderate density of IRF to MERGL was localized in TPv and hypothalamic nuclei (HthN). The rombencephalon presented IRF to ME in the ventral part of MZ. At day 49, an increase in the density of IRF and IRN to LE, ME and MERGL localized in the ventral part of the brain was observed, especially a high density of IRF in the HthN. Our results show that enkephalins are present in early stages of ontogeny, suggesting an important role in the organization and development of the CNS.
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440.12

RAT PINEAL CELLS EXPRESSING PREPROENKEPHALIN mRNA ARE NOT SEROTONIN-PRODUCING PINEALOCYTES: EVIDENCE USING *IN SITU* HYBRIDIZATION COMBINED WITH IMMUNOCYTOCHEMISTRY FOR SEROTONIN X.-T. Wang*, G. D. Pappas, J. Sagen and J. R. Unnerstall, Dept. of Anat. & Cell Bio., Univ. of Illinois at Chicago College of Med., Chicago, IL 60612.

The localization and physiological function of bioactive peptides and growth factors in the mammalian pineal gland has received considerable attention. In the present study, the preproenkephalin (PPEnk) mRNA expressing cells have been identified in rat pineal gland using radioactive *in situ* hybridization histochemistry. Approximately seven percent of the cells in the pineal gland (7.5±0.86, mean±95% C.I.) express PPEnk mRNA. These cells are distributed throughout the pineal either as scattered single cells or small groups of cells with large round or oval nuclei. Using ABC immunocytochemistry for serotonin (5-HT), the majority of cells in the pineal gland are found to be 5-HT positive. However, using *in situ* hybridization combined with 5-HT immunocytochemistry in the same pineal sections, the PPEnk mRNA labeling cells are found not to be serotonin immunoreactive cells. These data indicate that the PPEnk mRNA is expressed in certain discrete subpopulation of cells in rat pineal gland and these cells are not serotonin-producing pinealocytes. The physiologic role of PPEnk-derived peptides in the pineal remains unknown. It is possible that these peptides are either synthesized and secreted as hormones or act as pineal paracrine signals. Supported by NIMH MH47491

440.13

TRANSIENT APPEARANCE DURING TOOTH TURNOVER OF GALANIN PEPTIDE AND C-FOS PROTEIN IN TRIGEMINAL GANGLION NEURONS PROJECTING TO GINGIVA AND TOOTH PULPS IN A POLYPHYODONT SPECIES. F. TUISKU* AND K. EDOFF, DEPT. CELLBIOLOGY, FAC. HEALTH SCI., LINKÖPING UNIVERSITY, S-581 85 LINKÖPING, SWEDEN.

It has become appearant that neuropeptides and transcription factors contained in specific poulations of neurons are not necessarily detectable at all times. Changes in the levels of these factors commonly occur during development. In the polyphyodont cichlid *Tilapia mariae* nerve fibers influence the regular formation of new tooth germs. In view of this, it appeared of interest to analyse if the cytochemical characteristics of tooth-related trigeminal ganglion (TG) neurons in *T. mariae* change during tooth turnover (*i. e.* when the functional teeth are shed, a new generation erupts, and the development of a third one is intiated). In anesthetized animals fluorescent latex microspheres were injected into gingiva or tooth pulps. After a survival period the animals were reanesthetized and perfusion fixed. The TG was dissected out bilaterally and cryosectioned. Fluorescence microscopic analysis revealed retrogradely labeled TG neurons in the distal half of the ganglion. TG neurons projecting to the gingiva exhibited an unimodal size distribution with a diameter range of 8-14 µm (peak at 11 µm). TG neurons innervating tooth pulps displayed a bimodal size distribution with a diameter range of 10-30 µm (peaks at 12 and 25 µm). Following tracer analysis the same sections were processed for peroxidase-antiperoxidase immunohistochemistry against a selected set of neuropeptides or transcription factors. Light microscopic analysis revealed a galanin peptide (Gal)-like immunoreactivity (LI) in TG neurons projecting to gingiva and tooth pulps. This Gal-LI was exclusively present during tooth turnover. Other gingival and pulpal TG neurons displayed a c-fos-LI with the same temporal appearance as the Gal-LI. It is concluded that the cytochemical characteristics of some tooth-related TG neurons in *T. mariae* change during tooth turnover.

440.14

AUTORADIOGRAPHIC STUDY OF GALANIN BINDING IN THE PRIMATE BRAIN: REGIONAL DIFFERENCES IN THE RESPONSE TO GTP PRETREATMENT. J.B. Leverenz*, B. Planas, M.A. Raskind and M.A. Miller, Depts. Medicine and Psych & Behav.Sci., Univ. Washington, Seattle, WA 98195

In humans, galanin (GAL) is a 30 amino acid neuropeptide implicated in a variety of neuroendocrine and behavioral functions. GAL exerts its effects via interactions with a specific G-protein coupled receptor. In crude membrane preparations of human hypothalamus, guanine nucleotides reduce GAL binding by inducing dissociation of the ligand-receptor complexes. We have recently reported that preincubation of adult rat brain slices with guanosine-5'-triphosphate (GTP) enhances GAL binding in specific regions including hypothalamus (Planas et al., 1994). GTP pretreatment may enhance binding density in slices by inducing dissociation of endogenous GAL from its binding sites and thereby unmasking previously occupied receptors. Here, we assessed the effects of GTP preincubation on GAL binding in human and non-human primate brain slices. Fresh frozen coronal sections (20 µm) from normal human (n=4) and Macaca nemestrina (n=3) brains were sampled at the level of the paraventricular nucleus of the hypothalamus, amygdala, and hippocampus. Sections were preincubated with or without GTP (10^{-4} M) and then incubated in 0.25 nM ^{125}I-GAL (porcine). Non-specific binding was assessed by the addition of 0.25 µM unlabeled GAL (porcine). GAL binding in several hypothalamic regions was robustly enhanced following GTP pretreatment (e.g., human lateral hypothalamus R.O.D.: GTP pretreat 0.42 ±0.03, no GTP 0.11 ±0.03, $p \leq 0.001$). Preliminary findings in extrahypothalamic regions including the amygdala and hippocampus indicated that these regions differ from hypothalamus in their response to GTP pretreatment. This regional specificity of response to GTP parallels our previous observations in the adult rat and suggests that GAL release and receptor occupancy are also be increased in the primate hypothalamus compared to other brain regions. These findings indicate that pretreatment with GTP may be necessary for accurate assessment of GAL binding site density in primate brain slices and may provide information about receptor occupancy by endogenous GAL.

440.15

A RAT GALANIN RECEPTOR: ITS CLONING AND DISTRIBUTION IN RAT FOREBRAIN. E. Grafstein-Dunn, G. G. Cadd, D. K. Clifton, and R. A. Steiner*. Depts. of Ob-Gyn and PBio, University of Washington, Seattle, WA 98195.

The neuropeptide galanin (GAL) is thought to play an important modulatory role as a cotransmitter in a variety of classical and peptidergic pathways. In the hypothalamus, GAL is coexpressed with gonadotropin-releasing hormone (GnRH) and with growth hormone-releasing hormone (GHRH) and may modulate their release through undefined mechanisms. Although evidence for both pre- and post-synaptic actions of GAL has been found, GAL's target cells in the brain have not been clearly identified. Therefore, we have 1) cloned a fragment of a rat GAL receptor (Gal-R), 2) mapped its distribution in the forebrain, and 3) assessed possible coexpression of Gal-R in GnRH and GHRH neurons to determine whether GAL could directly regulate the activity of these cells. We cloned a 400 bp fragment of a GAL receptor cDNA by PCR from a rat brain cDNA library and found it to be 85% identical to the published human GAL receptor sequence. We synthesized a ^{33}P-labeled riboprobe and performed double-label *in situ* hybridization on brain sections of adult male and female rats. Neurons expressing Gal-R mRNA were identified in several regions previously reported to contain GAL binding sites. These included: preoptic regions, basal forebrain, hypothalamus, thalamus, amygdala, and hippocampus. We found no evidence for coexpression of Gal-R mRNA in GnRH or GHRH neurons in either sex. In conclusion, the distribution of Gal-R mRNA parallels that of GAL binding sites in several regions. A direct action of GAL on either GnRH or GHRH neurons does not seem likely since neither cell type expressed the Gal-R.

441.1

FOUR DOPAMINE D_1-LIKE RECEPTORS ARE EXPRESSED IN THE EEL BRAIN. Cardinaud, B., Kukstas, L.A.*, Vincent, J.D. & Vernier, P. Inst. Alfred Fessard, UPR 2212, CNRS, 91198, Gif/Yvette, France.

The phylogenetical analysis of bioamine receptor sequences suggests that the main classes of receptors were generated by gene duplication at a period close to the divergence of vertebrates from invertebrates. To test this hypothesis, we analyzed the molecular diversity of dopamine D_1-like receptors in a fish, the european eel *Anguilla anguilla*. The combined use of PCR cloning and screening of an eel brain cDNA library led us to isolate four subtypes of D_1-like receptor cDNA. Two of them are homologous to the vertebrate D_{1A} receptors (D_{1A} and $D_{1A'}$), one is a D_{1B} orthologue, and one is clearly homologous to the D_{1C} receptor found in fishes and amphibians. These results indicate that the three main classes of D_1-like receptors were generated before the divergence of fishes from the other vertebrates and that the D_{1A} receptor was duplicated in the *Anguilla* phylum. The $D_{1A'}$ receptor is expessed at a much lower level than its sister gene. The pharmacological profiles of these fish D_1 receptors, transiently transfected in COS cells, closely ressemble those of their vertebrate homologues. The D_{1A} and D_{1B} receptors are clearly discriminated by ligands such as butaclamol or flupentixol. *In-situ* hybridization of the D_{1A} receptors showed a distribution restricted to areas such as pituitary, stratum periventriculare, torus semicircularis and especially the ventricular wall. The analysis of the molecular data in terms of anatomical homology will be extended to gain a better understanding of the genetic events which gave rise to the bioamine receptor diversity in vertebrates.

441.2

THE D1 AGONIST SKF 82958 PREVENTS NALOXONE-INDUCED SEIZURES IN RATS INFECTED WITH BORNA DISEASE VIRUS. M. V. Solbrig, G. F. Koob and W. I. Lipkin*. Lab for Neurovirology, University of California, Irvine CA 92717

Borna disease (BD) virus is a neurotropic RNA virus that causes movement and behavior disorders in immunocompetent animals. Infected rats have orofacial dyskinesias resembling the medical condition, tardive dyskinesia, that may follow exposure to neuroleptic drugs. Because dopamine and opiate systems may mediate perioral movements in rats, the effects of a selective D1 agonist, SKF 82958, and the opiate antagonist, naloxone, were examined in the BD rat model of tardive dyskinesia.

BD rats had behavioral sensitivity to SKF 82958 (0.25, 0.50, and 1.00 mg/kg) as shown by continuous cage gnawing and self-biting. Naloxone (0.04, 0.20, and 1.00 mg/kg) caused myoclonic, generalized clonic, or atonic seizures, behavior arrest and staring spells, masking any effect on perioral movements. Naloxone-induced seizures were prevented by pretreatment with 1.00 mg/kg SKF 82958. These results demonstrate a convulsant action of naloxone in the BD rat and suggest that the seizures may be mediated by activity in D1 circuits.

441.3

DIFFERENTIAL DESENSITIZATION OF D_1 DOPAMINE RECEPTOR FOLLOWING A81686AND DOPAMINE TREATMENT. S.E.Côté*, P.J. Bédard, P. Falardeau, Médecine Génétique et Moléculaire, CHUL, Université Laval, Ste-Foy, Québec, G1V 4G2, Canada.

The motor effects of D1 receptor stimulation in Parkinson's disease seems to be a better therapeutic profil. However, specific D1 receptor stimulation leads to a rapid and extensive desensitization. Interestingly, this desensitization is different according to the agonist used. Desensitization mechanisms induced by a new selective dopamine agonist (A81686) compare to dopamine was investigated using mouse fibroblast Ltk- cells expressing human D1 receptors . Cells were treated with A81686 or dopamine and receptor levels, G protrein coupling and adenylyl cyclase activation were examined. Exposure of Ltk-#20 cells to 10µM dopamine or A81686 for 4 hours resulted in a 17% decrease of total number of D1 receptors, as assessed by [3H] SCH 23390 binding. Competition experiments of dopamine on [3H] SCH 23390 binding revealed no significant difference in the relative proportion of high affinity state receptors after pretreatment with dopamine (51%). In contrast, this proportion drop to 31% following A81686 treatment. Dopamine stimulated the activity of adenylyl cyclase in membranes of D1 receptor expressing cells (4.7-folds over basal). Following dopamine treatment, the potency of dopamine to stimulate AC remained comparable to untreated cells (4.2-fold over basal). In contrast, this potency was decreased after pretreatment with A81686 (1.8-fold over basal). These results showed that A81686 is more potent to induce D1 receptors desensitization than dopamine and suggested that the molecular mechanisms involved in desensitization can be dependent of the agonist used.

441.4

CHIMERIC DOPAMINE D1A, D1B AND D1C RECEPTORS: PHARMACOLOGICAL & FUNCTIONAL CHARACTERIZATION. K.S. Sugamori*, M. Chung, and H.B. Niznik *Dept. of Pharmacology and Psychiatry, University of Toronto, and the Molecular Neurobiology Laboratory, Clarke Institute of Psychiatry, Toronto, Ontario, M5T 1R8.*

We previously reported the cloning and characterization of three dopamine D1-like receptor subtypes (D1A, D1B, D1C) from *Xenopus laevis*. The isolation of the novel D1 receptor subtype, D1C, which lacks appreciable structural similarity but displays pharmacological homology to mammalian D1 receptors may facilitate the identification of regions or structural motifs regulating D1 ligand binding specificity and signal transduction. Using PCR-generated splicing by overlap extension, chimeras were constructed in which the variable carboxy tail regions were exchanged. Six chimeric receptors (D1A/D1B$_{CT}$, D1A/D1C$_{CT}$, D1B/D1A$_{CT}$, D1B/D1C$_{CT}$, D1C/D1A$_{CT}$, D1C/D1B$_{CT}$) have been generated, sequenced and expressed in COS-7 cells. Since the parent receptors differ in their affinities for the agonists DA and 6,7-ADTN, we have first determined the binding affinities (Ki's) for these chimeras. A rightward shift to wild type parent affinity for DA and ADTN was noted for the D1B/D1A$_{CT}$ and D1B/D1C$_{CT}$ chimeras indicating that sequences in the carboxy tail region of the D1B receptor may confer high affinity binding for certain D1 receptor agonists. The Kd for [^{3}H]SCH-23390 was unchanged for these chimeras. The reciprocal of this effect, however, could not be tested as the D1A/D1B$_{CT}$ and D1C/D1B$_{CT}$ receptor chimeras did not show any detectable [^{3}H]SCH-23390 binding, despite stimulation of cAMP accumulation in response to DA and high affinity SCH-23390 inhibition of DA-stimulated cAMP accumulation. None of the mutant receptors caused any appreciable shift in EC_{50} for DA-stimulated cAMP accumulation confirming that sequence-specific domains confering functional responsiveness may reside in the third intracellular loop. Since the carboxy tail regions may in turn play a role in receptor desensitization, the effect of short term desensitization is currently under investigation.

441.5

DOPAMINE D1 RECEPTORS ARE NOT INVOLVED IN THE ACUTE TOLERANCE TO NICOTINE IN THE RAT. X. Zhang, J.R. James*, C. Liu, Z.H. Gong, and A. Nordberg. Dept. of Clinical Neuroscience and Family Medicine, Division of Nicotine Research, Karloinska Institute, Huddinge University Hospital, 141 86, Sweden

Acute tolerance to nicotine was assessed in a two lever operant drug discrimination paradigm to study nicotine's mechanism(s) of action in the central nervous system. Rats were trained to discriminate nicotine (400 ug/kg, s.c.) from saline. A challenge dose of nicotine (800 ug/kg) administered 90-180 minutes prior to the training dose significantly attenuated drug lever responding in a subpopulation of the rats (desensitized) but not in another subpopulation (non-desensitized). Quantitative ^{125}I-SCH 23982 (dopamine D1 receptor antagonist) autoradiography revealed that there is no significant difference in the number of dopamine D1 receptors in caudate putamen (Cpu), accumbence nucleus (Acb), ventral tegmental area (VTA) and substantia nigra (SNR) between desensitized and non-desensitized rats. This result suggests that dopamine D1 receptors are not involved in the acute tolerance to nicotine in the rat.

441.6

SCH 23390 IS A PARTIAL AGONIST AT RAT D1A AND D1B DOPAMINE RECEPTORS EXPRESSED IN SF9 CELLS. [1]M.W. Martin*, [1]A.W. Scott, [1]S.A.Griffin, [2]S.Chumpradit, [2]H.F. Kung, [3]S.T. Elder, [3]R.H. Mach[2] and [1]R.R. Luedtke [1]Department of Pharmacology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107, [2]Department of Radiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 and [3]Department of Radiology, Bowman Gray School of Medicine, Winston-Salem, NC 27157.

The baculovirus system was used to express the rat D1a and the rat D1b receptors in Sf9 cells. Rat D1a and D1b dopamine receptors expressed in Sf9 cells appear to be linked to endogenous Gs protein since a dose dependent stimulation of adenylyl cyclase activity can be achieved using dopamine. The dopamine receptor antagonists cis-α-flupenthixol and fluphenazine inhibit a) dopamine dependent stimulation of adenylyl cyclase and b) basal enzyme activity in Sf9 cells expressing D1-like receptors. The benzazepine SCH 23390 stimulates adenylyl cyclase activity in Sf9 cells expressing rat D1a and D1b dopamine receptors with nanomolar EC_{50} values. A panel of benzazepines structurally related to 7-chloro-8-hydroxy-1-(3'-iodophenyl)-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (TISCH) were also agonists at rat D1a and D1b receptors and their potency for stimulation correlates with their affinity for D1-like receptors. *(Supported by NINDS 30507 and the National Alliance for Research on Schizophrenia and Depression).*

441.7

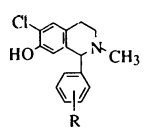
CONSTITUTIVE ACTIVITY OF THE D_1 DOPAMINE RECEPTOR BY MUTATIONS NEAR PROLINES W. Cho*, L. P. Taylor, A. Mansour and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

Proline is highly conserved in the α-helices of 7-transmembrane, guanine-nucleotide binding protein-coupled receptors. The peculiar properties of this imino acid have led to speculations that proline may be both structurally and dynamically important for 7TM function. As an alternative to the potentially deleterious consequences of proline-directed mutagenesis, substitutions were created in the X residue of X-pro peptide bonds (where X = the adjacent residue to the amino terminal side of proline) which may influence static geometries and possibly agonist-induced conformational changes at the X-pro peptide bond. In the fifth helix of the human D_1 receptor, isoleucine205 was substituted with either an alanine (I205A) or a tyrosine (I205Y). Similarly, in the sixth helix, leucine286 was substituted with either an alanine (L286A) or a tyrosine (L286Y). L286A resulted in a constitutively active receptor characterized by elevated basal cAMP levels and increased agonist potencies. This is the first report of a constitutively active receptor due to this novel point mutation. I205A resulted in subtle changes in the D_1 pharmacological profile and some deficits in signal transduction. I205Y and L286Y produced comparatively drastic impairments in both binding and signal transduction. While affinity shifts may reflect static alterations in ligand binding topography, modification of signal transduction properties may be due altered energetics of isomerizations or other conformational changes about the X-pro peptide bond necessary for linking agonist binding with G protein coupling.

441.8

PURSUIT OF A SELECTIVE D_5 ANTAGONIST. BT Hoffman, SD Wyrick, MA Mayleben, PS Charifson, DL Minor, RB Mailman*, Division of Medicinal Chemistry, School of Pharmacy, and Brain Development Research Center, School of Medicine, UNC-Chapel Hill, NC, 27599.

We have previously reported the binding affinities at the D1 dopamine receptor of several tetrahydroisoquinoline ring-contracted analogs of the D_1 antagonist SCH23390. These analogs were synthesized in order to further characterize the antagonist binding pharmacophore of the D_1 receptor. The binding affinities of these compounds for the D_1 receptor were determined by conducting competition binding studies using rat striatal membranes labeled with [^{3}H]SCH23390. (±)-N-methyl-6-chloro-7-hydroxy-1-phenyl-1,2,3,4,-tetrahydroisoquinoline (R=H) exhibited a K_i of 12.5 nM. In order to assess the effects on affinity of substituents on the appended phenyl ring , several analogs were synthesized. The (±)-*ortho*-methyl analog (R=CH_3) had a higher affinity than the unsubstituted (R=H) analog, with a K_i of 8.4 nM. Methyl substituents in other ring positions decreased affinity; the (±)-*meta*-methyl and (±)-*para*-methyl analogs had K_i's of 30 nM and 18 nM, respectively. An electron-withdrawing substituent in the *ortho* position decreased affinity, as was observed with the *ortho*-chloro analog (R=Cl), which had a K_i of 16 nM.



The D_1 and D_5 receptors are 80% homologous within the transmembrane regions, and radioligand binding experiments have shown that agonists and antagonists exhibit similar affinities for the D_1 and D_5 receptors. We are currently assessing the affinity of these compounds and novel analogs for the D_5 receptor. Discovery of a selective D_1 or D_5 ligand would be beneficial in delineating the pharmacological and biological differences between these dopamine receptor subtypes. This work was supported by PHS grant MH40537.

441.9

DINAPSOLINE: A NOVEL FULL D_1 AGONIST. D.E. Nichols[2], V.J. Watts[1], C.P. Lawler[1], D. Ghosh[2], C. Striplin[1]* and R.B. Mailman[1]. Univ. of North Carolina[1], Chapel Hill, NC 27599, and Purdue Univ.[2], W. Lafayette, IN. 47907.

Despite the widespread clinical use of dopaminergic ligands (e.g., Parkinson's Disease or schizophrenia), it is generally recognized that more selective drugs, or drugs with unusual functional effects, may be of great utility. Several years ago, we synthesized the first high affinity, bioavailable, full D_1 agonist dihydrexidine (DHX; *trans*-10,11-dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine, a drug later shown to have profound antiparkinsonian effects in MPTP-treated monkeys. DHX also provided a semi-rigid structural backbone that allowed refinement of a D_1 agonist pharmacophore model. We now report the pharmacological properties of 8,9-dihydroxy-2,3,7,11b-tetrahydro-1*H*-naph[1,2,3-*de*]isoquinoline (dinapsoline). This compound was designed by tethering the two phenyl rings of dihydrexidine through a methylene bridge and removing the C(7)-C(8) ethanobridge. Preliminary molecular modeling studies demonstrated that these modifications conserved the relative orientation of all the essential elements of the D_1 pharmacophore (position and orientation of the nitrogen, hydroxyls, and phenyl rings). Dinapsoline had slightly higher affinity than DHX at rat striatal D_1 receptors (K_i = 5.9 nM), and a Hill slope of 0.66, suggesting agonist characteristics. Consistent with this, in both rat striatum and C-6-mD_1 cells, dinapsoline was a full agonist with an EC50 of 30 nM. These data provide a powerful test of the model of the D_1 pharmacophore we have developed, and provide another tool for the functional study of D_1 receptors. Moreover, DHX is interesting in that it has relatively high D_2 affinity ($K_{0.5}$=100 nM) despite the accessory phenyl ring usually thought to convey D_1 selectivity. DHX is unusual in that it is an agonist at some D_2 receptors, but an antagonist at others (so called "functional selectivity"). Dinapsoline had even higher affinity for the D_2 receptor ($K_{0.5}$ = 31 nM) than DHX. Studies are underway to determine whether dinapsoline retains the D_2 "functional selectivity" of DHX.

441.10

A-77636 INDUCES PERSISTENT ACTIVATION AND PROLONGED DESENSITIZATION OF THE DOPAMINE D1 RECEPTOR C.W. Lin*, B.R. Bianchi, T.R. Miller, M.A. Stashko, P. Curzon, L. Hodges, K.E. Asin and Don Britton. Neuroscience Area, Abbott Labs, Abbott Park IL 60064

A-77636 is a D1 receptor selective agonist that was shown to elicit beneficial responses in animal models of Parkinson's disease (Eur. J. Pharmacol. 229: 203, 1992). However, A-77636 is of limited potential for PD therapy because it rapidly induces tolerance in vivo . To understand how A-77636 induces tolerance, we studied the in vitro properties of A-77636 and A-81686, a related compound that did not lead to tolerance after repeated administration. Using a neuroblastoma cell line, SK-N-MC, as a model for the D1 receptor, we found that A-77636 persistently activated the receptor. One h pretreatment with A-77636 resulted in significant residual cAMP production even after the drug solution was removed and the cells were washed. The residual cAMP activity was selectively inhibited by SCH 23390, a selective D1 antagonist. The residual cAMP activity declined with pretreatment time and, after 4 h pretreatment, little residual cAMP production was observed. A-77636-treated cells were devoid of agonist response 4 hr after drug removal. In contrast, A-81686 did not elicit residual cAMP production in SK-N-MC cells. Receptor binding studies showed that pre-incubation of rat striatal membranes with A-77636 led to a large decrease (>80%) in D1 receptor density, despite repeated washings. We conclude that A-77636 dissociates slowly from the D1 receptor. The continued activation of the D1 receptor leads to inability of the receptor to restore its responsiveness, and may explain the behavioral tolerance seen after A-77636 treatment .

441.11

DOPAMINE D1 RECEPTOR ACTIVATION PRODUCES OPPOSITE EFFECTS ON GABA RELEASE IN VENTRAL TEGMENTAL AREA BEFORE AND AFTER CHRONIC COCAINE. A. Bonci , D. Grandy * and J. T. Williams. Vollum Institute for Advanced Biomedical Research, Oregon Health Science University, Portland, OR 97201.

The dopamine D1-mediated modulation of the GABAB IPSP in ventral tegmental area of guinea pig was studied before and after chronic cocaine treatment. Electrically evoked GABAB-mediated IPSPs were isolated pharmacologically by applying 2-amino-5-phosphonopentanoic acid (AP-5), 6-cyano-2,3-dihydroxy-7-nitro-quinoxaline (CNQX), picrotoxin, strichnine and eticlopride. Our experiments show that in controls the D1 receptor activation produced an increase in the GABAB IPSP, while in animals tested one week after the last cocaine injection we observed a decrease on GABAB IPSP. The effect of forskolin, an adenylyl cyclase activator, was likewise opposite in the two groups of animals, producing in controls and in cocaine treated animals respectively an increase and a decrease in the GABAB IPSP amplitude. It is known from other works that increased extracellular levels of adenosine can result from metabolism of cAMP. It has also been demonstrated that presynaptic A1-adenosine receptor activation inhibits GABA release in VTA. The A1 adenosine antagonist, 8-CPT, and drugs able to decrease the extracellular adenosine levels such as RO-201724, probenecid and adenosine deaminase, reversed the inhibition of GABA release caused by D1 receptor activation or by forskolin. This suggests that one week after chronic cocaine treatment the extracellular levels of adenosine in ventral tegmental area are increased. These results support the hypothesis that the D1/adenosine balance in the ventral tegmental area is profoundly altered after chronic cocaine and that this interaction could play a crucial role in sensitization to psychostimulants.

441.12

SITE-DIRECTED MUTAGENESIS STUDY OF PALMITOYLATION SITES ON DOPAMINE D1 RECEPTOR. H. Jin, R. Zastawny, H. Moldofsky†, S. R. George and B. F. O'Dowd*. Departments of Pharmacology and †Psychiatry, University of Toronto; Addiction Research Foundation, Ontario, Canada.

Elimination of palmitoylation site(s) has been shown to produce different functional effects on several G protein coupled receptors. Two cysteines (Cys347 and Cys351) exist at the carboxyl terminus of the human D1 receptor. To investigate the role of palmitoylation in G protein-coupling and desensitization of D1 receptor, one or both cysteines were replaced by alanine using site-directed mutagenesis. In addition, a hemagglutinin epitope was tagged to both the wild type and mutant D1 receptors. DNA constructs encoding the epitope-tagged wild type and Cys347Ala mutant receptors were individually transfected into the BHK3 cell line. In the two stable cell lines (BHK14 for the wild type and BHK25 for the mutant) selected and characterized, Kd values of SCH23390 were: 590.9±65.1 pM for BHK14 and 527.9±75.5 pM for BHK25. Ki rank orders were the same for both clones: dopamine>SKF38393>5-HT>isoproterenol for agonists and SCH23390>butaclamol>haloperidol for antagonists. Therefore, introduction of both the epitope and mutation did not interfere with ligand binding. Two agonist affinity states were demonstrated by dopamine competition indicating the presence of G protein coupling, which was completely abolished in the presence of GppNHp. In addition, pretreatment of both cell lines with dopamine (10 μM for 15 min) also caused a complete conversion of high affinity sites to low affinity and a similar 2-3 fold increase in EC50 of adenylyl cyclase activity. Thus, the single mutation of Cys347Ala did not affect G protein coupling or agonist-induced adenylyl cyclase desensitization. Studies are currently being carried out to determine if the other single mutation (Cys351Ala) and the double mutation (Cys347Ala/Cys351Ala) will produce any different effects.

441.13

THE EFFECT OF GTP ON THE DISPLACEMENT OF [3H]SCH 23390 BY DA AGONISTS IN RHESUS MONKEY STRIATA. M.R. Weed, W.L. Woolverton* and I.A. Paul. Dept of Psychiatry, Univ of Miss Med Ctr, Jackson, MS 39216.

Guanyl nucleotides alter agonist displacement of antagonist binding to g-protein-coupled receptors by reducing the high affinity binding component. The efficacy of guanyl nucleotides to shift the displacement potency of an agonist may be related to the intrinsic activity of the agonist. The present study describes the effect of 100μM GTP on binding to the dopamine (DA) D1 receptor in rhesus monkey striata. Tissue was thawed in phosphate buffer (pH 7.4), homogenized, separated by centrifugation and used to initiate assays containing buffer, 4 mM MgCl2, 0.05 nM [3H]SCH 23390 and 16 concentrations of test compound. Assays were incubated at 37°C for 60 min, terminated by rapid filtration, soaked in 500μl of Packard Microscint cocktail, and bound radioactivity was measured using a Packard Top Count scintillation counter. 100μM cis-Flupentixol defined NSB. In the absence of GTP, D1 agonists (i.e. compounds which stimulate cAMP production in rat striata) displaced [3H]SCH 23390 in a manner best fit with a 2-site model (Prism, GraphPad). For agonists, GTP addition reduced the percent high affinity binding without changing the affinity of either binding site. One-site models were fit to displacement curves in the presence and absence of GTP and the ratio between these two IC50's is presented for each compound as the "fold-shift" induced by GTP. D1 agonists with higher efficacies in cAMP assays had larger shifts than lower-efficacy agonists and antagonists. These data are generally consistent with the hypothesis that an agonist's sensitivity to guanyl nucleotides is related to its intrinsic efficacy. However, the differences in the effect of GTP on D1 agonists were not as large as the differences of these same compounds to stimulate cAMP production in rat striata. Supported by DA- 05616 and DA-08731.

Compound	Fold Shift
Dopamine	6.14
SKF 81297	2.99
R(+)Br APB	2.56
SKF 82958	2.35
SKF 77434	2.33
SKF 38393	2.23
S(-)Br APB	1.84
SCH 39166	0.68
SCH 23390	0.51

441.14

DESENSITIZATION AND DOWN-REGULATION OF D_{1A} DOPAMINE RECEPTORS IN MUTANT CHO CELLS DEFICIENT IN CYCLIC AMP-DEPENDENT PROTEIN KINASE. Ana L. M. Ventura* and David R. Sibley. Molecular Neuropharmacology Section, ETB/NINDS/NIH, Bethesda, MD 20892.

In order to investigate the role of the cAMP-dependent protein kinase (PKA) in the desensitization and down-regulation of the D_{1A} dopamine receptor, we stably expressed the rat cDNA for this receptor in mutant CHO cell lines deficient in PKA activity. The 10260 mutant CHO cell line has been characterized as expressing less than 10% of type I and type II PKA activities relative to the parental 10001 CHO cell line. The 10248 mutant CHO line lacks type II PKA activity but expresses type I PKA. The transfected parental and mutant cell lines were found to express between 1-3 pmol/mg of D_{1A} receptor binding activity (Bmax) as determined using [^{3}H]SCH23390. All three cell lines demonstrated similar levels of dopamine-stimulated adenylyl cyclase activity. Treatment of all three CHO cells with dopamine resulted in a similar (>90%) desensitization of the dopamine-stimulated adenylyl cyclase response. Dopamine also promoted, in a time- and dose-dependent fashion, a >90% down-regulation of D_{1A} receptors in the parental cell line, but only a ~50% decrease in both mutant lines. Similarly, treatment of the cells with CPT-cAMP and 8-Bromo-cAMP, membrane permeable analogs of cAMP, decreased the receptor binding activity in the parental cell line by ~50%, however, the decrease was smaller for the 10260 mutant (~25%) and not significant (<5%) with mutant 10248. A similar result was obtained by treating the cells with forskolin which down-regulated the D_{1A} receptors by ~50% in the parental cells, ~25% in the 10260 mutant cells and ~10% in the 10248 cell line. Our results suggest that PKA significantly contributes to the down-regulation of D_{1A} receptors in CHO cells but may not be essential for desensitization of the adenylyl cyclase response by dopamine.

441.15

INVESTIGATION OF CYCLIC AMP-MEDIATED REGULATION OF THE RAT D_{1A} DOPAMINE RECEPTOR USING SITE-DIRECTED MUTAGENESIS TECHNIQUES. Dong Jiang and David R. Sibley*. Molecular Neuropharmacology Section, ETB/NINDS/NIH, Bethesda, MD 20892.

Previous investigations of D_{1A} receptor regulation have suggested a role for the cAMP-dependent protein kinase (PKA) in agonist-induced desensitization and down-regulation of receptor expression. Given the presence of at least three possible consensus recognition sites for PKA on the D_{1A} receptor protein, a reasonable hypothesis is that some of these PKA-mediated effects are due to direct phosphorylation of the receptor. In order to investigate this possibility, we have used site-directed mutagenesis techniques to alter each of three potential phosphorylation sites to determine the subsequent effects on agonist-induced regulation of the receptor. Using a PCR-based mutagenesis method, we modified the following amino acids as indicated: $Thr^{135}\rightarrow Val^{135}$, $Ser^{229}\rightarrow Ala^{229}$, and $Thr^{268}\rightarrow Val^{268}$. Residue 135 is found within the second intracellular loop of the receptor whereas residues 229 and 268 are present in the third intracellular loop. Preliminary characterization of the mutated receptors was achieved by lipofectamine-mediated transient transfection of COS cells. Both the wild type and mutant receptors were expressed at identical levels (Bmax values ranged from 1.5-4 pmol/mg between experiments) and there were no differences in the affinity of [^{3}H]SCH-23390 (K_D ~140 - 170 pM). Similarly, dopamine was found to compete for [^{3}H]SCH-23390 binding and stimulate cAMP production in a identical fashion for all of the receptors. These data suggest that the mutations have no effect on receptor expression, antagonist or agonist affinities, or on functional coupling with Gs. Preliminary experiments indicate that dopamine preincubation of the transfected COS cells results in desensitization of the wild type receptor with no alteration in [^{3}H]SCH-23390 binding. We are currently evaluating this response for the mutant receptors as well as preparing stably transfected cell lines for further analysis.

441.16

DOPAMINE D_1 RECEPTORS COUPLING TO ACTIVATION OF ADENYLYL CYCLASE IS MEDIATED BY Gs BUT NOT Golf PROTEINS. H.-Y. Wang, G.-P. Cai and E. Friedman. Department of Pharmacology, Medical College of Pennsylvania/EPPI, Philadelphia, PA 19129.

Previously, the possible role of Golf in dopamine D_1 receptor stimulated striatal adenylyl cyclase was suggested by the enrichment and its colocalization with D_1 binding sites in striatal tissues (Herve et al., 1993). The involvement of guanine nucleotide binding proteins, Gs and Golf in regulating the dopamine stimulated adenylyl cyclase was therefore investigated in rat striata. Incubation of striatal membranes with antisera against α-subunit of Gs but not Golf attenuated dopamine-stimulated cAMP accumulation by 50%. Furthermore, the association between those G proteins with dopamine D_1 receptor was examined by the ability of dopamine to stimulate [^{35}S]GTPγS and [α-^{32}P]GTP to the two Gα proteins in striatum. Dopamine increased [^{35}S]GTPγS to Gs but not to Golf. Analysis of immunoprecipitated [^{32}P]guanine nucleotide associated Gα protein revealed that dopamine activated Gs (45-KDa) but not Golf (42-KDa) in striatum. Similarly, antisera against Gαs but not Gαolf precipitated D_1 receptors as indicated by specific [^{3}H]SCH23390 binding. These results thus suggest that dopamine D_1 receptors are coupled with Gs but not with Golf to stimulate adenylyl cyclases.

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441.17

PRELIMINARY CHARACTERIZATION OF A MONOCLONAL ANTIBODY THAT BINDS A RECOMBINANT PROTEIN CORRESPONDING TO THE CARBOXY TERMINUS OF THE RAT D1b DOPAMINE RECEPTOR. R.R. Luedtke*, S.A.Griffin, X.Jin and S.Summers. Department of Pharmacology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107.

PCR was used to amplify a segment of DNA that codes for the carboxy terminus of the rat D1b dopamine receptor. *Bam* H1 restriction sites were engineered into the primers to facilitate subcloning the amplified fragment into the pET expression system vectors. Plasmid containing the receptor gene fragment was used to transform BL21plysS DE3 *E. Coli* cells. Several colonies were evaluated using analytical SDS-PAGE for the ability to express a recombinant peptide following induction with 1 mM isopropyl-β-D-thiogalactopyranoside. A recombinant protein corresponding to the carboxy terminus of the rat D1b receptor (rD1b-COOH) was partially purified by preparative SDS-PAGE and used to immunized BALB/c mice. Splenocytes from immunized mice were used for the preparation of hybridomas. Tissue culture media from the hybridomas was screened using recombinant protein with an ELISA and an immunoblot assay. One monoclonal antibody, designated SG5D1b bound rD1b-COOH but did not cross-react with the corresponding rD1a-COOH peptide. Research is ongoing to determine whether SG5D1b can specifically bind to the native receptor protein. *(Supported by NINDS 30507, the Scottish Rite Schizophrenia Research Program and the National Alliance for Research on Schizophrenia and Depression).*

441.18

CO-LOCALIZATION OF THE D_1 DOPAMINE RECEPTOR IN A SUBSET OF DARPP-32-CONTAINING NEURONS IN RAT CAUDATE-PUTAMEN. K.C. Langley*, C. Bergson‡ and C.C. Ouimet. Program in Neuroscience, Florida State University, Tallahassee, FL 32306, ‡Department of Pharmacology, Pennsylvania State College of Medicine, Hershey, PA 17033

DARPP-32 is a dopamine and cAMP-regulated phosphoprotein that is enriched in dopaminoceptive brain regions high in D_1 dopamine receptor binding. We used double-labeling immunofluorescence to localize both the D_1 receptor and DARPP-32 in the rat caudate-putamen. As expected, both D_1 and DARPP-32 antibodies heavily labeled fibers in the neuropil. Many medium-sized neuronal cell bodies were weakly immunoreactive for the D_1 receptor and a larger number were strongly immunoreactive for DARPP-32. All cell bodies immunopositive for the D_1 receptor appeared to be immunopositive for DARPP-32. The D_1 receptor was not detectable, however, in a subpopulation of DARPP-32-containing cell bodies.

These findings are consistent with the known regulation of DARPP-32 phosphorylation by D_1 receptor activation. The lack of detectable D_1 receptor in a subset of DARPP-32-positive cell bodies supports the idea that in these cells other receptors may regulate DARPP-32 phosphorylation.

441.19

DOPAMINE (D1) RECEPTOR IN THE CHICK FOREBRAIN. A LIGHT- AND ELECTRONMICROSCOPIC STUDY: R. Schnabel[1], S. Jiang[1], K. Braun[1], H. Stark[1]*, H. C. Hemmings[2] and P. Greengard[3]. [1]Fed. Inst. for Neurobiology, Magdeburg, Germany, [2]Cornell Medical Center, New York, [3]Rockefeller University, New York.

Auditory imprinting induces an increased metabolic activity in associative forebrain regions. In the mediorostral neostriatum/hyperstriatum ventrale (MNH) the type I neurons show a reduction of spine synapses and prolonged postsynaptic densities of the remaining synapses. From other experiments we have some evidence for the involvement of dopamine in this synaptic selection process.
The physiological effects of dopamine are mediated by two distinct receptor types. We compared the localization of D1 receptors in imprinting relevant forebrain areas of the chick detected by ligand autoradiography with the immunohistochemical demonstration of D1 receptors. Using autoradiography the highest D1 receptor densities were observed in the basal ganglia (lobus parolfactorius, paleostriatum augmentatum), amygdala (dorsal archistriatum) and dorsal and lateral caudal parts of the neostriatum and hyperstriatum ventrale. The MNH revealed moderate labeling. In general, the autoradiographical results are in accordance with the immunohistochemical pattern. Discrepancies were found in the HV including the hyperstriatal part of the MNH, which shows a high ligand binding but only few immunoreactive cell bodies and fibers. in the MNH and caudal neostriatum the immunoreaction product was found postsynaptically in symmetric and asymmetric synapses which are located on cell bodies and dendritic shafts and spines.
This work was supported by grants GSF 07 NBL 06 and 0310651 of the BMBF and Schn 505/1-2 of DFG.

SEROTONIN RECEPTORS: STRUCTURE AND STRUCTURE/FUNCTION

442.1

RARE NON-CONSERVATIVE AMINO ACID SUBSTITUTION IN THE 5-HT_7 RECEPTOR GENE FOUND IN ALCOHOLICS WITH ANTISOCIAL PERSONALITY DISORDER. U. Pesonen*, M. Koulu, M. Eggert, M. Virkkunen, M. Linnoila and D. Goldman. Laboratory of Neurogenetics, DICBR, NIAAA, Rockville, MD, USA.

Reduced central serotonergic neurotransmission has been associated with increased aggression and impaired impulse control in humans. The distribution and pharmacological properties of the serotonin 5-HT_7 receptor suggest that it may play a role in psychiatric disorders. We screened using PCR-SSCP method the coding sequence of the 5-HT_7 receptor gene for sequence variants within a Finnish population of alcoholics with violent behavior. Subjects included in the study are alcoholic offenders who were ordered to undergo forensic psychiatric examination. The controls are a random sample of healthy volunteers. In three patients with antisocial personality disorder we discovered a non-conservative amino acid substitution in the putative third intracellular loop of the receptor protein while none of the ethnical matched healthy controls had this mutation. The study provides evidence for the involvement of the 5-HT_7 receptor in antisocial personality disorder in violent alcoholic offenders.

442.2

STRUCTURE AND FUNCTION OF NATURALLY OCCURRING MUTATIONS IN THE HUMAN SEROTONIN$_{1A}$ RECEPTOR GENE. Bita Nakhai, David A. Nielsen, Bridget Giblin, Alessandro Rotondo, Gary Jenkins and David Goldman*. Section of Molecular Genetics, LN, NIAAA, Bethesda, MD 20892.

The serotonin 1A (5-hydroxytryptamine) receptor gene codes for the 5-HT_{1A} receptor. It is expressed both presynaptically in serotonergic cell bodies and in dendrites of the dorsal raphe nucleus and postsynaptically in hippocampus. Serotonin plays a key role in many physiological and behavioral functions including aggressive behavior, intolerance to delay, impulsivity, appetite and control of temperature and sleep. Serotonergic activity is regulated, in part, by binding of serotonin to the 5-HT_{1A} receptor.

Two relatively rare polymorphisms were identified in the human 5-HT_{1A} gene by SSCP analysis. Both amino acid substitutions alter the extracellular amino terminal domain. The polymorphisms encode amino acid substitutions in the 5-HT_{1A} receptor of a glycine to serine at amino acid 22 and an isoleucine to valine at amino acid 28, respectively. The polymorphic 5-HT_{1A} alleles were found in American and Finnish Caucasians and in Native Americans. To study the biological function and biochemical properties of these allelic variants we subcloned them in a eukaryotic expression vector, under the control of the CMV promoter. They were further transiently transfected into mammalian COS-7 cells to characterize 5HT_{1A} receptor function by various methods including receptor binding assays. The allelic variants did not show a remarkable difference in binding to the 5-HT_{1A} specific ligand 8-OH DPAT compared to the wild type receptor. Studies of the stably expressed variants are under way and these studies will allow us to assess effects on signal transduction and receptor regulation.

442.3

GENOMIC CLONING AND HETEROLOGOUS EXPRESSION OF A RECOMBINANT GUINEA PIG SEROTONIN 5-$HT_{1D\alpha}$-RECEPTOR. W.H.M.L. Luyten*, I. Van de Weyer, G. Nobels, P. Van Gompel, W. Gommeren, A. Lesage and J.E. Leysen. Dept. of Biochem. Pharmacol., Janssen Research Foundation, Beerse, B2340 Belgium.

Significant differences exist between the pharmacological properties of 5-HT_{1D}-receptors (Rs) in various species, rendering extrapolation of e.g. rodent data to humans hazardous. Human 5-$HT_{1D\alpha}$- and $_{1D\beta}$-R subtypes are very similar pharmacologically, but the ligand-binding properties of the latter differ significantly from those of their rat or mouse homologues, which are therefore called 5-HT_{1B}-Rs. Guinea pigs are also used frequently for pharmacological studies but their 5-HT_{1D}-Rs have not yet been cloned or expressed; only small partial sequences of their 5-$HT_{1D\alpha}$-R have been published so far (C.R. Acad. Sc. III **314**: 429 ('92); Proc. Natl. Acad. Sc. U.S.A. **91**: 3666 ('94)).

PCR-primers based on the published guinea pig 5-$HT_{1D\alpha}$-R sequence were used to amplify a ±350 bp fragment of the coding region (including transmembrane domains II through IV) starting from cDNA prepared from total RNA of guinea pig trigeminal ganglion and cerebellum. This fragment was verified by restriction digests and labelled with ^{32}P-dCTP by random-priming. A λEMBL-3 guinea pig genomic library was screened with this probe and two clearly positive plaques were identified amongst 750,000 plated (±3.5 genome equivalents). Both λ-clones were plaque-purified and partially sequenced by primer-walking (cycle-sequencing followed by resolving the reaction products on an ABI 373 automated fluorescent sequencer). The coding region was subsequently PCR-amplified with Pfu (Stratagene), subcloned into the SmaI site of pBluescript (Stratagene) and the sequence verified once more. The insert was then subcloned into the pSVL expression vector (Pharmacia) yielding pSVL/5-$HT_{1D\alpha}$-R. Membranes from COS-cells transiently transfected with pSVL/5-$HT_{1D\alpha}$-R were used for radioligand binding studies and the data were compared with those from guinea pig cerebellum membranes. The same insert was subcloned into the pRc/CMV vector (Invitrogen) and CHO-K1 cells were permanently transfected with this construct. Membranes prepared from transfectants were used for radioligand binding studies and inhibition of forskolin-stimulated cAMP formation was measured in intact CHO-cells.

442.4

CLONING AND CHARACTERIZATION OF RABBIT 5-$HT_{1D\alpha}$ AND 5-$HT_{1D\beta}$ RECEPTOR SUBTYPES: A COMPARISON OF THE PHARMACOLOGICAL PROFILE TO THE HUMAN SPECIES HOMOLOGUES. S.A. Kucharewicz, J.A. Bard, R.L. Weinshank, T.A. Branchek*. Synaptic Pharmaceutical Corporation, Paramus, NJ 07652.

Species differences in the pharmacology of 5-HT_{1D} binding sites have long been observed. In order to further explore this issue, we have cloned the rabbit 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ genes, transiently transfected them into Cos-7 cells, and assessed membrane preparations in relationship to the pharmacology of the previously cloned human homologues. The K_d of [^{3}H]-5-HT determined from saturation experiments for the rabbit 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors was 4.02 nM and 6.46 nM, respectively. The B_{max} for rabbit 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ was determined to be 235 fmol/mg and 740 fmol/mg protein respectively. The rank order of binding affinities determined from competition experiments is as follows: rabbit 1Dα, 5-CT>5-HT>5-CH_3O-tryptamine =5-CH_3-tryptamine>sumatriptan>α-CH_3-5-HT=8-OH DPAT>mCPP; rabbit 1Dβ, 5-CT =5-HT>5-CH_3O-tryptamine>5-CH_3-tryptamine>sumatriptan>α-CH_3 5-HT>8-OH DPAT >mCPP; human 1Dα, 5-CT>5-HT>sumatriptan=5-CH_3O-tryptamine>8-OH DPAT>α-CH_3 5-HT>5-CH_3 tryptamine=mCPP; human 1Dβ, 5-CT>5-HT>sumatriptan>5-CH_3O-tryptamine>α-CH_3-5-HT>8-OH DPAT>mCPP>5-CH_3 tryptamine. The differences in the rank order of binding affinities observed when comparing the cloned rabbit to the cloned human 5-HT_{1D} subtypes may be attributed to species differences in the amino acid sequences within the transmembrane domains of the species homologues. Correlation plots for rabbit vs. human 5-$HT_{1D\alpha}$ ($r = 0.82$) and 5-$HT_{1D\beta}$ ($r = 0.80$) demonstrates the similarity of the pharmacology of the species homologues. Finally, the rabbit 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors show considerable similarity in their pharmacological profiles ($r = 0.86$).

442.5

SITE-DIRECTED MUTAGENESIS OF THE HUMAN 5-HT1Dβ RECEPTOR FOR IDENTIFICATION OF AMINO ACIDS INVOLVED IN LIGAND BINDING. C. Grånäs*, G. Nordvall#, D. Larhammar. Dept. of Medical Pharmacology, Box 593, #Dept. of Organic Pharmaceutical Chemistry, Uppsala University, S-751 24 Uppsala, Sweden.

The precise structure of the 5-HT1Dβ receptor and the molecular mechanisms for ligand binding are unknown. We have made a three-dimensional model of the 5-HT1Dβ receptor based on the high-resolution structure of bacteriorhodopsin and energy minimized this model with ligands docked into the binding pocket. To evaluate the usefulness of this model and to determine the ligand-binding properties of the 5-HT1Dβ receptor we have performed site-directed mutagenesis in TM4 (Ser181 to Ala, Phe185 to Ala or Met), TM5 (Ser212 to Ala), TM6 (Phe331 to Ala or Tyr, Ser334 to Ala) and TM7 (Phe354 to Ala or Tyr). According to the model these amino acids may all interact with ligands. The indol part of 5-HT may interact with Ser181 and there may be a H-bond to sumatriptan with Ser212. An aromate ring interaction with Phe331 is predicted and Ser334 might have a specific interaction with sumatriptan, hence a mutation to Ala (as in the 5-HT1A receptor) should give more 5-HT1A-like binding properties. Phe354 is adjacent to Asn/Thr355 that accounts for the pharmacological differences between the human HT1Dβ and rodent 5-HT1B receptor. Phe185 differs between 5-HT1 receptor subtypes; we have introduced the Leu present at the corresponding position of the 5-HT1Dα receptor. The influence of these residues on binding of various ligands and the accuracy of the model predictions will be reported.

442.6

MOLECULAR CLONING OF PLANARIAN SEROTONIN RECEPTORS O.Saitoh[1], H.Orii[2], K.Agata[2], K.Watanabe[2] and H.Nakata[1]*. [1]Dept. of Mol. & Cell. Neurobiol., Tokyo Metropolitan Inst. for Neuroscience, Fuchu-shi, Tokyo 183 and [2]Dept. of Life Science, Fac. of Science, Himeji Inst. Tech., Hyougo 678-12, Japan.

Planarians are well known for their ability of regeneration. Although previous studies suggest a significant role of serotonin for the planarian regeneration, its molecular and cellular mechanisms are not well understood. We therefore explored the possibility that the planarians express novel serotonin receptors which are responsible for the regeneration process. We first analyzed ligand binding properties of crude planarian membranes and found that they possessed high affinity binding sites with the serotonergic ligand, [3H]LSD (Kd=2.6 nM). The ligand binding specificity of the planarian membranes was similar to that of Drosophila serotonin receptor 1 (5HT-dro1). We then tried to clone the corresponding receptor gene by PCR using degenerate primers. Four individual clones which contained consensus sequences of the G-protein coupled receptors were amplified from planarian cDNA. Judging from the homology comparison, all the four clones (PLAR1-4) appeared to be classified into the serotonin receptor family. A full-length cDNA clone for PLAR4 clone, which seems to be most abundantly expressed in planarians, was isolated from a planarian cDNA library. This cDNA clone encoded 478 amino acids that can be organized into seven transmembrane stretches. This amino acid sequence also showed a significant similarity with a human 5-HT1A serotonin receptor. Its physiological functions are currently under investigation.

442.7

MOLECULAR CLONING AND PHARMACOLOGICAL ANALYSIS OF NEUROTRANSMITTER RECEPTORS FROM THE CENTRAL NERVOUS SYSTEM OF LYMNAEA STAGNALIS. C. C. Gerhardt, J. Leysen1, R. J. Planta, E. Vreugdenhil and H. van Heerikhuizen*. Graduate School of Neurosciences Amsterdam, Research Institute Neurosciences Vrije Universiteit, Faculty of Chemistry, Department of Biochemistry and Molecular Biology, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands. 1Department of Biochemistry and Pharmacology, Janssen Research Foundation, Turnhoutseweg 30, B2340 Beerse, Belgium.

G-protein-mediated signal-transduction pathways are crucial in neuronal communication. To study the in vivo molecular mechanisms underlying these pathways we have adopted the giant neurons of the pond snail Lymnaea stagnalisas a model system. Because of their large size these neurons are extremely suitable for perturbation of signaling components by injection of specific 'molecular tools' (eg. expression plasmids, antisense oligonucleotides, antibodies, etc.). As a first step to generate such tools we have applied the PCR technique to amplify cDNA fragments encoding neurotransmitter receptors. Here we present data on the cloning and functional expression of three such receptors.

One of these receptors exhibits the highest similarity to the vertebrate subfamily of 5-HT2 receptors and was therefore designated 5-HTlym2. The identity of 5-HTlym2 was further investigated by its expression in HEK293 cells. As with the vertebrate 5-HT2 receptors, application of 5-HT induces an increase in intracellular IP3 concentration. Furthermore, [3H] mesulergine, a specific antagonist for mammalian 5-HT2C receptors, binds with high affinity to 5-HTlym2. This binding can be competed for by several mixed 5-HT2A/2B/2C antagonists and agonists, showing a pharmacological profile most closely related to 5-HT2B and 5-HT2C receptors

Two other neurotransmitter receptors, GRL004 and GRL007, exhibit significant sequence similarity with the tyramine receptor from Drosophila and mammalian adrenergic receptors. Expression studies show that [3H] rauwolscine, an antagonist for mammalian a-adrenergic receptors, binds to both receptors in the nanomolar range. Both adrenaline and noradrenaline are able to elicit an increase in intracellular cAMP levels. However, octopamine (the putative evolutionary precursor to adrenaline and nor-adrenaline) induces a strong increase in cAMP in GRL007 but not in GRL004, indicating that the former is likely to be the Lymnaea octopamine receptor.

442.8

IDENTIFICATION OF RAT SEROTONIN 5-HT$_{2C}$ RECEPTORS AS GLYCOPROTEINS CONTAINING N-LINKED OLIGOSACCHARIDES. J. R. Backstrom*, R. S. Westphal, H. Canton, and E. Sanders-Bush. Dept. of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232.

Antibodies against a portion of the rat 5-HT$_{2C}$ receptor third intracellular loop were generated and used to identify receptors solubilized from cell lines and rat brain. Western blots of CHAPS-soluble proteins were probed with affinity-purified anti-2C antibodies. The specificity of anti-2C was demonstrated with extracts prepared from NIH/3T3 fibroblasts which stably express functional rat 5-HT$_{2C}$ or 5-HT$_{2A}$ receptors. Extracts from the 5-HT$_{2C}$ cell line, but not the 5-HT$_{2A}$ cell line, contained immunoreactive proteins with masses of 51-52 kDa and 58-68 kDa. In the brain, the highest relative amount of immunoreactive proteins was identified in choroid plexus, with masses of 51 kDa and 58-62 kDa. The 5-HT$_{2C}$ receptor cell line (3T3/2C) was grown in the presence of tunicamycin to metabolically inhibit N-linked glycosylation. Proteins from the cell extracts were detected with masses of 40 and 41 kDa. Extracts prepared from 3T3/2C cells (grown in the absence of tunicamycin) and from choroid plexus were incubated with N-glycosidase F to enzymatically remove available N-linked sugars. Immunoreactive proteins were detected with masses of 41 and 42 kDa from 3T3/2C cells and 41 kDa from choroid plexus. Neuraminidase reduced the mass of the 51 and 58-62 kDa proteins from the choroid plexus to 50 and 54-58 kDa. In contrast, the proteins from 3T3/2C cells were not affected by treatment with neuraminidase. These results demonstrate that 5-HT$_{2C}$ receptors contain N-linked sugars and suggest that 5-HT$_{2C}$ receptors in the choroid plexus contain sialic acid residues. (Supported by NIH research grant MH34007).

442.9

IDENTIFICATION, MOLECULAR CLONING, AND DISTRIBUTION OF A SHORT VARIANT OF THE SEROTONIN 5-HT$_{2C}$ RECEPTOR PRODUCED BY ALTERNATIVE SPLICING. H. Canton*, R.B. Emeson, E.L. Barker, J.T. Lu, M.S. Chang, and E. Sanders-Bush. Dept. of Pharmacology, Vanderbilt University Medical School, Nashville, TN 37232.

S1 nuclease protection of the rat serotonin2C (5-HT$_{2C}$) receptor mRNA revealed a second short transcript. The short form was expressed in the same structures as the 5-HT$_{2C}$ mRNA, for example, choroid plexus, striatum, hippocampus, hypothalamus, olfactory tubercles, and spinal cord. However, the ratio of the short form over 5-HT$_{2C}$ mRNA varied from 0.6 in an epithelial tissue (choroid plexus) to 0.25 in striatum showing differential regulation. Cloning and sequencing revealed a second clone with a 95 nucleotide deletion in the region coding for the putative second intracellular loop and the fourth transmembrane domain of the 5-HT$_{2C}$ receptor. This deletion leads to a frameshift in the downstream sequence codin region and a premature stop codon. The deduced protein contains 172 amino-acids, with 153 residues at the amino-terminal identical to the 5-HT$_{2C}$ receptor, and 19 carboxy-terminal amino-acids that are specific. Expression of the short form in different cell types failed to reveal any serotonergic ligand binding. The two transcripts arise from a single gene by alternative splicing using two different donor sites and a common acceptor. PCR amplification of mouse or human brain cDNAs showed the occurrence of the same splicing pattern. Due to nucleotide sequence differences, the human short form encodes a putative 248 amino-acid protein with 96 specific residues on the amino-terminus. In conclusion, this study demonstrates the occurrence in rat, mouse and human of two 5-HT$_{2C}$ mRNA spliced-variants with apparent tissue-specific regulations. The functional significance of the short form remains to be established (supported by NIH research grant MH 34007).

442.10

SERINE[159] OF THE HUMAN 5HT$_{2A}$ RECEPTOR INTERACTS WITH 5HT BUT NOT LSD. N.Almaula*, B.J.Ebersole, D. Zhang, H.Weinstein and S.C.Sealfon. Mount Sinai School of Medicine, New York, N.Y. 10029.

The 5HT$_2$ receptor subtypes have been implicated in mediating the effects of hallucinogenic drugs of abuse. Mutant receptors were constructed to identify the determinants underlying the specificity of ligand interaction with the human 5HT$_{2A}$ receptor. We have identified a serine residue in transmembrane helix 3 which differentially affects the binding affinity of 5HT, tryptamine and LSD. Substitution of S3.36(159) by alanine causes an approximately 10 fold decrease in the affinity of 5HT and tryptamine, whereas the binding affinity of LSD remains unaffected. Replacement by cysteine has an intermediate effect on 5HT and tryptamine affinity whereas the affinity of the structurally unrelated ligand DOI for both mutant receptors was decreased approximately 20 fold. Ketanserin had wild-type high affinity for both mutant receptors. These results are interpreted with respect to a three dimensional molecular model of the human 5HT$_{2A}$ receptor, in terms of the different orientations of the various agonists. (Supported by NIH-NIDA DA09088, K05 DA00060 and T32 DA07135).

442.11

DETERMINANTS OF COUPLING EFFICIENCY AND SIGNAL SPECIFICITY IN THE TMH 6 ADJACENT DOMAIN OF THE HUMAN 5-HT_{2A} RECEPTOR.

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Departments of Anesthesiology, Pharmacology, Neurology , Physiology and Biophysics, and Fishberg Center in Neurobiology, Mount Sinai School of Medicine New York, NY.

Chimeras of the third cytoplasmic loop of several G-protein coupled receptors have been found to alter coupling specificity and one chimera involving only the membrane proximal segment of TMH6 of the β-adrenergic / α1 adrenergic receptor generated a constituitively active β-adrenergic receptor (Samama et al., J.Biol.Chem.268:4625, 1993). In the 5-HT receptors, there are three residues which differ in this domain between the human 5-HT_{2A} and 5-HT_{1A} subtypes. We studied the effects of exchanging these residues in the 5-HT_{2A} receptor for the corresponding 5-HT_{1A} receptor residues. A V6.36(324)T mutation caused no change in E_{max} or EC_{50} for 5-HT-stimulated phosphatidylinositol (PI) turnover , nor in binding affinities for [^{3}H]-Ketanserin or 5-HT in comparison to wild type receptor expressed transiently in COS-1 cells. Simultaneous alteration of all three residues in which the receptor subtypes differ [A6.33(321)T, C6.34(322)V, and V6.36(324)T] did not result in changes in binding affinities or receptor expression levels, but did lead to a 100-fold increase in EC_{50} and 50% reduction in E_{max} for 5-HT-stimulated PI turnover. Neither of these constructs showed significant changes in the basal level of PI turnover. These results and studies on other systems implicate this domain as a critical structural determinant in the efficiency of receptor coupling. Some mutations increase efficiency and generate constituitively active mutants whereas others, like the triple amino acid mutation, decrease coupling efficiency. The effect of these and additional mutations in this domain on coupling efficiency and specificity will be presented from the exploration of effects on PI turnover and cAMP accumulation. Supported by NIH grants DA09088, DA09083, KO5 DA00060, and NIDA training grant DA07135.

442.12

IDENTIFICATION OF RESIDUES WHICH ALTER KETANSERIN BINDING AFFINITY TO 5-HT2A RECEPTORS: Comparison with binding models for ketanserin.

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The elucidation of the mode(s) of drug binding to neurotransmitter receptors remains a major unsolved problem for modern molecular pharmacology. The approach we have taken to clarify this issue is to combine molecular modelling and molecular biology techniques. We now report our results in which more than 40 mutant 5-HT2A receptors were constructed and characterized and in which the results were compared with previously published models of ketanserin binding to 5-HT2A receptors. In addition, several ketanserin analogues were tested at mutant receptors to clarify the mode(s) of binding at the implicated sites. Chimeric receptor studies suggested that ketanserin bound to multiple helices; specific interactions were implicated between helices II-III and VI-VII. Point mutations of both conserved and non-conserved residues identified at least 5 sites which altered ketanserin's affinity for the 5-HT2A receptor (F339, M132, W336, W367, Y370). Preliminary results suggest that the nature of the substitutent on the benzyl moiety strongly affects binding to F339. The results were compared with a model for ketanserin binding (Khristianssen et al, 1994) and, in general, a good correspondence was found comparing residues predicted to be important for ketanserin binding and residues found to alter ketanserin's affinity for the 5-HT2A receptor. It was significant that residues which were found experimentally to affect ketanserin's affinity for the 5-HT2A receptor were found clustered around the predicted central core of the receptor. Supported by GM52213 (BLR).

SEROTONIN RECEPTORS: 5-HT_2

443.1

THE EFFECTS OF SELECTED 5-HT RECEPTOR AGONISTS ON NEURONAL NETWORK ACTIVITY IN MURINE SPINAL CORD CULTURES.

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The effects of 5-HT_{1A} and 5-HT_2 receptor activation on mouse spinal neurons were tested in 4-8 week old, spontaneously active monolayer networks. The cultures were grown on photoetched electrode arrays featuring 64 thin film electrodes for extracellular recordings. Spike activity was integrated for display and analysis. Bath applications of the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-N-proplamino)tetralin-HBr (8-OH-DPAT, 10-30 μM) resulted in prolonged episodes of repetitive bursting. Compared to pre-test activity, the 8-OH-DPAT-induced pattern had increased burst durations and increased interburst intervals, which produced a lower frequency of bursting. Applications of the 5-HT_2 receptor agonist [1(2,5-dimethyoxy-4-iodophenyl)-2-amino-propane-HCL (DOI, 0.2 mM) resulted in a cessation of activity that was reversible with a medium change. The 8-OH-DPAT results are consistent with the reported inhibitory action of 5-HT_{1A} agonists. DOI's blockade of activity is surprising, but may indicate 5-HT_2-receptor activation of inhibitory interneurons. Supported by the Federation of North Texas Area Universities, RO1 HD 28419 and GM 08256.

443.2

SEROTONIN EXCITES DORSAL VAGAL MOTONEURONS VIA 5-HT_{2A} RECEPTORS.

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In the rat, *in vitro* studies have shown that serotonin (5-HT) increases the excitability of dorsal vagal motoneurons (DVMs) by eliciting a direct, postsynaptic slow depolarization mediated via a reduction in K^+ conductance (Albert & Brooks, J. Physiol. 481, 23P, 1994). This study investigates in greater detail the receptor subtypes involved using selective 5-HT antagonists.

Whole-cell patch recordings in current-clamp mode were made from visually identified DVMs in 175-200 μm thick brainstem slices from 17-21 day old rats. The DVMs were identified as clusters of fusiform shaped cells between 20-30 μm in diameter.

From a holding potential of -60 mV control responses to bath applications (5ml/min) of 5-HT were obtained (6.9 ± 0.27 mV, n=35, 20 μM). Antagonists were preincubated for between 5-60 mins. The selective 5-$HT_{2A/2C}$ antagonists ketanserin (1 μM) and LY 53,857 (1 μM) both significantly blocked the response to 5-HT (20 μM) as did the 5-$HT_{1A/2A}$ antagonist, spiperone (1 μM), whereas pindobind.5-HT_{1A} (5 μM), a selective 5-HT_{1A} antagonist, had no effect. The selective 5-HT_3 antagonists MDL 72222 (10 μM) and ICS-205-930 (1 μM) had a small partial blocking effect. ICS-205-930 at higher concentrations (10 μM) produced an increase in this partial antagonism, however, complete block was never obtained.

We conclude that 5-HT increases the excitability of DVMs via a 5-HT_2 receptor, probably the 5-HT_{2A} subtype. Also, a small component could involve a 5-HT_3 response, although this requires further analysis. The involvement of 5-HT_4 receptors in this response is unlikely as ICS-205-930 was only a partial antagonist at high concentrations.

The research was supported by the Wellcome Trust.

443.3

INHIBITION OF THE 5HT_{2A} RECEPTOR-MEDIATED STIMULATION OF PHOSPHOINOSITIDE (PI) HYDROLYSIS BY AN ANTISENSE OLIGODEOXYNUCLEOTIDE IN A NEURONAL CELL LINE.

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The A1A1 variant cell line, is a descendent of the A1A1 clone which is derived from 16 day rat cortical cultures by retroviral transduction of the wildtype simian virus 40. The A1A1 variant cells endogenously express the 5HT_{2A}, adenosine-2, and bradykinin receptors and exhibit an amplified second messenger response as compared to the parent line (see Berg et al. Mol. Pharm. 45:826, 1994). In A1A1 variant cells quipazine acted as a partial agonist (intrinsic activity of 0.75 in reference to the full agonist 5HT) with an EC_{50} of 713 nM and E_{max} 119% above basal. Cells were treated with vehicle, antisense or control oligos (0.2μM cumulative) for 5 days in a serum-free medium. Cells were pre-labelled with 2 μCi of ^{3}H-myoinositol for PI hydrolysis assays or 1 μCi of ^{3}H-thymidine to assess oligo-induced cytotoxicity. On the sixth day ^{3}H-thymidine uptake was measured or cells were stimulated with quipazine and total inositol phosphate (IP) accumulation determined. The antisense oligo treatment decreased the efficacy of the partial agonist quipazine (antisense: E_{max} = 55.3 ± 4.4 % of control E_{max}) with no change in the potency (antisense: pEC_{50} = 5.71 ± 0.21; vehicle: pEC_{50} = 6.15 ± 0.16; p≤0.1). The sense, random and mismatch controls had no affect on the E_{max} or EC_{50} of quipazine to stimulate PI hydrolysis. ^{3}H-thymidine uptake was unaffected by the presence of either the antisense or control oligos. The data indicate that an 18-base antisense oligo attenuates 5HT_{2A} receptor-mediated second messenger function without affecting cell proliferation. Currently, the effect of the antisense oligo on receptor number, the bradykinin receptor response and ribonuclease protection assays are being performed. Antisense oligodeoxynucleotides may prove to be more selective tools than traditional agonists and antagonists for investigations into the role of central 5HT_{2A} receptors *in vivo*. Supported by USPHS grant MH52369.

443.4

5-$HT_{2A/2C}$ ACTIVATION PRODUCES VARIABLE EFFECTS ON SEROTONIN FIBER GROWTH AND SURVIVAL AFTER LESIONING BY PCA OR MDMA.

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Substituted amphetamines (SA) like 3,4-methylenedioxymethamphetamine (MDMA) and p-chloroamphetamine (pCA) are specific serotonergic (5-HT) neurotoxins which promote the release of monoamines (5-HT and DA). We have proposed the involvement of the 5-HT-2a/2c receptor in the mechanisms that promote the degeneration of fine 5-HT axons in the forebrain. This receptor has been shown to become transiently downregulated after chronic MDMA-exposure; possibly through increased synaptic 5-HT levels. MDMA and 5-HT have also been shown to increase cerebral glycogenolysis and promote PKC activation in whole brain and cortical astrocytes via this receptor. Consequently, SA's may deplete 5-HT synapses of vital energy reserves after the prolonged activation of this receptor. We now examine the effect of 5-HT-2a/2c receptor activation ***after*** a SA-mediated de-afferentation of 5-HT fibers, to observe how an increase in glucose availability to injured 5-HT axons influences their re-innervation.

Male Sprague-Dawley rats were injected (s.c.) with MDMA (6 x 20 mg/kg), pCA (1 x 10 mg/kg) or saline. One week later, rats received saline or the 5-HT-2 agonist, (±)-DOI•HCl (0.5, 2.5, or 5.0 mg/kg), for 3 days. Seven days later, the density and activity of forebrain and brainstem 5-HT nerve terminals were quantified by ^{3}H-paroxetine (^{3}H-PAR) binding and ^{3}H-5-HT uptake.

MDMA and pCA exposure significantly reduced the density of forebrain (cortical) ^{3}H-PAR binding sites by 45.5% and 85.3%, respectively. Post-lesion DOI exposure significantly **(p≤ 0.01)** returned both the activity and density of forebrain 5-HT axons after MDMA to levels comparable to saline treated controls. Conversely, DOI ***potentiated*** the loss of CTX and HIP 5-HT terminals after pCA in a dose-dependent fashion. However, in the brainstem, where the extent of the pCA lesion is lower (- 40%), DOI post-treatment only enhanced neurotoxicity by 29.3% compared to pCA alone. These results indicate that 5-HT axons lesioned by MDMA or pCA show differences in their potential for and rate of re-innervation. This effect may be related to the extent of the initial denervation, and to the density of astrocytes which increase glucose availability to damaged fibers through activation of the 5-HT-2 receptor. (NIDA# 271-90-7403)

443.5

PCA AND MDMA ACTIVATE RAT GLYCOGEN PHOSPHORYLASE *IN VIVO* VIA THE 5-HT$_{2A/C}$ RECEPTOR. J.C. Poblete*, J.C. Rios', E.C. Azmitia', and C.W. Harley². 1-Biology Dept., New York Univ., NY, NY 10003; 2-Psych Dept., Memorial Univ., St. John's, Newfoundland, Canada A1B 3X9.

Glycogen levels in the brain are regulated by neurotransmitters, neuropeptides, and ions Glycogen is mainly localized in astroglial cells in the CNS. Functional serotonin (5-HT) receptors are expressed in these astrocytes, and 5-HT is known to stimulate glycogenolysis in astrocytes. Substituted amphetamines (SA), like 3,4-methylenedioxymethamphetamine (MDMA) and p-chloroamphetamine (PCA), are potent 5-HT releasers that also inhibit 5-HT reuptake and monoamine oxidase-A (MAO-A) activity. As a consequence of MDMA or PCA administration, extracellular levels of 5-HT are increased acutely. Recently, we reported that 5-HT and MDMA activate glycogen phosphorylase (GPase) in cortical astroglial-rich primary cultures, which can be attenuated by mianserin, a 5-HT$_2$ antagonist. In addition, 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane (DOB), a 5-HT$_2$ agonist, also activates GPase in these cultures. These results indicate that astroglial glycogenolysis is controlled by the 5-HT$_{2A/C}$ receptor.

In the present study, the activation of GPase *in vivo* was assessed after the administration of PCA (10 mg/kg, sc.) or (±)MDMA (40 mg/kg, sc.) into adult Sprague-Dawley rats. Active glycogen phosphorylase (αGPase) was assayed histochemically 15 min. and 30 min. after MDMA and PCA, respectively. The relative optical densities (ROD) were determined using a computer-assisted densitometric software (Optimas). PCA and MDMA increased αGPase activity in layers 4, 5b and 6 of the neocortex compared to corresponding saline-treated controls. In addition, PCA produced a longer activation profile than MDMA. The activation of GPase was most significant in the parietal cortex, in which PCA increased the ROD in layer 4 (26 %), layer 5b (29 %), and layer 6 (69 %).

Our results indicate that 5-HT releasers, like PCA and MDMA, activate glycogen phosphorylase *in vivo*. The pattern of αGPase activation parallels the cortical distribution of 5-HT$_{2A}$ mRNA, and the decreases in 5-HT-IR axons produced by PCA and MDMA. These findings suggest that increased extracellular 5-HT activates GPase in cortical astrocytes via the 5-HT$_{2A}$ receptor. The possible relationship between GPase activation and SA-induced neuropathology will be discussed.

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443.6

IN VIVO STUDIES OF 5-HT2A RECEPTORS USING [C-11]-LABELLED MDL 100907. C.A. Mathis*, J.M. Gerdes, N.R. Simpson, J.C. Price, Y. Huang, K. Mahmood, K. Urso, M.A. Mintun. PET Facility, University of Pittsburgh Medical Center, Pittsburgh, PA 15213

MDL 100907, a potent and selective 5-HT2A antagonist, was radiolabelled with carbon-11 (20 min half-life) at either the 2- or 3-methoxy positions and evaluated in rats and rhesus monkeys. MDL 100907 is highly selective for the 5-HT2A receptor, possessing a 300-fold in vitro binding selectivity for this site over 5-HT2C, alpha-1, and D2 receptors. The less active C-11-labelled (S)-(-)-enantiomer (termed MDL 100009) was also evaluated.

In vivo distribution studies in male Sprague-Dawley rats indicated that both C-11-labelled MDL 100907 radioligands were taken up and regionally distributed in rat brain in a manner consistent with the known distribution of 5-HT2A receptors. Frontal cortex-to-cerebellum ratios (a measure of total-to-nonspecific binding) reached values >10:1 at 90 min post injection. Their specific uptake was blocked by pretreatment with altanserin (2 mg/kg) 5 min prior to their iv injection. The in vivo brain distribution of C-11-labelled MDL 100009 indicated the lack of regional localization in brain areas known to contain high concentrations of 5-HT2A receptors such as the frontal cortex.

In vivo PET imaging studies in rhesus monkeys with the two C-11-MDL 100907 radioligands resulted in high uptake and regional localization for both compounds consistent with the known distribution of 5-HT2A receptors in primate brain. The regional brain concentrations of these two radioligands in monkeys were similar with frontal cortex-to-cerebellum ratios >3:1 at 90 min post injection. Radiolabelled metabolites in the plasma of monkeys were determined for both of the C-11-MDL 100907 radioligands, and the 2-methoxy-labelled compound led to the formation of a lipophilic metabolite which was absent with the 3 methoxy-labelled compound. Injection of C-11-MDL 100009 in monkeys resulted in a uniform brain distribution without regional localization consistent with the lower affinity of this enantiomer for 5-HT2A receptors. These results indicate that C-11-MDL 100907 labelled in the 3-methoxy position is a useful agent to image 5-HT2A receptors in vivo using PET.

443.7

NOVEL STRUCTURAL CLASS OF COMPOUNDS INHIBITING SEROTONIN INDUCED RAT PAW INFLAMMATION; IDENTIFICATION OF 5HT$_2$ SUBTYPE RECEPTOR ANTAGONISTS. G. Koppel, D. Nelson, J. Reel, R. Simon, D. B. Wainscott, C. Whitesitt, H. Cole and H. Bryant*. Lilly Research Laboratories, CNS & Endocrine Divisions, Indianapolis, IN 46285.

Serotonin (5HT) is an important mediator of acute inflammatory responses due to its role in altering vascular tone and permeability which contribute to fluid extravasation. Intraplantar injection of 5HT produces a rapid, concentration dependent increase in paw weight and volume which is prevented by non-selective 5HT$_2$ antagonists (i.e. ketanserin, mianserin). While the pro-inflammatory activity of 5HT is likely mediated by 5HT$_2$ receptors, the subtype involved is unclear. Here we report the effects of a new structural class with relative selectivity for 5HT$_{2A}$ receptors which show potent activity at inhibiting 5HT-induced paw edema in the rat. Radioligand binding studies were performed with 125I DOI on human 5HT$_{2A}$ and 5HT$_{2C}$ receptors and with ^{3}H-5HT on human 5HT$_{2B}$ receptors. LY314228, LY320951 and LY320954 displayed higher affinity for the 5HT$_{2A}$ receptor. When compared to binding at other 5HT receptor subtypes, LY314228 had a 21 fold greater affinity for the 5HT$_{2A}$ site over the 5HT$_{2B}$ site, and 2.4 fold greater at the 5HT$_{2C}$ site. The compounds were also evaluated for effects on paw swelling in SD rats. With this model, all three compounds displayed potent inhibition of 5HT-induced swelling with ED$_{50}$'s of 2, 3.1 and 5.5 mg/kg (for LY314228, LY320951 and LY320954, respectively). These studies demonstrate the anti-inflammatory activity of a novel class of pharmacophores for the 5HT receptor family which show greater relative affinities for the 5HT$_{2A}$ receptor subclass.

443.8

HALLUCINOGENS ARE POTENT PARTIAL AGONISTS AT 5-HT$_{2A}$ RECEPTORS ON INTERNEURONS IN RAT PIRIFORM CORTEX.

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A subpopulation of interneurons in rat piriform cortex are excited by 5-HT via 5-HT$_{2A}$ receptors. Since controversy exists regarding whether LSD is a partial agonist (Sanders-Bush et al.,1988) vs antagonist (Pierce & Peroutka, 1990) at cortical 5-HT$_{2A}$ receptors, we explored whether LSD and a phenethylamine hallucinogen, DOI, are agonists, partial agonists or antagonists at 5-HT$_{2A}$ receptors on interneurons in piriform cortex. Extracellular studies were performed on interneurons excited by 5-HT near the borders of layers II and III while intracellular voltage-clamp studies were performed on pyramidal neurons in layer II. Low concentrations of LSD (10-100 nM) and DOI (0.3-3 μM) applied for 10 min excited almost every interneuron that was excited by 5-HT. The maximal excitation achieved with LSD and DOI was 44 and 54%, respectively, of that observed after a near maximal concentration of 5-HT (100 μM). Consistent with a partial agonist action, only the highest concentration of the hallucinogens occluded the maximal excitation by 5-HT. The excitation by NE was not affected by the hallucinogens while the excitation by the excitatory amino acid agonist AMPA was potentiated. MDL 100,907 (30 nM), a selective 5-HT$_{2A}$ antagonist, blocked the excitation of interneurons by LSD and DOI as well as the potentiation of excitation by AMPA. The excitation of interneurons by 5-HT and DOI induced inhibitory post-synaptic currents (IPSPs) in pyramidal cells. As expected from the extracellular studies, DOI (10 μM) induced fewer IPSCs than did 5-HT (100 μM). These findings suggest that the effects of hallucinogens at 5-HT$_{2A}$ receptors in the cortex are due to a potent partial agonist rather than antagonist action.

443.9

[^{3}H]RAUWOLSCINE AS AN ANTAGONIST RADIOLIGAND FOR THE CLONED HUMAN SEROTONIN$_{2B}$ (5-HT$_{2B}$) RECEPTOR. D.L. Nelson*, M. Baez, J.D. Kursar, D.A. Sasso, and D.B. Wainscott. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

We have previously reported the cloning and pharmacologic characterization of the cloned rat and human 5-HT$_{2B}$ receptors, using [^{3}H]5-HT. One limitation to the use of agonist radioligands is that they typically do not readily allow the study of the agonist low-affinity state of G protein-coupled receptors. Since no high affinity antagonist radioligand had been reported for the 5-HT$_{2B}$ receptor, the present work was undertaken. When examining the pharmacologic profile of the 5-HT$_{2B}$ receptor, it was found that rauwolscine had sufficiently high affinity for the human receptor (K_i human = 14.6 ± 1.2 nM, compared to K_i rat = 35.8 ± 3.8 nM). Therefore, [^{3}H]rauwolscine was examined for its usefulness as a radioligand for the cloned human 5-HT$_{2B}$ receptor expressed in AV12 cells. Initially, biphasic competition curves were obtained against [^{3}H]rauwolscine binding. This was due to [^{3}H]rauwolscine binding to an α_2-adrenergic receptor present in the AV12 cell line. Efaroxan was found to block [^{3}H]rauwolscine binding to the α_2-adrenergic receptor without significantly affecting binding to the 5-HT$_{2B}$ receptor and was therefore included in all subsequent studies. In saturation studies, [^{3}H]rauwolscine labeled a single population of binding sites, K_d = 3.32 ± 0.21 nM and B_{max} = 383 ± 69 fmol of 5-HT$_{2B}$ receptors / mg of protein. This compares to 331 ± 43 fmol of 5-HT$_{2B}$ receptors / mg of protein for [^{3}H]5-HT binding. Certain antagonist structures displayed higher affinity for [^{3}H]rauwolscine-than for [^{3}H]5-HTlabeled 5-HT$_{2B}$ receptors. Competition curves for antagonists had slope values of unity, which was not the case for agonists. [^{3}H]Rauwolscine is a useful antagonist radioligand for the cloned human 5-HT$_{2B}$ receptor and will aid in characterizing the properties of both the agonist high- and low-affinity states of this G protein-coupled receptor.

443.10

COMPARISON OF 5- and 7-SUBSTITUTED TRYPTAMINES AND DERIVED TETRAHYDRO-ß-CARBOLINES AT THE CLONED RAT SEROTONIN$_{2B}$ (5HT$_{2B}$) RECEPTOR. J.S. Nissen, J.J Droste, D.B. Wainscott, V.L. Lucaites, D.L. Nelson and J.E. Audia*. Lilly Research Laboratories, Eli Lilly & Company, Indianapolis, IN 46285.

Previous studies from these laboratories have demonstrated high affinity binding of 5-substituted tryptamines at the cloned rat 5HT$_{2B}$ receptor (Wainscott et al., *Mol. Pharmacol.* **1993**, *43*, 419). The current work broadens the investigation of structure activity relationships to include both 5- and 7-substituted tryptamines and their derived 6- and 8-substituted tetrahydro-ß-carbolines. Simple 5-substituents such as halogen (F, Cl, Br), OMe and OH confer high affinity upon the tryptamines. Analogous 6-substitution of the tetrahydro-ß-carbolines, however, gives rise to relatively low affinity binding at the 5HT$_{2B}$ receptor, with little correlation to the tryptamine SAR. High affinity binding was also observed with the 7-substituted tryptamines. In this case, conversion to the 8-substituted tetrahydro-ß-carbolines increased affinity approximately 2-3 fold relative to the tryptamines, while maintaining identical rank-order of potency. Functionally, these tryptamines showed agonist effects while the tetrahydro-ß-carbolines lacked intrinsic activity (IP$_3$ response). These studies provide insights into recognition and activation requirements for the rat serotonin 5HT$_{2B}$ receptor and the binding orientation of ligands at this site.

443.11

SB 206553: IN VITRO AND IN VIVO CHARACTERIZATION OF A POTENT 5-HT$_{2C/2B}$ RECEPTOR ANTAGONIST.

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We have previously reported on the synthesis and characterization of SB 200646A the first selective 5-HT$_{2C/2B}$ receptor antagonist (Forbes et al., 1994: *J. Med. Chem.*36,1104). We now report on the pharmacological characterization of a more potent, high affinity compound SB 206553A (5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-f]indole) with approximately 100 fold selectivity for the 5-HT$_{2C/2B}$ receptor over other neurotransmitter receptors. Characterisation of SB 206553 in functional studies measuring 5-HT-stimulated phosphoinositol hydrolysis as a model of 5-HT$_{2C}$ receptor activation revealed the compound to be a competitive antagonist with a pK$_B$ 8.8 compared to a pKi of 8.7 as measured against [^{3}H]-mesulergine binding in human HEK 293 cells transfected with the 5-HT$_{2C}$ receptor. SB 206553 was also shown to be a competitive antagonist (pA$_2$ 8.4) of 5-HT$_{2B}$ receptors in the rat stomach fundus assay. In vivo, SB206553 was approximately 3-4 fold more potent than SB 200646A at blocking m-CPP-induced hypolocomotion (ID$_{50}$ 5.5 vs 19.2 mg/kg p.o. respectively). Also, like SB 200646, SB206553 was shown to possess anxiolytic-like properties in rodent models. Thus SB 206553 represents an important tool to probe the therapeutic potential of 5-HT$_{2C}$/5-HT$_{2B}$ receptor antagonists.

443.12

FURTHER IN VIVO CHARACTERISATION OF SB 206553, A 5-HT$_{2C}$ / 5-HT$_{2B}$ RECEPTOR ANTAGONIST. G.A. Kennett, F. Bright, J. Cilia, D. C. Piper, N.Upton, M. L. Evans* and T.P. Blackburn, Dept of Psychiatry Research, SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW, U.K.

SB 206553 is a high affinity 5-HT$_{2C}$ and 5-HT$_{2B}$ receptor antagonist with over 100 fold selectivity over the 5-HT$_{2A}$ and other sites (see Blackburn et al., this meeting). It is also a potent antagonist (ID$_{50}$ 5.5 mg/kg p.o.) of mCPP-induced hypolocomotion, a model of 5-HT$_{2C}$ / 5-HT$_{2B}$ receptor function in vivo (Kennett et al., 1994, Brit. J. Pharmacol., 111: 797). SB 206553 increased total interaction scores in a 15 min rat social interaction test (method as in Kennett et al., 1994) under high light unfamiliar conditions over a dose range of 2-20 mg/kg p.o. given 1 h pretest. This profile may be consistent with anxiolysis. In a rat Geller-Seifter conflict test of anxiety (method as in Kennett et al., 1995, Psychopharmacol., 118: 178), SB 206553 increased punished responding between 2-40 mg/kg p.o. given 1 h pretest, but had little effect on unpunished responding. In a third model of anxiety using a second species, the marmoset conflict test (method as in Kennett et al., 1995), SB 206553 also increased suppressed responding. The anxiolytic-like profile of SB 206553 in three models of anxiety with different aversive and motivational components is compelling evidence that SB 206553 may be anxiolytic.

Recently, mutant mice lacking 5-HT$_{2C}$ receptors were reported to suffer from epilepsy and obesity (Tecott et al., 1995, Nature 374: 542). However, chronic administration of SB 206553 (5 or 40 mg/kg p.o. b.i.d. x 14) had no effect on rat body weight compared to vehicle treated controls, while acutely, 50 or 100 mg/kg p.o. 1 h pretest, had no effect in the mouse maximal electroshock (MES) threshold test (method as in Loscher and Schmidt 1988, Epilepsy Res., 2: 145).

443.13

REGULATION OF SEROTONIN 5-HT2 RECEPTORS BY A SIGMA LIGAND NE-100. N.Narita*, K.Hashimoto, S.Tomitaka, Y.Minabe and K.Yamazaki. Natl. Inst. Neurosci., NCNP, Tokyo 187 and Dept. of Psychiatry, Tokai Univ. Sch. of Med., Kanagawa, Japan.

There is evidence that sigma receptors may play a role in psychosis, and might be involved in the pathophysiology of schizophrenia. Some studies suggest that sigma ligands and serotonin 5-HT2 receptor antagonists lack the extrapyramidal side effects associated with dopamine D2 receptor antagonists. Therefore, sigma ligands and 5-HT2 receptor antagonists are a target for the development of atypical antipsychotic drugs. It is reported recently that NE-100 (Taisho Pharmaceutical. Co. Ltd.) is a novel selective and high potent ligand for sigma receptors. In this study, we examined the effects of NE-100 on the serotonergic neurons in rat brain. A single administration of NE-100 (3 and 10 mg/kg, i.p.) did not affect the contents of monoamines (5-HT and dopamine) and their metabolites (5-HIAA, DOPAC, HVA) in the brain. However, the Kd values of [3H]ketanserin binding to 5-HT2 receptors were increased significantly by a single injection of NE-100. Furthermore, subchronic administration of NE-100 (3 mg/kg, i.p., twice daily, 14 days) also did not affect the contents of monoamines and its metabolites. However, the Kd values of [3H]ketanserin binding were significantly increased at 24 hrs, 7 days and 14 days after final administration of NE-100. These results suggest that a sigma ligand NE-100 may regulate 5-HT2 receptor function in the brain. Thus, the regulation of 5-HT2 receptors by NE-100 suggests that this new potential antipsychotic drug may exert antipsychotic effects without producing significant extrapyramidal side effects.

443.14

OLANZAPINE EXHIBITS SIMILAR ACTIVITIES TO CLOZAPINE AT CLONED HUMAN SEROTONIN 2$_A$, 2$_B$, 2$_C$ AND MUSCARINIC RECEPTORS. V. L. Lucaites, F. P. Bymaster*, D. B. Wainscott, J. F. Falcone, M. Baez and D. L. Nelson. Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285

Antipsychotic agents are believed to control the positive symptoms of schizophrenia by blocking dopamine D$_2$ receptors. Control of the negative symptoms of schizophrenia is one of the benefits of the so-called "atypical" antipsychotics and may involve drug interaction at other neurotransmitter receptor systems. This study compares the binding and second messenger profiles of the atypical antipsychotics clozapine and olanzapine, as well as risperidone and haloperidol, at the human 5-HT$_2$ receptor family and the human muscarinic receptor subtypes. Clozapine and olanzapine showed no agonist activity at any serotonergic or muscarinic receptors. At the 5-HT$_{2A}$ receptor, olanzapine exhibited higher binding affinity (K$_i$) and greater potency in blocking 5-HT-stimulated increases in IP$_3$ (IC$_{50}$) than clozapine, with less difference at the 5-HT$_{2B}$ receptor.

	5-HT$_{2A}$ K$_i$ (nM)	5-HT$_{2A}$ IP$_3$ IC$_{50}$ (nM)	5-HT$_{2B}$ K$_i$ (nM)	5-HT$_{2B}$ IP$_3$ IC$_{50}$ (nM)
Olanzapine	2.5 ± 0.3	36.3 ± 11	11 ± 1.7	42 ± 7.8
Clozapine	6.5 ± 0.3	154 ± 22	7.3 ± 1.0	29 ± 7.4

Olanzapine showed lower binding affinity than clozapine (K$_i$= 2.5 ± 0.3 and 1.4 ± 0.3 nM) and less potency in blocking oxotremorine-M induced stimulation of [^{3}H]-arachidonic acid release in m1 muscarinic receptors (IC$_{50}$= 680 ± 295 and 272 ± 69 nM respectively) with similar results at m3 and m5 receptors. Thus olanzapine shows a profile similar to clozapine with these receptors.

443.15

ATYPICAL ANTIPSYCHOTICS BLOCK 5-HT-MEDIATED EXCITATIONS IN SEPTOHIPPOCAMPAL GABAERGIC NEURONS. W. Liu* and M. Alreja Dept. of Psychiatry, Yale Univ. Sch. of Medicine, CMHC, New Haven, CT 06508.

We recently reported that 5-HT excites GABAergic neurons in the rat medial septum/diagonal band complex (MSDB) via multiple 5-HT receptors, including the 5-HT$_{2A}$ subtype (Alreja, in press). Since a sub-population of MSDB GABAergic neurons projects to the hippocampus, we tested the effect of 5-HT on antidromically-activated septohippocampal neurons (SHNs) using extracellular recording techniques in a sagittal brain slice preparation that contained the MSDB and the dorsal fornix. Additionally, since atypical antipsychotics have been reported to target the 5-HT$_{2A}$ and other 5-HT receptor subtypes, we examined the effect of these drugs on 5-HT-mediated excitations in MSDB neurons.

The effect of bath-applied 5-HT (10-100 μM) was tested in a total of 119 neurons out of which 97 were antidromically identified as SHNs. Antidromic latency measurements indicated that the 5-HT-responsive SHNs had fast conducting fibers (presumably, GABAergic; mean conduction velocity - 1.62 ± 0.08 m/s; range - 0.2 to 3.7 m/s; n=76). 79% of the SHNs tested were excited by 5-HT, 18% were inhibited and 3% showed no response to 5-HT. A 20 min bath application of MDL 100,907 (30 nM), a highly selective and potent 5-HT$_{2A}$ receptor antagonist, blocked 5-HT-induced excitations in 78% of the SHNs tested with a mean pA$_2$ value of 8.51 ± 0.14 (n=19). The atypical antipsychotics, risperidone and clozapine also blocked 5-HT-mediated excitations with pA$_2$ values of 8.82 ± 0.17 (n=4) and 7.06 ± 0.11 (n=7), respectively. Haloperidol, a typical antipsychotic, blocked 5-HT-mediated excitations with a relatively lower pA$_2$ value of 5.49 ± 0.3 (n=3). In conclusion, 5-HT excites septohippocampal GABAergic neurons and the MSDB may represent an important site of action for the atypical antipsychotic drugs.

443.16

DIFFERENCES IN THE INOSITOL 1,4,5-TRISPHOSPHATE PROFILES OF TRANSFECTED HUMAN 5-HT$_{2A}$ AND 5-HT$_{2C}$ RECEPTORS, S.J. Briddon, R.A. Leslie* and J.M. Elliott, Dept. Clinical Pharmacology, University of Oxford, Radcliffe Infirmary, Oxford, England. OX2 6HE.

We have used the human neuroblastoma cell line SH-SY5Y, transfected with the cDNAs for the human 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors to study the adaptive response of these receptors to 5-HT stimulation. Clones were isolated which expressed similar numbers of receptors and gave a dose-dependent production of inositol 1,4,5-trisphosphate in response to 5-HT. The time-course of 5-HT stimulated Ins(1,4,5)P$_3$ production consisted, in both cases, of an initial peak falling rapidly to a plateau level of Ins(1,4,5)P$_3$ production. However, the peak in Ins(1,4,5)P$_3$ levels occurred earlier for the 5-HT$_{2C}$ receptor (≈5s) than for the 5-HT$_{2A}$ receptor (≈20s). A similar response has also been described for the endogenous muscarinic-m$_3$ receptor in this cell line, and is caused by a desensitization at the receptor level. We have confirmed this difference in desensitization between the two 5-HT$_2$ receptors by measuring the accumulation of total [^{3}H]inositol phosphates in the presence of lithium. For both receptors, prolonged exposure to 5-HT caused homologous desensitization of the resulting PI response which was both time and concentration dependent. When exposed to 1μM 5-HT for up to 120 min, both the rate and extent of 5-HT$_{2C}$ receptor desensitization was markedly greater than that for the 5-HT$_{2A}$ receptor (t$_{1/2}$= 12.5 min vs. t$_{1/2}$=110 min).

This data suggests that the human 5-HT$_{2C}$ receptor is more susceptible to agonist-induced desensitization than the 5-HT$_{2A}$ receptor when expressed in this cell line.

443.17

FURTHER CHARACTERIZATION OF AGONIST-INDUCED 5-HT$_{2A}$ RECEPTOR UP-REGULATION IN CEREBELLAR GRANULE CELLS. H. Li*, H. Chen, C. Hough, K.-G. Zhang and D.-M. Chuang Section on Molecular Neurobiology, Biological Psychiatry Branch, NIMH, NIH,Bethesda, MD 20892

We have reported that the levels of 5-HT$_{2A}$ receptor binding sites and mRNA are up-regulated following persistent stimulation of 5-HT$_{2A}$ receptors in cerebellar granule cells (CGC) (Mol. Pharmacol. 43:349-355, 1993). The present study was undertaken to further characterize this up-regulation process in CGC. The increase in the Bmax of 5-HT$_{2A}$ receptor binding sites induced by DOI, a 5-HT$_{2A}$ receptor agonist, was reversible; the up-regulated 5-HT$_{2A}$ receptor sites returned to basal level within 32 hours after DOI wash-out. The DOI up-regulation decreased, while basal level of 5-HT$_{2A}$ receptor sites increased with the age of CGC in culture. The up-regulation was blocked by ritanserin, a 5-HT$_{2A}$ receptor antagonist, indicating that it is receptor-mediated. The increase was also largely inhibited by depletion of extracellular Ca^{2+} or inclusion of SK&F 96365 or $LaCl_3$ during DOI stimulation. Moreover, calmidazolium prevents DOI-induced increase of 5-HT$_{2A}$ receptor sites and mRNA. These results suggest an involvement of Ca^{2+} influx and a Ca^{2+}/calmodulin dependent process in the agonist-induced 5-HT$_{2A}$ receptor up-regulation in CGC, possibly at the level of transcription.

443.18

DIFFERENTIAL REGULATION BY CORTICOSTEROIDS OF 5-HT$_{2A}$ RECEPTORS IN P-11 AND C-6 CELLS. E. Saiff*[1], J. Goska[1], E. Sibille[1], M. Toth[2], L. Pohorecky[2], D. Benjamin[3]. [1]Ramapo College, Mahwah, N.J. 07430. [2]Cornell University Medical College, N.Y., N.Y. 10024. [3]Rutgers University Center of Alcohol Studies, Piscataway, N.J. 08855.

Individual variations in the functioning of both the central serotonergic (5HT) system and the hypothalamic-pituitary-adrenal axis (HPA) have been implicated in the etiology of affective disorder, drug addiction and alcoholism. Understanding 5HT-HPA interactions is essential for the understanding and effective treatment of these disorders. Hypercortisolemia and an increase in 5-HT$_{2A}$ binding are frequently-reported biological correlates of depression. 5-HT release activates the HPA via 5-HT$_{2A}$ receptors, and there is evidence for 5-HT$_{2A}$ upregulation in response to glucocorticoids (GCCs) *in vivo*; we speculate that a high HPA response to chronic stress is the basis of a positive feedback cycle contributing to the pathogenesis of depression.

P-11 cells, isolated from rat pituitary tumor, and C-6 rat glioma cells express the 5-HT$_{2A}$ receptor and are convenient models for study of 5-HT$_{2A}$ regulation *in vitro*. We report that 50 hours of incubation with dexamethasone (DEX), or corticosterone upregulate the 5-HT$_{2A}$ receptor in P-11 cells two to three fold. 5-HT$_{2A}$ mRNA is also increased. DEX downregulates 5-HT$_{2A}$ receptors and mRNA approximately 50% in C-6 cells.

Demonstration of a cell-type dependent, differential regulation of 5-HT$_{2A}$ expression by GCCs may contribute to an eventual understanding of physiological mechanisms underlying the behavioral consequences of exposure to chronic stress.

443.19

EFFECT OF 5-HT2A RECEPTOR DOWNREGULATION ON 5-HT$_1$A RECEPTOR SENSITIVITY *IN VIVO*. K.A. Truett* and J.G. Hensler. Dept. of Pharmacology, Univ. of Texas Health Sci. Center, San Antonio, TX 78284.

Treatment of rats with antidepressant drugs, 5-HT$_1$A agonists, or 5-HT$_2$A agonists results in the downregulation and desensitization of 5-HT$_2$A receptors and the desensitization of 5-HT$_1$A responses. The goal of these studies was to determine whether the desensitization of 5-HT$_1$A receptors *in vivo* occurs as a result of 5-HT$_2$A receptor downregulation. Rats were treated with the 5-HT$_2$A receptor antagonist ketanserin (10 mg/kg, i.p., b.i.d., for 21 days). 5-HT$_1$A receptor sensitivity was assessed by measuring DPAT-induced hypothermia (0.05 mg/kg, sc.) twenty-four hours after the last ketanserin injection. [^{3}H]-ketanserin binding to 5-HT$_2$A receptors in cortical homogenates revealed a decrease in B_{max} (saline-treated: 166±10 fmol/mg protein; ketanserin-treated: 106±6 fmol/mg protein) with no change in K_D as a result of ketanserin treatment. Chronic ketanserin treatment did not alter DPAT-induced hypothermia (maximal decrease in body temperature for saline = 1.9±0.3°C; for ketanserin = 1.8±0.2°C; n=5). In a second study, rats were treated with mianserin, a 5-HT$_2$A antagonist that downregulates 5-HT$_2$A receptors after a single injection (10 mg/kg, sc.). [^{3}H]-ketanserin binding showed that a single injection of mianserin resulted in the downregulation of cortical 5-HT$_2$A receptors (B_{max}: saline-treated: 182±12 fmol/mg protein; mianserin-treated: 76±4 fmol/mg protein). Twenty-four hours after mianserin injection, DPAT-induced (1 mg/kg, sc.) forepaw treading was used as a measure of 5-HT$_1$A receptor sensitivity. Repeated measures ANOVA found no treatment effect of mianserin on DPAT-induced forepaw treading ($p = 0.13$; $n = 6$). These data indicate that 5-HT$_2$A receptor downregulation alone is not sufficient to cause 5-HT$_1$A receptor desensitization. Future studies will test whether 5-HT$_2$A receptor activation, which may subsequently downregulate 5-HT$_2$A receptors, is required to desensitize 5-HT$_1$A responses. Supported by USPHS grant MH52369.

443.20

DEVELOPMENT OF MONOCLONAL ANTIBODIES TO THE SEROTONIN 5-HT2A RECEPTOR. C. Wu, E. Yoder+, L. Shi, J. Wei, P. Dias, C.R. Monell, M. H. Ellisman+, and S. Singh*. PharMingen, San Diego, CA 92121 and +San Diego Microscopy and Imaging Resource, Department of Neurosciences, UCSD School of Medicine, La Jolla CA 92093.

Serotonin (5-Hydroxytryptamine; 5-HT) mediates many nervous system functions by its interaction with specific receptors. Physiological, pharmacological, and molecular cloning studies have identified fourteen serotonin receptors belonging to seven families. We have developed monoclonal antibodies (mAbs) directed against one of these receptors, the 5-HT2A. To generate mAbs against the 5-HT2A receptor (5-HT2AR), mice were immunized with GST-fusion proteins containing either the N- or C-terminus domain of the 5-HT2AR. The hybridomas were initially screened by ELISA against the corresponding recombinant proteins or the GST protein. Hybridomas positive for only GST-5-HT2AR were selected for reactivity in western blots to identify clones which specifically reacted with insect cell expressed recombinant proteins. Subsequently, 12 and 7 hybridomas containing antibodies against the C-terminus and the N-terminus, respectively were tested for suitability as immunocytochemical reagents by screening with cultured rat Schwann cells known to express the 5-HT2AR. Parallel coverslips were processed as controls. The expression of 5-HT receptors was monitored using calcium imaging. Several clones that labeled strongly and specifically were identified, and their IgG fractions were purified. Current studies test these purified antibodies in Schwann cell cultures and rat brain sections using immunofluorescence.

443.21

5HT$_2$ RECEPTORS AGONIST α-METHYL-5HT POTENTIATES THE GLYCINE-RECEPTOR MEDIATED Cl^- CURRENTS IN TRIGEMINAL NEURONS. S.L. Lai[1], J.M. Chung[2]* and L.-Y.M. Huang[2]. Koahsiung Medical College Hospital, Kaohsiung, Taiwan, R.O.C.[1] and Marine Biomedical Institute, Department of Physiology and Biophysics, The University of Texas Medical Branch, Galveston, Texas 77555-1069[2].

Serotonin (5HT) is released from nerve terminals in the descending pathways and inhibits the activities of dorsal horn neurons. To understand the mechanism actions of 5HT, we studied the effects of 5HT on glycine-receptor mediated Cl^- currents in medullary dorsal horn neurons isolated from the spinal trigeminal subnucleus caudalis. Whole cell currents were recorded using the patch clamp technique. The 5HT$_2$ agonist, α-methyl-5HT, increased glycine-receptor mediated Cl^- currents in 40% (24/60) of the cells. The agonist was effective at a concentration as low as 0.1 nM. The glycine responses rose by 40% at 100 nM α-methyl-5HT. The action of α-methyl-5HT was sustained. The glycine-activated currents stayed elevated for 20 min after the agonist was washed out. The enhancing effect of α-methyl-5HT could be blocked by a high concentration of the 5HT$_{1\&2}$ antagonist, spiperone (1 μM). The 5HT$_1$ antagonist, pindbind, had no effect on the action of α-methyl-5HT. These results suggest that α-methyl-5HT activates 5HT$_2$ receptors and brings about a sustained potentiation of glycine responses (Supported by NIH grants NS11255, NS30045, NS23061).

444.1

INTERCELLULAR CALCIUM WAVES PROPAGATED VIA GAP JUNCTIONS IN NEURONS A.C. Charles* and R.F. Tyndale Dept. Of Neurology, UCLA, Los Angeles, CA 90024 and Addiction Research Foundation and Dept. Of Pharmacology, Univ. Of Toronto, Canada M5S 2S1

Spontaneous waves of increased intracellular calcium concentration were rapidly propagated over groups of primary mouse cortical neurons and immortalized hypothalamic (GT1-1) neurons in culture. Ca^{2+} waves were propagated at a rate of 100-200 µm/sec over 10-200 cells. Ca^{2+} waves were abolished by the removal of extracellular calcium and by TTX. Similar intercellular Ca^{2+} waves were induced by mechanical stimulation of a single cell. GT1-1 neurons showed flourescence recovery after photobleaching of a single cell, and intercellular Ca^{2+} waves were abolished by the gap junction blocker octanol. By contrast, a different clone of the GT1 neurons (GT1-7) showed frequent spontaneous Ca^{2+} oscillations but no intercellular Ca^{2+} waves, no intercellular communication of the response to mechanical stimulation, and no fluorescence recovery after photobleaching. Comparison of expression of connexin mRNA in the GT1-1 and GT1-7 lines using RT-PCR revealed a 5-fold greater level of connexin26 mRNA in the GT1-1 line, but no difference in the levels of connexin32 or connexin43 mRNA. These results show that neurons are capable of extensive Ca^{2+} signaling via gap junctions, and suggest that connexin26 is the gap junction protein which enables intercellular Ca^{2+} signaling in GT1 neurons. Intercellular Ca^{2+} waves in cultured neurons may represent a model for gap-junctional signaling between neurons in the developing nervous system, and between subsets of neurons in the adult brain.

444.2

THYROID HORMONE CONTROL OF CALCIUM WAVES IN *XENOPUS LAEVIS* OOCYTES. L. M. John, J. D. Lechleiter, and P. Camacho*. Dep't of Neuroscience, Univ. of Virginia, Charlottesville, VA 22903.

Inositol 1,4,5 trisphosphate (IP_3)-induced intracellular Ca^{2+} release mediates the action of many neurotransmitter signalling pathways. Spiral Ca^{2+} wave propagation and annihilation of IP_3-induced Ca^{2+} release reveals an underlying excitable process which can be accounted for by the Ca^{2+} dependent properties of the IP_3-bound IP_3 receptor (IP_3R). We previously demonstrated the importance of cytosolic Ca^{2+} buffering on this dynamic process by overexpression of sarco-endoplasmic reticulum Ca^{2+}-ATPases (SERCAs) in *Xenopus* oocytes (Science **260**, 226-229). Thyroid hormones and growth hormones have been shown to increase the expression of Ca^{2+}-ATPases in smooth muscles and cardiac myocytes. Additionally, thyroid hormones have been implicated in the regulation of Ca^{2+} uptake and/or release in mitochondria. Given the importance of Ca^{2+} sequestration in the dynamics of Ca^{2+} wave activity, we examined the effects of thyroid hormones on IP_3-induced Ca^{2+} signalling in *Xenopus* oocytes. To accomplish this, oocytes were injected with mRNA encoding the *Xenopus* thyroid receptor (TRβA1) and assayed for IP_3-induced Ca^{2+} wave activity 1-3 days later using confocal imaging. Exposure of oocytes expressing TRβA1s to L-3,3',5-triiodothyronine (T_3, 50-100 µM for 20-60 min before recording) resulted in an increase in the number of oocytes exhibiting IP_3-induced regenerative Ca^{2+} wave activity (58%; n=65) compared with control, non-mRNA injected oocytes (21%; n=24). In addition, TRβA1 expressing oocytes showed an increase in Ca^{2+} wave amplitude from 0.77±0.49 (ΔF/F) to 0.95±0.41and an increase in interwave periods from 24.4±11.3 to 64.9±42s in the presence of T_3. We conclude that acute exposure to thyroid hormone dynamically modulates IP_3-mediating Ca^{2+} signalling. These data will be discussed in relation to the action of thyroid hormone on mitochondrial Ca^{2+} buffering. The work was supported by NIH GM48451 and AHA878.

444.3

ASSOCIATION OF TYPE I AND TYPE III INOSITOL 1,4,5 TRISPHOSPHATE RECEPTORS.
F.C. Nucifora Jr., A.H. Sharp, S.L. Milgram[1], R.E. Mains*, C.A. Ross. Laboratory of Molecular Neurobiology, Johns Hopkins University, School of Medicine, 720 Rutland Ave., Ross 615, Baltimore, MD 21205. 1. Department of Physiology University of North Carolina.

The Inositol 1,4,5-trisphosphate receptor (IP_3R) is an intracellular calcium channel involved in coupling cell membrane receptors to calcium signal transduction pathways within the cell. The IP_3R is believed to form a tetrameric structure to produce the calcium channel in endoplasmic reticulum membranes. Several isoforms (I, II, III) of IP_3Rs have been identified which are coded by separate genes, and are expressed in many tissues with differing patterns of cellular expression. We have generated specific affinity purified polyclonal anti-peptide antibodies to each of the three isoforms. Western Blot analysis of RINm5F and ATt20 cells shows high levels of endogenously expressed type I and type III IP_3R, but undetectable levels of type II. Co-immunoprecipitation experiments were performed by immunoprecipitating from these cells with the type I specific antibody and Western Blotting with the type III specific antibody, or by immunoprecipitating with the type III specific antibody and Western Blotting with the type I specific antibody. Both experiments yielded a band at 260 kDa, the appropriate size of both the type I and type III IP_3R. Immunocytochemistry performed on these cell lines with either antibody demonstrated similar ER staining patterns. The type III IP_3R was absent from the secretory granules of ATt20 cells. These data indicate that type I and type III IP_3Rs can associate into a molecular complex.

444.4

CONFOCAL MEASUREMENTS OF BASELINE NUCLEAR AND CYTOPLASMIC FLUORESCENCE: COMPARISON OF CA^{2+} INDICATOR AND NON-CA^{2+} INDICATOR DYES. M.N. Rand*, S. Agulian, & J.D. Kocsis, Dept. of Neurology and Sect. Neurobiology, Yale Medical School, New Haven, CT 06510; and VAMC, West Haven, CT 06516.

At baseline resting potentials, neurons which have been loaded with Ca^{2+} indicator dyes using a micropipette have higher levels of fluorescence in the nucleus than in the cytoplasm, and it has been suggested that this effect is due to the presence of more dye in the nucleus. To evaluate this idea confocal microscopy was used to compare nuclear to cytoplasmic fluorescence ratios (N/C ratios) of cultured adult rat dorsal root ganglion neurons filled by micropipette with either Ca^{2+} indicator or non-Ca^{2+} indicator dyes. Both 10 kD dextran-conjugated and free forms of the Ca^{2+} indicator and non-Ca^{2+} indicator dyes were used. In all cases, N/C ratios of Ca^{2+} indicator dyes were significantly higher than those of the non-Ca^{2+} indicator dyes (dextran-conjugated: 1.89 vs 1.11; free dye: 3.30 vs 1.58). N/C ratios of the non-Ca^{2+} indicator dyes remained constant whereas N/C ratios of Ca^{2+} indicator dyes varied significantly over time. Some of the neurons also developed blebs on their plasma membrane which filled with water, aqueous solutes and dye; for non-Ca^{2+} indicator dyes bleb fluorescence was always higher than nuclear fluorescence, and for Ca^{2+} indicator dyes nuclear fluorescence was always higher than bleb fluorescence. These results suggest that micropipette dye-loaded neurons have higher levels of nuclear Ca^{2+} at baseline resting potentials, and have important implications for the evaluation of depolarization-induced intracellular Ca^{2+} signals. Supported in part by the NIH and Department of Veterans Affairs.

444.5

CLONING AND SEQUENCING OF AN IP_3-RECEPTOR cDNA FROM LOBSTER OLFACTORY ORGAN. S.D. Munger *[1,2], B.W. Ache[1,2,3] and R.M. Greenberg[1]. Whitney Laboratory[1] and Depts. of Neuroscience[2] and Zoology[3], Univ. of Florida, St. Augustine, FL 32086.

Several lines of evidence suggest that inositol 1,4,5-trisphosphate (IP_3)-receptors (IP_3Rs) occur in the plasma membrane of neuronal and nonneuronal cells, but little is known about the structural similarities of these plasma membrane IP_3Rs to the better known intracellular IP_3Rs. IP_3 directly gates two types of ion channels in the plasma membrane of lobster olfactory receptor neurons (ORNs); these channels are functionally similar to IP_3Rs localized to endoplasmic reticulum (ER) and nuclear membranes in vertebrates (Fadool & Ache, *Neuron*, **9**: 907; Hatt & Ache, *PNAS*, **91**: 6264). A polyclonal antibody directed against the ER IP_3R of rat cerebellum recognizes membrane proteins of appropriate size in lobster ORNs; this antibody also perturbs function of the lobster IP_3Rs in excised patch recordings (Fadool & Ache, *ibid.*). We have exploited the suggested structural similarities between plasma membrane and ER IP_3Rs by amplifying a partial cDNA, homologous to known IP_3Rs, from reverse transcribed lobster olfactory organ RNA using degenerate primers and PCR. We extended the clone to the 3'-noncoding region using 3'-RACE (Rapid Amplification of cDNA Ends). We have constructed an IP_3R mini-cDNA library, and have isolated overlapping clones by plaque hybridization and PCR. The open reading frame of the cDNA isolated to date, which codes for 1400 amino acids and comprises *ca.* 50% of the anticipated coding region, exhibits 40-45% identity to known IP_3Rs. While Northern analysis demonstrates a low level of expression of a >10 kb message in the brain, but none in the nose, the more sensitive ribonuclease protection assay shows the message to be expressed in the nose and, at higher levels, in the brain. We are currently working to extend the cDNA to the 5'-end and to localize the receptor by *in situ* hybridization and immunohistochemistry. {This work supported by ONR grant N00014-90-J-1566}

444.6

PLATELET-ACTIVATING FACTOR INDUCED INTRACELLULAR CALCIUM OSCILLATIONS IN RAT HIPPOCAMPAL NEURONS. M. A. DeCoster, H. E. P. Bazan*, and N. G. Bazan. LSU Medical Center, Neuroscience Center, New Orleans, LA 70112-2234

As has been previously shown, we have found using confocal microscopy and fluorescent calcium indicators, that intracellular calcium concentration ($[Ca^{2+}]_i$) oscillates spontaneously in rat hippocampal neurons *in vitro*. While addition of glutamate (GLU) to these hippocampal cultures causes distinct $[Ca^{2+}]_i$ changes ranging from transient, single spikes (100-500 nM GLU) to sustained increases (20-80 µM GLU), GLU does not appear to induce $[Ca^{2+}]_i$ oscillations. We have investigated the ability of the potent lipid mediator platelet activating factor (PAF) to affect $[Ca^{2+}]_i$ dynamics in hippocampal neurons. When 4 µM methylcarbamyl PAF (mcPAF) was added to the hippocampal neurons, the average $[Ca^{2+}]_i$ was increased slightly in cells. Furthermore, the variance of fluorescence values after mcPAF additions was 8-fold higher than before additions, indicating an increase in oscillatory $[Ca^{2+}]_i$ dynamics induced by PAF. Neurons not spontaneously oscillating were observed to be induced to oscillate by PAF addition, and neurons spontaneously oscillating increased in oscillatory behavior upon PAF addition. In agreement with Bito *et al.* (Neuron, 9:285, 1992) we found that not all neurons responded to acute PAF application. In contrast, long-term effects of PAF treatment on hippocampal cultures appeared to affect the majority of cells. Overnight treatment with PAF (200-400 nM) and mcPAF (2-4 µM) reduced the neuronal $[Ca^{2+}]_i$ changes induced by GLU the next day when compared with cells pretreated with lysoPAF (2-4 µM) or the vehicle alone. In two cases, the $[Ca^{2+}]_i$ increases in response to 500 nM GLU were completely inhibited by PAF pretreatment. Since PAF has been shown to enhance hippocampal excitatory synaptic transmission (Clark et al., Neuron 9:1211, 1992) we postulate that induction of $[Ca^{2+}]_i$ oscillations by PAF may be an early signal of GLU release, resulting in GLU receptor desensitization (Supported by DAMD-17-93-V-3013).

444.7

LOCALIZATION OF THE RYANODINE RECEPTOR IN ALPHA MOTOR NEURONS IN THE CHICK SPINAL CORD. Y. Ouyang*, P. A. Cole, M. E. Martone, V. M. Edelman, J. A. Airey+, J. L. Sutko+ and M. H. Ellisman. Dept. of Neurosciences, Univ. California, San Diego, CA 92093-0608 and +Dept. of Pharmacology, Univ. Nevada, Reno, NV 89557-0046.

The ryanodine receptor is an intracellular calcium release channel localized to the membranes of the endoplasmic reticulum (ER) in neurons and other cell types. The subcellular localization of this channel has been most extensively studied in Purkinje neurons, which possess an abundant and elaborate intracellular membrane system that likely functions in the uptake, storage and release of intracellular calcium. Alpha motor neurons also possess abundant ER and are known to contain the ryanodine receptor (Ouyang et al., Brain Res. 620:269, 1993) but the subcellular distribution of this channel has not been investigated in this cell type. We employed light and electron microscopic immunolabeling techniques to investigate the organization of the ryanodine receptor in motor neurons in the chick spinal cord. Three monoclonal antibodies against the ryanodine receptor were employed: mAB34C which recognizes all forms of the ryanodine receptor thus far described in chick, mAB110F which specifically recognizes the alpha skeletal muscle isoform and mAB110E, which recognizes both the beta and the cardiac form but does not distinguish between the two. Immunofluorescence studies in chicks aged 1 day to 2 weeks indicated that motor neurons in the ventral horn contain the beta/cardiac but not the alpha skeletal muscle form of the ryanodine receptor. Labeling was seen throughout the cell soma but was most concentrated in a bright band underneath the plasma membrane. At the electron microscopic level, the prominent subplasmalemmal labeling was associated with the hypolemmal and subsurface cisternae. In order to determine whether the ryanodine receptor was localized within the motor nerve terminals, cryostat sections of muscle were double labeled using mAB34C labeled with FITC and rhodamine-labeled alpha bungarotoxin, to mark the neuromuscular junction (NMJ). Preliminary results indicate that labeling for the ryanodine receptor may be present within the pre-synaptic terminal at the NMJ. Thus, the ryanodine receptor may contribute to intracellular calcium regulation in motor neurons and their terminals.

444.8

CALCIUM OSCILLATIONS AND WAVES IN C2C12 CELLS. P. Lorenzon,[1] F. Eusebi,[2] A. Giovannelli,[2] D. Ragozzino[2] and F. Ruzzier[1]*. [1]Inst. of Physiology, Univ. of Trieste, I-34127 Trieste and [2]Lab. of Biophysics, C.R.S. Regina Elena, I-00158 Rome, Italy.

It is known that spontaneous oscillations and/or waves of $[Ca^{2+}]_i$ are present in many cells. Using confocal and video-microscopy techniques, we studied these two phenomena in the mouse C2C12 line myotubes. Oscillations have been observed in 20% of differentiated cells, with variable frequency in the range 0.01 - 2 Hz. About 80% of the cells showing oscillations had silent periods lasting several minutes. The activity was reversibly blocked using EGTA 5 mM, KCl 5 mM or TEA 10 mM. Ryanodine 1 μM, heparin 5 mg/ml or thapsigargin 1 μM did not allowed the appearance of oscillations. On the contrary, caffeine 2 - 5 mM inhibited pre-existing oscillations whilst inducing a transitory (10 - 50 s) activity in silent cells. In the majority of the cells, Ca^{2+} waves could be detected along the whole myotube when caffeine 40 mM was applied in the presence of EGTA. In some cells, sub maximal caffeine concentrations (2 - 5 mM) elicited waves confined in restricted regions. These observations suggest that spontaneous oscillations of $[Ca^{2+}]_i$ in C2C12 myotubes are related to two different mechanisms: changes in Ca^{2+} permeability of the cell membrane, and Ca^{2+} movements from intracellular stores. Furthermore, we suggest that the autoregenerative Ca^{2+} propagation is sustained by a calcium-induced calcium release mechanism.

444.9

CALCINEURIN ASSOCIATED WITH THE INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR-FKBP12 COMPLEX MODULATES CALCIUM FLUX. A. M. Cameron*, J. P. Steiner, A. J. Roskams, S. M. Ali, G. V. Ronnett, and S. H. Snyder. Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

FK506 Binding Protein 12 (FKBP) is a small, nearly ubiquitous cellular protein which possesses prolyl isomerase activity and was first identified as the target of the immunosuppressant drug FK506. When bound to FK506, FKBP binds to and inhibits the Ca^{2+} activated phosphatase calcineurin. In the absence of FK506, FKBP has been identified in complexes with the Ryanodine receptor (RyR) and Inositol trisphosphate receptor (IP_3R), both of which are large intracellular calcium release channels. FKBP modulates the Ca^{2+} release properties of these channels apparently through a mechanism independent of its prolyl isomerase activity. We now report that calcineurin is present in these Ca^{2+} channel-FKBP complexes and may be anchored to these channels by an interaction with FKBP. Calcineurin is catalytically active in these complexes and is thus able to dynamically modulate phosphorylation status of the IP_3R, most actively towards the protein kinase C (PKC) site of phosphorylation, suggesting a Ca^{2+} sensitive feedforward and feedback regulation of intracellular calcium flux and thus a possible substrate for observed intracellular Ca^{2+} oscillations.

444.10

CHARACTERIZATION OF THE RAFT-1 PROTEIN, THE MAMMALIAN TARGET OF THE G1-ARREST COMPLEX, FKBP12-RAPAMYCIN. D.M. Sabatini*, B. Pierchala, R. Barrow, D. J. Liss, N.A. Cohen and S.H. Snyder. Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore MD 21205.

The macrolide drugs rapamycin and FK506 were first identified as potent immunosuppressants which block T-cell mediated immunity. These drugs have been used as probes in a variety of cell types, including PC12 cells, for studying intermediate steps in signal transduction pathways from the cell surface to the nucleus.

FK506 acts by binding to a soluble intracellular receptor, FKBP12, and endowing this drug-receptor complex with the capacity to interact with and inhibit the Ca^{++} activated phosphatase, calcineurin. FKBP12 and calcineurin are enriched in the mammalian nervous system and have been implicated in the control of neurite outgrowth and neurotransmitter release.

Rapamycin also mediates its effects by binding to FKBP12. The rapamycin-FKBP12 complex does not interact with calcineurin, but instead with a protein that we have termed RAFT-1 (for Rapamycin And FKBP12 Target). We have recently purified the 220 kDa RAFT-1 protein and cloned its cDNA. We now show that RAFT-1 from brain has lipid kinase activity. Using *in situ* hybridization and immunochistochemistry we have localized the RAFT-1 mRNA and protein in the adult and embryonic central nervous system. In addition, we have identified the subcellular localization of RAFT-1 and are studying the effect of rapamycin on this localization.

444.11

NADH SELECTIVE STIMULATION OF INOSITOL 1, 4, 5-TRISPHOSPHATE RECEPTORS MEDIATES HYPOXIC MOBILIZATION OF CALCIUM. Adam I. Kaplin*, Solomon H. Snyder, and David J. Linden. Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

To evaluate the relationship of inositol 1, 4, 5-trisphosphate (IP_3) receptor-mediated signal transduction and cellular energy dynamics, we have characterized effects of nucleotides on IP_3 receptor (IP_3R)-mediated calcium (Ca^{2+}) flux in purified IP_3 receptors reconstituted in lipid vesicles (IP_3RV) and examined hypoxia-induced augmentation of intracellular Ca^{2+}. NADH increases IP_3-mediated Ca^{2+} flux in IP_3RV. This effect is highly specific for NADH and is quantal. Hypoxia elicited by brief exposure of PC12 cells or cerebellar Purkinje cells to cyanide elicits increases in internal $[Ca^{2+}]$. The augmented Ca^{2+} derives from IP_3-sensitive stores. Blockade of this effect by 2-deoxyglucose and inhibition of glyceraldehyde-3-phosphate dehydrogenase implicate enhanced glycolytic production of NADH in the Ca^{2+} stimulation. Internal $[Ca^{2+}]$ is markedly augmented by lactate, which elevates [NADH], and by direct intracellular injection of NADH. These findings indicate that direct regulation of IP_3R by NADH is responsible for elevated cytoplasmic $[Ca^{2+}]$ occurring in hypoxia. This link of IP_3R activity with cellular energy dynamics may be relevant to hypoxic damage and energy provision for IP_3 signaling processes.

444.12

THE ROLE OF INOSITOL TETRAKISPHOSPHATE IN CA^{2+} MOBILIZATION IN *XENOPUS* OOCYTES. B. Paul*, Y. Yao, R.F. Irvine, and I. Parker. Laboratory Cellular and Molecular Neurobiology, Dept. of Psychobiology, University of California Irvine, Irvine, CA., 92717, USA. The Babraham Institute, Babraham, Cambridge CB2 4AT, UK.

Although the Ca^{2+} mobilizing role of inositol 1,4,5-trisphosphate ($InsP_3$) in the phosphoinositide signaling pathway is well established, possible functions of inositol 1,3,4,5-tetrakisphosphate ($InsP_4$), formed via phosphorylation of $InsP_3$, are less clear. Studies of $InsP_4$ actions have been confounded by the finding that $InsP_4$ is back-converted to $InsP_3$ via $InsP_4$ 3-phosphatase (R.F. Irvine, 1992, *Inositol Phosphates and Ca^{2+} Signaling*, Raven Press, pp.161-185). We show that $InsP_4$ can mobilize Ca^{2+} from intracellular stores in Xenopus oocytes, even when co-injected with $InsP_6$, a highly effective substrate for the $InsP_4$ 3-phosphatase, which thereby acts as a potent inhibitor of its actions on $InsP_4$ (S.B. Shears, 1992, *ibid.*, pp.63-92). When injected alone, $InsP_4$ elicited Ca^{2+}-activated membrane Cl^- currents resulting from intracellular Ca^{2+} mobilization, whereas injections of $InsP_6$ (up to 0.75 pmol) failed to evoke responses. Co-injection of $InsP_4$ and $InsP_6$ (8-50 fold excess $InsP_6$) elicited currents of similar sizes to those evoked by comparable amounts of $InsP_4$ alone, indicating that previously reported mobilization of intracellular Ca^{2+} by $InsP_4$ (I. Parker & I. Ivorra, 1991, *J. Physiol.*, 433:207-27) arose through actions of $InsP_4$ itself, and not $InsP_3$ formed from it. Although $InsP_4$, injected together with $InsP_6$, evoked robust Ca^{2+}release from intracellular stores, it frequently failed to induce Ca^{2+} influx, as assessed by appearance of a transient inward (T_{in}) current in response to hyperpolarization. Thus, internal Ca^{2+} store depletion appears not to be a sufficient stimulus for plasma membrane Ca^{2+} influx. However, a role for $InsP_4$ in regulation of Ca^{2+} influx cannot be ruled out, since T_{in} currents were induced by $InsP_4$ following 'priming' injections of $InsP_3$ (I. Parker & R. Miledi, 1987, *Proc. R. Soc. Lond.* B, 232:59-70).

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444.13

NEURONAL CALCIUM HOMEOSTASIS AND THE REGULATION OF THE PLASMA MEMBRANE CALCIUM ATPASE: D.A. Zacharias* and E.E. Strehler Mayo Grad. School, Dept. of Molecular Neuroscience, Rochester, Mn 55905.

Ca2+ dyshomeostasis has been implicated as an underlying factor in the development and progression of neuronal degeneration occurring in disorders such as Alzheimers Disease (AD). Of all the cellular systems that participate in Ca2+ homeostasis, extrusion mechanisms have hitherto received little attention as a factor contributing to the Ca2+ dyshomeostasis that occurs in neurodegenerative diseases. ATP-driven plasma membrane Ca2+ ATPases (PMCAs) are responsible for the fine-tuning of [Ca2+]i and represent a ubiquitous system for transmembrane Ca2+ extrusion. Changes in the levels and distribution of differentially regulated PMCA isoforms in normal vs. diseased brains may provide clues regarding the reasons for the observed dysregulation of Ca2+ in the disease state. In the first part of this study, we present a comparison of normal and AD brains as analysed by reverse transcription (RT)-PCR, quantitative ribonuclease protection assay (Q-RPA) and *in situ* hybridization analysis of the PMCA expression. In the second part of the study, our aim is to identify some of the mechanisms by which altered PMCA expression may occur. We have developed a human-derived cell culture (IMR-32 neuroblastoma) system in which we are able to vary [Ca2+]i in a controlled fashion and analyse the changes that occur as a result. Our preliminary results indicate that Ca2+-related signalling pathways regulate the expression of the PMCA at the level of alternative splicing and that calcineurin may be involved in this regulation pathway.

444.14

TRANSFECTED PC-12 CELLS EXPRESS CALRETININ: DOES ALTERED Ca^{2+}-HOMEOSTASIS AFFECT PC-12 CELL DIFFERENTIATION, SURVIVAL, AND APOPTOSIS ?

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Calretinin (CR), a calcium binding protein homologous to calbindin D28k, is present in subsets of brain neurons. The role of this protein is unknown. We report that PC-12 cells do not express endogenous CR either before or after NGF-induced differentiation. We transfected PC12 cells to determine whether CR functions as a Ca^{2+} buffer to alter Ca^{2+}-homeostasis, and if CR affects neuronal differentiation, vulnerability to Ca^{2+} overload, or apoptosis. We devised a vector with the CMV promoter upstream of the CR coding region (pBK-CMVCR). Transformed *E. coli* induced with IPTG produced a 22 kDa protein that was recognized by CR-specific antisera and bound Ca^{2+} in overlay assays. Undifferentiated PC-12 cells transfected with Transfectam and pBK-CMVCR (minus the lacZ promoter and ATG) yielded CR-immunoreactive cells, found frequently grouped in pairs. To produce a line of stable transfectants, cells were treated with geneticin. Ca^{2+}-imaging and immunohistochemistry will be used to evaluate the fate of transfected PC-12 cells treated with A23187, excitatory amino acids or serum depletion. Changes in NGF-induced differentiation will also be analyzed.

444.15

INCREASED $^{45}Ca^{2+}$-ACCUMULATION AND INOSITOL 1,4,5-TRISPHOSPHATE (IP_3) RECEPTOR BINDING BY *ORTHO*-SUBSTITUTED POLYCHLORINATED BIPHENYLS (PCBs) IN RAT CEREBELLUM *IN VITRO*.

P.R.S. Kodavanti, W.R. Mundy, S. Willig, and H.A. Tilson* Neurotox. Div., NHEERL/USEPA, Research Triangle Park, NC 27711.

Previous reports from our laboratory indicate that *ortho*-substituted PCB congeners increase intracellular Ca^{2+}, alter agonist-stimulated phosphoinositide hydrolysis, and cause protein kinase C translocation at concentrations where no cytotoxicity is observed; the effects of non-*ortho*-substituted PCB congeners on these phenomena are minimal. The present research extends these findings and examines the possible sources of intracellular Ca^{2+}-rise following PCB exposure. We studied the effects of two PCB congeners on $^{45}Ca^{2+}$-accumulation in cerebellar granule cells to determine the contribution of Ca^{2+} from external source and inositol 1,4,5-trisphosphate (IP_3) receptor binding which is known to be associated with the Ca^{2+}-release from intracellular stores. $^{45}Ca^{2+}$-accumulation was studied by incubating cerebellar granule cells with radioactive $^{45}CaCl_2$. IP_3-receptor binding was studied by incubating brain cerebellar microsomes with radioactive IP_3,D-[inositol-1-^{3}H]. Both 2,2'-dichlorobiphenyl (DCB), an *ortho*-substituted non-coplanar congener and 3,3',4,4',5-pentachlorobiphenyl (PeCB), a non-*ortho*-substituted coplanar congener, increased $^{45}Ca^{2+}$-accumulation significantly at 100 μM indicating that both types of PCBs increase Ca^{2+}-accumulation in cerebellar granule cells from external source. However, DCB (at 30-100 μM), but not PeCB (even at 100 μM) enhanced IP_3-receptor binding significantly suggesting that interaction of *ortho* PCBs with cerebellar endoplasmic reticular membranes may be a mechanism by which *ortho* PCBs can mobilize intracellular Ca^{2+} and contribute to altered cellular Ca^{2+}-signaling. These results suggest that both Ca^{2+}-accumulation from extracellular source and Ca^{2+}-release from intracellular stores are involved in the intracellular Ca^{2+}-rise by *ortho* PCBs.

444.16

PROPERTIES OF CA^{2+} STORES IN TYPE 1 AND TYPE 2 ASTROCYTES.

P.B. Simpson, C.A. Sheppard and J. T. Russell* LCMN, NICHD, NIH.

In this study, we have investigated the Ca^{2+} stores present in cultured rat cortical type 1 and type 2 astrocytes. Immunocytochemical analysis with specific antibodies indicated that both type of astrocyte express subtypes of inositol trisphosphate receptors, ryanodine receptors (RyRs) and Ca^{2+}ATPases. Both cell types responded to bradykinin with a peak and plateau or oscillatory $[Ca^{2+}]_i$ elevation, which was markedly inhibited by pretreatment with cyclopiazonic acid (CPA). CPA alone evoked a transient $[Ca^{2+}]_i$ elevation in both types of glial cell in nominally Ca^{2+}-free medium, whereas a sustained elevation was apparent in normal $[Ca^{2+}]_i$ consistent with store depletion in both cell types activating Ca^{2+} entry. Despite widespread expression of RyRs, perfusion of type 1 astrocytes with caffeine evoked an elevation of $[Ca^{2+}]_i$ in only a small number of the cells examined (5 out of 143 cells, 4%). However, in type 2 cultures caffeine evoked a peak elevation in a much higher proportion of cells (16 out of 70 cells, 23%). This response was still present in type 2 astrocytes, and indeed often of greater magnitude, subsequent to activation of Ca^{2+} entry using kainate. These findings indicate that while type 1 and type 2 astrocyte Ca^{2+} stores appear to have considerable similarities in terms of their inositol trisphosphate receptor and capacitative entry properties, there is a marked difference in their responsiveness to caffeine. The reason for this difference, and its putative relevance for astrocyte RyR-mediated signalling, remains to be established.

444.17

DIRECT MEASUREMENT OF MITOCHONDRIAL Ca^{2+} IN NORMAL AND PYRUVATE DEHYDROGENASE-DEFICIENT HUMAN DIPLOID FIBROBLASTS. R.A. Padua*, M. Shen, C. Campbell and S.A. Thayer. Dept. of Pharmacology, Univ. of Minnesota, Minneapolis, MN, 55455.

Mitochondrial Ca^{2+} concentration ($[Ca^{2+}]_{mt}$) was directly measured in control and pyruvate dehydrogenase-deficient (pdh^-) human diploid fibroblasts expressing mitochondrial-targeted apoaequorin (mt-aq). In both cell types, bradykinin (BK, 100 nM) was able to elicit a reproducible cytosolic Ca^{2+} ($[Ca^{2+}]_i$) transient as shown by indo-1 microfluorimetric measurements. BK-induced $[Ca^{2+}]_i$ increases were not significantly different in the presence and absence of the mitochondrial uncoupler FCCP. In control fibroblasts, a transient increase in $[Ca^{2+}]_i$ induced by BK was paralleled by an increase in normalized luminescent signal (response counts/total counts; 0.237±0.031) indicative of a rise in $[Ca^{2+}]_{mt}$. FCCP blocked the BK-induced $[Ca^{2+}]_{mt}$ transient and this effect was reversible because subsequent BK application elicited a $[Ca^{2+}]_{mt}$ transient (0.097±0.012). In pdh^- fibroblasts, the BK-induced $[Ca^{2+}]_{mt}$ transients (0.027±0.003) was greatly reduced relative to control. However, mt-aq expression in pdh^- cells was 27% of control. During transfection, treatment of pdh^- cells with β-hydroxybutyrate (2 mM), an alternative substrate for aerobic metabolism, increased mt-aq expression by 35%, enhanced mitochondrial cytochrome C oxidase activity by 4-fold and restored BK-induced $[Ca^{2+}]_{mt}$ transients (0.072±0.013) to 30% of control. Taken together, our results suggest that cells with compromised aerobic metabolism have a reduced ability to sequester Ca^{2+} into mitochondria.

444.18

PDGF-BB INDUCED Ca^{2+} RESPONSES IN CELLS OF THE OLIGODENDROCYTE LINEAGE ARE MODULATED BY SPHINGOLIPIDS.

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CEINGE cl 3 is a cell clone which was obtained through the infection of rat embryo neural cells with the murine leukemia virus carryng the polyoma middle T oncogene. These cells stain with immunofluorescent antibodies directed against galactocerebroside C, myelin basic protein and cyclic nucleotide phosphodiesterase, three specific markers for oligodendroglia. Exposure of these cells to PDGF-BB (10ng/ml) for 5 minutes induced two types of $[Ca^{2+}]_i$ response, monitored through fura-2 single-cell videoimaging. The first type was observed in 56% of the responding cells with a delay of 187±6 seconds and was characterized by a non oscillatory increase in $[Ca^{2+}]_i$. The other 44% of the responding cells showed an oscillatory $[Ca^{2+}]_i$ response with a delay of 289±9 seconds. Both $[Ca^{2+}]_i$ responses were partially dependent on the presence of extracellular Ca^{2+}. It has been demonstrated in fibroblasts that PDGF-BB exposure induces the increase of the intracellular levels of sphingosine and sphingosine-1-phosphate (SPP), which appear to be correlated with cell proliferation. When CEINGE cl 3 were incubated for 10 minutes with 10μM DL-threo dihydrosphingosine, which inhibits the phosphorylation of sphingosine in SPP, 90 % of the responding cells showed oscillatory $[Ca^{2+}]_i$ responses to following PDGF-BB perfusion. Furthermore, when 10μM DL-threo dihydrosphingosine was added between two consecutive PDGF-BB pulses, cells showing a monophasic response to the first pulse responded with an oscillatory pattern to the second PDGF exposure. Finally, when sphingosine (10μM) and SPP (1μM) were separately perfused, more than 90% of the responsive cells showed an oscillatory and a non-oscillatory Ca^{2+} response, respectively. These results showed that in CEINGE cl3 cells Ca^{2+} signalling following PDGF-BB stimulation is differently modulated by sphingosine and SPP.

444.19

HISTAMINE H_1-RECEPTOR ACTIVATION INDUCES [^{3}H]GABA RELEASE FROM HUMAN U373 MG ASTROCYTOMA CELLS. L.E. Soria, D. Martinez-Fong* and J.A. Arias-Montaño. Departamento de Neurociencias, CINVESTAV-IPN, Apdo postal 14-740, 07000 México, D.F., Mexico.

Amino acidic neurotransmitters can be released from glial cells in a Ca^{2+}-independent manner (Bernath, Prog. Neurobiol.,38: 57-91,1992). Since glial and glia-derived cells often express histamine H_1-receptors coupled to phosphoinositide hydrolysis (Inagaki and Wada, Glia, 11: 102-109, 1994) we set out to study whether agonist-induced increase in intracelluar Ca^{2+} concentration ($[Ca^{2+}]_i$) results in neurotransmitter release from U373 MG cells, a cell line derived from a human astrocytoma. Cells grown in 12-well plates were incubated with [^{3}H]GABA (80 nM) for 30 min at 37°C. After washing out the excess of [^{3}H]GABA, the medium (1 ml) was changed every 2 min and radioactivity measured in each fraction. In the fraction exposed to histamine [^{3}H]GABA release was increased in a concentration dependent-manner (maximum 306 ± 23% of controls). Neither Ca^{2+} removal nor Cd^{2+} (100 μM) affected histamine-induced [^{3}H]GABA release. In cells labelled (24 hr) with [^{3}H]inositol and exposed to histamine for 2 min, total [^{3}H]inositol phosphates accumulation increased in a concentration dependent-manner (EC_{50}, 17 ± 2 μM, maximum 203 ± 4% of basal). Histamine (100 μM) also increased $[Ca^{2+}]_i$ (Δ $[Ca^{2+}]_i$ = 242 ± 12 nM) in a thapsigargin-sensitive manner. The H_1-selective antagonist mepyramine blocked the actions of histamine on [^{3}H]GABA release, [^{3}H]inositol phosphates accumulation and $[Ca^{2+}]_i$. Taken together our results indicate that Ca^{2+} ions released from intracellular stores are responsible for histamine H_1-receptor induced [^{3}H]GABA release from U373 MG cells.

BEHAVIORAL PHARMACOLOGY: SEROTONIN AND DOPAMINE

445.1

EFFECTS OF 5-HT_2 AND DA ANTAGONISM ON THE STIMULUS PROPERTIES OF LSD. W.B. West, D.M. Millus, J. Buggy *, and J.B. Appel, Behavioral Pharmacology Lab., Dept. of Psychology, Univ. of S. Carolina, Columbia, SC, 29208. Because the only drugs that substitute for LSD are 5-HT_2 agonists or congeners of LSD and the only drugs that block the LSD cue are 5-HT_2 antagonists, we concluded that the LSD cue is mediated primarily by the 5-HT_2 receptor. However, several findings suggest that other neuronal systems may at least modulate this effect: 1) Spiperone, probably the most selective 5-HT_2 antagonist does not block the LSD cue, and 2) both ritanserin and risperidone, which are potent and selective antagonists of LSD and have strong affinity for 5-HT_2 receptors, have substantial affinity for DA receptors. In light of these data, we studied the effects of LY 237733, a selective 5-HT_2 antagonist with only weak affinity for DA receptors, ritanserin, MDL 72222, a 5-HT_3 antagonist and haloperidol, a DA antagonist, on the stimulus effects of LSD. Rats were trained to discriminate i.p. injections of LSD (0.08 mg/kg) using a standard, two-lever drug discrimination paradigm. They were then given two kinds of combination (antagonism) tests: 1) several doses of each putative antagonist were given in combination with a single dose of LSD or, 2) a single dose of each antagonist was given in combination with three cumulative doses of LSD (0.02, 0.04, 0.08 mg/kg). Under both test conditions, sufficiently high doses of LY 237733 (4 mg/kg) and ritanserin (2 - 4 mg/kg) blocked the LSD cue completely; neither MDL 72222 nor haloperidol had any antagonistic effect. These results suggest that whatever affinity ritanserin (and probably risperidone) may have for DA is not as important as their actions at 5-HT_2 receptors in mediating the LSD cue. Drugs that act predominantly at either 5-HT_3 or DA receptors do not appear to play any role in the stimulus effects of LSD.

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445.2

DOPAMINERGIC AND SEROTONERGIC PROPERTIES OF FLUOXETINE. B.B. Simon and J.B. Appel *, Behavioral Pharmacology Lab., Dept. of Psychology, Univ. of S. Carolina, Columbia, SC, 29208.

Fluoxetine (Prozac) has been used to treat a variety of complaints including depression, obsessive-compulsive disorders, and drug abuse, each of which has been attributed to disturbances in central reward mechanisms. Since these mechanisms are thought to be dopaminergic, the reason why their behavioral expression is altered by a selective 5-HT reuptake inhibitor is unclear. This problem was analyzed indirectly in the present experiment by training rats to discriminate i.p. injections of a 5-HT agonist, LSD (0.08 mg/kg, n = 11) and a DA agonist, cocaine (10 mg/kg; n = 16) from saline in a standard two - lever, drug discrimination situation. All animals were then tested with fluoxetine (0.625 - 10 mg/kg) alone and in combination (0.625 - 2.5 mg/kg) with the training drugs.

A 2 x 5 mixed analysis of variance on percentage of drug-appropriate responding indicated that fluoxetine did not substitute for either LSD or cocaine at any of the doses tested [$F_{(4,56)} = 1.52$, $p > .21$]. Moderate dose of fluoxetine (1.25 mg/kg) appeared to potentiate the discriminability of cocaine (2.5 mg/kg) of but not of LSD (0.02 mg/kg). These data suggest that inhibition of 5-HT reuptake has little, if any effect on the behavioral effects of LSD, which probably involve 5-HT_{2C} receptors. The ability of fluoxetine to potentiate the cocaine cue (but not to substitute for cocaine) suggests that fluoxetine does not inhibit DA reuptake but may have dopaminergic actions such as increasing DA receptor density. Alternatively, cocaine may have serotonergic properties, which result in the combination of relatively low doses of fluoxetine and cocaine being additive or synergistic.

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445.3

SEROTONIN-2A ANTAGONISTS DECREASE APOMORPHINE-INDUCED STEREOTYPIC BEHAVIOR WITHOUT AFFECTING LOCOMOTOR ACTIVITY. P. Hicks*, K. Young, R. Zavodny and T. Richter. Department of Psychiatry and Behavioral Sciences, Scott and White Clinic, Temple, TX 76508

We have previously demonstrated the role of serotonin-2C (5-HT-2C) receptors in modulation of apomorphine-induced locomotor activity (AILA) at an apomorphine dose of 0.25 mg/kg. The 5-HT-2A/2C antagonist mesulergine potentiated AILA and suppressed apomorphine-induced stereotypic behaviors (AIS). To further characterize the role of the 5-HT-2A and 5-HT-2C receptors in modulation of AIS, we have studied the effects of several substances active at these receptor sites.

Male Sprague-Dawley rats were given apomorphine (0.5 mg/kg) and test compounds (vehicle or serotonergic agents) subcutaneously at time zero. Locomotor activity was monitored in 5-min intervals for 30 min (San Diego Instruments). Baseline crossings in the 15-25 min post-treatment interval were used for comparisons. Stereotypic behavior was rated at 5-min intervals for 30 min by an observer blind to the treatment using a 0-5 scale. The sum total of stereotypic behavior scores for all six observation intervals was used for comparisons.

The 5-HT-2A antagonists ketanserin (0.02 mg/kg), MDL 100,907 (0.02 and 0.1 mg/kg) and ritanserin (0.02 mg/kg) all suppressed AIS without significant effects on locomotor behavior. The specific 5-HT-2C antagonist SER 082 had no effect on either AILA or AIS.

These findings suggest that serotonergic modulation of locomotor activity and stereotypic behavior is complex. The 5-HT-2A receptor appears to be more important as a modulator of stereotypic behaviors. The lack of effect of the specific 5-HT-2C antagonist SER 082 on either AILA or AIS suggests that the previous findings of potentiation of AILA with mesulergine may reflect activity at a receptor site other than the 5-HT-2A or 5-HT-2C receptor.

445.4

DIFFERENT EFFECTS OF TROPISETRON (ICS 205-930) AND ONDANSETRON IN LEARNING AND MEMORY PARADIGMS. BEHAVIORAL EVIDENCE FOR DIVERSE 5-HT_3 RECEPTOR SUBTYPES? N. Pitsikas* and F. Borsini. Department of Biology, Boehringer Ingelheim Italia S.p.A. 20139 Milan, Italy.

The 5-HT_3 receptor antagonists tropisetron (ICS 205-930) and ondansetron were tested in different memory tasks in which scopolamine (0.2 mg/kg, SC) was used as amnestic agent. Pretreatment with ondansetron (0.01 and 1 μg/kg, IP) but not with tropisetron (1, 10 and 30 μg/kg, IP) reversed scopolamine-induced memory deficits in the step-through passive avoidance task.

When the effects of these 5-HT_3 receptor antagonists on cognition were assessed in the Morris water maze, ondansetron (0.01, 1 and 10 μg/kg, IP) did not antagonize the amnesia induced by scopolamine. On the contrary, pretreatment with tropisetron (10 and 30 μg/kg, but not 1 μg/kg, IP) counteracted the learning and memory impairment due to scopolamine treatment.

It is suggested that different subtypes of the 5-HT_3 receptor may underlie the different effects on cognition displayed by compounds which belong to the same pharmacological class.

445.5

COMPARISON OF PUNISHED RESPONDING AND CONDITIONED SUPPRESSION IN PIGEONS AND RATS. C.D. Overshiner, M.J. Benvenga, and J.D. Leander* Lilly Research Laboratories, Eli Lilly & Co., Lilly Corporate Center, Indianapolis, IN 46207

Two procedures, punished responding and CER, that are used to study anxiolytic activity of drugs were examined in two species, rat and pigeon. Chlordiazepoxide, 8OHDPAT and pentobarbital significantly increased punished responding in both species. In the CER paradigm, chlordiazepoxide, 8OHDPAT, and pentobarbital slightly increased suppressed responding in both species, with only chlordiazepoxide in the pigeon producing a statistically significant increase in suppressed responding. WAY100635, a 5HT 1a antagonist, did not significantly affect responding in either procedure or species. Buspirone and diazepam, tested only in rats, significantly increased punished responding, with only the highest dose of diazepam producing a significant increase in suppressed responding.

Punished responding was a more sensitive measure of anxiolytic effects than conditioned suppression in both species. Effects of compounds on conditioned suppression paralleled that of punished responding but with less magnitude and higher variability.

445.6

DRUG DISCRIMINATION STUDIES OF THE INTEROCEPTIVE CUES PRODUCED BY SELECTIVE SEROTONIN UPTAKE INHIBITORS AND SELECTIVE SEROTONIN RELEASING AGENTS. D. Marona-Lewicka* and D.E. Nichols. Departments of Pharmacology and Toxicology and Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, Indiana 47907

MMAI (5-Methoxy-6-methyl-2-aminoindan) is a non-neurotoxic, highly selective neuronal serotonin (5-HT) releasing agent. MMAI and other 5-HT releasing agents produce a robust discriminative cue in drug discrimination (DD) studies. In vitro, 5-HT release can be blocked by selective 5-HT uptake inhibitors. However, in the present DD studies, pretreatment with fluoxetine, sertraline, or citalopram 60 minutes before the training drugs MMAI or (+)-MBDB produced only slight inhibition of the discriminative cue. In substitution tests, sertraline and citalopram partially mimicked the training drugs, whereas only 40% substitution occurred with fluoxetine in MMAI or (+)-MBDB trained rats. Since acute systemic administration of 5-HT releasing agents is known preferentially to increase extracellular 5-HT in forebrain structures, while the extracellular 5-HT increase induced by a single dose of an uptake inhibitor is several-fold higher in the raphe nuclei than in the frontal cortex, activation of forebrain serotonergic systems may be an important factor for the discriminative stimulus properties of drugs with 5-HT releasing and uptake blocking properties.

445.7

BESIPIRDINE HCL (HP 749) AND HP 184 REDUCE SCHEDULE-INDUCED POLYDIPSIA (SIP) IN THE ABSENCE OF SELECTIVE SEROTONIN (5HT) UPTAKE INHIBITION. A.T. Woods, C.P. Smith, R. Corbett, G. Bores, W. Petko, J.E. Roehr, L. Davis, J.T. Klein, R.C. Effland and S. Kongsamut. Neuroscience Product Group Unit. Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ 08876.

The polydipsia (SIP) model may have relevance for the prediction of efficacy of compounds useful in the treatment of obsessive compulsive disorder. Previous results (Woods et al., *Psychopharmacol.* 112: 195-198, 1993) have shown that only selective 5HT re-uptake inhibitors such as clomipramine and fluoxetine decrease SIP after a 14-21 day treatment. In these experiments, food-deprived rats were exposed to a fixed-time feeding schedule (FT=60sec) for 150 min per day. After 3 weeks of training, rats considered polydipsic drank >60 ml of water during the 150 min session (vs. 17 ml for food deprived controls). Besipirdine and HP 184 both decreased SIP beginning with the first treatment and this reduction was maintained for 28 days. Notably, neither compound was a selective 5HT uptake inhibitor. Besipirdine is a weak, non-selective inhibitor of biogenic amine uptake, and HP 184 has no uptake inhibitory properties at all. However, besipirdine and HP 184 did enhance the release of [^{3}H]5HT from rat hippocampal slices, but these effects were not blocked by the selective 5HT uptake inhibitor citalopram (10 μM). Thus, for besipirdine and HP 184, mechanisms independent of selective 5HT uptake inhibition can successfully reduce SIP.

445.8

COMPARATIVE ANTIDEPRESSANT- AND ANTIANXIETY-LIKE EFFECTS OF 5-HT_{1A} AGONISTS IN MICE AND RATS. Roger D. Porsolt, Joseph G. Wettstein* and Sylvain Roux. I.T.E.M.-Labo, 93 avenue de Fontainebleau, 94276 Le Kremlin-Bicêtre, France.

One primary indication for 5-HT_{1A} agonists is the treatment of anxiety yet such compounds may also possess antidepressant activity. The purpose of the present study was to examine the behavioral effects of two novel 5-HT_{1A} agonists flesinoxan and alnespirone (S 20499), the prototype 5-HT_{1A} agonists buspirone and 8-OH-DPAT, and the conventional drugs imipramine and diazepam in procedures designed to detect antidepressant and anxiolytic activity. In the mouse tail suspension test, all 5-HT_{1A} agonists along with diazepam increased and imipramine decreased the duration of immobility. In the rat behavioral despair test, imipramine and the 5-HT_{1A} agonists (except buspirone) decreased immobility duration. In the mouse behavioral despair test, imipramine clearly decreased, alnespirone and 8-OH-DPAT partially decreased, and flesinoxan increased immobility; buspirone had no effect. In the Cook-Davidson conflict procedure, diazepam but not buspirone increased suppressed responding whereas both diazepam and buspirone increased suppressed drinking in the Vogel conflict test. Together, these results support the idea that the mouse tail suspension test is sensitive to an anxiolytic-like action of 5-HT_{1A} agonists. In comparison, the behavioral despair test with rats may be useful in detecting potential antidepressant activity of such compounds.

445.9

EFFECTS OF ACUTE TREATMENT WITH INDIRECT-ACTING SEROTONERGIC AGONISTS ON ANXIETY-LIKE (CONFLICT) BEHAVIOR IN MAUDSLEY RATS. M.W. Lewis, D.E. Nichols, M.A. Ritter, J.S. Verbanac and R.L. Commissaris*, Purdue Univ., West Lafayette, IND and Wayne State University, Detroit, MI.

The Maudsley Reactive (MR; 'anxious') and NonReactive (MNRA; 'non-anxious') rat strains may represent a genetically-based model for differences in anxiety-like behavior. Serotonin (5HT) has been suggested to play a role anxiety. The present studies examined the effects of acute treatment with the 5HT reuptake blocker fluoxetine (FLU) and the 5HT releaser 5-methoxy-6-methyl-2-aminoindan (MMAI) on anxiety-like behavior in the Maudsley rats. MR and MNRA rats were trained in a conditioned suppression of drinking conflict paradigm. After several weeks of baseline conflict sessions, rats received challenges with MMAI (0.0625 - 8.0 mg/kg) and FLU (2.5 - 20 mg/kg) over the course of several weeks. In addition, challenges with phenobarbital (PHENO: 10, 20 mg/kg) and alprazolam (ALP: 0.25, 0.5 mg/kg) served as reference anticonflict standards. As expected, conflict-trained MNRA rats accepted more shocks (i.e., were less 'anxious') than MR rats in non-drug conflict sessions; PHENO or ALP produced robust increases in shocks received in both rat strains. At low doses, neither MMAI nor FLU significantly affected shocks received or water intake. Higher doses of both agents significantly reduced water intake (i.e., disrupted behavior) with a concomitant decrease in shocks received. There were no MR -v- MNRA strain differences in the response to either agent for either shocks received or water intake. These data suggest that acute manipulations of 5HT neurotransmission via reuptake blockade or increased release do not affect conflict behavior. Moreover, these data suggest that the Maudsley rats do not differ in their sensitivity to acute treatments which alter 5HT neurotransmission.

445.10

INVOLVEMENT OF SEROTONIN $_{1A}$ RECEPTORS SUPPRESS DEFENSIVE RAGE BEHAVIOR IN THE CAT. M.B. Shaikh*, N. Simpson, and A. Siegel. Lab. of Limbic System and Behavior, Dept. of Neurosciences, N.J. Medical School, UMDNJ, Newark, N.J. 07103.

The midbrain periaqueductal gray (PAG), a primary brainstem site for the integration of defensive rage behavior (DR), receives converging inputs from medial hypothalamus (MH) which mediates DR and serotoninergic inputs from the raphe system. The present study was designed to test the hypothesis that activation of 5-HT_{1A} receptors within the PAG inhibits DR elicited from the MH. Stimulating electrodes were implanted into DR sites within MH and cannula electrodes were implanted into DR sites in the PAG for drug infusion. Following microinfusion of the selective agonist for 5-HT_{1A} receptors, 8-OHDPAT (50 pmole, 2.0, 3.0 nmole) into the PAG, response latencies for MH-elicited DR were significantly increased above baseline at the highest dose level ($p<0.01$) in a dose- and time -dependent manner at 5 min postinjection. At a dose of 2.0 nmole, microinjections of 8-OHDPAT also resulted in an increase in response latencies but of lesser magnitude. Pretreatment with the 5-HT_{1A} receptor antagonist, p-MPPI, (1.5, 3.0 nmole), blocked the suppressive effects of 8-OHDPAT. The results demonstrate the inhibitory role of 5-$HT1_A$ receptors within the PAG in modulating defensive rage behavior.

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445.11

EFFECTS OF DOPAMINE AGONISTS AND ANTAGONISTS ON BODY TEMPERATURE, ACTIVITY AND ULTRASONIC VOCALIZATIONS OF RAT PUPS. R. E. Brown*, F. Dastur, D. Wiles and I. McGregor(1), Depts of Psychology, Dalhousie University, Halifax, N.S. Canada B3H 4J1 and (1) University of Sydney, Sydney, NSW 2006, Australia.

As D1 agonists induce hyperthermia, while D2 and D3 agonists induce hypothermia, we predicted that the D1 agonist SKF 81297 (SKF) would reduce USVs while D2 (Quinpirole) and D3 agonists (7-OH-DPAT) would increase USVs in 11-13 day old rat pups. The axillary temperature of pups given SKF (2.5 mg/kg) was lower than saline injected controls and the pups emitted fewer ultrasonic vocalizations (USVs) and had decreased activity levels in a 5 min. test. As SKF increases body temperature in adults, the reduced body temperature in pups was unexpected. SCH 23390 (0.3 mg/kg) but not Raclopride (0.3 mg/kg) reversed the effect on temperature. Quinpirole decreased body temperature at low (0.3 mg/kg) and high (3.0 mg/kg) doses and both doses increased activity levels, but only the high dose reduced USVs. These effects were reversed by Raclopride, but not SCH. 7-OH-DPAT reduced temperature at high (1. mg/kg) but not low doses (0.1 mg/kg). Both doses increased activity levels and this effect was reversed by both antagonists. Only the high dose of 7-OH-DPAT reduced USVs and this effect was reversed by Raclopride but not by SCH. Neither SCH (0.3 mg/kg) nor Raclopride (0.3 mg/kg) alone altered body temperature or USVs, but SCH reduced activity levels. The drop in body temperature during the 5 min. test was not the most important stimulus for emission of USVs. The reduction in USVs by the D1, D2 and D3 agonists suggests that the antianxiety or antidepressant actions of these drugs, their reinforcing properties, or their effects on respiration alter USV emission in infant rats, rather than their thermoregulatory actions.

445.12

IMPROVED SENSITIVITY OF A WATER WHEEL AUTOMATED BEHAVIORAL DESPAIR TEST USING WISTAR-KYOTO RATS. F.W. Ninteman*, D.J. Johnston, J.N. Wiley, A.E. Corbin, and T.G. Heffner, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Co., Ann Arbor, MI 48105.

Although the forced swim behavioral despair test has been used to identify antidepressant drugs, selective serotonin reuptake inhibitors (SSRI) are often inactive in this test. In order to improve the sensitivity of the test, we compared the behavior of Wistar-Kyoto (WKY) and Sprague-Dawley (SD) rats in an automated behavioral despair test that employs a wire mesh wheel mounted in a tank of water to enable recording of escape activity as wheel rotations. Drugs were given after a 15 min exposure to the apparatus on day 1 and again 24 hrs later, one hour prior to a 15 min test. The control behavior of WKY rats was significantly lower than that of SD rats (approx. 6 vs 50 rotations). WKY rats showed larger percentage increases in rotations than SD rats in response to the antidepressants, imipramine, desipramine, phenylzine, and mianserin. WKY rats, but not SD rats, also showed a significant increase in rotations after the SSRI fluoxetine. The SSRI paroxetine also produced significant effects in WKY rats. While the anxiolytic, diazepam, appeared to be a false-positive in SD rats, WKY rats did not show this effect. Both WKY and SD rats showed reduced rotations in response to the antipsychotic, haloperidol. Although amphetamine, scopolamine, and phencyclidine increased rotations in both WKY and SD rats, these drugs could be distinguished from antidepressants by their stimulant profiles in locomotor tests. These data indicate that WKY rats enhance the sensitivity and selectivity of the behavioral despair test and support previous suggestions that WKY rats represent a potential animal model of depression.

445.13

TRIHEXYPHENIDYL ATTENUATES DEFICITS PRODUCED BY CHRONIC DOSING OF RACLOPRIDE BUT NOT SCH 23390 IN RATS TRAINED TO PERFORM A SUSTAINED ATTENTION TASK. B.J. Brockel*, S.C. Fowler. University of Mississippi, University, MS 38677.

Sixty rats were trained to respond to 1 of 3 briefly presented light stimuli with a nose poke response. Using a between groups design, each subject received either vehicle or its own dose of SCH 23390 (0.02-0.16 mg/kg) or raclopride (0.2-0.8 mg/kg) daily for 7-21 days. After chronic dosing, the effects of chronic treatment were challenged with the anticholinergic trihexyphenidyl (0.5 mg/kg). Chronic SCH 23390 and raclopride treatment lengthened reaction time, increased errors of omission, and decreased earned reinforcers. Head exits, a possible model of akathisia, were increased by raclopride but not by SCH 23390. Trihexyphenidyl attenuated reaction time deficits produced by raclopride but not by SCH 23390. The results of this study indicate that both SCH 23390 and raclopride could have antipsychotic properties. Raclopride may be more likely to produce motor side effects such as parkinsonism and akathisia, but anticholinergic cotreatment may not alleviate side effects produced by SCH 23390. Supported by MH43429.

445.14

EVIDENCE FOR GENETICALLY MEDIATED DYSFUNCTION OF THE CENTRAL DOPAMINERGIC SYSTEM IN THE STARGAZER RAT. J.W. Brock* and C.R. Ashby, Jr. Brain and Development Research Center, The University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599; Medical Department, Brookhaven National Laboratory, Upton, NY, 11973. The stargazer rat is an autosomal recessive mutant (homozygous *stg/stg*) that displays abnormal behavior, characterized by stereotypic head-movement, circling, and a high level of ambulatory activity. Heterozygous (*stg/+*) littermates display normal spontaneous behaviors. In this study, stargazers and their unaffected littermates were compared in their behavioral responses to both stimulation and inhibition of dopamine D_2/D_3 receptors, using quinpirole and haloperidol. Stargazers were observed to yawn a significantly fewer number of times than littermates in response to (-) quinpirole (50 μg/kg i.p.). Halopcridol (HAL, 0.1 and 0.3 mg/kg s.c.) caused a significant decrease in stereotypic head-movement in the mutants that was both time- and dose-dependent. In normal littermates, HAL inhibited locomotor activity and produced catalepsy in a time- and dose-dependent manner. In stargazers, both doses of HAL inhibited locomotor activity to a similar degree as in the normal littermates. However, no catalepsy was detectable in the mutants using 0.1 mg/kg of HAL, and 0.3 mg/kg was only weakly cataleptogenic. Overall, the spectrum of abnormal behaviors expressed by the stargazers and the present evidence of D_2/D_3 receptor subsensitivity suggest that stargazers possess a genetically mediated dysfunction of the central dopaminergic system.

445.15

CARBAMAZEPINE REDUCES DOPAMINE-MEDIATED BEHAVIOR IN NORMAL AND CHRONIC NEUROLEPTIC-TREATED RATS: IMPLICATIONS FOR TREATMENT OF TARDIVE DYSKINESIA. T. Wigal[1], G.J. LaHoste[2], B.H. King[4], N. Khouzam[1], S.J. O'Dell[3]*, F.M. Crinella[1] and J.M. Swanson[1]. Depts. of [1]Pediatrics, [2]Phys. Med. & Rehab., and [3]Psychobiology, Univ. California, Irvine, CA 92717; [4]Dept. Psychiatry, Univ. Calif. Los Angeles.

We have recently found in developmentally disabled patients that tardive dyskinesia (TD) following withdrawal from long-term neuroleptic treatment occurs less frequently in patients that had concurrently received anticonvulsants, principally carbamazepine (CBZ). To determine whether this protective effect is due to anticonvulsant treatment *per se*, we employed an animal model of TD, based on chronic treatment with the neuroleptic haloperidol (HAL). In this model rats develop up-regulated dopamine D2 receptors and behavioral supersensitivity, effects which many investigators believe underlie TD. Twenty-nine adult rats received in their drinking water: HAL (1.2 mg/kg/day; n=6), HAL+CBZ (1.2 + 25 mg/kg/day; n=7), CBZ (25 mg/kg/day; n=6), 1/2HAL (0.6 mg/kg/day; n=5), or lactic acid (0.77%; n=5). After eight weeks, drugs were withdrawn and 96 h later all rats were tested for behavioral responsivity to combined treatment with the selective D1 agonist SKF 38393 (20 mg/kg, i.p.) and the selective D2 agonist quinpirole (0.125 mg/kg, i.p.). Chronic HAL treatment resulted in elevated stereotypy rating scores, as expected, for both the full and the half dose ($P < 0.04$). Chronic CBZ significantly reduced stereotypy scores regardless of whether HAL was given concomitantly or not ($P < 0.01$). The present behavioral data parallel the human data and suggest that carbamazepine may be effective in lowering the incidence of TD and in treating hyperdopaminergic states. To help elucidate the bioloigcal basis of these findings, we are currently assessing whether CBZ alters the effects of HAL on striatal D2 receptor binding.

445.16

CLOSE CORRELATION BETWEEN INTRASTRIATAL CONCENTRATION OF SULPIRIDE AND CATALEPSY AS STUDIED BY IN VIVO VOLTAMMETRY. H.P.M. Horikawa*[1,2], T. Nakazato[2] and O. Hikosaka[2] [1]Dept. of Neurochem. Brain Res. Inst. Niigata Univ.1-757 Asahimachi-dori, Niigata, Japan, [2]Dept. of Physiol., Juntendo Univ. Sch. of Med., 2-1-1 Hongo, Tokyo Japan.

To investigate the correlation between intrastriatal dopamine(DA) and its behavioral effects, we injected a dopamine D2 antagonist(sulpiride) locally into the rat striatum and measured its kinetics using in vivo voltammetry and examined behavioral changes due to the injection. First, we found that sulpiride could be measured in vitro with this technique. The measured current intensity selective for sulpiride increased dose-dependently ($1x10^{-7}$ M to $2x10^{-6}$ M). We then applied this method to the in vivo examination. Sulpiride(10^{-3} M, 7.5 μl) was administered into the striatum of freely moving rats over 30 min. Sulpiride began to be detected approximately 12 min after the start of injection, and remained higher than the baseline for more than 3 h. Sulpiride- induced catalepsy appeared 45 min after the start of injection and continued for 3 h. For comparison, DA and 1- methyl- 4- phenyl- pyridinium(MPP^+) were administered intrastriatally, and these drugs disappeared soon after the end of injection. When MPP^+ and its uptake inhibitor were co- administered intrastriatally, the concentration of extracellular MPP^+ remained high for a longer period than in the case of MPP^+ only. These results indicate that the time course of exogenous sulpiride in the striatum was closely correlated with that of cataleptic changes. The effects were prolonged probably because sulpiride is taken up or metabolized only slowly.

445.17

EFFECTS OF CLOZAPINE AND A DOPAMINE D1 ANTAGONIST ON DIZOCILPINE-INDUCED LOCOMOTION AND STEREOTYPY IN RATS.

H.A. Al-Amin, and S.B. Schwarzkopf*. Departments of Psychiatry and Psychology, University of Rochester, Rochester, NY 14620.

Dizocilpine (MK-801) is a PCP analog that acts as a noncompetitive NMDA receptor antagonist. It induces specific abnormalities of sensory gating, locomotion and stereotypy that have been used to develop animal models of schizophrenia and to test the efficacy of neuroleptics. Dopaminergic and serotoninergic systems are known to modulate the MK-801-induced hypermotility and stereotypy.

This study evaluated the time course and dose response of the effects of MK-801 on automated motility measures in rats. Testing started immediately after injection of MK-801 and lasted for 30 minutes. MK-801 0.2 and 0.5 mg/kg but not 0.05mg/kg significantly increased motility and stereotypic behaviors. Effects of MK-801 0.2mg/kg were very similar to 0.5mg/kg. The MK-801-induced behaviors were not evident until 12-18 min after injection with the effect stabilizing between 18-24 min. The selective D1 antagonist SCH23390 (SCH) 0.05mg/kg blocked the effects of MK-801 on all behavioral measures. This blockade was evident during the whole session. Pretreatment with Clozapine (CLOZ) 10mg/kg reduced the hyperactivity induced by MK-801 0.2mg/kg and had a similar pattern as SCH. The relative blockade of MK-801-induced stereotypy by CLOZ was significantly less than that by SCH.

These findings demonstrate that MK-801 effects on stereotypy and locomotion reach their maximum in about 20 minutes and that doses above 0.2mg/kg do not increase the magnitude of these effects in rats. These data also support the role of D1 receptors in modulating the activity of noncompetitive NMDA receptors. The results further support the differential effects of CLOZ on locomotion and stereotypy induced by MK-801.

445.18

PHARMACOLOGICAL CHARACTERISATION OF VARIOUS DOPAMINE (DA) AGONISTS IN TWO PUTATIVE MODELS OF D_3 RECEPTOR FUNCTION: COMPARISON WITH EFFECT ON PREPULSE INHIBITION (PPI).

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We have evaluated the potency of various dopamine (DA) agonists, 7-OH-DPAT (7-OH), quinpirole, quinelorane, PD128907 (PD), apomorphine (apo) and SKF38393 (SKF) in two putative models of D_3 function: DA agonist-induced hypothermia (Millan et al (1994) Eur. J. Pharmacol. 260: R3-R5) and 7-OH-DPAT-induced drug discrimination (McElroy (1994) Pharmacol. Biochem. Behav. 48: 531-533). Drug potencies in these models were compared with that required to disrupt prepulse inhibition (PPI). The objective being to establish whether D_3 receptors may be involved in the modulation of PPI.

Male, Wister rats (250-400g) were used throughout. The high affinity D_3 agonist, 7-OH inhibited PPI (0.03-1mg/kg sc) and reduced body temperature (0.03-0.3mg/kg sc). Both responses were blocked following pretreatment with the $D_{2/3}$ antagonist raclopride (0.3mg/kg sc). Rats were successfully trained to discriminate 7-OH (0.03mg/kg) from saline using a two lever operant procedure (final ratio, FR10). Raclopride (0.03mg/kg) attenuated this discrimination (veh/7-OH: 91±4%, rac/7-OH: 53±9%). In tests of substitution, quinpirole, PD, quinelorane, apo all completely generalised to the 7-OH cue. The rank order of potency for generalisation to the 7-OH cue was similar to that required to reduce body temperature and disrupt PPI; i.e. quinelorane>quinpirole=7-OH>PD=apo>>SKF. Thus the similar order of potencies in the PPI model may be consistent with a D_3 receptor mediated event, although a D_2 receptor involvement cannot be excluded.

445.19

DIFFERENCES BETWEEN THE HIGH POTENCIES OF THE D1/D2 ANTAGONISTS HALOPERIDOL AND *cis*-FLUPENTHIXOL TO AFFECT BEHAVIORAL VIGILANCE IN INTACT RATS.

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The determination of the role of mesolimbic dopamine in attentional functions is of significance for the understanding of the potential consequences of dopaminergic dysfunctions on attention. Our hypothesis suggests that cortical cholinergic hyperactivity is necessarily associated with an overactive dopaminergic transmission in the nucleus accumbens (via GABAergic projections to the basal forebrain), and that the attentional dysfunctions that are due to cortical cholinergic hyperfunction contribute to the emergence of psychotic symptoms in schizophrenia (Sarter, Psychopharmacol., 1994, 114:539-550; Holley et al., Psychopharmacol., 1995, in press). In a first step to study the role of dopaminergic systems in attention, the effects of the D1/D2 antagonists haloperidol and *cis*(Z)-flupenthixol on behavioral vigilance were studied using the task previously characterized (McGaughy et al., Psychopharmacol., 1995, 117:340-357). Administration of haloperidol (0.001 - 0.05 mg/kg; 2.6 - 133 µmol) potently impaired the animals' ability to detect visual signals (presented for 25 - 500 msec) and to correctly reject non-signal trials. Although flupenthixol has significantly greater affinity for D1 and D2 receptors than haloperidol, no significant effects were found (0.01 - 0.05 mg/kg; 19 - 98 µmol). These findings question the role of D1/D2 receptors in the effects of haloperidol.

445.20

PINEALECTOMY, MELATONIN AND 5-HYDROXYTRYPTOPHOL EFFECTS ON ANXIETY IN RATS.

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Anticonvulsive, hypnotic and sedative effects had been described after melatonin (MEL) systemic administration. However, the mechanism of action used by MEL to produce such effects are unknown. A GABA-ergic like mechanism has been proposed, because MEL enhances GABA binding "In Vitro" and elicits some biochemical effects those produced by diazepam (DIAZ), chlordiazepoxide (CDP) and buspirone (BUS). These data suggest MEL could exert other effects of this kind of drugs like, anxiolysis. In the present study we analyze comparatively the anxiolytic action of MEL, 5-hydroxytryptofol (5-Hol), DIAZ and CDP, in pinealectomized and control rats. Male Wistar rats (180-230 g) were deprived of water for 48 h before testing on Vogel conflict chamber. A drinkometer circuit was connected between a drinking tube of a water bottle and the grid of the floor of a Plexiglas box, the rat completed the circuit whenever it licked the tube. Each time, the rat closed the circuit, an electrical pulse (0.7 mA/2 sec), was delivered. The effects of two classical anxiolytic drugs, DIAZ (2 and 5 mg/Kg) and CDP (5 and 10 mg/Kg), were compared with MEL (1 and 2 mg/KG) and 5-Hol (1 and 2 mg/KG). Anxiolytic drugs as well as MEL and 5-Hol, produced a dose dependent increase in the number shocks, being MEL more potent. When the drugs were applied in pinealectomized rats, the effects were more significant. These results suggest that the two pineal indols used in this work, are involved in the modulation of the stress responses.

CATECHOLAMINES: BIOSYNTHETIC ENZYMES

446.1

MELATONIN INCREASES THE IN SITU ACTIVITY OF TYROSINE HYDROXYLASE IN THE MEDIAN EMINENCE/ARCUATE REGION OF CASTRATED SYRIAN HAMSTERS.

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Daily late afternoon melatonin injections have been shown to increase the *in situ* activity of tyrosine hydroxylase (TH) and to decrease dopamine (DA) content in the median eminence (ME)/arcuate region of the mediobasal hypothalamus (MBH) of male Syrian hamsters (Alexiuk & Vriend, 1994). In the present study, the effects of melatonin injections (for 9.5 weeks) on TH activity were examined in the ME/arcuate region and in the neurointermediate lobe (NIL) of the pituitary of gonadectomized (GX) male hamsters. The tissues were sampled from animals sacrificed at nighttime, since previous data showed that the most significant effect of melatonin on DA levels occurred during the dark phase of the light-dark cycle. TH activity was determined by measuring the accumulation of L-DOPA following administration of the aromatic L-amino acid decarboxylase inhibitor, NSD-1015. As previously shown in intact hamsters, a marked elevation ($p < 0.001$) in ME/arcuate TH activity was demonstrated in castrated hamsters injected with melatonin, compared to saline-treated GX controls. This melatonin-induced increase in TH occurred concomitantly with a significant reduction in DA content of the ME/arcuate region ($p < 0.001$) and of the NIL ($p < 0.01$). However, no significant effect of melatonin on TH activity of the NIL was demonstrated. Melatonin administration had no detectable effects on nighttime norepinephrine concentrations. The present data are consistent with the interpretation that melatonin increased the synthesis/release (turnover) of tuberoinfundibular DA neurons of the MBH. Since all of the animals in this study were castrated, the effects of melatonin on TH activity and on DA content could not be secondary to melatonin-induced changes in circulating levels of testosterone.

446.2

IN VIVO AND *IN SITU* STUDY OF TYROSINE HYDROXYLASE (TH) ACTIVITY MODULATIONS IN THE RAT BRAIN USING [3',5'-$^3H_2$]-α-FLUOROMETHYL-TYROSINE ($^3H_2$-α-FMT).

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TH activity can be modified by changes in its specific activity (SA) or in the levels of active enzyme. We have developed a methodology which allows to measure with an excellent anatomical resolution TH enzymatic activity and protein quantity by quantitative radioautography and immunoautoradiography respectively, from adjacent sections taken at serial intervals along the longitudinal extent of a same brain. SA of TH was estimated by the slope of correlation curves established between TH quantity and activity. To evaluate TH activity, we have used the $^3H_2$-α-FMT, which is transformed by TH into $^3H_2$-α-fluoromethyl-dopa. This last compound is suggested to be a potent and irreversible substrate for aromatic amino acid decarboxylase. Indeed: (1) intracisternal injection of 20 µCi of $^3H_2$-α-FMT was followed by a rapid disappearance of $^3H_2$-α-FMT ($t_{1/2} = 1.5$ *h*) and by specific accumulation of radioactivity into the locus caeruleus (LC) cell body limits ; (2) following hemilateral 6-hydroxydopamine-induced lesion of the nigrostriatal pathway, the bilateral injection of $^3H_2$-α-FMT into the neostriatum revealed a specific accumulation of radioactivity in the intact side only. The study of the tissue distribution of SA of TH in the LC region revealed that this SA was 48% higher in the LC cell body limits (PKA) than in the pericaerulean neuropil (PCN). Only 12% of TH contained in the PCN exhibited a detectable level of enzymatic activity. We have finally examined short (15 min) and long term (72 h) increases in TH activity in the LC region, following Vindébumol treatment (30 mg/kg, i.p.), which is also known to induce long term increase in TH protein levels. Two distinct mechanisms have been observed: short term effect was due to an increase in the SA of TH in the PKA only ; long term effect was due to an increase in active TH protein quantity in the PCN only.

446.3

POSTNATAL DEVELOPMENT OF THE DOPAMINERGIC INNERVATION OF MONKEY ENTORHINAL CORTEX. S.L. Erickson*, M. Akil, A.I. Levey and D.A. Lewis. Depts. of Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA, 15260 and Dept. of Neurology, Emory University, Atlanta, GA, 30322.

The rostral portions of the entorhinal cortex (ERC) of the adult monkey receive a dense dopaminergic innervation (Akil and Lewis, *Cerebral Cortex*, 1993). The postnatal maturation of the dopaminergic innervation of limbic cortical areas has not been examined, but the development of this afferent system in primate frontal cortex has been shown to be protracted and region-specific (Rosenberg and Lewis, *J. Comp. Neurol.*, 1995). Antibodies against the dopamine transporter and tyrosine hydroxylase (TH) were used to study the laminar distribution and density of dopaminergic axons in the rostral subdivision ER in 22 rhesus monkeys (*Macaca mulatta*) ranging in age from 2 days to over 18 years. With both antibodies, the density of labeled axons in layers I and III increased from birth to peak at the middle of the first year of life, then declined over the next year to relatively stable levels, which were maintained through adulthood. In contrast, the density of labeled axons in layer VI appeared to remain stable throughout postnatal development. Preliminary quantitative measurements of TH-labeled axons revealed that these developmental changes were more pronounced in layer III, with peak values representing a 10-fold increase over those observed in the newborn, whereas the peak in layer I represents a 3-fold increase. In contrast to frontal cortex, no increase in DA axon density was observed in the ERC in the animals 2-3 years of age, the peripubertal period. These findings suggest that the role of dopamine in the development of the ERC may be functionally distinct from that in frontal cortex.

446.4

LIGHT AND ELECTRON MICROSCOPIC COMPARISON OF AXONS IMMUNOREACTIVE FOR DOPAMINE TRANSPORTER OR TYROSINE HYDROXYLASE IN MONKEY NEOCORTEX. R.E. Whitehead*, D.S. Melchitzky, S.R. Sesack, A.I. Levey and D.A. Lewis. Depts. of Psychiatry and Neuroscience, University of Pittsburgh, PA 15213 and Dept. of Neurology, Emory University, Atlanta, GA 30322.

The influence of dopamine (DA) on neocortical function is in part determined by the activity of two major proteins: tyrosine hydroxylase (TH), the rate-limiting enzyme in DA biosynthesis, and the specific dopamine transporter (DAT), the uptake carrier that terminates the action of DA. We used immunocytochemistry to compare the light microscopic organization and ultrastructural features of axons and terminals immunoreactive for DAT or TH in the neocortex of adult cynomolgus (*Macaca fascicularis)* monkeys. By light microscopy, the majority of DAT-IR axons were similar in morphology to TH-IR axons, although DAT-IR axons generally appeared to be less varicose. The density of DAT-IR axons was greatest in motor regions, intermediate in association areas, and lowest in primary sensory areas. DAT-labeled axons were distributed across all layers in the densely innervated regions, and typically were located in layers 1-2 and 5-6 in the other regions. With few exceptions, the relative regional density and laminar distribution of DAT-IR axons were quite similar to those of TH-IR axons. At the ultrastructural level, both DAT- and TH-IR terminals formed primarily symmetric synapses. However, TH label was seen in both axons and terminals, whereas DAT label was more often found in pre-terminal axons. These findings suggest that the use of specific antibodies directed against proteins with different roles in DA neurotransmission may provide new insights into the function of cortical DA systems in normal development and psychopathology.

446.5

MEASURES OF TYROSINE HYDROXYLASE PROTEIN SYNTHESIS AND TURNOVER IN THE RAT LOCUS COERULEUS NUCLEUS USING A PULSE-RADIOLABELING PARADIGM. T.G. Sherman*, J.T. Curtis, S. Ito, and A.F. Syed. Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

The expression of tyrosine hydroxylase (TH) is important for the functioning of noradrenergic neurons of the locus coeruleus (LC). A variety of stimuli, such as stress, have been shown to alter the level of TH mRNA and TH protein in these neurons; however, the impact of these treatments on the synthesis, stability, and transport of TH to nerve terminals have never been directly examined. In order to address these issues, we have developed a strategy for studies on the synthesis, turnover, and transport of TH in the LC that involves the pulse-radiolabeling of TH *in vivo*. 90 µCi of [35]S-methionine (1175 Ci/mmol) in 2 µl is infused during 20 min into the left LC of halothane anesthetized male Sprague-Dawley rats. Following injection, the rat is allowed to recover for 30 min to 7 d prior to sacrifice. The LC is dissected from a 2 mm frozen section of pons. The relative specific radioactivity of TH [(radiolabeled TH)/(total TH)] in the LC is determined by the quantitative immunoprecipitation of tissue TH, autoradiography, and indirect Western quantitation. The incorporation of [35]S-methionine into immunoprecipitable TH is ~70% of maximal levels at 30 min after injection. Levels of radiolabled TH plateau within 3 hrs and remain stable for ~1 d. After 1d, levels of radiolabeled TH in the LC decrease with first-order kinetics with a half-life of ~2 days ($k = 0.36/d$). The relative contributions of axonal transport and protein turnover to this first-order decay have yet to be determined. These data demonstrate the feasibility of a pulse-labeling strategy for studies of TH expression in nuclei of the CNS, and will permit a comprehensive examination of the contributions of TH to catecholamine expression. (Supported by NINDS NS19608 and NIMH MH29670 grants.)

446.6

TYROSINE HYDROXYLASE (TH) PHENOTYPE EXPRESSION WITHIN CONTROL AND VINDEBURNOL-TREATED BALB/C (C) AND C57BLACK/6 (B6) MOUSE LOCUS COERULEUS (LC) . D. Marcel, N. Ginovart, L. Bezin, J.F. Pujol* and D. Weissmann , Lab. CNRS-UCBL UMR105, Fac. de Médecine A. Carrel, rue G. Paradin, F-69008 Lyon, France.

TH phenotype expression and its plasticity after Vindéburnol treatment (known to induce long term increase in TH level within the LC) was investigated in the two compartments of the LC of pure inbred strains of mice, the perikarya-delimited space and the surrounding neuropile. Serial coronal sections were selected along the caudo-rostral extent of the LC of control and Vindéburnol-treated C and B6 mice ; and were processed for TH immunocytochemistry or TH immunoradioautography. Three days after the single intraperitoneal injection of the drug (20mg/kg), we observed : 1) an increase in the number of cells expressing the enzyme in the C strain only, which equalized the catecholaminergic neuronal population of the two strains; 2) an increase in the enzyme level in the two compartments of the structure of both strains. In the perikarya-delimited space, two mechanisms of this pharmacologically-induced protein modulation were demonstrated: in C strain, the increase was obviously explained by the subset of additional TH expressing cells; whereas in B6 strain, the mean TH quantity provided by each cell was increased. Furthermore, a similar response to Vindéburnol treatment was observed in the surrounding neuropile of the two strains: a significant increase in the TH level was measured, resulting in a significant increase in the immunoradiolabeled volume. These results demonstrate 1) a different regulation of the TH phenotype expression between the perikarya area and the surrounding neuropile of the LC of each strain ; 2) a strain-dependant difference in the response to the Vindéburnol treatment.

446.7

TYROSINE HYDROXYLASE IMMUNOREACTIVE CELLS IN THE RAT NEOCORTEX AND HIPPOCAMPUS A. H. Siddiqui*, W. H. Pilcher, S. A. Joseph. Division of Neurological Surgery, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Catecholamines have been implicated as integral neurochemicals in a variety of neuronal functions including; motor, biobehavioral, neuroendocrine, and cognition among others. Their presence in brain is primarily identified by immunocytochemical localization of their rate limiting enzyme Tyrosine Hydroxylase.

We have identified Tyrosine Hydroxylase immunoreactive cells in colchicine treated adult rat neocortex and hippocampus. Similar findings have been reported in human, monkey and developing rat brain. We have also demonstrated a non colchicine enhanced tyrosine hydroxylase immunoreactivity in neonatal rat cortex which appears to dissappear during early stages of development. Our results indicate that these cells persist throughout adulthood and form part of an endogenous cortical catecholaminergic system in addition to the exogenous fiber projections from the locus coeruleus, ventral tegmental area and the zona incerta as determined from our tract tracing studies.

These findings assume greater importance in light of the recently reported work by Zhu et al who report that patients with complex partial seizures express abberent hippocampal tyrosine hydroxylase immunoreactive cells as compared to controls. These findings have been confirmed and extended in our lab. Anti epileptogenic characteristics of catecholamines have been well recognized in neuropharmacological, and neurophysiological studies utilizing catecholaminergic cell groups in the brain stem and various agonists and antagonists. Our results indicate the presence of this inherent cortical catecholaminergic system in normal adult rats and provides basis for studies which address these cortical cells and their possible role in modulation of epilepsy.

446.8

TYROSINE HYDROXYLASE AND AROMATIC L-AMINO ACID DECARBOXYLASE ACTIVITIES DURING ETHANOL WITHDRAWAL. N.H. Neff*, M. Hadjiconstantinou, T.A. Wemlinger and Z.L. Rossetti. Department of Pharmacology and Psychiatry and The Neuroscience Program, The Ohio State University College of Medicine, Columbus, OH 43210, U.S.A.

The mechanism(s) underlying the long-lasting inhibition of dopaminergic (DA) function during ethanol withdrawal is unclear. To study whether ethanol withdrawal was associated with alterations in the DA biosynthetic pathway we measured tyrosine hydroxylase (TH) and aromatic L-amino acid decarboxylase (AAAD) activities in terminal areas of the DA system of ethanol-withdrawn rats. Ten hr following withdrawal, i.e. the time at which overt physical signs of withdrawal were manifested, TH activity was increased to 195 % of control in the nucleus accumbens (NAS) and was unchanged in the striatum (CS). In contrast, AAAD activity was reduced to 49% in NAS and to 83% in CS. Concomitant with increased TH and decreased AAAD was an increase of approximately 200% of control in mRNA for the two enzymes in the midbrain. All parameters returned to control values 36 hr following withdrawal. It is possible that AAAD may become rate-limiting under these conditions and the increase in mRNA in the midbrain for the two enzymes may represent a compensatory response to maintain a normal level of DA function. The reduction of AAAD activity provides additional evidence of functional modulation of this enzyme and may represent another mechanism for the reduction of DA availability during ethanol withdrawal. Supported by Grant NS34571.

446.9

MODULATION OF TYROSINE HYDROXYLASE AND AROMATIC L-AMINO ACID DECARBOXYLASE IN MOUSE STRIATUM AFTER INHIBITION OF MONOAMINE OXIDASE S. Cho*, M. Hadjiconstantinou and N.H. Neff Dept. Pharmacol., Neurosci. Program, Ohio State Univ. Coll. of Med., Columbus, OH 43210

After the administration of the monoamine oxidase (MAO) inhibitors clorgyline (MAO-A), or pargyline (MAO-A and -B) there is a transient reduction of tyrosine hydroxylase (TH) and a long-lasting reduction of aromatic L-amino acid decarboxylase (AAAD) activity in the mouse striatum. Deprenyl, a MAO-B inhibitor, had no effect on these two enzymes except at high doses, doses that apparently inhibit MAO-A. The MAO inhibitors altered the expression of TH and AAAD mRNA in the midbrain. Pargyline and high doses of deprenyl increased AAAD mRNA, while clorgyline initially decrease and then increase expression. In contrast, TH mRNA transiently decreased following clorgyline or deprenyl (a high dose) administration. The acidic metabolites of dopamine appeared most effected by pargyline and clorgyline, suggesting deamination of striatal dopamine by MAO-A. Our results suggest that striatal TH and AAAD are modulated differently in response to perturbation of dopamine metabolism. The observation that MAO inhibitors decrease AAAD activity might have clinical implications, as current therapy for Parkinson's disease might include deprenyl alone or combined with L-DOPA. Supported, in part, by Grant NS34571.

446.10

AROMATIC L-AMINO ACID DECARBOXYLASE ACTIVITY IS REGULATED VIA PHOSPHORYLATION M.D.Berry*, M.Hadjiconstantinou and N.H.Neff. Dept. Pharmacology and Psychiatry and the Neuroscience Program, Ohio State University College of Medicine, Columbus, Ohio 43210.

Recent studies have suggested that aromatic L-amino acid decarboxylase (AAAD) activity can be modified by treatment with dopamine receptor agonists and antagonists. Similar changes have also been shown to occur following administration of compounds which stimulate/inhibit protein kinases, suggesting that regulation may occur via phosphorylation. We investigated this possibility directly by examining the effects of cAMP-dependant protein kinase (PKA) on the phosphorylation and activity of AAAD. Crude P_2 synaptosomal tissue preparations from rat striatum were incubated at 37°C under a variety of phosphorylating conditions. AAAD protein was identified by immunoblot and AAAD bands were cut out and counted for ^{32}P. Purified PKA catalytic sub-unit caused a concentration-dependant increase in ^{32}P incorporation into AAAD. Increases were also seen following incubation with okadaic acid, forskolin or 8-bromo-cAMP. The effects of forskolin and 8-bromo-cAMP were prevented by the protein kinase inhibitor H-7. Incorporation of ^{32}P was increased following prior dephosphorylation. Using l-dopa as a substrate, conditions which increased phosphorylation were also found to increase AAAD activity. These studies provide the first direct demonstration that AAAD activity can be regulated by phosphorylation. That such a regulation of AAAD activity occurs under physiological conditions is suggested by the effect of okadaic acid alone on phosphorylation and AAAD activity, and the effect of prior dephosphorylation on ^{32}P incorporation. Such a regulation of AAAD may be of importance in the normal control of monoaminergic neurotransmission, and in Parkinson's disease, during l-dopa administration. Supported in part by NINDS grant NS 34571.

446.11

PROTEIN TYROSINE PHOSPHORYLATION IN THREE MODELS OF NIGROSTRIATAL DOPAMINE DEPLETION: WEAVER MOUSE, MPTP AND 6-OHDA. J.A. Richter*, B. Ghetti and D.J. Bare. Indiana University Sch. Med., Indianapolis, IN 46202.

The weaver mutant mouse has a 70% loss of nigrostriatal dopaminergic neurons and of striatal dopamine. We have found that the tyrosine kinase inhibitor genistein stimulates fractional dopamine release from striatal slices in vitro and does so to a greater extent from weaver compared to normal striatal slices (Soc. Neurosci. Abs. 20:285,1994). Therefore we examined the phosphorylation of proteins on tyrosine in weaver and normal mouse striatum. Striatal proteins were extracted in lysis buffer and separated by SDS-PAGE. In a Western blot striatal proteins were reacted with the mouse monoclonal anti-phosphotyrosine antibody (4G10) and HRP-conjugated secondary antibody and processed for detection by enhanced chemiluminescence. We found no differences in the level of phosphorylation on tyrosine in weaver striatum compared to normal mouse striatum. For comparison we treated mice with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) given s.c. Striatal dopamine depletions of 80-92% by MPTP did not result in alterations of phosphotyrosine levels in striatal proteins. In contrast, in preliminary studies, unilateral nigral injections of 6-OHDA (6-hydroxy-dopamine) in rats resulting in >99% loss of dopamine on the lesioned side resulted in an increase in pp180 and perhaps other bands as previously shown by Girault et al. (1992). Further experiments will evaluate whether the key difference is the species, the degree of dopamine depletion or the 6-OHDA itself. Supported by USPHS R01-NS14426.

446.12

PHOSPHORYLATION-SPECIFIC ANTIBODIES TO THE SERINE 19 SITE IN TYROSINE HYDROXYLASE (TH). M. Goldstein*, K. Harada, J.Y. Lew, J. Wu, J.W. Haycock**. Neurochem. Res. Labs., NYU Med. Ctr., New York, NY and **Dept. Biochem. Molec. Biol., LSU Med. Ctr., New Orleans, LA

Activation of catecholaminergic cells increase the phosphorylation of TH at Ser19 (mediated by Ca/CaM PKII), Ser31 (mediated by ERK), and Ser40 (mediated by PKA). For the study of multiple-site TH phosphorylation, immunoblot analyses with antibodies (Abs) that selectively recognize the phospho-forms of each site offer a facile alternative to HPLC/ß detection of tryptic peptides from ^{32}P-TH, isolated by immunoprecipitation after Pi prelabeling of cells/tissue. In a previous report, Abs specific for Ser40-P were developed (Goldstein. et al., J. Neurochem. 64:2281,1995). In the present report, we describe analogous Abs that are specific for Ser19-P. Rabbits were immunized with a chemically phosphorylated, synthetic Ser19 peptide (ARAVSEQDAK) conjugated to KLH. In immunoblots of extracts from AtT20 cells expressing recombinant TH (rTH), robust labeling of wild type rTH by anti-Ser19-P was observed with okadaic acid-treated (but not untreated) cells, whereas labeling of the site-mutant S19L rTH was not detected with either treated or untreated cells. The reactivities of anti-Ser19-P and anti-Ser40-P were compared in blots of extracts from PC12 cells. Elevated KCL (40mM, 30s) produced a large increase in anti-Ser19-P labeling but only a small increase in anti-Ser40-P. Conversely, forskolin (5µM, 10min) selectively increased anti-Ser40-P reactivity. Combined treatment increased labeling by both of the Abs. Such data indicate that the site-specific, phospho-specific Abs will be useful for studying multiple-site TH phosphorylation.

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446.13

THE EFFECTS OF OKADAIC ACID, HIGH POTASSIUM, OR FORSKOLIN ON *IN SITU* L-DOPA SYNTHESIS AND ON PHOSPHORYLATION OF TYROSINE HYDROXYLASE (TH) IN ATT20 CELLS. K. Harada*, J.Y. Lew, M. Goldstein, J. Wu and J.W. Haycock**, Neurochem. Res. Labs., NYU Med. Ctr., New York, NY, ** Dept. Biochem. Molec Biol., LSU Med. Ctr., New Orleans, LA

It was reported that protein phosphatase 2A (PP2A) is involved in dephosphorylation of TH (Haavik, et al, FEBS Letters, 251, 36-42, 1989), and therefore we have studied effects of okadaic acid (OKA), a PP2A inhibitor, on phosphorylation of TH and on biosynthesis of L-dopa in AtT20 cells which express recombinant TH. The cultured cells were treated for various time periods with OKA, high potassium (60mM), or forskolin (1µM) alone or in combination. The exposure of the cells for 10min to OKA had no effect, while high potassium or forskolin increased L-dopa biosynthesis. The treatment of cells with OKA in combination with high potassium had no additional effect on the high potassium-elicited increase of L-dopa production. The effects of these treatments on phosphorylation of TH at serine 40 (Ser40) were determined by Western analysis using anti-pTH32-47, an antibody which selectively recognizes the TH phosphorylated at Ser40 (Goldstein, et al, J. Neurochem. 64, 2281-87, 1995). The exposure of the cells to OKA for 10min did not increase, while that for 20min increased the phosphorylation of TH. High potassium treatment for 10min increased phosphorylation of TH, but the increase was diminished in the cells treated for 20min. The exposure of the cells to high potassium in combination with OKA for 20min resulted in a greater increase of phosphorylated TH at Ser40 than individual treatments. The potentiating effect of this combination treatment was not detected in the cells exposed for 10min. These results show that OKA affects L-dopa biosynthesis and phosphorylation of TH at Ser40 in the cells treated for 20min. Our findings suggest that the levels of phosphorylated TH at Ser40 are critical for dephosphorylation, and therefore we postulate that PP2A plays a role in the regulation of TH under activated condition.

446.14

PHOSPHORYLATION OF TYROSINE HYDROXYLASE ISOFORMS IN HUMAN NEUROBLASTOMA CELLS. John W. Haycock*. Dept. Biochem. & Molec. Biology, LSU Medical Center, New Orleans, LA 70119.

In most mammalian species, tyrosine hydroxylase (TH) is a M,~240,000 homotetramer, and activation of catecholaminergic cells increases the phosphorylation of TH subunits at Ser19 (mediated by Ca/CaM PKII), Ser31 (mediated by ERK), and Ser40 (mediated by PKA). In primates and humans, alternative RNA splicing produces an additional TH isoform (TH-2) which contains four extra amino acids (-Val-Arg-Gly-Gln-) between Met30 and Ser31, thus creating a consensus recognition motif for Ca/CaM PKII (-R-X-Y-S-) at the ERK site of TH-1. In order to determine whether alternative splicing changes the signaling pathway involved in regulating the phosphorylation of this site, two human neuroblastoma cell lines [BE(2)M17. CHP234] were preincubated with ^{32}P, and then treated with activators of the three signaling pathways. ^{32}P incorporation into TH-1 and TH-2 at the multiple sites was analyzed by on-line radiochemical detection of tryptic TH phosphopeptides separated by HPLC. Veratridine and forskolin increased Ser19 and Ser40 (TH-1, TH-2) phosphorylation, respectively, and phorbol dibutyrate increased ^{32}P incorporation into Ser31 of TH-1. ^{32}P incorporation into "Ser31" of TH-2 was disproportionately low relative to the amount of TH-2 protein present, irrespective of the treatment condition. Thus, rather than altering its regulation, alternative splicing appears to eliminate this residue as a phosphorylation site in TH-2, the major alternative splicing variant present in humans and monkeys. [Supported by NS25134]

446.15

NEUROPEPTIDE Y INHIBITS NICOTINIC RECEPTOR STIMULATED TYROSINE HYDROXYLASE PHOSPHORYLATION. J. Zheng and T.D. Hexum*. Department of Pharmacology, University of Nebraska College of Med., Omaha, NE 68198-6260.

Increased catecholamine secretion is accompanied by a corresponding increase in catecholamine biosynthesis to keep pace with secretory demands of the neuron. The increase in biosynthesis occurs, in part through the activation of tyrosine hydroxylase. The immediate activation of tyrosine hydroxylase after neuronal stimulation occurs as a result of the receptor-mediated phosphorylation of the enzyme resulting in the activation of various protein kinases. The extent of enzyme phosphorylation correlates directly with an increase in enzyme activation. Correspondingly, inhibition of enzyme phosphorylation should result in decreased enzyme activity. We have demonstrated that neuropeptide Y (NPY) inhibits forskolin-stimulated cyclic AMP accumulation in chromaffin cells through a pertussis toxin-sensitive process (Zhu, J., W. Li, M.L. Toews and T.D. Hexum, 1992, J. Pharmacol. Exp. Ther., 263:1479-1486). Chromaffin cells were prelabeled with [^{32}P]PO_4 (90 min, 37°C) washed and incubated in the presence or absence of nicotine (1-100 μM) for 4 min. The medium was aspirated and the cells solubilized in 1% $NaDodSO_4$ containing 1 mM EDTA, pH 8. Proteins (50 μg) were separated by polyacrylamide gel (7.5%) electrophoresis. After autoradiography of stained and dried gels, [^{32}P] incorporation was quantitated by phosphor imagery. This procedure characteristically labels several proteins of which the 100 kDa, 87 kDa, 74 kDa, 60 kDa and 55 kDa are the most prominent. The 60 kDa protein, tyrosine hydroxylase, is easily identified by its characteristic increase in [^{32}P] incorporation in the presence of nicotine. The [^{32}P] labeling of tyrosine hydroxylase was confirmed by Western blot analysis and immunoprecipitation. A significant increase in [^{32}P] incorporation into tyrosine hydroxylase occurred in the presence of increasing nicotine concentrations compared to that seen in the absence of nicotine. NPY (0.3-300 nM) produced a concentration dependent, pertussis toxin-sensitive, decrease in [^{32}P] incorporation into tyrosine hydroxylase (IC_{50} = 3.02 nM) when present during nicotine (100 μM) stimulation. (Supported by NIH grant # NS26479.)

446.16

PROTEIN SYNTHESIS INHIBITORS STABILIZE TYROSINE HYDROXYLASE (TH) IN ISOLATED BOVINE CHROMAFFIN CELLS. E. Fernandez and G.L. Craviso*. Dept. Pharmacology, Univ. Nevada Sch. Med., Reno, NV 89557.

Reports of independent regulation of TH mRNA and TH protein levels raise the issue of post-translational control of TH turnover. To address this possibility, we have been studying the regulation of TH half-life ($t_{1/2}$) in primary cultures of bovine adrenal chromaffin cells. Using two different methodological strategies, approach to steady-state and pulse-chase analyses, an apparent $t_{1/2}$ value of 17-30 hours was estimated for TH in non-stimulated chromaffin cells. To establish whether ongoing protein synthesis has a role in regulating TH turnover, TH levels were examined in chromaffin cells treated with two mechanistically distinct inhibitors of protein synthesis, puromycin (100 μM) and cycloheximide (20 μM). After 3 days of treatment, neither inhibitor reduced the activity of TH or the amount of the 59-60 kD TH subunit, even though incorporation of [^{3}H]leucine into trichloroacetic acid precipitable protein was inhibited 90- 97% at 1-2 hours as well as throughout the duration of the treatment. This lack of effect on TH level contrasted with the decline in glucose-6-phosphate dehydrogenase, an enzyme reported to have a similar subunit size (63 kD) and a similar $t_{1/2}$ (15 hrs) as TH. These results suggest that TH is selectively stabilized under conditions in which protein synthesis is blocked. One possibility that could explain TH stabilization is that protein synthesis inhibitors prevent the synthesis of a factor(s) that is involved in regulating TH turnover. Supported by the AHA Nevada Affiliate and the AAUW.

446.17

TYROSINE HYDROXYLASE IMMUNOREACTIVITY (TH-IR) MAY NOT RELIABLY MARK CATECHOLAMINE NEURONS IN THE MEDULLA. G. Aston-Jones* and Y. Zhu, Div. Behavioral Neurobiology, Dept. Psychiatry, Hahnemann University, Philadelphia, PA 19102.

Brains of 30 adult male Sprague-Dawley rats were prepared for immunohistochemistry using antibodies against TH, dopamine beta hydroxylase (DBH), phenylethanolamine-N-methyl transferase (PNMT), dopamine (DA), or dihydroxyphenylalanine (L-DOPA). Double labeling was conducted using either rhodamine + fluorescein or DAB + SG substrate (Vector).

Numerous neurons in the ventral and dorsal medulla stained for TH but not for DBH, DA or L-DOPA. Virtually all TH-ir neurons in the caudal ventral medulla (A1 cell group) also stained for DBH. However, cell counts in 3 rats revealed that 7%, 21% and 53% of TH+ neurons in the caudal, mid- and rostral levels of the nucleus paragigantocellularis (PGi), respectively, did not stain for DBH. Thus, in the ventral medulla TH+/DBH- cells were most frequent at rostral levels where more than half of the TH+ neurons in the area of the C1 cell group did not stain for DBH. Such TH+ neurons also did not stain for DA or L-DOPA, indicating that they may not be catecholaminergic. Triple labeling with retrograde transport of Fluoro-Gold (FG) indicated that many of these TH-only neurons projected to the spinal cord. Many TH+/DBH- neurons were also identified in the Xth cranial nucleus, whereas virtually all TH+ neurons in the NTS also stained for DBH. Some TH+/DBH- neurons in X stained for DA, indicating that a small population of dopaminergic neurons may exist in this cranial nucleus. It appeared that many TH+/DBH- neurons in the ventral and dorsal medulla may also stain for PNMT. A group of neurons in the medial A5 region stained for DBH but not TH, and other cells in the caudal NTS were PNMT+ but TH-.

These results indicate that immunoreactivity for TH, DBH or PNMT may not reliably identify catecholaminergic neurons in certain regions of the medulla. While it is possible that these neurons contain undetectable low levels of other catecholamine chemicals, these findings identify populations of neurons with unusual properties. Supported by PHS grants NS 24698 and DA 06214.

446.18

A NOVEL SUBSTRATE FOR Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II IN BOVINE ADRENAL MEDULLARY CELLS. **N. Yanagihara*, H. Yamamoto,[1] Y. Ohishi,[1] E. Miyamoto,[1] M. Tsutsui, Y. Toyohira, Y. Uezono and F. Izumi. Dept. of Pharmacol. Univ. of Occup.& Env. Health, Sch. of Med., Kitakyushu 807 and [1]Dept. of Pharmacol. Univ. of Kumamoto Sch. of Med., Kumamoto 860, Japan.**

We have recently reported the isolation of a novel substrate (70-kDa protein) for Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) from bovine adrenal medullary cells (Mol. Pharmacol., 46, 423-430, 1994). In the present study, we examined the interactions among CaM kinase II, the 70-kDa protein and tyrosine hydroxylase (TH), and studied whether the 70-kDa protein would be phosphorylated by several other protein kinases.

1) When TH and the 70-kDa prtoein were incubated with CaM kinase II, TH protein enhanced the phosphorylation of the 70-kDa protein by CaM kinase II, but the 70-kDa protein attenuated the phosphorylation of TH. The autophosphorylation of CaM kinase II was stimulated by the presence of TH or the 70-kDa protein.

2) The 70-kDa protein was phosphorylated by cyclic AMP-dependent protein kinase and protein kinase C.

These results suggest that interactions among CaM kinase II, TH and the 70-kDa protein occur during the stimulation of adrenal medullary cells and that the 70-kDa protein is the substrate for several multifunctional protein kinases.

446.19

TETRABENAZINE BINDING TO SYNAPTIC LIKE MICROVESICLES FROM ADRENAL MEDULLA. I. Llona[1], W.G. Annaert[2], W.P. De Potter[2], Gysling K.[3]* [1]Facultad de Ciencias Médicas, Universidad de Santiago de Chile, [3]Lab. Farmacologia Bioquimica, Facultad de Ciencias Biológicas, Universidad Católica de Chile, [2]Lab. Neurofarmacologie, Dept Geneeskunde, Universiteit Antwerpen (UIA), Belgie.

We have previously described the presence of catecholamines in a synaptic like microvesicles (SLMV) enriched fraction from bovine adrenal medulla (Annaert et al., (1993) J. Neurochem. 60,1746). However, in PC12 cells SLMV store acetylcholine but not monoamines. In order to asses the presence of the catecholamine carrier in SLMV we studied the binding of [^{3}H]-dihydro-tetrabenazine (TBZ) to a SLMV purified fraction from adrenal medulla.

SLMV fraction was obtained after centrifugation in sucrose and urografin gradients. SLMV can be separated from plasma membranes, chromaffin granules (CG) and Golgi membranes as indicated by the distribution of synaptophysin (P38), 5' nucleotidase, dopamine ß hydroxilase (DßH) and cytochrome b561 (CYT) and galactosyl transferase.

Membrane fragments from CG equilibrated at lower density than P38. TBZ binding presented a bimodal distribution with a peak coincident with CG markers (CYT and DßH) and the other peak with P38. The specific activity of TBZ binding in SLMV was lower compared to CG, however the dissociation constant determined by kinetic analysis was similar. These results indicate that SLMV contain a catecholamine carrier similar to CG, supporting the presence of catecholamines in SLMV

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446.20

MULTIPLE FORMS OF PHENYLETHANOLAMINE N-METHYL TRANSFERASE IN RAT ADRENAL AND BRAIN. J.L. Rozycki, T.D. Gbadebo, and J.K. Stewart.* Dept. of Biology, Virginia Commonwealth Univ., Richmond, VA 23284

Recently investigators detected two closely migrating forms of PNMT mRNA in the rat adrenal, but it is unclear whether there is more than one form of the enzyme in rat adrenal or brain. In this study, four different antibodies to bovine PNMT detected a major and minor immunoreactive protein (M_r 31,500 and 30,500, respectively) on Western blots of rat adrenal samples. Optical density (O.D.) of each of these proteins correlated significantly with PNMT activity, and O.D. of other adrenal proteins cross-reacting with one of the antibodies exhibited no correlation with enzymatic activity. Neither of the adrenal proteins was observed on Western blots of rat brainstem, but two of the antibodies detected a lower molecular weight protein (M_r 29,500) in brainstem samples. The brainstem protein was not evident in cerebellum, a tissue with little or no PNMT activity. All immunoreactive proteins were present in both frozen and freshly prepared samples homogenized with or without proteolytic inhibitors or dithiothreitol. These findings provide immunochemical evidence for two closely migrating forms of PNMT in the rat adrenal and a third form of PNMT in the rat brainstem.

447.1

UTILIZING "CAGED-DOPAMINE" IN MEASUREMENT OF DOPAMINE UPTAKE IN BRAIN SLICES. T.H Lee, K. Gee, E.H. Ellinwood, and F.J. Seidler*. Dept. of Psychiatry and Pharmacology, Duke U. Med. Ctr., Durham, NC 27710 and Molecular Probes, Eugene, OR, 97402.

In order to facilitate examining altered dopamine (DA) uptake kinetics following chronic cocaine pretreatment, we have developed a methodology which evokes an "instantaneous" rise in extracellular DA concentrations without electrical stimulation. The uptake can be then measured in "real-time," using fast scan cyclic voltammetry at a 10-Hz sampling rate. In a recording chamber mounted on a Nikon Optiphot epifluorescent microscope, coronal rat brain slices containing both the caudate nucleus and nucleus accumbens were perfused with artificial CSF containing 150 - 200 µM of "caged-DA" (MPR 71012 synthesized by K.G.). UV illumination (0.5-1 sec) focused at the tip of the recording electrode (exposure timing controlled by an Uniblitz shutter) produced an "instantaneous" rise in extracellular DA concentrations; a peak DA concentration, 4-8 µM, was detected immediately following shutter closure(within 100 msec). This rise was followed by uptake with a "half-width" time of 1.2 ± 0.08 sec. The uptake could be inhibited by cocaine in a concentration-dependent manner (1.9 ± 0.2 and 2.9 ± 0.3 sec at 10 and 20 M, respectively). The main advantages of our methodology include: (1) similar initial DA concentrations (by varying UV exposure time) achievable for uptake measurement regardless of endogenous DA content in the region (e.g., caudate vs. prefrontal cortex) and (2) elimination of diffusional delay (seconds to minutes) inherent in pressure and microiontophoretic application techniques. Regional differences in uptake kinetics and effects of various uptake blockers will be presented.

447.2

EXPERIMENTAL STUDY TOWARDS DEVELOPING DOPAMINE RELEASING DRUG DELIVERY SYSYTEM (DDS) **K.Nishino; M.Kowada, Y. Tabata, Y Ikada**
Dept. of Neurosurgery, Akita University & Kakunodate General Hospital , Akita, Japan Research Center for Biomedical Engineering, Kyoto University
50 mg/ml of Dopamine(DA) was encapsulated in silicon polymer(DK 36 fluid, DDS-1) or biodegradable polylactate polymer (DDS-2) to develop DA releasing drug delivery system and was determined in vitro and vivo as for duration and characteristic of DA release to test whether DDS was implantable in the brain or not. Both DDS was immersed in test tubes filled with 1 ml of lactate Ringer solution. Every day solution of the test tube was exchanged for from a week to 5 months. The samples were stored at - 40 ° C deep freezer until the amount of DA release was measured by HPLC and ECD. Paradigm of In vitro DA release from DDS-1 was sequentially determined by microdialysis technique In test tube for a couple of hours. In vitro DDS-1(N=3) released DA for a week and DDS-2 (N=5) for more than five months at 20 ° C. The amount of DA release at 20 ° C was 10 times higher than at 4 ° C. In vitro microdialysis indicated that that DDS-1 showed DA release at constant rate for at least a couple of hours. DDS-2 was microscopically implanted in the right frontal (N=2) and the parietal paraventricular region(N=5) of male SD rat (BW; 250-350 gm) subjected to stereotactic insertion and fixation of steel catheter into right caudate-putamen under nembutal anesthesia. Then the extracellular levels of DA were sequentially monitored with In vivo microdialysis system(Eicom Co Ltd, Tokyo, Japan). Using another animal with inserted probe, the sequential monitor of extracellular DA level was performed after intraventricular injection of 10 µl of 50 uM DA. Extracellular DA levels were 1.07 ± 0. 22 (m ± SEM, pg/10 µl) at 4 th day post surgery In normal control (N=3), 5.4 ± 1.8 (N=3) at 4 th day and 37.1 ± 16.6 (N=5) at 7- 18 days in paraventricular implantation group, while 1.2 (N=2) at 4 th day post frontal implantation. The intraventricular injection of DA caused rapid increase in extracellular level of DA in the right caudate putamen without marked changes in metabolite levels od catecholamine. We conclude that DA was stable after polymer encapsulation and its implantation of ventricle was helpful to boost extracellular DA level in the caudate-putamen.

447.3

IN VIVO DIFFERENTIATION OF DA SYNTHESIS FROM DA RELEASE ALTERATIONS USING PUSH-PULL SUPERFUSION AND DIFFERENTIAL PULSE AMPEROMETRY. V. Leviel*, V. Olivier and B. Guibert. Inst. A. Fessard, CNRS, 91198 Gif sur Yvette, France.

A push-pull canula supplied with artificial CSF was implanted in the striatum of anesthetized rats, and the basal extracellular dopamine (DA) was assayed in the superfusates using HPLC and electrochemical detection. Simultaneously a carbon fiber electrode was implanted in close proximity (300 µm) of the canula and the evoked DA release was detected by differential pulse amperometry (DPA) during stimulation of the DA axons at the level of the medial forebrain bundle (MFB). The absence of calcium in the superfusing fluid induced a complete disappearance of the response to MFB stimulation detected by DPA. The same effect resulted from an i.p. injection of reserpine (10 mg/kg). This confirmed previous results showing that DPA allows to detect the release of DA from vesicular stores by a calcium dependent mechanism. In contrast, the DA collected through the push-pull canula during the same period of time was only reduced by 50% (Ca0) and 40% (reserpine). This demonstrates, that DA detected in extracellular space by superfusion with a push-pull canula is only partially coming from vesicular stores. In addition, the absence of calcium ions produced a decrease of the DA release that was non additive with the effect of a-methyl-p-tyrosine (0.1 mM), blocking the DA synthesis. This suggests that superfusion without calcium reduces extracellular DA by a mechanism different from exocytosys and involving alterations of DA synthesis. These results show that basal extracellular DA, coming from a non exocytotic process of release, and highly sensitive to DA synthesis, can be monitored simultaneously with the firing-dependent release.

447.4

SIMULTANEOUS MEASUREMENT OF SUBFEMTOMOLE AMOUNTS OF NOREPINEPHRINE, DOPAMINE AND SEROTONIN IN MICRODIALYSIS SAMPLES USING HPLC-EC **P.K. Mishra[1], R.L. Burger[1], J.K. Cullison[2], M.L. Bowers[2], I.N. Acworth[2] and P.C. Jobe[1]-**
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Among the *in-vivo* sampling methods, voltammetry offers extremely high temporal resolution but it lacks specificity and does not allow simultaneous quantitation of several neurotransmitters. These factors limit its applicability to selected areas of the brain that have a dominant neurotransmitter. In contrast, samples from *in-vivo* microdialysis are analyzed *ex-vivo* and therefore several compounds can be measured concurrently from a single dialysate. However, microdialysis has been regarded as lacking in temporal resolution . We now report successful, reproducible, and simultaneous analysis of subfemtomole quantities of monoamines and metabolites. This assay allows routine quantitation of these compounds in one minute microdialysates.

A microbore-microflow HPLC system (1 mm X 150 mm column at 75 µl/min) was used with a 5 µl injection loop and a low dispersion sample valve. A high sensitivity microbore electrochemical detector cell with glassy carbon electrode was used for detection. Detectable levels of norepinephrine, dopamine and serotonin were observed in one minute basal microdialysate samples from superior colliculus and cortex using a 3 mm loop-type microdialysis probe. The samples were collected at 4.5 µl/min and acidified continuously with 0.5 µl/min 0.01N $HClO_4$ using a 'Y'-connector. Factors influencing HPLC-EC in this assay are: pH of the samples, chemically reactive components of the injection valve and duration of exposure of dialysate to non-inert components. While variations in sample pH produce subtle effects on the retention of components, certain injection valve materials can produce artifact peaks and contribute to a large solvent front. Proper handling of samples insures the stability of monoamines in the microdialysate, which otherwise degrade rapidly at low volume and in nanomolar concentration.

447.5

INTERACTIONS BETWEEN (-)DS 121 AND COCAINE ON GLUCOSE METABOLISM AND EXTRACELLULAR DOPAMINE IN THE RAT BRAIN Susanne Koch*[1,2], Montford F. Piercey[1], Kjell A. Svensson[1], and Matthew P. Galloway[2], The Upjohn Co[1], CNS Res, Kalamazoo, and Wayne State U Sch Med[2], CCN Program, Dept Psych & Behav Neurosci, Detroit, MI

The phenylpiperidine (-)DS 121 is a dopamine (DA) antagonist that has a preferential action at terminal DA autoreceptors and stimulates locomotor activity. Interestingly, (-)DS 121 decreases cocaine self-administration in rats and is not self-administered. In the present study, (-)DS 121 was tested for its ability to affect cocaine-induced changes in extracellular DA and local cerebral glucose utilization (LCGU) in rats. Using Sokoloff's 2-deoxyglucose (2-DG) autoradiography technique, cocaine administration (5mg/kg, iv) increased LCGU in the frontal cortex, motor cortex, caudate, substantia nigra reticulata and mammillary body. By itself, (-) DS 121 (15mg/kg, ip) did not significantly alter metabolism in any brain region, however, in combination with cocaine, (-)DS 121 antagonized the stimulant effects of cocaine on LCGU, particularly in cortical areas. Using *in vivo* microdialysis in freely moving rats, administration of either cocaine (5mg/kg, iv) or (-) DS 121 (15mg/kg, ip) increased extracellular striatal DA. When combined with cocaine, (-) DS 121 prolonged the cocaine-induced increase in extracellular striatal DA. Our results with 2-DG support the hypothesis that (-) DS 121 blocks postsynaptic DA sites. Furthermore, prolongation of cocaine's effect on striatal DA may reflect (-)DS 121 antagonism of terminal DA autoreceptors. Because (-)DS 121 has both cocaine-mimetic and cocaine antagonist properties, it may be beneficial in treating both cocaine craving and binging. Supported by The Upjohn Co., DA 04120, and the Joe Young Sr. Research Fund.

447.6

INCREASES IN EXTRACELLULAR DOPAMINE FOLLOWING ELEVATED KCL - A NORMALIZING FACTOR FOR DRUG RESPONSES? T.L. Ripley, P.K. Randall* and R.A. Gonzales. Division of Pharmacology, University of Texas, Austin, TX 78712

We have previously shown that there is a good correlation between increases in extracellular dopamine (DA) following stimulation with high potassium (K^+) and that induced by other mechanisms causing depolarization. Here we investigate whether this correlation remains for drugs affecting extracellular DA by different mechanisms. Male Sprague-Dawley rats (250-280g) were implanted with a guide cannula in the left striatum 7-10 days before experimentation. Concentric dialysis probes (diameter 270µm: length 3mm) were inserted 18h before the experiment. Following 3 basal samples (sample = 15min, 2µl/min. ACSF 4mM K^+), 50mM K^+ ACSF was perfused for 10min, followed an hour later by a 10min perfusion with the test drug (n=3-6). The change in DA from basal (pg/µl) was calculated for the K^+ and drug induced responses, and the Pearson correlation coefficient (r) calculated. Calcium free ACSF decreased extracellular DA by at least 50%.

Test drug	r	Test drug	r
50mM K^+	0.940	5% Ethanol	1.000
10µM Amphetamine	-0.594	1mM NMDA	0.990
20µM Cocaine	0.322	10mM NMDA	0.962
1µM Tetrodotoxin	0.205		

The increase in DA following the first 50mM K^+ perfusion was not significantly different between the test groups ($p>0.05$. ANOVA). The mean value across all the groups was 0.608±0.083 pg/µl (n=27). A high positive correlation was seen when the second drug administered was K^+, NMDA or ethanol. The magnitude of the test drug response altered the slope of the correlation, irrespective of the correlation value. The other drugs investigated showed little to moderate correlation to the initial K^+ response at the concentrations tested, amphetamine being the only drug to show a negative correlation. Haloperidol was also tested (5µM) but the increase in extracellular DA followed a much slower time course and therefore was not included in the analysis. These results suggest that not all drugs that change extracellular DA can be correlated with the animal's response to K^+. Further work will reveal if those drugs that correlate with the K^+ response act through similar neuronal mechanisms. Work supported by NIAAA (AA08484 and AA00147).

447.7

IN-VIVO MONITORING OF NEOSTRIATAL DOPAMINE ACTIVITY AFTER NASAL DRUG ADMINISTRATION IN THE RAT: RELEVANCE TO PARKINSON'S DISEASE AND ADDICTION

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Pharmacological treatments aimed at the brain, like DOPA for the treatment of Parkinson's disease, are usually performed by administering drugs orally or iv; however, limited effectiveness and inconveniences for the patient call for using other ways of drug delivery. Here, the nasal route may be an interesting approach, not the least, since its effectiveness to affect brain mechanisms is vividly illustrated by drug abuse which is often performed via the nose. However, regarding the neurochemical effectiveness of nasal drug intake, experimental evidence is sparse. Thus, we used in-vivo microdialysis to monitor neurochemical activity of the neostriatum in urethane-anesthetized rats, which received drug treatments ip or nasally. When using the nasal route, saline-dissolved drugs (5-10µl) were administered into one nostril using a blunt microsyringe. In a first study, where methyl-DOPA (50mg/kg) was given without prior inhibition of peripheral DOPA decarboxylase, both, ip and nasal administration of methyl-DOPA increased extracellular DOPAC and HVA levels in the neostriatum by about 200-500%. In a next study with prior enzyme inhibition (benzerazide), the effects on DA metabolites were confirmed and, furthermore, pronounced increases of extracellular DA were found. Thus, nasal administration of methyl-DOPA or similar drugs may serve as an interesting drug route in the treatment of Parkinson's disease. Further studies with nasal administration of amphetamine (5mg/kg) and cocaine (15 mg/kg) yielded huge increases of extracellular DA in the neostriatum. These data indicate that studying neurochemical effects after nasal drug administration may be useful with respect to further improving drug therapies against brain disease, and may also serve as an interesting approach for studying neuronal mechanisms of drug abuse.

447.8

D2 RECEPTOR MODULATION OF SOMATODENDRITIC RELEASE OF DOPAMINE IN THE SUBSTANTIA NIGRA: THE ROLE OF NIGRAL AND EXTRANIGRAL SITES.

Marten J. Heeringa* and Elizabeth D. Abercrombie. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102, USA.

Dopaminergic neurons of the substantia nigra (SN) can release dopamine (DA) at both the nerve terminal level in striatum and at the somatodendritic level in SN. Based on the effects of systemic administration of D2 antagonists, somatodendritic DA release in the SN has been suggested to be under tonic autoreceptor control. However, this approach does not discriminate between different sites of action of the antagonists. In the present studies, we used *in vivo* microdialysis in awake rats to examine the effects of systemic administration of haloperidol (HAL) and the effects of local administration of this drug, in both SN and striatum, on somatodendritic DA release in SN. Systemic administration of HAL (0.5 mg/kg i.p.) increased DA output in SN by 34% (from 1.1±0.2 to 1.5±0.3 pg/20µl; n=4). This effect was maximal since a higher dose (1 mg/kg i.p.) did not induce a further increase. Local administration of HAL (1µM) in SN via the microdialysis probe did not alter the DA output in SN. However, this treatment abolished the decrease in DA output induced by local administration of the D2 agonist quinpirole (1µM), thus demonstrating effective pharmacological blockade of D2 receptors. In contrast to local administration in the SN, local application of HAL in striatum via microinjection (50µg/2.5µl) increased DA output in SN by 27% (from 1.7±0.2 to 2.2±0.2 pg/20µl; n=5), similar to the increase observed after systemic administration of the drug. This effect was maximal since a higher dose (75µg/2.5µl) of HAL caused a similar increase. The effect of local HAL (50µg/2.5µl) in striatum on DA output in SN was not further increased by simultaneous administration of HAL (0.5mg/kg) via the systemic route (30% of basal values; from 1.3±0.1 to 1.7±0.1 pg/20µl; n=4). These data fail to support the concept of autoregulation of somatodendritic DA release in the SN by local D2 receptors, at least under resting conditions. The effect of systemic administration of D2 antagonists on the release of somatodendritic DA in SN results from D2 receptor blockade in striatum, rather than from a blockade of nigral D2 receptors (support: USPHS Grants NS19608/DA08086).

447.9

REGULATION OF STRIATAL ACETYLCHOLINE RELEASE: ROLE OF DOPAMINE D1/D2 RECEPTORS AND EFFECT OF CHRONIC AMPHETAMINE

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A reciprocal relationship between acetylcholine (ACh) and dopamine (DA) appears to modulate basal ganglia-related behavior. Striatal ACh efflux is directly inhibited by D2 receptor activation but increased by activity at extrastriatal D1 receptors[1,2]. These opposing DAergic influences on ACh suggest a D1/D2 balance exists; thus while systemic D2 agonists inhibit ACh release, amphetamine (AMPH), activating both D1 and D2 receptors, has little effect. We hypothesize that chronic AMPH produces behavioral sensitization via changes in striatal DA/ACh interactions, determined by alterations in the D1/D2 balance. Male rats received AMPH (4mg/kg i.p.; b.i.d., 14 days) or were untreated (controls). Behavioral sensitization, defined as a shift from hyperactivity to predominant stereotypy in response to AMPH, was produced. After chronic treatment, microdialysis was used to monitor striatal ACh efflux (10 nM neostigmine). In control rats, striatal AMPH infusion (10 µM) decreased ACh output by 35%, from 59±8 to 38±5 fmol/20µl. This local inhibitory effect was reversed (63±5 fmol/20µl) by systemic AMPH (2 mg/kg i.p.)[2]. In AMPH-treated rats, ACh output was decreased by 61%, from 46±1 to 18±1 fmol/20µl, by local AMPH application. This decrease was significantly greater than that observed in control rats. Systemic AMPH also reversed the inhibitory local effect of the drug in the AMPH-treated group (44±3 fmol/20µl). Basal DA levels in the two groups did not differ. Extracellular DA increased in response to local AMPH ~30-fold in control rats and ~23-fold in AMPH-treated rats. These results suggest chronic AMPH produces a shift in the balance of D1/D2 receptor influences upon striatal ACh efflux in favor of D2-mediated inhibition. This shift did not result from increased DA release but may instead reflect changes in D1/D2 receptor mechanisms. An apparent hyperdopaminergic state, in terms of behavior, may thus be brought about by altered DA/ACh interactions in the absence of changes in striatal DA itself. (Supported by NIDA 08086)

[1]Damsma, Robertson, Tham & Fibiger, 1991. JPET 259:1064-1072.
[2]DeBoer & Abercrombie, 1994. Soc. Neurosci. Abs. 20:126.17.

447.10

STEREOISOMERS OF CARBIDINE AS D3 DOPAMINE RECEPTOR PREFERRING ANTAGONISTS

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Carbidine ((+) - cis - 3,6 - dimethyl - 1,2,3,4,4a,9b - hexahydro - γ - carboline dihydrochloride) was introduced into clinical use as an atypical neuroleptic drug with stimulatory and thymoleptic properties. The drug in clinical trials has been shown to possess antidepressive action alongside its antipsychotic efficacy and has been used in the treatment of patients with some forms of schizophrenia and alcoholism. Trans - isomer of carbidine synthesized according to V.A.Zagorevsky et al. (Zh. Vses. Khim. Obshchest., 27(1),pp.102-104, 1982) has been found to possess a higher potency in behavioral and neurochemical studies as regard to dopamine (DA) receptor blocking ability vs cis-carbidine. In microdialysis studies both compounds were shown to increase DA release more than its synthesis/metabolism and to produce certain behavioral activation in rats at low doses. Cis- and trans-isomers of carbidine were studied in the screening tests for the native rat striatal DA D2 using [^{3}H]spiperone and bovine D3 receptor using [^{3}H]7-OH-DPAT. In two independent experiments, cis-carbidine showed IC_{50} ~10µM against [^{3}H]spiperone binding to rat striatal membranes (maximal inhibition of 0 and 13% at 10 µM) and against [^{3}H]7-OH-DPAT binding to membranes from the bovine caudate (maximal inhibition of 46.6 and 38.8% at 10µM). Trans-carbidine showed IC_{50} values of 4.5 and 4.9 µM against [^{3}H]spiperone and 0.33 and 0.20 µM against [^{3}H]7-OH-DPAT. Thus binding data as well as behavioural and in vivo neurochemical profiles of the drugs demonstrate D3 vs D2 DA receptor preference of both cis- and trans-isomers of carbidine.

447.11

MODULATION OF NITRIC OXIDE-MEDIATED STRIATAL DOPAMINE RELEASE *IN VIVO*.

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The existence of both nitric oxide synthase (NOS) and excitatory amino acid transmitter mediated nitric oxide (NO) release in the striatum has suggested a role for NO in modulating striatal function. Using microdialysis, we report that both NOS substrate and NO-donors increase striatal dopamine (DA) release *in vivo*. Intra-striatal infusion with N^G-hydroxy-L-arginine (100*µM* for 120 min) increased DA release to 57± 5% above basal levels. Perfusion with sodium nitroprusside (SNP) (cumulative-dose 1.7 mM) increased extracellular (EC) DA to 331± 76% over basal concentrations. Local perfusion of (±)-S-nitroso-N-acetylpenicillamine (SNAP, 1mM), elevated EC DA levels to 472± 120% above baseline levels. This effect of SNAP was not mimicked by perfusion with 1mM (±)-penicillamine indicating that this NO-carrier compound is not responsible for SNAP-mediated DA release. The DA releasing effect of SNAP was abolished in rats pretreated with reserpine (5 mg/kg) implicating a vesicular dependent release process. The guanylyl cyclase inhibitor LY-83583 (100*µM* administered 100 minutes prior to and during the SNAP pulse), elevated EC DA levels approximately 6-fold by itself and potentiated the DA releasing effect of SNAP to 2598± 551% above basal DA levels. While similar pretreatments with antagonists such as methiothepin (10*µM*), bicuculline (100*µM*), or atropine (100*µM*) were without effect, the NMDA antagonists MK-801 (10*µM*) and ±CPP (100*µM*) blocked SNAP-mediated DA release. These results suggest that both endogenous and exogenous NO releases vesicular stores of striatal DA via an NMDA-receptor mediated mechanism and independent of guanylyl cyclase activation. Supported by NIDA-04120 and Joe Young Sr. Research Fund.

447.12

UNUSUAL NEUROCHEMICAL PROPERTIES OF THE NOVEL ANTIPSYCHOTIC OPC-14597 IN RAT STRIATUM.

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OPC-14597 (***OPC***) is a novel antipsychotic from a unique structural class. It binds with high affinity to rat striatal D_2-like dopamine receptors, and exhibits functional antagonist properties at measures of postsynaptic receptor function. ***OPC*** also has nanomolar affinity for cloned D_2 receptors where it behaves functionally as an antagonist. Preliminary clinical data also indicate that this drug may be a new atypical antipsychotic having good efficacy, yet little tendency to induce extrapyramidal side effects. Thus, it was surprising that in rat brain slices ***OPC*** actually had, at high concentrations, a modest inhibitory effect on dopamine synthesis, suggesting possible agonist presynaptic actions. The present studies were aimed at investigating the actions of ***OPC*** on another measure of presynaptic function, dopamine release. The effects of ***OPC*** on dopamine release in rat striatum were compared to those of the D_2 selective antagonist sulpiride using two distinct preparations. The first of these utilized in vivo cerebral microdialysis in freely moving animals. We have previously shown that ***OPC*** (12 mg/kg) had little or no effect on the concentration of DA or its metabolites HVA and DOPAC, whereas haloperidol markedly increased all three. In the present studies, 1 µM ***OPC*** was infused directly into the dialysis cannula. Sulpiride, as expected, increased the efflux of dopamine and its metabolites DOPAC and HVA. Surprisingly, ***OPC*** increased dopamine overflow but, unlike sulpiride, had no effect whatsoever on the overflow of HVA and DOPAC. This was investigated further using superfused rat striatal slices preloaded with [^{3}H]dopamine. As expected, apomorphine inhibited [^{3}H] efflux, and this effect was blocked by sulpiride. Yet whereas sulpiride alone had no effect, ***OPC*** [1 µM] alone dramatically increased basal [^{3}H]dopamine release. These data demonstrate that ***OPC*** has neurochemical functional effects distinct from those of other antipsychotic candidates, and that this may play a role in its actions in vivo.

447.13

EVALUATION OF D_3 RECEPTOR FUNCTION IN VIVO: A MICRODIALYSIS STUDY *C.Routledge, L.Thorn, T. Ashmeade and S.Taylor. SmithKline Beecham Pharmaceuticals, Discovery Research, New Frontiers Science Park North, Third Avenue, Harlow, Essex, England, CM19 5AW

It was recently suggested that dopamine D_3 receptors may function as autoreceptors on nigrostriatal and mesolimbic dopaminergic neurones (Sokoloff *et al.*, 1990, Nature 347: 146), this was supported by data demonstrating that the D_3 receptor agonist 7-OH-DPAT induced a decrease in dopamine release in the striatum (Damsma *et al.*, 1993, Eur. J. Pharm. 249: R9). In the present study we have further characterised D_3 receptor function by determining the effects of the D_3 receptor agonists quinelorane and quinpirole (EC_{50} D_2/D_3=21.4; EC_{50} D_2/D_3=3.3 respectively, Sautel *et al.*, 1995, NeuroReports 6: 329) on dopamine release in the nucleus accumbens and striatum (the nucleus accumbens having a higher D_3/D_2 receptor density than the striatum) using microdialysis in the conscious rat.

Quinelorane (30 ug/kg s.c.) significantly ($p<0.01$) decreased dopamine release in both the nucleus accumbens and the striatum to 40.0±7.0 and 45.0±7.0 % of preinjection control levels respectively but was without effect at lower doses (0.1-10.0 ug/kg s.c.). Maximum effects were observed 60 min post-drug administration. Similarly quinpirole (30 ug/kg s.c.) significantly ($p<0.01$) decreased dopamine release in both the nucleus accumbens and striatum to 42.0±2.0 and 26.4±12.0 respectively but was without effect at lower doses (0.0-10.0 ug/kg s.c.). Again maximum effects were observed at 60 min post-drug administration. That there was no differentiation between the effects of these ligands in the nucleus accumbens and striatum, and the relatively high doses of agonists used to elicit the decrease in dopamine release suggest that this response may be mediated via D_2 rather than D_3 receptors.

447.14

DIFFERENCES IN EFFECTS OF PUTATIVE D3 DOPAMINE RECEPTOR AGONIST 7-OH-DPAT ON DOPAMINE RELEASE AND SYNTHESIS IN RAT BASAL GANGLIA *IN VIVO* T.D.Sotnikova*, R.R.Gainetdinov, T.V.Grekhova and K.S.Raevsky. Inst.of Pharmacology RAMS, Baltiyskaya 8, Moscow, 125315, Russia.

To study the role of D2 and D3 dopamine (DA) autoreceptors in presynaptic regulation of DA synthesis and release in mesolimbic and nigrostriatal targeting regions we investigated *in vivo* neurochemical effects of putative D3 DA receptor agonist 7-OH-DPAT using transcerebral microdialysis on awake rats. The drug dose-dependently decreases DA release, metabolism and synthesis of DA in nucleus accumbens (NAC) and dorsal striatum (DS). However the potency of 7-OH DPAT in decreasing of DA release was higher in the NAC vs. DS. DA metabolism assesed by DOPAC level and synthesis determined as extracellular L-DOPA following NSD-1015 pertusion (10^{-5} M) were decreased at higher dose ranges of 7-OH-DPAT. Importantly, that no difference between DS and NAC in this regard was observed. The hypomotility induced by 7-OH-DPAT well coressponds to decrease of DA release in NAC. Infusion of the drug into the brain also resulted in preferential decrease of DA release in NAC vs. DS. Pretreatment with 7-OH-DPAT at putative D3 DA receptor "selective" dose 0.03 mg/kg i.p. prior haloperidol or (+) AJ76 was found to prevent the increase of striatal DA release but not DOPAC level produced by both DA receptor antagonists. The present results give further support to the hypothesis that DA D3 terminal autoreceptor preferentially involved in regulation of DA release while D2 one regulates DA synthesis in rat basal ganglia *in vivo*.

I.p. administration of 7-OH-DPAT	ED50 (mg/kg)
Inhibition of locomotor activity	0.0044
Inhibition of DA release in NAC	0.0096
Inhibition of DA release in DS	0.068
Inhibition of DOPA output in NAC	0.124
Inhibition of DOPA output in DS	0.101
Local application of 7-OH-DPAT	**EC50 (nM)**
Inhibition of DA release in NAC	1.9
Inhibition of DA release in DS	11.3

447.15

BURST STIMULATION OF THE MEDIAL FOREBRAIN BUNDLE SELECTIVELY INCREASES FOS IMMUNOREACTIVITY IN THE LIMBIC FOREBRAIN OF THE RAT. K. Chergui, G.G. Nomikos, J.M. Mathé, F. Gonon and T.H. Svensson*. Dept. of Physiology & Pharmacology, Div. of Pharmacology, Karolinska Institutet, S-171 77 Stockholm, Sweden.

The present study was designed to evaluate the postsynaptic, functional consequences of different presynaptic activity patterns in midbrain dopamine (DA) systems using electrical stimulation of the rat medial forebrain bundle (MFB) and subsequent determination of c-*fos* expression, used as a marker for neuronal activation, in DA target areas, by means of Fos immunohistochemistry. Nerve terminal DA release evoked by electrical stimulation of the MFB was monitored in the same animals using *in vivo* voltammetry. A 5 Hz stimulation consisting of 60 trains of 5 pulses and lasting 1 min was applied to the MFB. This stimulation was repeated 15 times every 3 min. Its pattern was defined by the interpulse interval which was either 70 ms or 200 ms for burst or regularly spaced stimulation, respectively. Our results show that burst stimulation of the MFB, which increases release of DA in target areas, increases the basal expression of Fos in the stimulated hemisphere, while regular stimulation does not affect expression of this protein. Moreover, the increased expression of Fos induced by burst stimulation is restricted to limbic related structures, i.e. nucleus accumbens shell and intermediate aspect of the lateral septum, and the major island of Calleja, but is not observed in motor related structures (nucleus accumbens core and striatum). Finally, pretreatment with the D1-DA receptor antagonist, SCH23390 (0.1 mg/kg i.p.), blocked the increased expression of Fos induced by burst stimulation of the MFB, suggesting a role for these receptors in the observed effects. The present data indicate that, rather than the absolute mean discharge rate of midbrain DA neurons, the temporal organization of the action potentials they generate conveys information to their target areas.

447.16

CHRONIC CLOZAPINE ADMINISTRATION REDUCES STIMULUS-EVOKED DOPAMINE OVERFLOW IN THE RAT NUCLEUS ACCUMBENS IN VIVO. K.J. Feasey-Truger, C. Alzheimer and G. ten Bruggencate*, *Institute of Physiology, University of Munich, Pettenkoferstr. 12, 80336 Munich, Germany.*

The antipsychotic effects of neuroleptic drugs appear to arise from long-term adaptive changes in the mesolimbic dopamine (DA) system. A number of studies have shown that chronic treatment with the atypical neuroleptic, clozapine (CLOZ) selectively decreases **basal** DA levels in the nucleus accumbens (Acb), but not in the striatum. In this study, we have used fast cyclic voltammetry (FCV) to examine the effects of chronic CLOZ treatment on **stimulus-induced** i.e. impulse-dependent mesolimbic DA overflow in the Acb of the anaesthetized rat in vivo.

Treated rats (n=7) received CLOZ (20 mg/kg/d) in their drinking water for 21 days; control rats (n=8) received tap water alone. Electrochemical studies were performed under urethane anaesthesia. DA overflow was evoked via stimulus trains delivered every 5 min to the VTA (350 µs, 60 pulses, 60 Hz), and detected via a carbon fibre microelectrode positioned in the ipsilateral Acb. DA overflow in response to a range of stimulus intensities (0.2-1.0 mA) was compared in each group. Evoked DA overflow was significantly reduced in treated rats ($p<0.0001$). The effects of the DA autoreceptor agonist, quinpirole (QUIN), were also studied in treated and control rats. Quin (1.0 mg/kg, i.p.) significantly reduced evoked DA overflow in both groups: however, the magnitude and time course of the reduction in DA overflow did not differ significantly between the groups.

These results show for the first time that chronic CLOZ administration reduces stimulus-induced DA overflow in the rat Acb in vivo. This effect does not appear to be due to DA autoreceptor supersensitivity, since the QUIN-induced decrease in DA overflow was unchanged in CLOZ-treated rats. The mechanism underlying the observed reduction in DA overflow will be the subject of further study.

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447.17

EFFECT OF A TETRODOTOXIN PULSE VIA A MICRODIALYSIS PROBE ON DOPAMINE AND SEROTONIN SYNTHESIS AND METABOLISM IN RAT STRIATUM. K. W. Perry and R. W. Fuller*, Lilly Research Laboratories, Eli Lilly and Co., Lilly Corporate Center, Indianapolis, IN 46285.

Tetrodotoxin (TTX) has been widely used in microdialysis experiments to demonstrate that the substance being measured is released from neurons, because TTX is known to block the impulse-induced release of neurotransmitters (Westerink et al, Naunyn-Schmied. Arch. Pharmacol. 336, 502-507, 1987). Consistent with previous literature, we found that there was an immediate decline in extracellular dopamine and serotonin in rat striatal microdialysate upon infusion of TTX (3 µM) through the probe. The duration of the decrease was dependent on the length of the TTX pulse (15 or 60 min). Both serotonin and dopamine gradually returned to normal after the TTX infusions ended with serotonin recovering faster than dopamine. Extracellular concentrations of DOPAC did not change significantly while the TTX was present in the perfusate, but when TTX infusions ended extracellular DOPAC increased to approximately 300 and 400% over baseline after infusion durations of 15 and 60 min, respectively. Extracellular 5-HIAA decreased by 9% and 17% after TTX infusions of 15 and 60 min respectively, then returned to baseline. To determine if the changes observed in the dopamine and serotonin metabolites reflected changes in synthesis, a decarboxylase inhibitor was included in the perfusate along with TTX which enabled the measurement of L-DOPA and 5-HTP. Extracellular levels of L-DOPA measured in this way increased to 170% of baseline in the presence of TTX and returned toward normal after TTX infusion had ended. Extracellular levels of 5-HTP were measured at the same time and, after a 30 min delay, decreased by about 25%. The increase in dopamine synthesis after TTX infusion probably occurred due to a lack of dopamine at the presynaptic autoreceptor. In contrast, serotonin synthesis was decreased after TTX, indicating that the serotonin autoreceptor does not exert an influence similar to the dopamine autoreceptor in the rat striatum.

447.18

EFFECTS OF THE SIGMA LIGAND PENTAZOCINE ON THE EXTRACELLULAR CONCENTRATION OF DOPAMINE (DA) IN THE STRIATUM OF THE RAT. Gary A. Gudelsky*, College of Pharmacy, University of Cincinnati Medical Center, Cincinnati, OH. 45267

Sigma (σ) binding sites are characterized by their high affinity for certain opiates, antipsychotic drugs, steroids and guanidines. Data from behavioral and neurochemical studies are supportive of the view that σ ligands interact with dopaminergic systems in the brain. The present studies were undertaken to assess the effects of the σ ligand pentazocine on the release of DA from nigrostriatal neurons using in vivo microdialysis. The systemic administration of (+)-pentazocine (10-20 mg/kg, sc) produced approximately a 50% increase in the extracellular concentration of DA in the striatum; (-)-pentazocine was less effective. The administration of (+)-pentazocine (1 mM) through the dialysis probe for 120 min resulted in a biphasic effect on DA release: a brief increase of 75% was followed by a prolonged decrease of approximately 70%. The local perfusion of (-)-pentazocine (1 mM) also produced a biphasic response similar in magnitude to that produced by the (+)-isomer. Furthermore, the intrastriatal administration of the NMDA antagonist CPP (100 µM) diminished the initial increase but not the subsequent decrease in DA release elicited by the intrastriatal administration of (+)-pentazocine. These data are suggestive that DA release may be modulated by multiple σ receptor subtypes and that NMDA receptors also may mediate the stimulatory effect of σ ligands on DA release in the striatum.

447.19

METHYLENEDIOXYMETHAMPHETAMINE (MDMA)-INDUCED DOPAMINE RELEASE IN RATS: EFFECTS OF RESERPINE PRETREATMENT AND ALTERATIONS IN CORE BODY TEMPERATURE. K.E. Sabol*, M. Balcells, L.S. Seiden. Dept. Pharm./Phys. Sci., University of Chicago, Chicago, IL 60637

MDMA increases dopamine release. Pretreatment with reserpine (RES), which depletes vesicular dopamine stores, attenuates the MDMA-induced dopamine release. RES also decreases core body temperature. The purpose of this report was to determine whether the RES-induced reduction in body temperature is responsible for the attenuation in MDMA-induced dopamine release. Using in vivo dialysis, dopamine release was measured in three treatments groups: 1.) MDMA alone (N=5). 2.) MDMA plus RES (N=5), and 3.) MDMA, RES, plus heat (N=8). Animals were implanted with guide cannulae (above striatum) one week before testing. The evening before testing, dialysis probes were lowered into the guides, and the animals were connected to the dialysis perfusion system. RES (10.0 mg/kg) was administered 18 hr prior to MDMA (10.0 mg/kg) treatment. At the start of the experiment the average core temperature of all the RES pretreated rats was 32.2 (± 0.5 SEM) degrees C. In the group receiving heat treatment, core temperature was maintained between 37 and 39 degrees C (range of normal body temperature) using a heat lamp. As was previously found, RES significantly attenuated the MDMA-induced dopamine release. When the core temperature in RES pretreated animals was maintained at 38 degrees C, the MDMA-induced dopamine release was intermediate between the MDMA alone and the RES pretreated groups. The heated reserpinized group however, was not significantly different from either the RES (not heated) pretreated or the MDMA alone groups. The heated reserpinized group demonstrated substantial variability, with some animals overlapping with the RES (not heated) rats and some animals overlapping with the MDMA alone groups. Although cooling may be responsible for some of the attenuation of MDMA-induced dopamine release in reserpinized animals, other important variables appear to be involved (Supported by NIDA DA-00085 & RSA 10562, L.S. Seiden).

447.20

RESERPINE DOES NOT AFFECT THE ACCUMULATION OF 3-METHOXYTYRAMINE FOLLOWING PARGYLINE IN THE DIALYSATE FROM THE SUBSTANTIA NIGRA. A. Elverfors*, J. Jonason and H. Nissbrandt. Department of Pharmacology, Göteborg University, Medicinaregatan 7, S-413 90 Göteborg, Sweden.

Indirect biochemical methods, as accumulation of the dopamine (DA) metabolite 3-methoxytyramine (3-MT) after monoamine oxidase inhibition has been used as an indirect measurment of DA release. Results obtained with this indirect method are in accordance with results obtained with more direct methods, as microdialysis technique, when DA release is measured in in terminal regions, as the striatum, but not when measured in somatodendritic regions, as the substantia nigra (SN). This study was undertaken to further investigate the discrepancy in the SN between the different methods to measure DA release. The DA and 3-MT concentrations were measured by microdialysis in freely moving rats in the SN and in the striatum following pargyline (75 mg/kg) treatment and reserpine (5 mg/kg) plus pargyline treatment. Pargyline treatment only increased the DA concentrations to 180% of basal levels in the striatum and to 310% of basal levels in the SN. Combined treatment with pargyline and reserpine decreased the DA concentrations to 6% of basal levels in the striatum and to 13% of basal level in the SN. In the striatum, the 3-MT concentrations in the dialysate decreased when the animals were treated with reserpine plus pargyline as compared to when treated with pargyline only. However, in the SN the 3-MT concentrations following pargyline plus reserpine was equal with the concentrations following pargyline only. This suggest that pargyline-induced 3-MT accumulation in the SN does not reflect extracellular concentrations of DA. A possible explanation for this discrepancy between the striatum and the SN could be that the metabolic enzyme COMT is located in dopaminergic neurons in the SN but not in the striatum.

CATECHOLAMINES: ELECTROPHYSIOLOGICAL STUDIES

448.1

DIFFERENTIAL EFFECTS OF ACUTE AND CHRONIC FLUOXETINE ON THE ELECTRICAL ACTIVITY OF DOPAMINERGIC NEURONS IN THE VENTRAL TEGMENTAL AREA. E. Esposito* and S. Prisco. Istituto di Ricerche Farmacologiche "Mario Negri", Consorzio "Mario Negri" Sud, Santa Maria Imbaro (Chieti), Italy.

There is extensive evidence that the activity of midbrain dopamine (DA) neurons is modulated by the serotonergic system. Electrophysiological studies have shown that serotonin (5-HT) exerts an inhibitory action on the firing rate of DA neurons in the ventral tegmental area (VTA) and the substantia nigra, pars compacta (SNc). Thus, it is conceivable that selective 5-HT reuptake inhibitors, such as the antidepressant fluoxetine, may affect DA function. In order to investigate this point, we have studied the effects of acute and chronic administration of fluoxetine on the electrical activity of DA neurons in both the VTA and the SNc. Extracellular single unit recordings were performed *in vivo* on male Sprague Dawley rats, anesthetized with chloral hydrate. DA neurons in the VTA and SNc were identified by their location, waveform, firing rate and pattern. Acute i.v. injection of fluoxetine (20-1280 μg/kg) caused a dose-dependent inhibition of the firing rate of VTA DA neurons, but it did not affect the activity of DA cells in the SNc. Pretreatment with the specific 5-HT2c receptor antagonist mesulergine (80 μg/kg, i.v.) blocked the inhibitory effect of fluoxetine on VTA DA cells. Selective lesions of 5-HT neurons by the neurotoxin 5,7-dihydroxytryptamine abolished the reduction of VTA DA activity induced by fluoxetine. In another series of experiments, i.p. fluoxetine (10 mg/kg) or saline was administered once daily for 21 consecutive days. Acute i.v. administration of fluoxetine (20-1280 μg/kg, 72 hours after the last i.p. injection) did not modify the basal firing rate of VTA DA neurons in treated rats, whereas it induced the typical inhibitory effect in control animals. A group of rats chronically treated with fluoxetine, received i.v. m-chlorophenylpiperazine (10-320 μg/kg), a 5-HT2c receptor agonist. This drug significantly inhibited VTA DA function in control rats, but it did not modify the basal activity of DA cells in animals given chronic fluoxetine. On the basis of these findings it is possible to argue that prolonged administration of fluoxetine induces a down-regulation of 5-HT2c receptors.

448.2

THE ELECTROPHYSIOLOGICAL EFFECTS OF MONOAMINO OXIDASE INHIBITORS ON RAT DOPAMINERGIC NEURONS INTRACELLULARLY RECORDED *IN VITRO*. N. B. Mercuri, P. Calabresi, A. Bonci, A. Pisani, R. Vagnozzi*, G. Bernardi. Clinica Neurologica, Univ. Tor Vergata, 00173, Roma, Italy.

The effects of unspecific and specific monoamino oxidase (MAO) inhibitors were studied on the spontaneous firing of the dopaminergic neurons intracellularly recorded in slices of the ventral midbrain. The non-specific MAO inhibitor pargyline superfused at a concentration of 30- 100 μM irreversibly inhibited within 5 - 15 minutes of application the spontaneous firing discharge of the neurons either in the substantia nigra pars compacta and/or in the ventral tegmental area. The D2 receptor antagonist sulpiride (100 - 300 nM) reversed the effect of pargyline. The selective inhibitor for MAO A, clorgyline (30 - 100 μ M) slight depressed the firing rate of the DA neurons. Contrariwise, the MAO B inhibitor deprenyl (30 - 100 μM) reduced or blocked the spontaneous discharge of dopaminergic cells and this effect was at least in part reversible. Our electrophysiological data suggest that only a mixed inhibition of type A and type B MAO is able to profoundly inhibit the dopamine-containing neurones of the rat mesencephalon. This effect is probably due to an increased action of endogenous dopamine on DA autoreceptors The transient inhibition produced by l-deprenyl could be due to an amphetamine-like effect of this compound.

448.3

CHARACTERIZATION OF HUMAN MIDBRAIN NEURONS IN CULTURE. W.-X. Shi, H.G. Foellmer, C.L. Pun, D.E. Redmond, Jr and B.S. Bunney*. Depts of Psychiatry and Pharmacology, Yale Univ Sch of Med, New Haven, CT 06510

Our knowledge of brain neurons has come largely from studies in non-human species. To study human neurons directly, we have developed a technique for culturing human fetal midbrain neurons and have begun to compare their properties with those of rat neurons. Human fetal tissue was collected following termination of pregnancy (7-11 wks; Redmond et al., *Science* **242**:768, 1988). Ventral mesecephalon neurons from either freshly collected or cryopreserved fetal tissue were cultured using methods similar to those previously described for rat neurons (Rayport et al.,*J Neurosci,* **12**:4264, 1992). They had cell bodies 5.8-14.7 μm in diameter (mean±SEM=8.35±0.31, n=43), exhibiting different shapes, and extensive processes. Whole cell recordings were made from 33 human neurons cultured for an average of 20 days. All fired action potentials either spontaneously or during current injection. TTX (1μg/ml, n=5) blocked the action potential and Co^{2+} (2 mM) reduced its size (7/8 cells), suggesting the involvement of both Na^{+} and Ca^{2+} in the action potential of these cells. Spontaneous synaptic potentials (both IPSPs and EPSPs) were frequently observed, indicating that human fetal neurons were capable of making synaptic connections in the culture. Application of glutamate and GABA evoked robust responses in all cells tested, suggesting the presence of receptors for both transmitters on these neurons.

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448.4

FURTHER CHARACTERIZATION OF DOPAMINE-INDUCED DEPOLARIZATION OF NEURONS IN THE PREFRONTAL CORTEX. X.-F. Liang, B.S. Bunney and W.-X. Shi*. Depts of Psychiatry and Pharmacology, Yale Univ Sch of Med, New Haven, CT 06510

Last year, we reported that dopamine (DA) dose-dependently depolarized both pyramidal and non-pyramidal neurons in the prefrontal cortex (PFC) and increased their excitability (Shi and Bunney, *Soc Neurosci Abstr* **20**:1354, 1994). However, this effect was not mimicked by either D1- or D2-like receptor agonists or blocked by D1- or D2-like receptor antagonists. It was, thus, suggested that the depolarizing effect of DA in the PFC may be mediated non-specifically by non-DA receptors, such as those for norepinephrine (NE) and serotonin (5-HT). To test this possibility, the effect of DA on PFC neurons was reexamined in the presence of the antagonists for these non-DA receptors. PFC slices (400 μm) were prepared from male Sprague-Dawley rats weighting between 75 and170 g and maintained in an interface slice chamber. Neurons in layers V and VI of the medial PFC were recorded in whole cell mode and drugs were applied through the bath. Confirming the previous finding, DA dose-despendently depolarized most neurons tested. Surprisingly, this effect was not blocked by either the α antagonist phentolamine (10 μM, n=8) or the β antagonist alprenolol (10 μM, n=12), or a mix of the two (n=3). The effect also persisted in the presence of high concentrations of clozapine (10 μM, n=5; 100 μM, n=11), an atypical antipsychotic drug that blocks multiple receptors including those of DA and 5-HT. These results again (Sesack & Bunney,*JPET* **248**:1323, 1989) suggest the existence of an as yet uncharacterized class of DA receptors in the PFC, which may be responsible for the observed depolarizing effect of DA.

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448.5

REPETITIVE FIRING PROPERTIES OF NIGRAL DOPAMINE (DA)-CONTAINING NEURONS *IN VITRO*. H.X. Ping and P.D. Shepard* MD Psychiatric Res. Cntr. & Univ. of MD School of Med., Baltimore, MD 21228.

DA-containing neurons *in vivo* exhibit an adaptive response to long duration depolarizing pulses consisting of a progressive reduction in firing frequency (Grace & Bunney, 1984). This phenomena, referred to as spike frequency adaptation (SFA), is reported to be considerably less pronounced *in vitro*. In the present study, intracellular recording techniques were used to investigate the voltage dependence and pharmacological sensitivity of spike frequency modulation exhibited by these neurons *in vitro*. In agreement with previous studies, DA neurons showed little evidence of SFA in response to low intensity stimuli applied during spontaneous spiking. Further increases in stimulus strength reduced the intervals between initial spikes in the train after which firing frequency rapidly adapted to a steady state value that was maintained for the duration of the stimulus. In marked contrast to this response, 14 of 18 cells depolarized from a holding potential of -64 mV exhibited a progressive increase in firing frequency. Bath application of TEA (20 mM, n=5) increased spike width as well as the number of spikes evoked in response to membrane depolarization but had no effect on the temporal organization of spike trains. Cd^{2+} (200 μM; n=5) prevented the paradoxical increase in firing frequency but failed to induce SFA. These effects were accompanied by an increase in spike width and a reduction in the amplitude of the post-spike afterhyperpolarization (AHPs; 17.6 ± 3% decrease). A further reduction in AHPs amplitude was observed following 100 nM apamin (88.7 ± 8.7%) and was associated with both a marked increase in neuronal excitability and the appearance of SFA. These data suggest that the repetitive firing properties of DA neurons are modulated principally by AHPs. It is further suggested that loss of SFA *in vitro* may reflect an increase in the apamin-sensitive conductance responsible for generating AHPs.

448.6

DIFFERING EFFECTS OF HALOPERIDOL VERSUS MK-801 ON NONLINEAR SEQUENTIAL STRUCTURE OF DOPAMINE NEURON FIRING PATTERNS. R.E. Hoffman*, W.-X. Shi, B.S. Bunney. Dept. of Psychiatry, Yale Univ. Sch. of Med. New Haven, CT 06520.

The acute effects of systemic haloperidol on deterministic structure of interspike interval (ISI) patterns of dopamine (DA) neurons were studied using an analysis of dynamical complexity. Data consisted in single unit extracellular recordings of A9 and A10 DA neurons in rats anesthetized with chloral hydrate. Haloperidol (.5mg/kg) produced increased nonlinear sequential structure of single unit extracellular ISI firing patterns in 6/6 A9 and 5/6 A10 DA neurons. Nonlinear organization is known to enhance flexibility of dynamical systems. Our preliminary investigation of MK-801 (.2mg/kg), a drug with psychotomimetic properties, demonstrated an opposite effect, namely reductions in nonlinear firing pattern structure in 5/6 A9 cells. Altered nonlinear structure was not secondary to activation *per se* since both haloperidol and MK-801 increased firing rate of DA cells. Is it possible that antipsychotic effects of haloperidol include enhancement of nonlinear organization and functional flexibility of neural networks incorporating DA cells?

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448.7

THE EFFECTS OF PD 128907 ON THE FIRING ACTIVITY OF SUBSTANTIA NIGRA ZONA COMPACTA DOPAMINE (DA) AND ZONA RETICULATA (ZR) NEURONS L.T. Meltzer*, C.L. Christoffersen and K.A. Serpa. Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Co., Ann Arbor, MI 48105.

PD 128907(R-(+)-trans-3,4,4a,10b-tetrahydro-4-propyl-2H,5H-[4,3-b]-1,4-oxazin-9-ol) has an 18-fold higher affinity for DA D3 vs. DA D2 receptors in vitro (DeMattos. et al., Soc. Neurosci. Abst. 19:77,1993). Thus. it represents a potential tool for discriminating the relative functional roles of D3 versus D2 receptors. In the present studies we compared the inhibitory effects of PD 128907 (PD), quinpirole (Q; 9-fold D3/D2) and apomorphine (A, 3-fold D2/D3), administered i.v. in a cumulative dose-response, on the firing activity of substantia nigra DA and ZR neurons in chloral hydrate anesthetized rats. The ability of various DA antagonists to selectively block the effects of PD versus A, both tested at 0.1 mg/kg i.p., was also evaluated.

PD, Q and A all produced similar, complete inhibitory effects on DA neuronal activity. The dose-response curves for all three agents overlapped. Thus, D3 versus D2 selectivity did not alter the DA autoreceptor agonist effects.

A and PD, both at 0.1 mg/kg i.p, produced similar decreases in DA neuronal activity. Pretreatment with haloperidol (0.03 & 0.1 mg/kg i.p.; 5-fold D2/D3), (-)-sulpiride (10 mg/kg i.p.; 2-fold D2/D3) or remoxipride (2 mg/kg i.p.; 10-fold D2/D3) produced similar antagonism of the PD- or A-induced inhibition of DA neuronal activity. Thus, these studies utilizing antagonists with varying selectivity for D2 and D3 receptors did not reveal a preferential role for D2 or D3 receptors in mediating the agonist effects of A or PD.

PD and Q produced a dose-related biphasic effect on ZR neuronal firing. Relatively low doses (0.5-16 µg/kg i.v.) produced an increase in activity, which was returned to or below baseline with higher doses (> 32 µg/kg i.v.). However, in control recordings there was also a slow spontaneous increase in neuronal firing. Thus, only relatively high doses of PD and Q, which are unlikely to show D3 vs D2 receptor selectivity, produced measurable effects on ZR neuronal activity. H (0.3 mg/kg i.p.), antagonized the secondary decrease in activity. In conclusion, these studies did not clearly differentiate between D2 or D3 mediated actions of DA agonists.

448.8

IBOTENIC ACID LESIONS OF THE VENTRAL HIPPOCAMPUS INCREASES THE PROPORTION OF BURSTING MIDBRAIN DOPAMINE NEURONS IN THE A10 AREA OF THE RAT. **L.Wang, J. Zhang and D.K. Pitts*. Dept. of Pharmaceutical Sciences, College of Pharmacy, Wayne State University, Detroit, MI 48202.**

An excitotoxic lesion was produced in 6-week-old rats by infusing ibotenic acid into the ventral hippocampus (VH). One experimental group had bilateral microinjections of ibotenic acid (n=18) into the VH while the other had bilateral vehicle (saline; n=16) injections. After a one month recovery period rats were anesthetized with chloral hydrate and prepared for standard *in vivo* extracellular electrophysiological recording. The number of spontaneously active midbrain dopamine (DA) neurons in the A9 and A10 areas was determined within a stereotaxically defined block of tissue created by 9 equally separated (200μm) electrode tracks per nucleus. No significant treatment-dependent difference in the number of DA neurons per electrode track was found (ANOVA, P>0.20 for main effects and interaction). A three-way frequency (log-linear) analysis was used to examine the distribution of cells according to three factors: cell type (A9/A10), treatment group (lesion/control) and discharge pattern (bursting/non-bursting). Significant marginal/partial associations were found for the cell type x discharge pattern (P<0.001/ P<0.001) and treatment x discharge pattern (P<0.01/P<0.02) interactions. This result indicated that overall there was a greater proportion of bursting neurons in the A10 (75%) vs A9 (59%) cell groups, and that for the A10 cell group, but *not* the A9 cell group, there was a greater proportion of bursting neurons in lesioned animals (81%) relative to control animals (68%). No significant treatment-dependent differences were found for seven parameters used to describe the pattern displayed by bursting neurons. These results suggest that bilateral VH lesions may increase the proportion of bursting DA neurons associated with the mesolimbic and/or mesocortical DA pathways. **Supported by MH47857 (DKP).**

448.9

LACK OF PACEMAKER ACTIVITY IN THE NIGRAL DOPAMINE NEURONS OF NEONATES: EXTRACELLULAR ELECTROPHYSIOLOGICAL STUDIES IN VITRO. **J. Zhang*, M.W. Lewis and D.K. Pitts. Department of Pharmaceutical Sciences, College of Pharmacy, Wayne State University Detroit, MI 48202.**

Midbrain slices containing the substantia nigra and ventral tegmental area were obtained from rats anesthetized with chloral hydrate (400 mg/kg i.p.). The activity of single nigral dopamine (DA)-containing neurons was sampled *in vitro* from midbrain slices at the postnatal ages of 1-2 days old, 1-, 2- and 4-weeks old, and from juveniles (approximately 7 weeks old) using standard electrophysiological techniques. The distribution of spontaneously active DA neurons was found to differ in an age-dependent manner. A proportionately greater number of spontaneously active DA neurons was found in both the pars reticulata and pars compacta of midbrain slices from animals 2-weeks-old or less than from slices in juvenile rats. In the slices from animals 2-weeks-old or less there was also a greater proportion of spontaneously active non-DA neurons in both the pars reticulata and pars compacta relative to juveniles. The discharge rate of the nigral DA neurons progressively increased in slices from 1-2 day-old to juvenile animals in an age-dependent manner (P<0.001). The nigral DA neurons from slices older than 2-weeks-old discharged in a very regular pattern characteristic of pacemaker activity. However, as earlier postnatal ages were examined the discharge pattern of DA neurons became progressively less regular. The average coefficient of variation (CV) for interspike intervals of nigral DA neurons from slices less than 2-weeks old was significantly (P<0.001) greater (2x to 6x) than the average CV of DA neurons from older slices. Preliminary evidence suggests that DA neurons from 1-week-old slices have somatodendritic autoreceptors that are less sensitive to the inhibitory effects of DA agonists than those from juvenile animals. These studies suggest that electrophysiological properties intrinsic to DA neurons are still developing during the postnatal period. **Supported by MH47857 (DKP).**

448.10

PRENATAL ETHANOL EXPOSURE DECREASES THE ELECTRICAL ACTIVITY OF A9 AND A10 DOPAMINE NEURONS IN ADULT RATS. Roh-Yu Shen*. Cellular and Clinical Neurobiology Program, Department of Psychiatry and Behavioral Neuroscience, Wayne State University School of Medicine. Detroit, MI 48201.

Prenatal ethanol exposure has been observed to alter dopamine (DA) content, turnover, and receptors in the striatum as well as DA receptor-mediated behaviors. Because the function of the midbrain DA systems is related to the electrical activity of DA neurons, we investigated the effect of prenatal ethanol exposure on the electrical activity of DA neurons in the substantia nigra (A9) and ventral tegmental area (A10). Pregnant rats were intubated by 0, 3, or 5 g/kg of ethanol once daily from gestation day 8 - 20. Rats in the *ad lib* group received no intubation. At 2-4 months of age, male offspring from each group were anesthetized with chloral hydrate and the electrical activity of A9 and A10 DA neurons was sampled by extracellular single-unit recording and cells-per-track sampling techniques. There were no differences in the electrical activity of DA neurons between the *ad lib* and 0 g groups in either A9 or A10. In A9, prenatal ethanol treatment decreased the number of spontaneously active DA neurons by 30%. In A10, the numbers of spontaneously active DA neurons were reduced by 25% and 47% in 3 g and 5 g groups respectively. The average firing rates of DA neurons were not changed by prenatal ethanol exposure. These observations suggest that the decreased DA content and turnover in the striatum after prenatal ethanol exposure may result from decreased electrical activity of midbrain DA neurons. The hypofunction in midbrain systems also support the view that the treatment effect of amphetamine-like stimulants on attention problems often observed in fetal alcohol children is due to their ability to increase the release of DA. [Supported by AA07606]

448.11

CYCLIC AMP-SENSITIVE SUBCONDUCTANCE STATE OF DOPAMINE-MODULATED K^+ CHANNELS ON RAT CAUDATE-PUTAMEN NEURONS. Gabriela J. Greif*, Yong-Jian Lin, and Jonathan E. Freedman. Dept. Pharmaceutical Sciences, Northeastern Univ., Boston, MA 02115.

We are characterizing the properties of an 85 pS K^+ channel which is modulated by D_2-like dopamine receptors on freshly dissociated rat corpus striatum neurons, using cell-attached patch-clamp recording. Previous studies indicated that this channel has an absolute requirement for the presence of D_2-like dopamine agonists within the patch pipette for activation, but can also be activated by the G-protein stimulating peptide mastoparan. Agonists applied outside the cell-attached patch pipette were ineffective at channel activation, arguing against a direct role for a diffusible second messenger in receptor-channel coupling. We now report that the membrane-permeable cyclic AMP analog Sp-cAMPS and the cAMP blocker Rp-cAMPS (each at 100 μM) were ineffective at either activating or inhibiting this channel, making it unlikely that changes in cAMP levels mediate this action of D_2 receptors. However, Sp-cAMPS (but not Rp-cAMPS) reduced channel closed time, while increasing channel dwell time in a subconductance state. This implies a more subtle role for cAMP in dopamine receptor signal transduction than previously anticipated, and suggests the existence of another receptor on these cells acting cooperatively with D_2 receptors via the cAMP system. If this other receptor were a D_1 receptor, our results could provide a simple molecular model for D_1-D_2 receptor cooperativity. (Supported by MH-48545.)

448.12

D_2 DOPAMINE-MODULATED K^+ CHANNEL EXPRESSION ON RAT STRIATOPALLIDAL AND STRIATONIGRAL NEURONS. Lynn P. Martin*, Gabriela J. Greif, Jonathan E. Freedman and Barbara L. Waszczak. Dept. Pharmaceutical Sciences, Northeastern Univ., Boston, MA 02115.

Medium spiny neurons of the caudate-putamen can be classified as projecting to either the globus pallidus or the substantia nigra. The distribution of D_1-like and D_2-like dopamine receptors between these two cell populations is a matter of controversy. We have previously used cell-attached patch-clamp recording to describe an 85 pS K^+ channel which is modulated by D_2-like dopamine receptors on a subpopulation of freshly dissociated rat corpus striatum neurons. This channel was observed in about half of patches from cells with a characteristic shape, which were immunoreactive for neuron-specific enolase and for GABA. We are now using retrograde labeling with fluorescent latex microspheres in order to identify projection neurons during our electrophysiological recordings. We found that the 85 pS channel was expressed in a significant proportion of recordings from neurons projecting to the globus pallidus, demonstrating the presence of this channel on medium spiny neurons, and consistent with some models of D_1-D_2 receptor distribution. There were often multiple channels per patch when present. We are currently assessing whether the D_2-modulated K^+ channel is also expressed on neurons projecting to the substantia nigra.

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448.13

THE FUNCTIONAL PHARMACOLOGY OF D3 RECEPTORS: SINGLE NEURON ANALYSIS OF THE VENTRAL PALLIDUM. A. Javed*, F. Rehman, B.A. Heidenreich, E.R. Larson[1], M.A. Ariano[1] and T.C. Napier. Dept. Pharmacol., Loyola Univ. Chicago, Sch. Med., Maywood, IL 60153; and [1]Dept. Neurosci., Univ. of Health Sci./Chicago Med. Sch., North Chicago, IL 60064

Using polyclonal antibodies selectively generated against unique peptide fragments of the native dopamine (DA) D3 receptor protein, D3 receptors were identified in both perikarya and fibers throughout the basal forebrain, including the ventral pallidum (VP). To ascertain if D3 receptors regulate VP neuronal firing, extracellular single-unit recordings were obtained from >200 neurons in chloral hydrate-anesthetized rats and responses to D3-preferring agents were monitored. Intravenous administration of the D3-preferring agonists PD128907 (30μg/kg) or 7OHDPAT (0.3-38μg/kg) altered firing in 74% and 56% of the neurons tested, respectively. Both rate increases and decreases were observed. The D3-preferring antagonists (+)AJ76 (0.6mg/kg) or U99194A (0.14mg/kg), antagonized these effects in 70% and 73% of the neurons, respectively. In contrast, 0.6mg/kg of (+)AJ76 attenuated only 12% of the VP responses to the D2 agonist, U91356 (30μg/kg); 1.2mg/kg (+)AJ76 was less specific, in that this dose antagonized 80% of the responses to 100μg/kg of U91356. To verify that the activated receptors were located within the VP itself, microiontophoresis was used to locally apply DA agonists. Iontophoresis of PD128907 altered firing in 48% of the VP neurons tested. Sixty percent of these were antagonized by 0.6mg/kg i.v. (+)AJ76. Responses evoked by PD128907 differed from those evoked by iontophoresis of DA or the D2/D3 agonist quinpirole. These results support an important functional role for the D3 DA receptor subtype in the regulation of basal forebrain neuronal activity, and demonstrate that at the appropriate concentrations, D3-preferring agents can be selective for this receptor subtype. Work made possible by gifts from UpJohn and RBI, and grants MH45180 to TCN and NS23079 to MAA.

448.14

ELECTROPHYSIOLOGICAL EVIDENCE ASSOCIATING LIMBIC PREFERENCE WITH PRAMIPEXOLE, A D_3-PREFERRING DOPAMINE AGONIST. W.E.Hoffmann, M.W.Smith, D.K.Hyslop and M.F.Piercey, The Upjohn Company, Kalamazoo, MI 49001

In situ mRNA hybridization experiments suggest a preferential distribution of D_3 receptors in the mesolimbic dopaminergic (DA) pathways (Bouthenet *et al.*, Br Res 564:203, 1991). Pramipexole (PPx), a D_3-preferring DA agonist, was evaluated for its effects on firing of neurons in substantia nigra pars compacta (SNPC), ventral tegmental area (VTA), substantia nigra pars reticulata (SNPR) and nucleus accumbens (NA). PPx was equipotent in inhibiting SNPC and mesolimbic VTA DA neurons. PPx effects were antagonized by haloperidol, a D_2-preferring DA antagonist (Sokoloff *et al.*, Nature 347:146, 1990) and U-99194A, a D_3 dopamine antagonist (Waters *et al.*, J. Neural Transm 94:11, 1993). In SNPC, U-99194A was more potent in antagonizing PPx than in blocking U-91356A, a D_2-preferring DA agonist. In SNPR, the output target of striatal efferents, PPx inhibited approximately half the cells; a few were stimulated. PPx doses required in SNPR were much larger than those required in SNPC. Even large doses of haloperidol only partially reversed PPx inhibitions in SNPR. However, U-99194A was as potent in reversing PPx inhibitions in SNPR as it was in SNPC. In the NA, PPx again inhibited about half the cells, although approximately 20% were stimulated. PPx was more potent in depressing NA neurons than in depressing SNPR neurons; potencies for depressing NA approximated those for depressing VTA. U-99194A tended to more commonly antagonize PPx inhibitory effects than did haloperidol. Overall, the data suggest that pramipexole has a mesolimbic preference and exerts most of its biological effects via the D_3 receptor.

448.15

MODULATORY ACTION OF DOPAMINE ON STRIATAL AND ACCUMBAL NEURONS: IONTOPHORESIS IN AWAKE, UNRESTRAINED RATS. G. V. Rebec* and E. A. Kiyatkin. Prog. in Neural Science, Dept. Psychology, Indiana University, Bloomington, IN 47405

Although monitoring dopamine (DA) neuronal activity or fluctuations in terminal DA release may help identify the role of the DA system in goal-directed behavior, it is equally important to know the effects of released DA on its targets under natural conditions. To assess this issue, single-unit recording was combined with iontophoresis to study the effects of DA and glutamate (Glu) on spontaneous impulse activity in the striatum and nucleus accumbens in awake, unrestrained rats.

Approximately half of the recorded striatal and accumbal cells were inhibited by a brief DA application (20-30 s) at low ejection currents (10-30 nA). Although small in magnitude, this effect occurred with short onset and offset latencies and was dependent upon basal impulse activity and ongoing behavior. The DA-induced inhibition was attenuated or disappeared entirely when neurons were phasically activated during spontaneous motor behavior. Prolonged DA application (120-180 s) not only inhibited basal impulse activity but enhanced the signal-to-noise ratio of Glu responses. In most cases, this DA-induced enhancement was associated with: a depression of both basal impulse activity and Glu responses (40%), an inhibition of basal impulse activity only (37%), or with a slight increase in Glu-response magnitude (23%). The enhancing effect was maximal at relatively low DA ejecting currents (10-30 nA), on cells with moderate rate of basal impulse activity, which had an initially small and moderate magnitude of Glu responses. Because Glu afferents appear to provide the major excitatory input to striatal and accumbal cells from widespread areas of cerebral cortex, a DA-induced modulation of Glu responses may serve as a mechanism for enhancing the transmission of a wide range of behaviorally significant information.

Supported by NIDA (DA 02451).

448.16

DIHYDREXIDINE DEMONSTRATES FUNCTIONAL SELECTIVITY AT D_2 DOPAMINE RECEPTOR MEDIATED PROCESSES. H.P. Smith[1]*, G.S. Oxford[1], D.E. Nichols[2], R.B. Mailman[1] and C.P. Lawler[1]. Univ. of North Carolina[1], Chapel Hill, NC 27599, and Purdue Univ.[2], W. Lafayette, IN 47907

Dihydrexidine (DHX), a member of the novel hexahydrobenzo[a]phenanthridine dopamine agonists, is best known as a full intrinsic activity D_1 agonist (Mottola et al., 1992) that has potent antiparkinsonian effects in MPTP-treated primates (Taylor et al., 1991). Although ten-fold D_1 selective, DHX also has high affinity for the D_2 receptor ($K_{0.5}$ 80 nM), yet its functional profile is unusual: In all assays that represent functions mediated by "postsynaptic" D_2 receptors (e.g., inhibition of adenylyl cyclase activity in striatal slices), DHX demonstrates clear agonist activity. Conversely, in D_2-mediated "presynaptic" functions (e.g., inhibition of nigral cell firing, or inhibition of dopamine synthesis or release), DHX is inactive even though it binds with similar affinity to both pre- and post-synaptic receptors. Despite this unusual functional profile, DHX apparently binds equally well to both pre- and post-synaptic receptors. Thus, we hypothesized that the functional selectivity of DHX at D_2 receptors may depend on the nature of the transduction mechanism (e.g., D_2 coupled cyclase vs. D_2 mediated opening of inwardly-rectifying potassium ion channels) This hypothesis was tested by performing full DHX dose-response experiments using both whole-cell and perforated patch clamp methods in single pituitary lactotrophs in vitro. Compared to dopamine, DHX demonstrates only partial agonist efficacy to elicit potassium currents, with a peak current value only one-fourth that of dopamine. As with dopamine, this response is specifically blocked by the selective D_2 antagonist (-)-sulpiride (30 μM). The combination of DHX plus dopamine results in a significant decrease in the current caused by dopamine alone, consistent with the idea that the drugs are competing for the same receptor. If DHX inhibits cAMP synthesis in these cells as it does in striatum, it would provide a mechanistic explanation for the unusual functional discrimination of this class of agonists

448.17

ACTIVATION OF DARPP-32, AN INHIBITOR OF PROTEIN PHOSPHATASE 1, MODULATES THE VOLTAGE-GATED SODIUM CURRENT IN STRIATAL NEURONS. S.N. Schiffmann*[1], P.-M. Lledo[2], F. Desdouits[3], P. Greengard[4], J.J. Vanderhaeghen[1], J.-D. Vincent[2] & J.-A. Girault[3] [1]Brain Research Unit, ULB, Brussels, Belgium, [2]Inst. A. Fessard, CNRS, Gif-sur-Yvette, France, [3]INSERM U114, Paris, France, [4]Rockefeller University, NY, NY, USA

Dopamine D1 receptor stimulation reduces sodium current (I_{Na}) by promoting activation of cAMP-dependent protein kinase (PKA) in striatal neurons which results in a reduction of neuronal excitability (Schiffmann *et al.*, J. Physiol. 483:95-107, 1995). From these results, we concluded that direct phosphorylation of voltage-gated sodium channels by PKA leads to this reduction. It seems possible that phosphorylation of DARPP-32 (*d*opamine- and cAMP-regulated *p*hosphoprotein, Mr =32000) by PKA in response to D1 receptor stimulation might result in a similar effect through its inhibition of protein phosphatase 1 (PP1) and therefore the stabilization of phosphorylated states of the sodium channels. Therefore, we used whole-cell patch-clamp recordings of striatal neurons to study the effect of phospho-DARPP-32 and dephospho-DARPP-32 on I_{Na}. Phospho- or non-phospho- recombinant DARPP-32 (0.5 mg/ml) was loaded into the cytosol of neurons by simple diffusion from the pipette solution. Loading of phospho-DARPP-32 resulted in a time-dependent decrease in I_{Na} (19.2±3.0%, mean±S.E.M.) whilst dephospho-DARPP-32 did not significantly reduce I_{Na} (3.7±1.3%). To ascertain whether this effect may result from an inhibition of PP1 activity, okadaïc acid (1 µM) was similarly loaded into striatal neurons and this led to a significant decrease of I_{Na} (23.1±2.9%) as compared to a control solution (3.6±0.7%). We therefore propose that, in striatal neurons, phospho-DARPP-32 reduces sodium current by inhibiting dephosphorylation of voltage-gated sodium channels and that this contributes to the regulation of these channels by D1 receptors.

RECEPTOR MODULATION, UP- AND DOWN-REGULATION II

449.1

ADENOSINE A2a RECEPTORS SELECTIVELY AND ANTAGONISTICALLY REGULATE DOPAMINE D2 (LONG-FORM) RECEPTORS IN STABLY COTRANSFECTED FIBROBLAST CELLS. S. Ferré*, S. Dasgupta, S. -N. Yang, P. B. Hedlund, U. -B. Finnman, S. Ahlberg, E. Arenas, B. B. Fredholm and K. Fuxe, Department of Neuroscience, Karolinska Institute, Stockholm.

In membrane preparations from rat striatum, where adenosine A2a and dopamine D2 receptors are coexpressed, stimulation of A2a receptors was found to decrease the affinity of D2 receptors. We now demonstrate the existence of this antagonistic interaction at the cellular level, by using a fibroblast cell line stably transfected with the human D2 (long-form) and the dog A2a receptors. Stimulation of A2a receptors with the selective A2a agonist CGS 21680 decreased the affinity of D2 receptors 2-3 fold, as shown in competition experiments with dopamine versus the selective D2 antagonist [3H]raclopide. CGS 21680 was however ineffective in control cells expressing D2 but not A2a receptors. The effect of CGS 21680 was counteracted by the adenosine antagonist 8-phenyltheophylline and was independent of the effect of the GTP analogue Gpp(NH)p suggesting that a GTP-independent mechanism mediates this A2a-D2 receptor interaction. In cotransfected cells both A2a and D2 receptors were found to be coupled to adenylate-cyclase. CGS 21680 induced a small but significant increase and dopamine induced a significant decrease of cAMP acumulation. In control cells forskolin did not induce any change in the binding characteristics of D2 receptors. Furthermore, activation of the constitutively expressed A2b receptors with the non-selective adenosine agonist NECA, which also induced a high cAMP accumulation, did not modify dopamine D2 binding. Therefore, the A2a-induced modulation of D2 receptor affinity seems to be independent of adenylate cyclase activation. With calcium image analysis experiments a functional A2a-D2 interaction was also demonstrated in cotransfected cells. CGS 21680 was found to counteract the increase in intracellular calcium levels mediated by the selective D2 agonist quinpirole.

449.2

STIMULATION OF ADENOSINE A_2 RECEPTORS INDUCES FOS-LIKE IMMUNOREACTIVITY PREFERENTIALLY IN THE NUCLEUS ACCUMBENS: POSSIBLE ANTIPSYCHOTIC EFFECTS. M. Morelli*, A. Pinna, J. Wardas+. Dpt. Toxicology, Univ. of Cagliari, Italy + Inst. Pharmac. Polish Acad. Science, Cracow, Poland.

Administration of typical neuroleptics like haloperidol is known to induce Fos-like immunoreactivity (FLI) in several rat brain areas including the nucleus accumbens and the striatum, while atypical neuroleptics like clozapine induced FLI in the nucleus accumbens but not in the striatum. It was therefore proposed that the different clinical profile of haloperidol and clozapine might be related to their different targets in the brain (Robertson and Fibiger, 1992). Adenosine A_2 receptor agonists, like neuroleptics, antagonize the motor hyperactivity induced by dopamine agonists, moreover A_2 receptors are strictly localized in areas rich of dopamine innervation like the striatum, nucleus accumbens and olfactory tubercle. Here we show that administration of the selective A_2 agonist CGS 21680 dose-dependently (5-20 mg/kg s.c.) induced FLI in the shell of rat nucleus accumbens while even at the higher dose used, no FLI was observed in the core of the nucleus accumbens and in the striatum. CGS 21680-induced FLI was abolished by a 6-hydroxydopamine lesion of the medial-forebrain-bundle and by stimulation of dopamine D2 receptors. The results suggest that A_2 receptor agonists, like atypical neuroleptics, might have antipsychotic effects without producing extrapyramidal side effects.

449.3

REGULATION OF A1 ADENOSINE RECEPTOR EXPRESSION IN CEREBELLAR GRANULE CELLS AFFECTS SENSITIVITY TO ADENOSINE ANALOG-INDUCED INHIBITION OF ADENYLYL CYCLASE. B.D. Hettinger-Smith*, M. Leid and T.F. Murray, College of Pharmacy, Oregon State University, Corvallis, OR 97331.

Adenosine acts as a neuromodulator in the central nervous system via its actions at A1, A2a, A2b, and A3 adenosine receptors which belong to the superfamily of G protein coupled receptors. The A1 adenosine receptor (A1AR) is antagonized by caffeine and related methylxanthines and is coupled to the inhibition of adenylyl cyclase. Chronic exposure to caffeine leads to a tolerance to its stimulatory effects and the basis of this tolerance may, in part, be due to an upregulation of A1AR. Primary cultures of cerebellar granule cells from 8 day old Sprague Dawley rat pups were established and used to study A1AR regulation in response to chronic exposure to agonists and antagonists. In addition, the sensitivity of adenylyl cyclase to A1AR agonist-induced inhibition was assessed. A 72-hour treatment of cultures with the A1AR agonist, cyclopentyladenosine (CPA) resulted in a decreased Bmax with no change in receptor affinity as measured by saturation binding of [3H]dipropylcyclopentylxanthine (DPCPX, an A1 selective antagonist) in cerebellar granule cell membranes. Down regulation could also be demonstrated in whole cell binding experiments. Desensitization of the CPA-induced decrease in adenylyl cyclase activity accompanied this down regulation of A1AR. Chronic antagonism of A1AR by caffeine or 8-parasulfophenyltheophylline (8-pSPT) resulted in an increased Bmax with no change in affinity of A1AR; a sensitization to CPA-induced decrease in adenylyl cyclase activity accompanied this upregulation. Parallel A1AR regulation studies in P19 cells stably transfected with A1AR have been performed. (Supported by NIDA grant F31 DA05631-01 to BHS.

449.4

REGULATION OF TYPE-1 AND TYPE-2 ANGIOTENSIN II RECEPTOR mRNA DURING DIFFERENTIATION OF NG108-15 NEUROBLASTOMA CELLS. W.R. Schelman, S.G. Soltys, J.L. Kurth, R.L. Berdeaux and J.A. Weyhenmeyer*. Dept. of Cell and Structural Biology, University of Illinois, Urbana, IL, 61801.

The murine neuroblastoma x glioma NG108-15 cell line possesses both AT_1 and AT_2 angiotensin II (AngII) receptor subtypes. We have previously shown that AT_1 and AT_2 binding is increased following DMSO differentiation of NG108-15 cells. *Binding studies performed on membranes from undifferentiated and differentiated (maintained in medium containing 1.5% DMSO and 0.5% FBS for 4 days) NG108-15 cells revealed a single* K_D of 0.64 ± 0.12 nM, and B_{max}s of 26.8 ± 0.12 fmol/mg protein and 607 ± 139 fmol/mg protein, respectively. Further, undifferentiated cells predominantly express the AT_1 receptor subtype, while differentiated cells contain a majority of AT_2 receptors suggesting that AT receptors play a role in neuronal growth and differentiation. In the present studies, we investigated the regulation of AT_1 and AT_2 mRNA during DMSO differentiation of NG108 cells. Northern blot analysis was used to determine AT_2 mRNA levels in cells treated from 0-4 days. AT_2 levels increased threefold from 0 to 2 days of treatment. Transcript levels then remained relatively constant between days 2-4. To examine AT_1 mRNA levels, a competitive PCR method was used. Preliminary data suggests that AT_1 mRNA is also increased during DMSO differentiation; however, an exact time-course has not been established. Taken together, these findings suggest that the increase in AT binding during differentiation is due to *de novo* transcription and, presumably, translation. (Supported by NSF. W.R.S. is supported by an American Heart Association Student Stipend).

449.5

REPEATED POSTNATAL PCP EXPOSURE DOES NOT ALTER SIGMA RECEPTOR BINDING IN RAT CEREBELLUM C.-S. Li, H.-J. He* and R. Sircar, Laboratory for Developmental Neuroscience, Departments of Psychiatry and Neurology, Albert Einstein College of Medicine, Bronx, New York.

We have earlier demonstrated that postnatal PCP treatment in rats produce long-term changes in seizure susceptibility. Although PCP binds to specific brain PCP receptor, at high concentrations it is known to interact with a large number of molecular target sites - σ binding site, monoamine uptake sites, K^+ channel, acetylcholine receptor, γ-aminobutyric acid (GABA) regulated chloride channel. To test the hypothesis that the behavioral effects of postnatal PCP exposure are mediated via the sigma receptor, we carried out experiments to measure sigma receptor binding in postnatal PCP-treated rats and compared the data from those obtained from saline-treated controls. Rat pups were treated with PCP (5 mg/kg/day) for 11 days from postnatal days 5 to 15, intraperitoneally. Control pups received equivalent volume of saline. On postnatal day 21, groups of saline- and PCP-treated rats were sacrificed, their brains removed, dissected and cerebellum immediately frozen. [^{3}H](+) pentazocine binding was measured in drug-treated and control rat cerebellum. Saturation analyses of the binding data showed no significant difference in any of the binding characteristics between chronic PCP-treated rats and saline-treated controls suggesting that mechanisms other than alterations in σ_1 binding underlie the behavioral effects of repeated postnatal PCP administration in immature rats.

Supported by: National Institute on Drug Abuse

449.6

CHRONIC NEONATAL PCP DIFFERENTIALLY REGULATES mRNA LEVELS FOR NMDA RECEPTOR SUBUNITS R. Sircar*[1], P. Tollesa[2], M.K. Ticku[2] [1]Laboratory for Developmental Neuroscience, Departments of Psychiatry and Neurology, Albert Einstein College of Medicine, Bronx, New York; [2]Department of Pharmacology, University of Texas Health Science Center, San Antonio.

The NMDA receptor has been implicated in developmental plasticity. We have earlier demonstrated that postnatal PCP treatment in rats produce long-term changes in seizure susceptibility. In Day 21 rats, [^{3}H]MK-801 binding was decreased in selective brain areas. To test the hypothesis that this selective decrease in [^{3}H]MK-801 binding is due to the downregulation of NMDA receptor subunits, NMDAR1, NMDAR2A-C mRNA levels in specific brain regions in PCP-treated and control rats were measured. Rat pups were treated with PCP (5 mg/kg/day) for 11 days from postnatal days 5 to 15, intraperitoneally. Control pups received saline (1 ml/100 g). On postnatal day 21, groups of saline- and PCP-treated rats were sacrificed, their brains removed, dissected and brain regions immediately frozen. Using the RNase protection assay we compared the levels of different NMDAR subunit mRNAs in postnatal PCP-treated rat brain with those from saline-treated controls. Our results indicate, that in both male and female PCP-treated pups mRNA encoding for NMDAR2B subunit was selectively reduced in the frontal cortex. There was no significant effect on the NMDAR1 subunit levels in the hippocampus, striatum and frontal cortex. Also, we did not find any change in the NMDAR2A or 2C levels in any of the regions studied. These results suggest that the decrease in [^{3}H]MK-801 binding in the frontal cortex seen in neonatal PCP-treated rats is associated with the downregulation of the gene encoding for specific NMDAR subunits.

Supported by: National Institute on Drug Abuse

449.7

MECHANISM OF HISTAMINE H_2 RECEPTOR DOWNREGULATION IN CHINESE HAMSTER OVARY CELLS. E. Roovers, M.J. Smit*, J. Blauw, A. Alewijnse, H. Timmerman, and R. Leurs Leiden/Amsterdam Center for Drug Research, Department of Pharmacochemistry, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands.

Under several pathophysiological conditions, where histamine is released in large quantities, or during treatment with histaminergic drugs in various disorders, regulatory processes such as desensitization and downregulation may become apparent.

Exposure of Chinese Hamster Ovary cells (CHO) expressing the histamine H_2 receptor to histamine leads to a dose dependent and time dependent decrease in the number of cell surface receptors (up to 44% reduction after 24 hours of exposure) without affecting receptor affinity. RNA slot blotting hybridization analysis was used to evaluate the levels of receptor mRNA. Stimulation of the cells with histamine caused a rapid decrease in histamine H_2 receptor mRNA levels (up to 80% reduction within 4 hours), which is due, at least partly, to an increased turnover of the transcript. Stimulation with forskolin also induced downregulation of the H_2 receptor and its mRNA in a manner similar to the histamine-induced downregulation. This suggests the involvement of protein kinase A in H_2 receptor downregulation. Yet, stimulation of a mutant H_2 receptor with a truncated C-terminus displacing normal adenyl cyclase activity, was even more susceptible to histamine-induced downregulation than the wild type receptor. This suggests that an other signaling pathway is involved in H_2 receptor downregulation.

To investigate whether this alternative pathway is G-protein dependent or independent, we are currently in the process of generating receptor mutants that are unable to couple to G-proteins.

449.8

REGULATION OF THE HUMAN H_1 HISTAMINE RECEPTOR IN HeLa CELLS AND IN STABLY TRANSFECTED CHO-K1 CELLS. Steven L. Max* and Claire M. Fraser, Department of Cellular and Molecular Biology, The Institute for Genomic Research, 932 Clopper Road, Gaithersburg, MD 20878.

A gene encoding the human H_1 histamine receptor was recently cloned from a human genomic library prepared from blood leukocytes. The coding region was cloned into the mammalian expression vector, pcDNA3, and transfected into CHO-K1 cells. Membranes prepared from the transfected cells specifically bound the radioligand ^{3}H-pyrilamine with a K_d of 0.6 nM and B_{max} values ranging from 150-1800 fmol/mg protein. A stably transfected CHO clone expressing the H_1 receptor (CHO/H_1R) at a density of 150 fmol/mg protein was selected and compared to human HeLa cells which endogenously express the H_1 receptor at a similar level. Exposure to the agonist histamine resulted in a dose-dependent increase in inositol phosphates production with an EC_{50} of 0.75 μM. The maximum response in the HeLa cell line was 5-fold, whereas, in CHO/H_1R cells, the maximum resonse was 2-fold. Pre-treatment of the cells with 1 mM histamine for 30 min resulted in a 50 percent reduction in the maximal response in the transfected CHO line, while the maximal response in the HeLa cells was reduced to basal levels. Long-term exposure (24 hrs) of HeLa cells and CHO/H_1R cells to histamine in the presence of 0.2 mM sodium metabisulfite resulted in 80 percent and 60 percent decreases, respectively, in the density of binding sites for ^{3}H-pyrilamine as compared to untreated cells. A ribonuclease protection assay employing an H_1-specific ^{32}P-antisense RNA probe was used to evaluate the levels of receptor mRNA in histamine-treated vs. control HeLa cells. There appeared to be a significant decrease (65 percent) in steady-state levels of H_1 receptor mRNA in the treated HeLa cells. Overall, these results indicate that although CHO/H_1R cells express a functional protein, they are coupled to second messenger systems less efficiently than the naturally expressed receptor in HeLa cells. Furthermore, H_1 histamine receptor down-regulation may be due, in part, to changes in mRNA levels.

449.9

THE SYNTHESIS AND BIOLOGICAL ACTIVITY OF A NOVEL CLASS OF ANTAGONISTS FOR THE BRAIN CB1 CANNABINOID RECEPTOR. K. Fahey, M. Chaney, G. Cullinan, D. Hunden, D. Johnson, G. Koppel*. Lilly Research Laboratories, CNS & Endocrine Divisions, Indianapolis, IN 46285. C.C. Felder[1], K.E. Joyce[1], E.M. Briley[1], K. Mackie[3], J.N. Crawley[2], B.R. Martin[4], M.J. Brownstein[1]. [1]Laboratory of Cell Biology, National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892. [2]Section on Behavioral Neuropharmacology, National Institute of Mental Health, Bethesda, MD 20892. [3]Department of Physiology and Anesthesiology, University of Washington, Seattle, WA 98195. [4]Department of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA 23298-0613.

A new structural series of compounds were synthesized and their binding affinities to the brain CB1 receptor were determined. In addition, their ability to functionally antagonize the anandamide mediated adenylate cyclase inhibition in CHO cells stably expressing the CB1 receptor were evaluated. The lead compound in the series, LY320135, is a selective antagonist for the brain CB1 receptor, having a 16 fold higher affinity for the CB1 compared to the peripheral CB2 receptor. The Ki values for LY320135 at the CB1 and CB2 receptors, transfected and stably expressed in cell lines, was 203 nM and 3340 nM, respectively. Similar Ki values were measured in binding studies performed on cerebellum and spleen membrane preparations endogenously expressing the CB1(204 nM) and CB2(>1 μM) receptors, respectively. Finally, molecular modeling comparisons with other selective CB1 binding substrates have been completed. LY320135, also, potently reversed cannabinoid-mediated modulation of K^+ and Ca^{2+} currents.

449.10

NEUROTROPHIN AND RETINOID REGULATION OF NEURONAL B_2 BRADYKININ RECEPTOR EXPRESSION IN DEFINED AFFINITY FORMS. Y.-J. I. Jong and N. L. Baenziger*, Dept. of Anatomy and Neurobiology, Washington Univ., St. Louis, MO 63110.

We have defined regulation of expression of B_2 subtype receptors for the neuropeptide bradykinin (BK) upon differentiation of human and rat neuronal cell lines. In SH-SY5Y neuroblastoma and PC12 pheochromocytoma cells we identify B_2 receptors equivalent to and recognized by MAbs selective for Intermediate (I, K_d 5.6 nM) and Low (L, K_d 42 nM) affinity B_2 receptor forms in human lung fibroblasts (Jong et al, Proc Natl. Acad. Sci. 90:10994,1994). Receptors of 78-82 kDa comparable to the human fibroblast receptors are immunoprecipitated from detergent lysates of neuronal cells with I and L form specific MAbs, and ^{3}H-BK binding reveals 2 sites with respective K_d values of 3 nM and 55 nM, corresponding to known *in vivo* BK levels. A 2- to 3-fold induction of BK receptors accompanies neuronal differentiation by NGF and retinoic acid (RA), in a cell- and context-specific manner. In PC12 cells only the I form is enhanced; L form expression remains unchanged from its initial constitutive levels. In SH-SY5Y cells both I and L forms increase upon neuronal differentiation. The time course of BK receptor augmentation relative to neurite outgrowth also differs in the 2 cell lines. It directly matches the pace of neurite outgrowth in PC12. However it preceeds the bulk of neurite outgrowth in SH-SY5Y; augmented expression is minimal at 7-17 hr of differentiation, then increases sharply to maximal levels from 17-24 hr, whereas neurite outgrowth is maximal at 72-96 hr. Induction thus represents a mechanism of cross-talk between the BK branch of the G-protein-coupled receptor family, the Trk family of membrane Tyr kinase receptors, and the RA family of cytosolic translocating - nuclear receptors which exert their activity at the transcriptional level. BK receptor induction by NGF and RA in SH-SY5Y cells is not additive, indicating that NGF and RA actions both funnel into a final common pathway governing BK receptor expression.

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449.11

AGONIST-INDUCED DESENSITIZATION OF P_{2U} PURINOCEPTORS. S.M. Sromek*#, E.R. Lazarowski‡ and T.K. Harden‡. Curr. of Neurobiology# and Dept. of Pharmacology‡, Univ. of North Carolina, Chapel Hill NC 27599.

The P_{2U} purinergic receptor is a 377 amino acid G protein-coupled receptor which is activated by ATP and UTP and stimulates phospholipase C (PLC). Preincubation of CF/T43 human airway epithelial cells with P_{2U} receptor agonists results in desensitization, *i.e.* uncoupling of the receptor from PLC activation. Recovery of agonist-promoted PLC response is rapid (<10 min) after drug washout. When stably expressed in 1321N1 human astrocytoma cells, the human P_{2U} receptor displays a similar pharmacological profile for PLC activation compared with CF/T43 cells, although agonists are 30- to 100-fold more potent than with the natively expressed receptor. Pretreatment of P_{2U}-1321N1 cells with agonist for 8 h results in a decrease in apparent potency of agonists without decreasing the maximal effect on PLC.

An epitope-tagged P_{2U} receptor has been created by addition of 9 amino acids at the amino terminus. This receptor is currently being expressed in 1321N1 cells to determine the fidelity with which this receptor reflects the signalling properties of the native receptor. The tagged receptor will be utilized to study the role of receptor trafficking in P_{2U} purinoceptor desensitization and resensitization.

449.12

CARBAMAZEPINE ACTS THROUGH THE PERIPHERAL BENZODIAZEPINE RECEPTOR TO UP-REGULATE β-ADRENERGIC RECEPTOR mRNA.
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We have previous shown that chronic treatment (6 days) of C_6 glioma cells with carbamazepine (CBZ) causes a time- and concentration-dependent increase in the expression of β-adrenergic receptor (β-AR) protein and mRNA. To determine the mechanism of this up-regulation, we investigated the possibility that CBZ acts at the peripheral benzodiazepine receptor to change β-AR mRNA levels. C_6 glioma cells were treated for 7 days with ligands of the peripheral benzodiazepine receptor, PK 11195, RO 5-4864, and FGIN 1-27, at concentrations ranging from 1 nM to 10 μM. All three ligands caused concentration-dependent increases in levels of both β_1- and β_2-AR mRNA at concentrations between 1 and 100 nM. Above this, the effect of the ligands to cause β-AR mRNA up-regulation diminished in a concentration-dependent manner. The addition of 50 μM CBZ to these same treatments antagonized the up-regulation of β-AR mRNA at low concentrations of ligand and potentiated the diminution of their effect at high concentrations. There were some differences in the effects of CBZ and the peripheral benzodiazepine ligands on β_1- and β_2-AR mRNA levels. Simultaneously, CBZ displayed a clear ability to alter the level of β-AR mRNA on its own. These results suggest that CBZ acts as a partial agonist at the peripheral benzodiazepine receptor to cause β-AR mRNA up-regulation.

449.13

DOPAMINE AND 5-HT MODULATION OF SYNAPTIC TRANSMISSION IN THE LAMPREY SPINAL CORD. M. Wikström, A. El Manira, W. Zhang, R.H. Hill and S. Grillner*. Nobel Institute for Neurophysiology, Department of Neuroscience. Karolinska Institute, S - 171 77 Stockholm, Sweden

Using the lamprey spinal cord *in vitro* preparation we have investigated the effects of 5-HT on the synaptic transmission from primary sensory neurons to relay giant interneurons as well as the effects of dopamine (DA) on the reticulospinal input to the spinal cord. Both 5-HT and DA are important modulatory transmitters in the lamprey spinal cord. To a large degree these transmitters acts in a complementary fashion on the cellular and locomotor network levels . DA and 5-HT are colocalized and co-released from a ventromedial plexus (Schotland et al. *Nature* 374, 266-269, 1995). 5-HT immunoreactive fibers (without DA-ir) are also present in the dorsal column and dorsolateral areas. Application of 5-HT depressed the glutamatergic monosynaptic excitatory postsynaptic potentials (EPSP) recorded in giant interneurons which were evoked by stimulation of the dorsal column or the dorsal roots. 5-HT had no effect on the input resistance of giant interneurons, suggesting that 5-HT acts presynaptically on sensory axons to depress synaptic transmission. 5-HT has a similar effect at synapses between reticulospinal axons and motoneurons in the ventral spinal cord which are located close to the 5-HT/DA plexus (Buchanan and Grillner. *Neurosci. Lett.* 112, 71-74, 1991 ; Shupliakov et al. *Eur. J. Neurosci.* in press). DA also modulates synaptic efficacy between reticulospinal axons and spinal neurons. The amplitude of monosynaptic postsynaptic potentials (PSP) evoked by stimulation of reticulospinal axons is reduced upon application of DA. The effect is considered to be presynaptic as no significant change in input resistance occurs in the target cells. The selective D_2 receptor agonist R(-)-2,10,11-Trihydroxy-N-propyl-noraporphine HBr mimicked the effects mediated by DA. DA reduces calcium-currents in spinal neurons through activation of dopamine D_2 receptors which might be a mechanism through which transmitter release is modulated also at the presynaptic terminal. DA and 5-HT thus both act to reduces glutamatergic synaptic transmission from reticulospinal axons.

449.14

DECREASED EXPRESSION OF DOPAMINE D3 RECEPTORS IN THE LIMBIC SUBREGIONS OF RAT BRAIN FOLLOWING NEONATAL EXCITOTOXIC LESION OF THE VENTRAL HIPPOCAMPUS. G. Flores*, D. Barbeau, J.J. Liang, R. Quirion and L.K. Srivastava. Douglas Hospital Research Center, McGill University, Montreal, Qc, Canada, H4H 1R3.

Neonatal, bilateral lesion of the ventral hippocampus in rats has been proposed as a model of schizophrenia as these animals show postpubertal hypersensitivity to stress and dopamine agonists that can be reversed by neuroleptic treatment (Lipska et al. Neuropsychopharmacology, 9, 67, 1993). To investigate the mechanisms of abnormal dopamine related behaviours in these animals, we have evaluated the expression of dopamine D1, D2 and D3 receptors in various striatal and limbic subregions of rats that had received bilateral ibotenic acid lesion of the ventral hippocampus at postnatal day 7 (PD7). Locomotor activity of the hippocampal and sham-lesioned rats were measured at prepubertal (PD35) and postpubertal (PD56) ages. In accordance with previous reports, an increased locomotor activity was observed at PD56 in the hippocampal-lesioned group following exposure to novelty and swim-stress test, as well as after D-amphetamine (1 mg/kg) or apomorphine (0.5 mg/kg) injections. The density of dopamine receptor subtypes were then estimated by ligand autoradiography at PD41 and PD62. The density of dopamine D3 receptors, as measured by [^{3}H]7-OH-DPAT binding, is markedly reduced in the nucleus accumbens and the island of Calleja of hippocampal-lesioned rats with the changes being more pronounced at PD62. A small, but significant, increase in D1 receptors ([^{3}H]-SCH23390 binding) was also observed in the dorsal striatum of PD62 hippocampal-lesioned animals, while no significant changes in [^{3}H]spiperone binding (representing total D2-like receptors) were noted. In view of the inhibitory role of D3 receptors in locomotion, the results suggest that some of the delayed behavioral changes seen in the neonatal hippocampal-lesioned rats may be mediated by decreased D3 receptor levels. (Supported by the MRC, FRSQ and the Low-Beer Foundation).

449.15

CORTICAL NMDA RECEPTOR LEVELS ARE INCREASED IN ADULT RATS FOLLOWING NEONATAL EXCITOTOXIC LESIONS OF THE VENTRAL HIPPOCAMPUS Z. Albo, G. Flores, D. Barbeau, R. Quirion and L.K. Srivastava*. Douglas Hospital Research Center, Departments of Psychiatry, and Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada, H4H 1R3.

Neonatal bilateral excitotoxic lesion of the ventral hippocampus in rats results in hyperlocomotor behavior induced by dopamine agonists, and hyperresponsiveness to stress-induced behaviors in adult animals (Lipska et al, Neuropsychopharm. 9:67,1993). These neonatally hippocampal-lesioned rats have accordingly been proposed as an animal model of schizophrenia. Glutamate plays a prominent role in locomotor behavior and has been implicated in schizophrenia (Ellison, Brain Res. Rev.,23:250, 1995). We have compared the level of N-methyl-D-aspartate (NMDA) receptors in cortical areas, striatum and nucleus accumbens of rats that underwent either bilateral ibotenic acid lesions or sham-lesion of the ventral hippocampus at postnatal day 7 (PD7). Hippocampal-lesioned rats at PD56 but not PD35, showed increased locomotor activities induced by swim-stress or exposure to a novel environment, as well as after D-amphetamine or apomorphine injections. The density of NMDA receptors as measured by ligand autoradiography using [^{125}I]MK801 (200pM), was found to be significantly increased in the inner layers of frontal and cingulate cortices at PD62 in the hippocampal-lesioned group compared to the sham-lesioned controls of same age. No significant difference in binding was observed between sham and lesioned groups at PD41. Other brain areas studied showed no significant change in [^{125}I]MK801 binding between the two groups. The results suggest that alterations in the levels of cortical NMDA receptors could underlie some of the behavioral changes at adulthood observed in neonatally hippocampal-lesioned rats. (Supported by the MRC, the FRSQ and the Low-Beer Foundation).

449.16

DOWN-REGULATION OF GABA ρ RECEPTOR CHANNELS BY PROTEIN KINASE C. T. Kusama[1]*, M. Sakurai[1], Y. Kizawa[1], G.R. Uhl[2] and H. Murakami[1]. [1]Dept. of Physiol. Anat., Nihon Univ. Coll. of Pharmacy, Chiba 274, Japan; [2]Mol. Nurobiol., ARC/NIDA/NIH & Nsci., JHUSM, Baltimore, MD 21224.

Several ligand gated ion channels are robustly regulated by phosphorylation events. In the present study, we investigated the effect of protein kinase C (PKC) activators on GABA ρ1 and ρ2 receptor, newly cloned from human retina, in expressing *Xenopus* oocytes under voltage clamp.

Phorbol 12-myristate 13-acetate (PMA), exogenously applied for 3 min, inhibited more than 80 % of the ρ1 and ρ2 receptor currents. This inhibition developed slowly, reaching a maximum about 40-70 min, and was concentration-dependent, with EC_{50} for PMA of 6 nM. PMA inhibition decrease GABA efficacy, but not its potency. 100 nM phorbol 12-monomyristate, an inactive analog of PMA, did not exhibit such an inhibitory effect. 3 min application of 10 nM mezerein, a non-phorbol ester PKC activator, also inhibited the ρ1 responses more effectively than 25 nM PMA. However, 1 mM 8-chlorophenylthio-cyclic AMP, an effective activator of oocyte protein kinase A, failed to effect ρ1 responses. The inhibition by 25 nM PMA was blocked by 20 min pretreatment with 1 μM staurosporine, a PKC inhibitor.

These results suggest that GABA ρ receptor function may be dramatically regulated by PKC-mediated phosphorylation events in retinal and other neurons that express ρ1 and/or ρ2 receptors. Current mutagenesis experiments may determine the PKC site responsible for down-regulation of ρ receptors.

449.17

FLUNITRAZEPAM INDUCES A BI-PHASIC DOWN-REGULATION OF THE $GABA_A$ RECEPTOR α_1 SUBUNIT PROTEIN. M.J. Brown and D.R. Bristow*. Division of Neuroscience, University of Manchester, Manchester, M13 9PT .

Prolonged benzodiazepine administration results in tolerance to some of their therapeutic effects. Studies on the molecular mechanisms underlying tolerance to benzodiazepines have reported a down-regulation of certain $GABA_A$ receptor subunit mRNA species (α_1 and γ_2). In the present study, the short-term effect on the expression of the $GABA_A$ receptor α_1 subunit protein by the benzodiazepine agonist, flunitrazepam, was examined in primary cultures of cerebellar granule cells.

Cerebellar granule cells were prepared and treated with 1 μM flunitrazepam (1-48 hours) and then the protein extracted. Proteins were separated by 10% (w/v) SDS-PAGE, Western blotted onto nitrocellulose membrane and probed with subunit-specific α_1 antibody. Membranes were incubated with HRP secondary antibody, and protein bands detected using ECL. The optical density of the protein bands were quantitated using a OmniMedia flatbed 600dpi scanner.

This study demonstrates that after 1, 2, 4, and 8 hours stimulation with 1μM flunitrazepam, the α_1 subunit protein is reduced (-24±16, -64±25, -26±11 and -51±14, mean±SEM, respectively, compared to controls, n=4-8, $p<0.05$, Wilcoxon Rank). Following 12 hours exposure, the α_1 subunit protein expression recovers to control values (0±22%, mean±SEM, n=4). However, by 24, 30 and 48 hours, the α_1 subunit protein is reduced again (-38±12%,-26±10% and -40±3%, mean±SEM, respectively, compared to controls, n=4-11, $p<0.05$, Wilcoxon Rank). This study will further explore the mechanism underlying the two phase response to benzodiazepines by examining the α_1 subunit RNA over a similar time course of flunitrazepam treatment. This study provides a better understanding of the molecular events occurring at the $GABA_A$ receptor upon benzodiazepine stimulation.

449.18

REGULATION OF MUSCARINIC M1 AND ADRENERGIC α1 RECEPTOR EXPRESSION IN LIVING VISUAL CORTEX NEURONAL CULTURES. Y.H. Wang*, Q. Gu and M. S. Cynader. Dept. of Ophthalmology, Univ. of British Columbia, Vancouver, B.C. Canada V5Z 3N9

We have demonstrated the regulation of expression and distribution of muscarinc m1 receptors and adrenergic α1 receptors, respectively, in living visual cortex neuronal cultures by using confocal microscopy and fluorescently labelled analogues of receptor-specific ligands. Here, our goals were to study the specificity of receptor regulation to the blockade of a particular receptor class. We approached this by comparing the effects of treatment with the α1 receptor antagonist prazosin and m1 receptor antagonist pirenzepine on the expression of α1 and m1 receptors in cultured cortical neurons. Neurons were either pretreated with (a) 10 nM pirenzepine alone, (b) 50 nM prazosin alone, and (c) 50 nM prazosin and 10 nM pirenzepine for 5 days in culture. The results showed that blockade of muscarinic receptors with pirenzepine upregulates muscarinic receptor expression selectively without changing α1 receptor expression. Conversely blockade of α1 receptors upregulates α1 expression but not muscarinic receptor expression. This implies that the expression levels of m1 and α1 receptors are both regulated through specific signal transduction pathway. We have previously found that tetrodotoxin (TTX) treatment decreases expression of muscarinic recepor in cultured neurons.

To study the interactions between neuronal activity and receptor activation (or blockade) on receptor number, we compared the effects of TTX, prazosin, and noradrenaline on the expression of α1 and m1 receptors in our cultured neurons. In these experiments, neurons were pretreated either with (a) 0.1 μM TTX (b) 0.1 μM TTX and 50 nM prazosin and (c) 0.1 μM TTX and 1 μM noradrenaline for 7 days in culture. The results show that TTX exposure reduces both α1 and m1 receptor expression regardless of the presence of the receptor agonists or antagonists. This implies that the activity-dependent regulation of receptor number is predominantly regulated by neuronal activity rather than receptor occupancy.

449.19

LACK OF RECYCLING OF INTERNALIZED NEUROTENSIN RECEPTORS IN PRIMARY CULTURED NEURONS.

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Lab. of Neurochemistry (1080), Université Catholique de Louvain, B-1200, Brussels, Belgium. (SPON: SONA)

Both short-term and long-term regulation processes induced by neurotensin on its receptor were studied in rat primary cultured neurons. After incubation of intact neurons with unlabelled neurotensin (3 nM) for 1 or 24 h at 37°C, the [^{3}H]-neurotensin specific binding measured in cell homogenates was decreased to about 35 and 65% of control values respectively. In these experiments, the decreases in binding corresponded to reductions of B_{max} value, without significant changes in the affinity. The neurotensin-induced receptor down-regulation is thought to result from receptor degradation since it was reduced by lysosomotropic drugs and because no agonist-induced change in neurotensin mRNA level could be measured. After short term incubations, internalized receptors slowly reappeared at the cell surface during further incubation in the absence of the peptide. Such receptor reappearance did not occur in the presence of the protein synthesis inhibitor cycloheximide and is therefore thought to result from the new-synthesis and not from the recycling of internalized receptors. These results indicate that the neurotensin-induced receptor internalization in cultured neurons is irreversible and that it is followed by a down-regulation of the receptor through a degradative process.

449.20

FACTORS REGULATING THE EXPRESSION OF THE Y1 RECEPTOR FOR NEUROPEPTIDE Y IN PC12 CELLS.

J. C. Bournat and J. M. Allen*. Division of Biochemistry and Molecular Biology, Davidson Building, University of Glasgow, Glasgow G12 8QQ, Scotland, UK.

The region of the Y1 receptor gene that lies 5' to the first exon has been attached to the reporter function, luciferase, such that the expression of this enzyme has been placed under the control of the 5' flanking region of the Y1 receptor. It is thus possible to measure the activity of the gene and the factors that regulate its expression in cells that constitutively express the endogenous gene. Luciferase activity can be readily measured in cell extracts by providing substrate and measuring luminescence in a luminometer. The fusion gene has been transfected into PC12 cells, which express low levels of the Y1 receptor. Activity of the gene promoter region was measured by analysing the activity of the luciferase enzyme in cell extracts 48 hours after transfection. Very low levels of luciferase activity were observed in cells transfected with the promoterless control plasmid indicating that the vector itself contained no sequences that activate gene expression in PC12 cells. Maximal activity was assessed by transfecting PC12 cells with a control plasmid in which the luciferase has been placed under the control of a viral promoter. Transfection of the fusion gene (in which the enzyme was placed under the control of the 5' flanking region of the Y1 receptor) resulted in slightly higher levels of luciferase activity compared to the promoterless plasmid but were considerably less than the maximal activity. These results indicate a low basal level of Y1 receptor gene transcription in these cells. We have started to analyse the factors that regulate Y1 receptor gene activity in PC12 cells. Preliminary data indicates that the gene for the Y1 receptor is regulated in a similar fashion to that for the peptide itself. Thus, Nerve Growth Factor and activators of cAMP dependent protein kinase both stimulate Y1 receptor gene transcription.

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NEUROENDOCRINE REGULATION: VASOPRESSIN AND OXYTOCIN

450.1

SINOAORTIC DENERVATION INCREASES OSMOTICALLY STIMULATED PEPTIDE RELEASE WITHIN SUPRAOPTIC NUCLEUS (SON). M.F. Callahan*, M. Ludwig, C. Barrett, and M. Morris. Dept. of Physiology/ Pharmacology, Wake Forest University Medical Center, Winston-Salem, NC, 27157-1083.

Removal of baroreceptor function by sinoaortic denervation (SAD) results in increased plasma peptide response to osmotic stimulation. We examined whether chronic SAD alters vasopressin (VP) and oxytocin (OT) release into the SON. Urethane anesthetized SAD or sham operated male Sprague Dawley rats were given arterial and venous catheters and bilateral microdialysis probes into the SON. Baroreceptor testing was accomplished by examining bradycardia following phenylephrine induced pressor responses. Successful SAD (< 0.5 bpm bradycardia/mmHg increase in arterial pressure) was observed in 7 of 9 SAD rats. Dialysis probes were perfused with artificial CSF (3μl/min) over nine 30-minute periods. After a one-hour baseline, animals were given an i.p. osmotic challenge (3.5M, 600μl/100g body wt). The osmotic stimulus caused an increase in VP and OT peptide release in both groups of animals with the peak occurring during 30-60 minutes post stimulation. In sham operated rats, dialysate VP increased from 0.28±0.15 pg/sample in baseline to 4.4±3 following stimulation. SAD rats showed an exaggerated response to osmotic stimulation with dialysate VP increasing from a baseline of 0.15±0.02 to 9.3±2.8 pg/sample. OT release was also exaggerated in the SAD group. This supports previous research which showed that intranuclear peptide release to direct osmotic stimulation was also exaggerated in SAD rats. These results indicate that intranuclear peptide release in response to osmotic stimulation is under control of arterial baroreceptor function. *(Supported by NHLBI Grant # HL-43178).*

450.2

MECHANISMS INVOLVED IN SUCKLING-INDUCED INHIBITION OF MILK EJECTION IN RATS. **M.T. Morales, E. Shapiro and F. Mena. (SPON: S. Díaz-Cintra*). Centro de Neurobiología, Universidad Nacional Autónoma de México, México D.F. 04510, México.**

Milk ejection in rats results from interactions of antagonistic mechanisms activated by suckling, i.e., oxytocin (OT) and catecholamines (CAs), from neural or adrenal origin, upon the motor apparatus of the mammary gland. We know from previous work that neural regulation of the gland (facilitation or inhibition of milk ejection) may be activated by central administration of CAs or by peptides like prolactin (PRL), but it is unclear the role that suckling may have on its activation. In the present study, we analyzed whether suckling may inhibit milk ejection induced by IV doses of OT in anesthetized lactating rats, and to what extent it may result from adrenal or neural CAs. Methods: Wistar lactating rats (pp days 12-16) chronically implanted with cannulae into each lateral cerebral ventricle (ICV) were used. The day of the experiment the mothers were separated from their pups for 8h and then anesthetized with urethane. Groups of rats were either hypophysectomized, adrenalectomized, spinal cord transected (T3-T4), or had the nipple area locally anesthetized before suckling. IV doses of OT (0.8 mU every two min) were injected to the mother during 10 min periods of suckling by the pups. Milk yield was determined by weighing the litter after each period of suckling. Results: 1) Milk yield was greatly depressed in either control intact, sham operated or saline ICV-injected rats. This inhibition was counteracted by IV or ICV administration of the ß-adrenergic blocker propranolol, spinal-cord transection or local anesthesia of the nipple area; and was partially prevented by bilateral adrenalectomy. 2) During suckling, no facilitation of milk ejection occurred after ICV administration of PRL. In fact, inhibition instead of facilitation was observed when suckling was applied after PRL, and this effect was counteracted by IP propranolol. These results indicate that suckling inhibits milk ejection in the anesthetized rat through the reflex activation of central ß-adrenergic mechanisms, involving both, adrenal and neural CAs. Also, they suggest that the inhibition of PRL on milk ejection occurred by potentiation of suckling stimulation.

450.3

EFFECT OF BILATERAL ADMINISTRATION OF ß-ACTIN ANTISENSE INTO THE SUPRAOPTIC NUCLEI ON THE MILK-EJECTION REFLEX IN THE CONSCIOUS RAT. Q.J. Pittman*, Y. Takahashi & B.C. Wilson. Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada T2N-4N1.

Antisense oligonucleotides (oligos) are used experimentally as specific inhibitors of gene expression. Bilateral injection of antisense directed against oxytocin (OT) mRNA into the supraoptic nuclei (SON) was recently shown to reduce pup weight gain and reduce the total number of milk-ejections of conscious dams 4 hours post-injection. While these effects were specific for OT-antisense (OT-as) and not mimicked by VP-as or mixed bases (which would not hybridize in an OT cell), the question arose if similar effects would result from hybridization with a different active transcript in the OT cell. Nineteen mer antisense (TCGTCATCCATGGCGAACT) or sense (AGTTCGCCATGGATGACGA, as control) oligos were directed against a region of the mRNA spanning the start codon of the precursor of the microfilament protein, ß-actin. Injections (1µg/0.5µl) were given bilaterally into the SON of conscious Day 9 lactating rats implanted with guide cannulae. Four hours post-injection, 10 pups were re-introduced into the cage of each dam and assessments were made of maternal behaviour, pup weight gain and the number of milk-ejections occurring in a 30 min period after suckling was established. There was no significant ($p>0.05$) difference between maternal behavior, pup weight gain or the number of milk-ejections of dams receiving sense or antisense for ß-actin into the SON (verified histologically) or rats receiving injections of either substance into adjacent brain tissue. These data indicate that short latency actions of antisense nucleotides may be specific to secreted protein and are not seen after hybridization with mRNA for another cellular constituent. Our data also suggests that short-term disruption of ß-actin synthesis does not affect milk delivery to rat pups.

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450.4

RESPONSE OF PLASMA AVP AND *FOS* IMMUNOREACTIVITY TO INTRAGASTRIC HYPERTONIC SALINE IN CONSCIOUS RATS

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Some studies have suggested peripheral osmoreceptor control of arginine vasopressin (AVP) release in the rat. The afferent pathway mediating this response is unclear. Experiments were conducted to examine the effects of intragastric administration of isotonic (290 mOsm/L) and hypertonic (600 mOsm/L) saline on plasma AVP (PAVP) and *fos* immunoreactivity within various central nuclei. In the first study, rats were chronically instrumented with arterial and gastric catheters. Two days later, rats received an intragastric infusion of either isotonic (n = 7) or hypertonic (n = 9) saline (.59 ml/min.). Blood samples were taken before and after infusion to measure plasma osmolality (POSM) and PAVP. Isotonic saline did not affect PAVP (4.1 ± 1.4 to 3.9 ± 0.4 pg/ml), while hypertonic saline resulted in a significant increase in PAVP (3.5 ± 1.1 to 5.8 ± 1.7 pg/ml; $P < 0.05$). Hypertonic saline did not affect POSM, indicating that the central osmoreceptors were not stimulated. In a second study, chronically instrumented rats (n = 10) were examined for changes in *fos* immunoreactivity in selected brain nuclei in response to intragastric isotonic and hypertonic saline infusions. Hypertonic saline increased *fos* immunoreactivity in the area postrema, nucleus tractus solitarius, lateral parabrachial nucleus, and the paraventricular and supraoptic hypothalamic nuclei. These results indicate that intragastric hypertonic saline stimulates AVP release in the absence of central osmoreceptor activation, which is consistent with the hypothesized peripheral osmoreceptor control of AVP release. Additionally, *fos* immunoreactivity results indicate that the hypertonic gastric saline activates central nuclei apart from the hypothalamic nuclei associated with AVP release, including the area postrema, nucleus tractus solitarius and lateral parabrachial nucleus. Whether these nuclei are associated with the observed AVP release is unknown, and their exact role in the response to gastric hypertonic saline remains to be determined.

450.5

OXYTOCIN AND VASOPRESSIN GENE EXPRESSION IN THE INTERMEDIATE LOBE OF RAT PITUITARY. Q. Sun, S. Pretel*, D. Piekut. Dept. of Neurobiology & Anatomy, Univ. of Roch., Rochester, NY 14642

One important function of the pituitary is to modulate the body's stress-response through secretion of ACTH. Corticotrophin releasing factor, oxytocin (OX) and vasopressin (VAS) are known to stimulate ACTH release; their synthesis and release are increased in many stressful conditions. Two pathways by which OX and VAS may modulate the corticotrophin cells in the anterior lobe of the pituitary have been hypothesized. One is via the long portal veins, through which OX and VAS, released from parvocellular neurons into the median eminence, are carried to the anterior lobe. The other is via the short portal veins, which transport the OX and VAS, released by magnocellular neurons at the posterior lobe, to the anterior lobe. The presence of OX and VAS peptide in posterior lobe is well known. Recently the presence of VAS, but not OX mRNA, has also been reported; however, the location of VAS mRNA in the pituitary is unclear. The goal of the current study was to localize the expression of OX and VAS mRNA, as well as their peptide, in specific components of the pituitary of normal and kainate treated rats. In situ hybridization was performed on frozen sections with [35]S-labeled oligonucleotide probes (generously provided by Dr. S. Young, NIH). In this study, strong OX and VAS immunoreactivity (ir) was demonstrated in nerve endings and Harris bodies in the posterior lobe; no mRNA for either OX or VAS was present. In the anterior lobe, only a few weakly labeled OX-ir cells were found; no VAS-ir cells, and no OX or VAS mRNA labeling was present. However, in the intermediate lobe, positive labeling for OX and VAS mRNA was present in the majority of cells in normal and kainate treated rats. The OX-ir and VAS-ir labeling were weak, but clearly detectable. Since the intermediate lobe has much less blood supply, its secretion of OX and VAS might stimulate the ACTH release through paracrine mechanism. Supported by NIH grant NS 18626

450.6

PROFILE OF CATECHOLAMINE AND AMINO ACID NEUROTRANSMITTER CONCENTRATIONS IN RAT SUPRAOPTIC NUCLEUS DURING PARTURITION D. Voisin[1], C. Chapman[1], A. Douglas[2] and A. Herbison[1]. (SPON: Brain Research Association) [1]Lab. of Neuroendocrinology, Babraham Institute, Cambridge and [2]Dept. Physiology, University of Edinburgh, U.K.

The magnocellular oxytocin neurones of the supraoptic (SON) and paraventricular nuclei of the hypothalamus are believed to play an important role in the initiation of parturition in the rat. However, little is known about the neural inputs regulating oxytocin neurone activity at the time of parturition. In the present study we have used the technique of intracerebral microdialysis to monitor the extracellular concentrations of a variety of catecholamines and amino acid neurotransmitters within the SON prior to and during parturition.

Microdialysis probes were implanted under halothane anaesthesia into the SON of pregnant rats (n=5) on the day preceding parturition and 15 min dialysate samples were collected from freely moving rats over the following two days until 3h after birth of the last pup. Dialysate samples were analysed by electrochemical and fluorescence HPLC for glutamate, aspartate, GABA, norepinephrine, dopamine and serotonin.

On the day of parturition, extracellular concentrations of norepinephrine were significantly ($p<0.05$) increased in the hour leading up to and including the birth of the first 2-3 pups but then fell to basal levels only to increase again in a transient fashion at the termination of parturition. Dopamine levels remained unchanged but increases in serotonin were noted during parturition in some rats. An increase in glutamate levels was observed immediately preceding and during the initiation of parturition although this change was not significant (n=4). No differences in aspartate or GABA levels were observed at this time although GABA concentrations were elevated in an inconsistent manner during the course of parturition.

These results indicate that norepinephrine, and possibly glutamate, may be involved in the activation of SON oxytocin neurones at the initiation of parturition but not during its maintenance.

450.7

EFFECTS OF ESTROGEN ON NORADRENERGIC AND VASOPRESSIN CELL RESPONSES TO HYPOXIA. K.M. Buller* & T.A. Day. Dept. of Physiology & Pharmacology, University of Queensland, Australia.

This laboratory has recently shown that in male rats systemic hypoxia produces an activation of neurosecretory vasopressin (VP) cells that is mediated by an input from the A1 noradrenergic (NA) cell group of the caudal medulla. In the female rat this system may be influenced by the ovarian hormone estrogen (E). Thus, in the ovariectomized (OVX) rat, the excitability of A1 cells is reportedly enhanced by E administration, while in the intact rat it has been shown that the VP response to haemorrhage varies in accord with circulating E levels. We therefore hypothesized that variations in E levels might both alter the basal activity of brainstem NA and hypothalamic VP cells and influence the responsiveness of these cells to systemic hypoxia (10% O_2). Using the *c*-fos neuronal activity mapping technique we have investigated in the OVX rat the effects of both a single E bolus (100 µg/kg, s.c.) and repeated daily E injections (day 1, 5 µg; day 2, 50 µg; day 3, 50 µg), compared to injections of vehicle at matching times. Neither single nor multiple injections of E (n = 4) significantly changed the basal levels of *c*-fos expression in A1 or A2 NA cells, or supraoptic nucleus VP cells. With regard to responsiveness to 10 % O_2, single E treatment had no significant effect on A1, A2 or SON *c*-fos expression. Multiple injections of E also failed to alter the A2 cell group response to hypoxia, but significantly increased the A1 cell response (40.7 ± 3.9 % versus 19.2 ± 9.4 % of caudal ventrolateral medulla tyrosine hydroxylase positive cells being Fos-positive). However, despite the well established link between A1 cell activation and VP cell activity, multiple injections of E had no significant effect upon the frequency of *c*-fos expression in supraoptic VP cells.

450.8

EFFECTS OF SELECTIVE HYPOVOLAEMIA & HYPOTENSION ON A1 NORADRENERGIC & NEUROSECRETORY VASOPRESSIN CELLS. D.W. Smith*, K.M. Buller and T.A. Day. Dept. of Physiology & Pharmacology, University of Queensland, Australia.

Hypotensive haemorrhage elicits a vasopressin (VP) cell response that depends on a relay from the A1 noradrenergic cell group. There has been considerable debate as to the importance of arterial baroreceptors versus cardiac volume receptors in this response. By applying stimuli that recruit receptors selectively, we have investigated their contributions to A1 and neurosecretory cell responses to the component stimuli of haemorrhage. Animals received either i.p injections of polethylene glycol (PEG; 20-30% w/v in dH_2O; 20 ml/kg; n=6) or the ganglionic blocker pentolinium (PENT; 10 mg/kg; n=5). Controls received similar volumes of isotonic saline. After 2 h animals were sacrificed and brains processed for Fos, VP, oxytocin (OT) and tyrosine hydroxylase immunocytochemistry. Parallel groups of animals, chronically instrumented for blood pressure recording, received identical treatments. PEG, but not PENT treated animals, showed a significant reduction in plasma volume (20%) as estimated by haematocrit. PENT, but not PEG treated animals, showed a significant reduction in MAP (40 mmHg), suggesting that PEG is a non-hypotensive hypovolaemic stimulus and PENT is an iso-volaemic hypotensive stimulus. Both stimuli caused significant increases in VP and OT neurosecretory cell *c-fos* expression, although these responses were substantially greater in the supraoptic nucleus. Both stimuli significantly increased A1 noradrenergic cell group *c-fos* expression with a similar distribution of activated cells along the rostro-caudal axis of the A1 column. These data suggest that both arterial baroreceptor and cardiac volume receptor populations contribute to the VP and OT responses to haemorrhage and that the A1 cell group has a role in these responses.

450.9

FUNCTIONAL ROLE OF ATRIAL NATRIURETIC PEPTIDE RECEPTORS ON NEURONAL AND GLIAL ELEMENTS IN THE RAT NEUROHYPOPHYSIS. M. Hlubek, K.D. Ramsell and P. Cobbett.* Dept. Pharmacol./Toxicol. and Neuroscience Program, Michigan State Univ., E. Lansing, MI 48824.

Activation of atrial natriuretic peptide (ANP) receptors increases cGMP within axon terminals and pituicytes (astrocytes) of the neurohypophysis (Rambotti et al., Brain Res:644,52-58,1994). We have therefore investigated the effect of ANP on vasopressin (AVP) secretion from neurosecretosomes (isolated neurohypophysial axon terminals) and on the morphology of cultured pituicytes. ANP (1μM) significantly elevated AVP secretion evoked by potassium-induced depolarizations of neurosecretosomes but had no effect on basal secretion of AVP. In contrast, 8bromo-cGMP (20-100μM) did not appear to affect depolarization-evoked secretion but appeared to increase basal secretion. In cultures of pituicytes, the fraction of stellate cells was significantly increased by incubation in medium containing ANP (1μM) or 8bromo-cGMP (100μM) compared to control medium. These data indicate that ANP may act at the level of the neurohypophysis to modulate AVP secretion. Supported by NINDS (NS28206).

450.10

ELECTROPHYSIOLOGICAL DIFFERENCES BETWEEN OXYTOCIN AND VASOPRESSIN NEURONS IN FEMALE RATS. J.E.Stern* and W.E.Armstrong. Dept of Anat. & Neurobiol., Univ. Tenn., Memphis, TN 38163.

Oxytocin (OT) and vasopressin (VP) neurons display different firing patterns during hormone release. The extent to which these patterns result from intrinsic membrane properties, particularly in OT neurons, is incompletely known. In addition, most intracellular recording studies in rats have focused on males. In the present study, the membrane properties of immunohistochemically identified supraoptic nucleus neurons in hypothalamo-neurohypophysial explants were studied in lactating and virgin female rats. Resting membrane potential and input resistance didn't differ between OT and VP neurons, but spike threshold was 5 mV more depolarized in OT neurons. When the great majority (94%) of OT, but not VP (93%) neurons were current-clamped at depolarized voltages (<-50 mV), small hyperpolarizing pulses revealed a time- and voltage-dependent outward rectification which decreased with increasing hyperpolarization. A rebound depolarization (RD) followed the offset of pulses associated with strong rectification. Both the outward rectification and RD were abolished by TEA (10 mM) and increased by 4-AP (6 mM). Ba^{2+} (0.1-1 mM) but not Cs^{+} (2 mM) blocked the outward rectification. The RD was partially blocked by Cd^{2+} (0.5 mM) but not by Ni^{2+} (0.2-0.5 mM). In general, the outward rectification and RD were reminiscent of behavior seen in sympathetic ganglion cells and attributed to M-current. An additional difference between OT and VP neurons was seen in the amount of transient outward rectification, which was stronger in VP neurons. These results indicate the presence of significant differences in the intrinsic membrane properties, probably K^{+} currents, between OT and VP neurons in both lactating and virgin female rats. Experiments in progress are testing whether these differences affect the firing pattern of these neurons, and whether they are amenable to neurotransmitter modulation. Supported by NIH NS 23941 (WEA) and the Neuroscience Center for Excellence.

450.11

CHANGES IN THE ELECTROPHYSIOLOGICAL PROPERTIES OF OXYTOCIN AND VASOPRESSIN NEURONS DURING LACTATION. W.E.Armstrong* and J.E.Stern. Dept of Anat. & Neurobiol., Univ. Tenn., Memphis, TN 38163.

During lactation, oxytocin (OT) neurons adopt a synchronized and intermittent high frequency bursting firing pattern, which optimizes the release of OT at the neural lobe and the resulting milk ejection. Furthermore, profound rearrangements in the ultrastructure of the supraoptic nucleus (SON) have been observed in association with parturition and lactation. In the present study we sought to determine whether the intrinsic membrane properties of supraoptic nucleus (SON) magnocellular neurons were altered as a function of lactation, and if so whether changes were selective to OT neurons. Conventional intracellular recordings from SON neurons were combined with immunohistochemical identification of OT and vasopressin (VP) neurons using hypothalamo-neurohypophysial explants taken from virgin diestrus and lactating rats, both of which have low circulating estrogens. Resting membrane potential, input resistance, and spike height were not different between lactators and diestrus females. OT, but not VP neurons showed a lactation-dependent increase in the amount of afterhyperpolarization per spike following spike trains. The presence of a depolarizing after-potential (DAP) was increased by 20% in OT neurons during lactation, but the differences were not statistically significant. The repetitive firing properties of SON neurons also changed during lactation. Neurons from lactating rats had a less steep F-I slope for early interspike intervals than those in diestrus rats, but reached significance only in OT neurons. Individual spikes were wider in OT neurons during lactation and showed more spike broadening during trains. In both cell types the number of spikes for a given positive current injection was decreased during lactation. These results indicate that the state of lactation can affect some properties of both neuron types but has a more pronounced effect on OT neurons. Supported by NIH NS 23941 (WEA) and the Neuroscience Center for Excellence.

450.12

INCREASED INTESTINAL TRANSIT AFTER LiCl TREATMENT IN RATS MAY BE MEDIATED BY OXYTOCIN. E.A. Baker, K.S. Curtis, J.G. Verbalis and E.M. Stricker*. Departments of Neuroscience and Medicine, University of Pittsburgh, Pittsburgh, PA 15260.

The ingestion of toxins by animals is known to reduce food intake and slow gastric emptying. These same adaptive responses can be produced by systemic administration of agents such as LiCl. This study evaluated intestinal transit after LiCl treatment in rats. Control rats given ip injections of isotonic NaCl solution required 7.3 ± 0.6 h (n=9) to pass a dye contained in a consumed meal; in contrast, rats injected ip with LiCl (3 mEq/kg) 45 min after eating had a significantly faster IT time of 4.0 ± 0.7 h (n=25; p<0.01 compared to controls). Previous studies indicated that LiCl-induced anorexia was potentiated by administration of the opioid antagonist naloxone, so we also evaluated the effects of this agent on LiCl-induced changes in IT. Whereas injection of either a low dose of LiCl (1.5 mEq/kg ip) or naloxone (2.5 mg/kg ip) separately had no effect on IT (8.8 ± 0.5 h, n=9, and 7.3 ± 0.4 h, n=9, respectively), combined treatment with naloxone and this low dose of LiCl significantly decreased IT time to 4.8 ± 0.9 h (n=9; p<0.01 compared to controls). Because LiCl stimulates pituitary OT secretion in rats, an effect which is potentiated by naloxone, we pretreated rats with the OT receptor antagonist [d(CH_2)$_5$, Tyr(OMe)^2, Orn^8] vasotocin (OVT; 50 μg/kg) 30 min before LiCl administration (3 mEq/kg); OVT pretreatment abolished the LiCl-induced changes in IT (8.0 ± 0.7 h, n=6; p<0.01 compared to rats treated with this dose of LiCl alone). Collectively, these results indicate that toxins such as LiCl increase intestinal transit in rats, and suggest that this effect may be mediated by the increase in plasma OT levels known to accompany the administration of LiCl and other toxins in this species.

450.13

INDUCTION OF FOS-LIKE IMMUNOREACTIVITY BY NITRIC OXIDE IN THE HYPOTHALAMIC MAGNOCELLULAR NEUROSECRETORY NEURONS OF THE RAT. E.Shen*, Z.J.Yang and X.Sun Shanghai Brain Research Institute, 320 Yueyang Rd, Shanghai 200031, China.

High concentration of nitric oxide synthase (NOS) in the hypothalamic magnocellular neurosecretory neurons (MCNs) in the rat has been documented. Its function remains unknown. In the present study we tried to investigate whether nitric oxide (NO) synthesized in the MCNs could induce c-fos or not. The substrate of NOS, L-arginine (10 nM, 5 μl), was slowly microinfused in a period of 50 min into the supraoptic nucleus (SON) of adult male Sprague-Dawley rats under sodium pentobarbital anesthesia. Eighty min after the start of microinfusion the rats were sacrificed. Fos-like immunoreactivity (FLI) could be seen in the nuclei of a subpopulation of MCNs in the SON. A majority of the FLI-positive nuclei were not in the NADPH-diaphorase-positive cells. Only about 7% were colocalized with them. The FLI-positive nuclei could be found in both the oxytocin cells and vasopressin cells. However the percentage of FLI expressed in the oxytocin cells was higher. It is suggested that the NO synthesized in the NOS-containing MCNs diffuses out of the cells and induces FLI in the nearby MCNs. Supported by the National Natural Science Foundation of China 39270241, 39270239, the Chinese Academy of Sciences ks852024.

450.14

Age Interfers with the Response of the Hypothalamo-neurohypophysial System to Hypertonic Saline Injection. Gwyneth Hill Beagley*, Department of Psychology, Alma College, Alma MI 48801

The Hypothalamo-neurohypophysial System (HNS) shows characteristic morphological changes in response to a large systemic injection of hypertonic saline. In the posterior pituitary, cytoplasmic processes of astrocytic glial cells (pituicytes) typically retract, allowing decreased enclosure of axons and increased apposition of neuropeptide terminals with the basal lamina (BL). This facilitates increased release of oxytocin and vasopressin into the bloodstream. The present study investigated the effect of age on the HNS response. Male Sprague-Dawley rats aged 4-5 months (young) and 20-24 months (old) were given 18 ml/kg injections of either hypertonic (1.5 M NaCl) or normal (0.15 M NaCl) saline and sacrificed after 5 h. HNSs were removed for examination with a transmission electron microscope. Morphological analysis of micrographs of the neural lobes showed:

1. Terminal apposition in the neural lobes of both groups of older rats was lower than in young control rats (old 0.15 M NaCl = 17.2±2%; old 1.5 M NaCl = 13±2.7%; young 0.15 M NaCl = 30±3%).
2. Pituicytes in the neural lobe of older rats did not retract from the BL in response to the hypertonic saline injection (pituicyte apposition in young 1.5 M NaCl rats = 41±1.9%; in old 1.5 M NaCl rats = 87±2.5%).
3. Many terminals in the hypertonically stimulated older rats were still enclosed by pituicytes, but were only partially filled with neuropeptide containing vesicles.

These findings suggest that although a hypertonic saline injection may stimulate neuropeptide release from the neural lobe of older rats, the pituicytes do not respond to the challenge with retraction characteristic of pituicytes in younger animals. This suggests increased age could result in decreased effctiveness in the response of the HNS to acute and chronic dehydration.

450.15

Oxytocin Receptor mRNA and Binding in the Ventromedial Hypothalamus of Male and Female Rats: Effects of Androgens and Progesterone Tracy L. Bale* and Daniel M. Dorsa, Depts. of Pharmacology and Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98195

In situ hybridization and radioligand binding techniques were used to demonstrate correlations between changes in oxytocin receptor (OR) mRNA and binding levels in the ventromedial hypothalamus (VMH) in both males and females in response to gonadal steroids. Previous reports have shown that estrogen (E) increases OR binding in the VMH in both males and females, and testosterone (T) also increases binding in males. The E induced increase in OR binding in females has been reported to be further modulated by progesterone (P), as P was shown to increase OR binding area. We have previously reported that E treatment of ovariectomized females results in a significant increase in OR mRNA expression in the VMH. The present studies were designed to 1) determine if the reported increases in OR binding in the VMH in males is preceded by increases in OR mRNA expression, 2) to determine the effect of P and E on OR mRNA in the VMH in females, and 3) to correlate these findings in the VMH with radioligand OR binding. Castrated males were treated with T or its metabolites, E and dihydrotestosterone (DHT). Autoradiographic results from in situ hybridization indicate that T and E can both significantly increase OR mRNA expression in the VMH. However, DHT when administered with E, reduced the induction observed with E alone. ^{125}I-OTA binding in the VMH confirmed this inhibition. Ovariectomized females were treated with E, P, or E and P (E/P). Results from this study confirmed our previous report of increases in OR mRNA expression with E treatment. P alone had no effect, nor did it alter expression seen with E alone (E/P). ^{125}I-OTA binding measured in adjacent sections of these animals showed no difference in OR binding site density or distribution between the two groups. These findings contrast with previous reports of an increase in the area involved in OR binding in the VMH. These results confirm a stimulatory role for E, but indicate a negative effect of DHT and no effect of P on OR regulation. We are presently sequencing the promoter region of the OR gene to identify enhancer elements which might mediate these hormonal effects. (Supported by NS-20311, NS-07332)

450.16

NITRIC OXIDE DEPOLARIZES PARVOCELLULAR, NOT MAGNOCELLULAR NEURONS IN PARAVENTRICULAR NUCLEUS. J.S Bains* and A.V. Ferguson, *Department of Physiology, Queen's University*, Kingston, ON. Canada K7L 3N6.

Nitric oxide (NO) has been implicated in playing a pivotal role in an increasing number of neuronal functions. The localization of NO synthase in the paraventricular nucleus of the hypothalamus (PVN), led us to investigate the putative role of NO within this structure. Previous work from our laboratory has reported that bath administration of NO depolarizes cells within PVN. Since PVN is a heterogenous structure composed of at least two different cell types, we have now extended this work to clarify the types of cells affected by NO and the underlying mechanisms responsible for the observed response.

Using patch electrodes filled with potassium-gluconate, recordings were made from PVN neurons in coronal brain slices (400 µm thickness) of adult, male Sprague-Dawley rats. Cells were classified into type I (putative magnocellular) or type II (putative parvocellular) according to the criteria of Tasker and Dudek (J.Physiol. 434: 273-294). Bath application of NO ($\leq 10^{-7}$) or the NO donor N-Acetyl-S-nitroso-D-penicillamine (SNAP) (10^{-3} - 10^{-4}) elicited a mean depolarization of 4.5 ± 1.1 mV (mean ± SEM) (n=19) and a mean decrease in input resistance of 140 ± 41 MΩ). Application of the NO precursor L-arginine (L-ARG) (n=14) also elicited a mean depolarization (5.2 ± 1.2 mV), and an accompanying decrease in input resistance (246 ± 71 MΩ). Type I cells, however, were unaffected by administration of NO, SNAP or L-ARG (n=10). Type II cells tested in TTX exhibited a mean depolarization of 6.2 ± 1.9 mV (n=6) in response to NO or SNAP, but did not respond in a zero calcium solution (n=5). Preliminary evidence suggests that NO actions may be the result of activation of cyclic GMP (cGMP), as SNAP has no effect on the membrane potential of type II cells in the presence of the cGMP inhibitor LY83583 (n=2).

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NEURAL-IMMUNE INTERACTIONS: PATHOLOGY

451.1

BRAIN MAST CELL DEGRANULATION BY COMPOUND 48/80 OPENS THE BLOOD-BRAIN BARRIER X. Zhuang[1]*, A.-J. Silverman[2], and R. Silver[1,3], [1]Dept. Psychology, [2]Dept. Anat. & Cell Biol., P&S, 10032, and [3]Barnard College, Columbia Univ., New York, NY 10027

Mast cells (MC) are well known for their production and secretion of vasoactive substances. MC are particularly numerous in the medial habenula (MH) of the ring dove at the peripubertal age of 5-7 mo. In this study we asked if MC activation causes alterations in the permeability of the blood-brain barrier (BBB). Thus, the mast cell degranulator, compound 48/80 (C48/80) was injected i.m. into 6 mo. old birds followed 2 hrs later by i.v. injection of the fluorescent dye, Evans blue (EB). This dye does not cross the BBB. Control animals were injected with saline followed by EB. All birds were perfused 30 min after EB injection. Frozen sections (50 micron) were examined for the distribution of EB in the brain parenchyma using fluorescence microscopy. The optical density of the fluorescent signal was quantified by digital imaging (Image NIH). Images of brain sections containing the MH, paraventricular nucleus (PVN), and a circumventricular organ - the lateral septal organ (LSO) were captured and stored. Brain sections were then stained with toluidine blue to delimit the foregoing nuclei for quantification. C48/80 treatment significantly increased the spread of the tracer in the MH ($p<0.01$). In contrast, there was no leakage of tracer into the PVN in either group. There was leakage of EB into the brain around the LSO in both groups but the extent of leakage does not differ between groups. Degranulation of mast cells after C48/80 treatment was confirmed histochemically and ultrastructurally. The result supports the hypothesis that mast cell activation can open the BBB. Supported by NSF grant SGER and NIMH grant 29380 (RS) and HD 10665 (AJS).

451.2

STUDY OF *DE NOVO* SYNTHESIS OF C1q PROTEIN IN KAINIC ACID TREATED RAT BRAIN.

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C1q has been found in it's activated form in senile plaques in Alzheimer disease (AD) (Eikelenboom et al., 1994; Johnson et al., 1992; Lampert-Etchells et al., 1993; Rogers et al., 1992). Aggregated ß-amyloid may be consequent, among other possibilities, to activation of C1q (Jiang et al., 1994). We are studying rodent models of these complex processes. Lesions of limbic and cortical pathways in rats result in increased levels of C1q protein and C1qB mRNA *in vivo* (Johnson et al., 1992; Rozovsky et al, 1994). The present studies test for *de novo* synthesis of C1q in KA treated rat brain. Increases in C1q protein are observed after 2 lesions: systemic KA (10mg/kg); and neocortical ablation. Batch chromatography modified from the methods of Tenner et al. (1981) revealed three C1q monomers of expected size (aprox. 26 kd) as identified on Western blots, which were identical to those from rat sera. Brains processed 3 days after KA had aprox. 6-fold increases in C1q vs. controls (KA+ pentobarbitol, pentobarbitol alone, saline(SAL)). Ten days after neocortical ablation, ipsilateral striatum only showed increased C1q. *De novo* synthesis was studied using ^{3}H-proline or ^{35}S-methionine. Pilot data indicate a 5 fold increase in incorporation of the ^{3}H-proline into the purified C1q fraction of KA slices, incubated for 3 h as compared to controls (KA 0 incorp. time; SAL 0 incorp. time; SAL 3 h incorp.). SDS-PAGE gels of purified C1q from KA slices incubated in ^{35}S-methionine resulted in labeled monomers in the 26Kd range. (Supported by AG 07909.)

451.3

Is Activated Complement Neurotoxic to Rat Hippocampal Neurons?
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Alzheimer disease (AD) is characterized by excessive deposition of the β-amyloid peptide in the central nervous system. Several proteins in addition to the β-amyloid protein have been found in close association with both diffuse and classical neuritic plaques. Immunocytochemical studies have demonstrated the presence of complement proteins. Senile plaques and extracellular tangles are decorated with complement proteins of the classical pathway indicating activation of this cascade in AD brain. It has been shown that the β-amyloid protein which is the chief component of the senile plaques is capable of activating the complement cascade.
To determine whether complement is neurotoxic, we treated neuronal hippocampal cultures with human serum. Hippocampal neurons were prepared from day 17 rat embryos and cultured on poly-D-lysine coated plastic in serum free neural basal medium with B27 supplements. Treatment with increasing concentrations of sera showed a concentration dependent increased neural toxicity, as indicated by reduced conversion of calcein AM to the fluorescent "live" dye calcein and by decreased reduction of the dye Alamar Blue, an indicator of mitochrondial function. The neurons appear compromised or stressed as indicated by Alamar Blue, yet the cells remain viable as indicated by Calcein AM. Sera depleted of the C1q and C3 component was also toxic to these cells suggesting that complement is not the onlycomponent of sera to be toxic to neurons. Further work will examine the effect of activated complement on rat hippocampal neurons and whether we can inhibit activation and slow the progression of neuronal death by complement.

451.4

INVOLVEMENT OF AMOEBOID MICROGLIA IN NEURODEGENERATION IN ORGANOTYPIC SLICE CULTURES OF MOUSE HIPPOCAMPUS B.W. Coltman* and C.F. Ide. Dept. of Cell and Molecular Biology, Tulane University, New Orleans, LA 70118

Microglia, the resident macrophages of the brain, are widely recognized as immunocompetent cells of the CNS. In response to injury or disease, microglia become activated and are drawn to the site of damage and acquire macrophage-like characteristics. Current *in vitro* models of neuroglial inflammatory responses offer a limited approach to the complex interactions between neurons and glia because functional neuroimmune networks are either absent or greatly simplified. This obstacle is overcome in slice cultures which may be manipulated *in vitro* while maintaining the complex *in situ* environment of the brain. To further investigate the role of microglia during neurodegenerative events, we have characterized the temporal and spatial distribution of activated and quiescent microglial cells associated with necrotic sites in culture using the *Griffonia simplicifolia* lectin. Initially, out of 76 cultures, spontaneous neurodegenerative sites occurred in approximately 43% of slices within six days *in vitro* (DIV). In a subset of the group that formed holes, after 3-4 DIV, 82% developed necrotic centers in CA3, 18% in CA1 and no necrotic sites were observed in the dentate gyrus. After 5-6 DIV, 87% of necrotic sites were found in CA3, 33% in CA1 and again, no necrotic centers were observed in the dentate gyrus. In all cultures, amoebiod microglia were closely associated with the retracting necrotic edge of the forming hole. Resting microglia were confined primarily to sites devoid of tissue. A temporal and spatial propensity for specific sites of neurodegenerative events occurs *in vitro*, and appears to follow *in vivo* patterns. Thus, organotypic slice cultures may serve as an excellent model for elucidating neuroglial inflammatory responses to disease or injury. Supported by DOD grant DOD93DNA-2 and DOE grant DE FG01-93 EW 53023.

451.5

Ultrastructural Localization of Quinolinate in Human Monocytes/Macrophages. V. Sung[1], C. Venkateshan[2], L. Williamson[3], M.G. Espey[1], E. Neale[3], C.G. Gibbs[2] Jr., J.R. Moffett[1]* and M.A.A. Namboodiri[1]. Dept. Biology, Georgetown[1] Univ., Washington, DC, LCNSS[2], NINDS, and LDN[3], NICHD, NIH Bethesda, MD 20892.

Quinolinate (QUIN), a tryptophan derived excitotoxin, was localized in human monocytes/ macrophages (MQ) by immunoelectron microscopy. A combined carbodiimide/glutaraldehyde/ formaldehyde based fixation procedure was developed for optimal retention of intracellular QUIN while minimizing loss of ultrastructure, and a silver enhanced colloidal gold detection was used to visualize QUIN. QUIN-immunoreactivity was detectable at the cell periphery and in association with the inner face of the plasma membrane in normal MQ, and increased significantly on treatment with 1) IFN-γ, an immune activator and 2) kynurenine, a precursor. Dense aggregations of gold particles representing QUIN were especially present in the pseudopodia of stimulated MQ. The specific localization of QUIN near the plasma membrane and its reported release suggest a possible role for QUIN as an immune regulator.

451.6

ENHANCEMENT OF MACROPHAGE FUNCTION BY DYNOPRHIN A.

M. Ichinose* and M. Sawada. Dept. Physiology, Shimane Med. Univ. Izumo 693, Japan.

The effects of the opioid peptide dynorphin A (DynA) on phagocytosis in peritoneal macrophages was examined by flow cytometry (FCM). DynA enhanced phagocytosis in a dose-dependent manner. Leucine-enkephalin (Leu-Enk), methionine-enkephalin (Met-Enk), β-neo-endorphin (βNeo-End), DynA(9-17) and DynA(13-17) had no such activity. α-Neo-endorphin (αNeo-End), dynorphin B (DynB), DynA(1-13) and DynA(6-17) enhanced phagocytosis less effectively than DynA. Naloxone did not inhibit the enhancement of phagocytosis induced by DynA. Unstimulated control phagocytosis was partially suppressed in Ca^{2+}-free EGTA-containing solution and even in this solution DynA enhanced phagocytosis. However, the enhancement by DynA was suppressed in EGTA- and BAPTA-AM-containing Ca^{2+}-free solution. The present study showed that enhancement of phagocytosis by DynA was independent of extracellular Ca^{2+} ($[Ca^{2+}]_o$) and dependent on intracellular Ca^{2+} ($[Ca^{2+}]_i$). The present results support DynA being one of the mediators from the nervous system that modulates the immune system.

451.7

MODULATION OF MACROPHAGE PHAGOCYTOSIS BY OPIOID PEPTIDE, ENDORPHIN.

M. Sawada* and M. Ichinose. Dept. Physiology, Shimane Med. Univ. Izumo 693, Japan.

The effects of β-Endorphin (βEnd) on phagocytosis in peritoneal macrophages were examined by using flow cytometry (FCM). βEnd enhanced phagocytosis in a dose-dependent manner. Leucine-enkephalin (Leu-Enk), methionine-enkephalin (Met-Enk), α-endorphin (αEnd), γ-endorphin (γEnd), αEnd(18-31) and βEnd(28-31) had no such activity. βEnd(1-27) and βEnd(6-31) enhanced phagocytosis less effectively than did βEnd. Naloxone did not inhibit the enhancement of phagocytosis induced by βEnd. Unstimulated control phagocytosis was partially suppressed in Ca^{2+}-free EGTA-containing solution and even in this solution βEnd enhanced phagocytosis. However, the enhancement was suppressed in the solution containing BAPTA-AM. The present study showed that βEnd enhanced extracellular Ca^{2+} ($[Ca^{2+}]_o$) dependent and independent phagocytosis and that the enhancement is largely dependent on intracellular Ca^{2+} ($[Ca^{2+}]_i$). These results support βEnd being one of the mediators that modulates the immune system.

451.8

FETAL THYMIC EXPRESSION OF CRF AND POMC IS AFFECTED BY MATERNAL ALCOHOL CONSUMPTION IN MALE RATS, SELECTIVELY.

S. Revskoy, I. Halasz and E. Redei*. Depts. of Pharmacology and Psychiatry, University of Pennsylvania, Philadelphia, PA 19104.

CRF and POMC have been shown to be expressed in the adult thymus. However, no data to date is available on the expression of these genes in the fetal thymus. Early environmental stressors such as maternal alcohol exposure deleteriously affect immune function of the offspring and alters the expression of CRF and POMC in the hypothalamus and pituitary. Thus, we have hypothesized that changes of thymic POMC and CRF expression can reflect the altered fetal thymic milieu in these animals. In the present study, we have measured thymic CRF and POMC mRNA levels on days 19 and 21 of embryonic life in male and female fetuses. Fetal thymi were obtained from fetuses of control dams maintained on liquid diet and from dams consuming an alcohol-containing liquid diet from day 8 of gestation. CRF and POMC mRNA levels were measured by reverse-transcription-polymerase chain reaction method in individual thymic tissue. The levels of expression were estimated as a ratio of the levels of CRF and POMC to GAPDH mRNAs, respectively. In general, CRF and POMC transcript levels were higher on embryonic day 19 compared to day 21. CRF gene expression was similar in the fetal rat thymus of both sexes. However, thymic POMC expression was substantially higher in males on day 19. Maternal alcohol exposure significantly increased CRF and lowered POMC expression in male thymus on day 19; this effect was not observed in females and it disappeared by day 21. Therefore, the data also suggest that fetal thymic CRF and POMC are developmentally regulated and that environmental stimuli can modulate their expression. The differential response of CRF and POMC genes in fetal thymus to alcohol exposure in utero suggest that POMC transcription in the fetal thymus is regulated differently from pituitary.

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451.9

IMMUNE EFFECTS OF PRENATAL ETHANOL EXPOSURE (PEE) ARE MODULATED BY NEUROACTIVE DRUGS IN C57BL/6 MICE.

B. Günther, P. Clausing*, A. Emmendörffer, and M.-L. Lohmann-Matthes. Dept. Lab. Animals, F.-S.-University, 07745 Jena, Germany.

In order to determine if an altered neurotransmission plays any role in fetal alcohol effects on the immune system, mice prenatally exposed to ethanol (PEE, 1.69 g/kg, po, twice daily, gestational days 14-18) were treated postnatally with the ß-adrenergic agonist isoproterenol (IPR, 6 μg/kg, im) or the α-adrenergic antagonist yohimbine (YO, 2.6 mg/kg im) prior to assessing contact sensitivity (CS) to picryl chloride and analysis of lymphocyte subsets in spleen and cervical lymph node (CLN) at 4 or 12 w of age. The norepinephrine concentration was also determined in these organs. In spleen PEE *increased* significantly the portion of the L3T4 and B220 subsets of 4 w old mice. Postnatal treatment with IPR or YO abolished this effect. PEE *decreased* these subset portions as well as Thy1.2 and the cell count in 12 w old mice. Postnatal treatment with IPR aggravated these decreases in the 12 w old subjects. In CLN PEE alone did not affect lymphocyte subsets in 4 w old mice. Postnatal treatment of such individuals with IPR or YO *decreased* the L3T4, Thy1.2, B220 portions and the cell count. In 12 w old mice these parameters were significantly increased after PEE. IPR and YO did not affect the alterations induced by PEE. CS was unaffected by PEE in 4 w old subjects, however in 12 w old PEE-mice CS was significantly decreased. There was a significant positive over all correlation ($r = 0.672$, $n = 52$) between the intensity of CS and the norepinephrine concentration in the CLN. It is concluded that the adrenergic system is involved in PEE induced immune changes in an age-related manner.

451.10

FETAL ALCOHOL EXPOSURE ATTENUATES INTERLEUKIN-1β-INDUCED FEVER.

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Exposure to alcohol in utero can lead to long-lasting impairments of immune functions and to decreased resistance to infectious agents. We have previously reported that fetal alcohol exposed (FAE) rats show markedly decreased lipopolysaccharide-induced fever. Based on this finding, as well as on data from other laboratories, we hypothesized that FAE impairs the communication between the immune and the nervous systems. In the present study we assessed this hypothesis by examining the effects of interleukin-1β on body temperature in FAE and control rats. Transmitters for biotelemetric recording of body temperature were calibrated and implanted i.p. in normal (N) adult male rats, age- and sex-matched offspring of dams fed a liquid diet supplemented with ethanol (35% caloric equivalent) during the last two weeks of gestation (FAE) and pair-fed control offspring (P). Baseline recording began 2 weeks after the operation. After 24 hr of baseline recording, all rats were injected with IL-1β (2 μg/kg, i.p.) And body temperature was recorded for an additional 24 hr. IL-1 produced a significant increase in body temperature, starting about 2 hr and ending 7 hr following the injection. The febrile response in FAE rats was significantly lower than in P and N rats. These results indicate that FAE produces an impairment in the pyrogenic effects of IL-1. This impairment may account for some of the decreased resistance to infections observed in FAE animals and humans.

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451.11

Retinoic Acid Potentiates the ability of Interferon β-1B to augment Non-Specific Suppressor T Cell function *in vitro* in Multiple Sclerosis. Z. X. Qu, M. A. Jensen and Barry G.W. Arnason*. Dept. of Neurology, Univ. Chicago. Chicago, IL 60637.

The Diverse effects of Retinoids on the immune system make them important immunomodulators. Interferons (IFNs) exert anti-viral, anti-proliferative and immunomodulatory effects. IFNs have been used in combination with vitamin A and it's analogues to treat various diseases. IFN β-1B is used to treat Multiple Sclerosis (MS). Deficient non-specific suppressor T-cell function in MS is partially restored by IFN β-1B. We studied the effect of IFN β-1B and Retinoic Acid (RA) on Con A-induced suppressor function *in vitro*. Peripheral blood mononuclear cells (PBMC) from 14 healthy controls (8 female and 6 male) and 14 patients (12 female and 2 male) with MS were incubated with IFN β-1B, RA (prepared in dimethyl sulfoxide) or both plus Con A for 48hrs and then treated with mitomycin C to arrest cell proliferation. MMC treated PBMC were washed three times and incubated with fresh PBMC (1×10^5/100 µl for both) in medium alone or with Con A for 72 hrs. Cultures were set up in quadruplicate in a final volume of 220 µl. One µCi of [^{3}H]-thymidine was added during the final 5 hrs of incubation. Incorporated radioactivity was measured using liquid scintillation spectroscopy. The results indicate that: 1. RA, at a non-toxic concentration ($<5\times10^{-5}$M), has no effect on Con A-induced PBMC proliferation; 2. IFN β-1B inhibits lymphocyte proliferation and enhances suppressor function in controls and in MS (P<0.001); 3. Suppressor function in controls is higher than in MS (17.13±2.81 v.s 9.01±1.83 (P<0.02)); 4. RA (5×10^{-5} - 5×10^{-7} M) does not augments suppressor function significantly; 5. RA (5×10^{-5} - 5×10^{-7} M) combined with IFN β-1B (100 to 1000 u/ml) significantly increases suppressor function compared to IFN β-1B alone (39.30± 2.73 v.s 31.33 ±2.53 in controls (P< 0.04); 35.69±3.13 v.s 23.77±2.54 in MS (P<0.005)).

451.12

THE IMPACT OF DIFFERENT VIRAL INFECTIONS ON CORTICOSTERONE SECRETION IN MICE A.H. Miller*, B.D. Pearce, T.L. Pisell, J.S. Orunge, H.C. Su, J.J. Leung, C.A. Biron, Emory Univ. Sch. of Med., Atlanta, GA 30322 and Brown Univ., Providence, RI 02912.

Although there is ample data to suggest that the neuroendocrine and immune systems reciprocally interact, little is known about the specifics of these interactions in the context of ongoing immune responses such as during a viral infection. We have been evaluating cytokine and cellular immune responses to different experimental infections with murine cytomegalovirus (MCMV) and 2 distinct clones of lymphocytic choriomeningitis virus (LCMV). The immune responses to these agents differ both qualitatively and quantitatively. To examine the impact of these infections on the neuroendocrine system, male C57BL/6 mice were infected ip with either MCMV or LCMV clone E350 or iv with LCMV clone 13, and plasma corticosterone was measured at the am nadir on days 1,2,3,5 and 7 post infection (n=3 or more per time point). Each viral infection exhibited a distinct pattern of corticosterone secretion. LCMV clone E350 gave a modest rise in corticosterone with a peak response of 2.3 ± 0.4 µg/dl on day 5 post infection. MCMV infection resulted in a peak response of 6.9 ± 1.9 µg/dl on day 2 post infection followed by a concentration of 6.2 ± 3.9 µg/dl on day 3 post infection. LCMV clone 13 gave the greatest corticosterone response with a sharp rise to 18.3 ± 2.8 µg/dl on day 7 post infection. Baseline corticosterone concentrations in uninfected animals were 1µg/dl or lower in all experiments. These results demonstrate that the corticosterone response to a viral infection is highly specific. Moreover, they suggest that within a given infection there may be critical periods when endogenous immune responses induce glucocorticoids which, in turn, serve to regulate particular cellular and/or cytokine responses to the virus. Supported by MH47674, MH00680 and CA41268.

451.13

DIFFERENTIAL UP-REGULATION OF ANTI-VIRAL STATE GENES IN NEURAL CELLS. L.A. Ward* and P.T. Massa, Ph.D

Neurons express high levels of interferon-beta (IFN-β) but not MHC class I molecules following viral infection. The non-coordinate induction of these two genes is the result of differential binding of transcription factors at cis-regulatory sites (κB and ISRE sites) common to the promoter regions of these and other anti-viral genes. In addition, the IFN-β produced by neurons acts in an autocrine fashion to induce an anti-viral state but not MHC class I gene expression. As a result, it was of interest to determine: 1) what anti-viral state genes are induced in neural cells following viral infection or IFN treatment, and 2) how these genes are differentially regulated via κB and ISRE binding regions. Therefore, we have examined the expression and regulation of the 2'-5' oligoadenylate synthetase (OAS) and inducible nitric oxide synthase (iNOS) genes within neural cells. Northern blot analysis of RNA derived from primary cultures of neurons and astrocytes show that both cell types are induced to express the OAS gene following viral infection or treatment with IFN-β. In contrast, astrocytes but not neurons are induced to express the iNOS gene following treatment with virus but not IFN-β. This suggests that multiple anti-viral genes can be differentially regulated in neural cells. The cis-regulatory regions and transcription factors responsible for the non-coordinate induction of these genes are being investigated. (Supported by the National Multiple Sclerosis Society.)

451.14

CORRELATION OF HERPEVIRUS NEUROVIRULENCE WITH ALTERATION OF INTRACEREBRAL CYTOKINE PRODUCTION. G. Lewandowski* Dept. of Neuropharmacology, The Scripps Research. Institute, La Jolla, CA 92037.

In general, herpes simplex virus type 2 (HSV-2) is more neurovirulent than closely related strains of HSV-1. The basis for the increased neurovirulence of type 2 is unclear. However, our previous work comparing an avirulent strain of HSV-1 and a extremely neurovirulent strain of HSV-2 suggest that the neurovirulence of HSV-2 results from deficiencies and-or alterations in intracerebral cytokine production. Moreover, the alteration of intracerebral cytokine production results from HSV-2-mediated suppression of class II major histocompatibility complex (MHC)-restricted antigen presentation.

To test this hypothesis the following parameters were used to evaluate several strains of HSV-1 and HSV-2: (i) lethality, (ii) neuroinvasiveness and extent of viral replication in the PNS and CNS, (iii) cellular localization of MHC class II proteins, and (iv) intracerebral cytokine production. Following ocular inoculation of immunocompetent mice, all strains of HSV-1 and HSV-2 invaded the trigeminal ganglia and brain. All strains of HSV-2 were neurovirulent with LD50 values ranging from 3 plaque forming units (pfu) to 66 pfu. Four of 7 strains of HSV-1 were avirulent with LD50's ranging from 7000 pfu to »8 x 10^5 pfu. Two strains of HSV-1 had LD50's that were within the HSV-2 range (19 and 60 pfu.). Expression of MHC class II antigens at the cell membrane was inhibited in CNS and PNS tissues from all mice infected with strains of HSV-2, but not in mice infected with avirulent strains of HSV-1. Most importantly, we observed that the production of intracerebral IFN-γ was significantly inhibited in mice infected with all strains of HSV-2, and in those mice infected with strains of HSV-1 that had low LD50 values (neurovirulent strains). In summary, our data suggest that the neurovirulence of HSV-2 (and some strains of HSV-1) may result from significantly suppressed production of intracerebral IFN-γ. The inhibition of intracerebral IFN-γ is likely caused by HSV-2-mediated suppression of class II MHC-restricted antigen presentation in the PNS and CNS.

(Supported by NIH grant R29MH51926)

451.15

SIGNIFICANCE OF CERVICAL LYMPH NODES IN BRAIN TUMOR IMMUNITY IN THE RAT. Y. Okamoto*, S. Kida, J. Yamashita, Dept. of Neurosurgery, Kanazawa Univ., School of Med., Kanazawa, Japan, 920

Recent physiological and anatomical studies have demonstrated that a major fraction of brain interstitial and cerebrospinal fluid drains into cervical lymph nodes in a number of experimental animals. To investigate the role of cervical lymph nodes in brain tumor immunity, temporal profiles of T lymphocyte subsets in brain tumor, cervical lymph nodes and other lymphoid tissues were analyzed by immunohistochemistry and flow cytometry. **Methods** : A total of 37 male Wistar rats were used. 1) Intracranial transplantation models (n=16) 2) Extracranial transplantation models (n=12) 3) Intracranial transplantation with cervical lymphadenectomy models (n=9). 1, 2, 3 and 4 weeks after transplantation, brain, cervical lymph nodes, peritoneal lymph nodes and spleen were removed. Immunohistochemistry was performed by using following antibodies : OX6 (MHC class II), ED-1 (macrophage, microglia), W3/25 (CD4+ T cells) and OX8 (CD8+ T cells). Tumor infiltrating lymphocytes were isolated and were analyzed by flow cytometry. **Results** : Two weeks after the transplantation of C6 glioma cells into the rat brain, expression of MHC class II was induced in the brain and all lymphoid tissues examined. However, the following activation of CD4+ or CD8+ cells was firmly confined to cervical lymph nodes, which coincided with the infiltration of such cells in the brain tumor 2 weeks after the transplantation. Extracranial transplantation of C6 glioma cells showed less reaction of cervical lymph nodes, in which delayed increase of the number of CD4+ cells was observed 4 weeks after the transplantation. In the animal group of cervical lymphadenectomy followed by intracerebral transplantation of C6 glioma cells, there was a delayed infiltration of CD4+ cells in the brain tumor 3 weeks after the transplantation, and the production of such cells was substituted by the spleen. These results suggest that cervical lymph nodes act as draining lymph nodes of the brain and play a crucial role of regional lymph nodes in brain tumor immunity.

451.16

INDUCTION OF INTERLEUKIN-1 BIOACTIVITY AFTER INTRACEREBRAL INFUSION OF NATIVE GP120 AND GP160. J.M. Weiss*, Z. Zhang, M. Emery, E. Lai, R. Bonsall, V.S. Kalyanaraman‡, and N. Quan. Dept. of Psychiatry and Behavioral Sciences, Emory Univ. Sch. of Med., Georgia Mental Health Institute, 1256 Briarcliff Rd, Atlanta, GA 30306. ‡Advanced BioScience Laboratories, Inc., Kensington, MD.

We reported previously that intracerebral infusion of recombinant gp120 (r-gp120 Intracel; formally American Bio-Technologies, Inc.) induced IL-1 bioactivity in rat brain (Sundar et al., (1991) *PNAS, 88*, 11246-11250). To test whether IL-1 is also induced by native HIV envelope proteins, we used native gp120 (n-gp120) and gp160 (n-gp160), which were purified from the conditioned medium of HIV-1_{451}-infected HUT78 cells (Advanced BioScience Laboratories, Inc). Under halothane anesthesia, 10 µl of one of the following solutions was slowly infused (over 10 min.) into the hippocampus of pathogen-free Sprague-Dawley rats: pyrogen-free saline, 0.1 or 0.5 µg r-gp120, 0.5 µg n-gp120, 0.5 µg n-gp160, or 1 ng LPS. Three hours later, animals were sacrificed, brains removed, and 5 mm^3 tissue surrounding the infusion site dissected. Samples were processed by liquid chromatography and IL-1-containing fractions analyzed by D10 assay (see Quan, N. et al., this meeting). IL-1 bioactivity was found in the extracellular fluid (EC) of tissue infused with LPS and all gp120 and gp160 preparations. IL-1 bioactivity was not found in the saline-infused tissue; the small amount of tissue in the sample likely accounts for absence of IL-1 bioactivity in saline-infused tissue. IL-1 bioactivity was confirmed by the blockade of the stimulation of D10 cells after addition of antibody to IL-1 receptor. To assess whether IL-1 bioactivity might have been due to endotoxin contamination, LPS, r-gp120, n-gp120, and n-gp160 were also infused into the brain of LPS-resistant C3H/HeJ mice. In these animals, IL-1 bioactivity was detected in gp120- and gp160- but not in LPS-infused tissue. Furthermore, infusion of carboxy methylated r-gp120 and r-gp160 (CM-gp120 and CM-gp160; Advanced BioScience Laboratories, Inc), which have original amino acid sequence but altered structure, produced no IL-1 bioactivity. Thus, native HIV envelope protein gp120 and gp160 induced brain IL-1 activity; this effect depends on the intact structure of these proteins. (Supported by NIH grant 50420)

451.17

DETECTION OF IL-1 BIOACTIVITY IN VARIOUS BRAIN REGIONS OF NORMAL HEALTHY RATS. N. Quan*, Z. Zhang, M. Emery, R. Bonsall, and J.M. Weiss. Dept. of Psychiatry and Behavioral Sciences, Emory Univ. Sch. of Med., Georgia Mental Health Institute, 1256 Briarcliff Rd., Atlanta, GA 30306.

Although interleukin-1(IL-1) has been implicated in an array of brain functions, it is generally not detected in brain unless viral or bacterial stimulation is present (e.g., by lipopolysaccharide). But in view of the potency of IL-1 in brain, small amounts of this cytokine may normally act in brain and such quantities can escape detection by assay methods usually employed. Bioassays that are currently most sensitive to IL-1 can be compromised by molecules in brain tissue other than IL-1 and attempts to purify IL-1 from brain tissue can result in significant loss of IL-1 from samples. In studies reported here, we have refined techniques sufficiently to reliably detect IL-1 bioactivity in both extracellular fluid (EC) and tissue lysate (LY) of normal, healthy rat brain. After pathogen-free Sprague-Dawley rats were perfused by cardiac puncture to eliminate blood from brain tissue, brainstem, cortex, diencephalon, and hippocampus were dissected immediately on ice [for dissection, see Quan et al. (1994) *J. Neuroimmunol., 49*, 125-134]. EC from brain regions was extracted by segregating brain cells with a nylon mesh in culture medium and collecting supernatant after centrifugation. Additional samples of LY were produced by ultrasonic sonification of tissue residue. IL-1 in EC and LY was isolated by running samples through a 9x95 mm G-50 sephadex mini-column equilibrated with culture medium after which fractions containing IL-1 were subjected to assay using D10 cells, which detects rhIL-1 at the level of 1 pg/ml in our hands. The short G-50 columns minimized loss of IL-1 (more than 40% IL-1 can be recovered from a starting sample load of 50 pg of IL-1). IL-1 bioactivity was reliably detectable, i.e., D10 growth was greater in IL-1 containing fractions relative to baseline (incubation with medium alone), in EC of all brain regions examined. Furthermore, IL-1 bioactivity was blockable in all brain regions by addition to the D10 cell bioassay of monoclonal antibody against type I IL-1 receptor. Hence, low levels of IL-1 appear to be present in most brain regions of normal healthy rats. (Supported by NIH grant 50420)

451.18

CHARACTERIZATION OF THE PERIPHERAL IMMUNE RESPONSE TO OVALBUMIN MICROINFUSED INTO THE NORMAL MOUSE BRAIN. J.T. Park, C.J. Harling-Berg, P.M. Knopf*. Physiology and Molecular Microbiology and Immunology, Brown University, Providence, RI 02912.

We have demonstrated in a normal rat model, that CNS immunity involves a significant, persistent and enhanced specific antibody response elicited in the periphery of rats receiving a microinfusion of antigen into brain (Cserr and Knopf, Imm. Today, 13(12): 507-512, 1992). Antigen was administered through an indwelling cannula implanted 7 days earlier to allow for the recovery of the blood-brain barrier. Our current studies extend findings to an outbred (CD1) mouse strain. The peripheral humoral and delayed-type hypersensitivity (DTH) responses to OVA infused into ventricular CSF were characterized and compared to subcutaneous (SC) injections of OVA in the presence and absence of complete adjuvant. Antibody and DTH responses were measured using an ELISA and standard ear-swelling assay, respectively. Following a CSF-infusion of OVA, serum anti-OVA antibody titers were elevated and sustained for at least 10 weeks post-immunization. A response was measured for all OVA doses: 10µg (n=12), 20µg (n=4), and 50µg (n=4). CSF immunizations yielded significantly ($P<0.01$) greater serum titers (mean log titer = 3.655) than comparable (50µg) SC-administered doses (n=6, mean log titer = 2.143). CSF and SC immunization protocols elicit predominantly IgG1 and IgG2b isotypes with relatively little IgM, IgG2a, IgG3 and IgA. However, immunization via CSF elicited a greater percentage of IgG2b than SC immunization. Both CSF and SC immunizations with OVA alone did not generate a DTH response as compared to negative controls at 21 days. Data indicates that the afferent limb of the immune response to brain-administered antigen is intact in the mouse. Results, consistent with our rat model, support the concept that CNS immunity favors a strong humoral response and down-regulates potentially damaging cell-mediated inflammatory responses.

451.19

EXPRESSION OF NITRIC OXIDE SYNTHASE TYPE II IN THE CNS DURING ACUTE AND PERSISTENT HEPATITIS VIRUS INFECTION. A. Loihl*, D. Grzybicki+, S. Kardos, S. Perlman# and S. Murphy. Depts. of Pharmacology, Pathology+ and Pediatrics#, Univ. of Iowa, Iowa City, IA 52242.

Transcriptional induction of nitric oxide synthase type II (NOS II) requires cellular exposure to proinflammatory cytokines, suggesting that NOS II is active under pathophysiologic conditions. In this study we determined whether, and in which cell types, NOS II was induced during acute and/or persistent mouse hepatitis virus (MHV-JHM) infection in the CNS.

Intranasal inoculation with MHV-JHM in C57BL/6 mice results in an acute, rapidly fatal encephalitis. We find expression of NOS II mRNA in brains of infected (but not uninfected) animals by RT-PCR and ribonuclease protection assay on day 6 after infection when all mice become clinically ill, and this continues through day 7, when all mice expire. Western blotting reveals NOS II protein in infected brain. Expression is in macrophages diffusely infiltrating in the late stages of infection. In persistent MHV-JHM infection NOS II mRNA and protein were expressed in brain and spinal cord of mice exhibiting hindlimb paralysis. Immunohistochemical studies are in progress to localize the cell type(s) expressing NOS II.

The results suggest that NO plays a role in the pathologies occuring with acute and persistent MHV infection. In acute infection, NO production does not appear to be a first-line defense mechanism.
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451.20

INDUCTION OF ICE mRNA AND ENZYME ACTIVITY IN THE RAT BRAIN AND ADRENAL GLAND. M. Schultzberg*, S. Tingsborg, C. Eriksson, M. Zetterström, K. Alheim and T. Bartfai, Dept. of Clin. Neurosci., Geriatric Med., Karolinska Institute, Novum & Dept. of Neuroschem. and Neurotoxicol., Stockholm Univ., Stockholm, Sweden.

The overexpression of interleukin-1ß converting enzyme (ICE) has been shown to cause apoptotic cell death in transfected fibroblasts and dorsal root ganglion neurons. The present study analyzes the distribution and inducibility of ICE enzyme activity and mRNA in the adult rat brain, the pituitary and the adrenal gland. Using an artificial fluorescence substrate, DABCYL-Tyr-Val-Ala-Asp-Ala-Pro-Val-EDANS, the specific activity of ICE, under non stimulated conditions, was highest in the hypothalamus, followed by the pituitary and hippocampus. The K_m values for ICE: 25±15 µM using the fluorescence substrate, were similar in all tissues. ICE enzyme activity was significantly induced in the pituitary 4 hours after intraperitoneal (i.p.) injection of *E. coli* lipopolysaccharides (LPS; 2 mg/kg), while the same treatment did not affect hypothalamic, and slightly suppressed hippocampal and adrenal gland enzyme activity. Constitutive expression of ICE mRNA was found in all brain regions, the pituitary and the adrenal gland, and an increase was found in the pituitary and adrenal gland after the LPS treatment, as was pro-interleukin-1ß (pro-IL-1ß) mRNA expression. ICE mRNA levels in the hypothalamus and hippocampus were not affected at the 4 hour time point after LPS injection. The results are discussed in relation to LPS-stimulated production of mature IL-1ß by ICE. The constitutive and regionally specific distribution of ICE in the rat brain suggests that ICE at least partly is neuronally expressed. This work was supported by grants from the Swedish Medical Research Council and Trion FOUAB.

NEURAL-IMMUNE INTERACTIONS: INFLAMMATION

452.1

DIFFERENTIAL RESPONSES TO BACTERIAL ENDOTOXIN OF SPLENIC AND RENAL SYMPATHETIC NERVES. B.J. MacNeil*, A.H. Jansen, A.H. Greenberg, and D.M. Nance. Dept. of Pathology and Manitoba Institute of Cell Biology, Univ. of Manitoba, Winnipeg, Manitoba, Canada R3E 0W3.

Regulatory interactions and neuroanatomical pathways exist between the sympathetic nervous system (SNS) and the immune system. It is not clear whether these pathways are selectively active in immune organs during immune responses. We tested whether systemic injection of endotoxin (LPS) induces sympathetic outflow to an immune organ (spleen). Sympathetic nerve activity was recorded in adult male rats from either the splenic or renal nerve. Splenic nerve activity increased in a dose dependent manner up to 175% of control 17.1 to 23.5 minutes following i.v. injections of LPS. In contrast, renal nerve recordings showed a significantly slower onset of 37.1 to 52.6 minutes at the same doses, despite a similar magnitude of increase. In addition, splenic nerve recordings of 8/8 rats responded to 10 µg of LPS, whereas only 4/11 positive renal nerve responses were observed at this dose. Spectral analysis of sympathetic nerve activity (35 contiguous 5 s windows, percent area under the average FFT curve calculated) revealed baseline splenic nerve activity had greater power in the 0-2 Hz bandwidth compared to renal nerve activity (0.333±0.013 vs 0.271±0.007, p=0.015). The renal nerve had a greater proportion of power in the 6-8 Hz range relative to the splenic nerve (0.135±0.003 vs 0.101±0.009, p=0.028). LPS enhanced these differences, albeit, nonsignificantly. In summary, splenic nerve activity, which differs in discharge pattern from the renal nerve, is more sensitive to LPS-induced sympathetic activation regarding the latency and rate of responses. Thus, differential sympathetic outflow can be directed to an immune organ in response to a stimulus known to activate the immune system. Supported by MRC (Canada), MHRC (Manitoba) and NIMH.

452.2

CENTRAL NITRIC OXIDE SYNTHASE INHIBITION BLOCKS ENDOTOXIN-INDUCED C-FOS PROTEIN IN THE HYPOTHALAMUS.

A.T.K. Jackson*, L. Janz, A.H. Greenberg and D.M. Nance, Depts. of Pathology and Physiology and Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, MB, Canada R3E 0W3

Endotoxin is a potent stimulator of numerous immunological and physiological systems. It has been shown that injection of lipopolysaccharide (LPS), a powerful bacterial endotoxin, results in the expression of the proto-oncogene c-fos in the brain (Wan et al, B.R.B. 32:581,1993). We report here that central injections of L-NAME, a nitric oxide synthase inhibitor, inhibited LPS-induced c-fos in the paraventricular nucleus and this inhibition was LPS dose-dependent. Male S-D rats were pretreated by i.c.v. infusion of L-NAME (50µg) and then treated with an i.v. LPS dose of either 10, 20, 40, 50, or 100µg. All doses of LPS induced c-fos in the brain, but only the 10 and 20µg doses were significantly inhibited by L-NAME. This suggests a functional role for central nitric oxide in low dose LPS-induced neuronal changes. The lack of inhibition at higher doses may be due to extra-immunological effects induced by high doses of LPS. In preliminary work, centrally administered PGE_2 induced c-fos in a pattern similar to that produced by i.v. LPS and this PGE_2-induced c-fos also was inhibited by i.c.v. L-NAME. This suggest a role for nitric oxide in the neuronal signalling produced by LPS, and that PGE_2 is a likely mediator in this pathway. Supported by MRC and NIMH.

452.3

CENTRAL CATECHOLAMINE INVOLVEMENT IN THE HYPOTHALAMIC INDUCTION OF C-FOS AFTER ENDOTOXIN TREATMENT.

D.M. Nance*, A.H. Greenberg and A.T.K. Jackson, Depts. of Pathology and Physiology and Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, MB, Canada R3E 0W3

Peripheral injection of lipopolysaccharide (LPS), a potent bacterial endotoxin, results in the central expression of the proto-oncogene c-fos. Areas of the brain that have increased c-fos expression include the paraventricular nucleus of the hypothalamus (PVN) and the catecholamine cell groups located in the dorsal and ventrolateral regions of the medulla (Wan et al, B.R.B. 32:581,1993). To investigate further the potential role of catecholamines in the the central expression of c-fos, unilateral posteriorlateral hypothalamic knife cuts were used to eliminate ascending catecholamine input to the hypothalamus. After i.v. LPS treatment (50µg), c-fos induction in the PVN was inhibited on the cut side of the brain and the knife-cuts produced an ipsilateral reduction in dopamine-ß-hydroxylase staining in the hypothalamus. Preliminary work with unilateral knife cuts of catecholamine tracts in the mesencephalic pontine region produce a similar ipsilateral reduction of endotoxin-induced c-fos staining in the PVN. These results suggest a functional neural role for catecholamines in the central processing of the LPS immune stimulus. Supported by MRC and NIMH.

452.4

ENDOTOXIN INCREASES NITRIC OXIDE SYNTHASE mRNA LEVELS IN THE PARAVENTRICULAR NUCLEUS OF THE RAT HYPOTHALAMUS.

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Nitric oxide synthase (NOS), the enzyme responsible for nitric oxide (NO) formation, is found in hypothalamic neurons that control ACTH secretion. This led to the hypothesis that brain NO may modulate the response of the hypothalamic-pituitary axis (H-P) to various stimuli. We tested this hypothesis by measuring changes in constitutive (c) NOS mRNA levels in the hypothalamus of rats systemically injected with endotoxin, a lipopolysaccharide (LPS) that releases endogenous cytokines, and analyzed these results in the context of the appearance of corticotropin-releasing factor (CRF) and vasopressin (VP).

Following LPS injection (100 µg/kg, iv), cNOS mRNA levels increased dramatically 3-4 h in the paraventricular nucleus. LPS treatment also rapidly upregulated CRF heteronuclear RNA, and increased mRNA levels of the immediate early genes, *c-fos* and NGFI-B, as well as CRF and its receptors. On the other hand, no changes in cytoplasmic VP mRNA levels were noted following LPS injection. At present, the prevailing concept is that iv administered LPS influences both the activity of nerve terminals and that of hypothalamic cell bodies, a phenomenon believed to reflect the concerted action of the various neurotransmitters and cytokines released by endotoxin. Our results indicate the involvement of several circuitries of potential importance for ACTH secretion in rats injected with endotoxin, though it is premature to invoke a causal relationship between the increased gene expression of cNOS and CRF.

These studies indicate that systemic LPS produces rapid increases in neuronal activity of hypothalamic regions known for their involvement in the responses of the H-P axis. While the role of CRF and its receptors is fairly well understood, the observation of increased cNOS gene expression following LPS treatment, and the presence of this enzyme in neurons that regulate ACTH secretion, bring support to the hypothesis that this gas also plays an important function in mediating the H-P axis response to an immune challenge.

452.5

THE ROLE OF INTERLEUKIN-6 IN THE NEUROCHEMICAL AND HPA CHANGES INDUCED BY ENDOTOXIN.

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Interleukin-6 (IL-6) is produced by immune cells early in the response to antigenic stimulation. IL-6 has been reported to activate the hypothalamo-pituitary-adrenocortical (HPA) axis. Our data show that IL-6 concentrations in the plasma rise after endotoxin (LPS) administration, with a peak at 2-3 hours. Administration of purified recombinant mouse IL-6 intraperitoneally activated the HPA axis as indicated by increases in plasma ACTH and corticosterone. IL-6 was significantly less potent than IL-1. The peak effect occurred at around one hour. We observed no effects on catecholamines or metabolites in the brain. However, tryptophan and 5-hydroxyindoleacetic acid (5-HIAA) were elevated at several time points after mIL-6 injection. Experiments with monoclonal antibodies to mouse IL-6 have indicated attenuation of the HPA response to LPS. These results suggest that IL-6 may play a role in the responses to LPS, but the lack of effect of IL-6 on cerebral norepinephrine, suggests that it is not the only factor.

Supported by a grant from NIMH (MH46261)

452.6

THE EFFECTS OF HIGH FAT DIET AND EXERCISE ON THE RESPONSE TO BACTERIAL ENDOTOXIN IN FEMALE SPRAGUE DAWLEY RATS.

P.J. Rowsey*, T.A. Lennie and B.L. Metzger. The University of Michigan, Ann Arbor, MI 48109-0482

The hypothesis that exercise induces clinically significant functional changes in the immune system is supported by data from several studies showing that moderate to intense exercise is associated with an increased number of lymphocytes, especially natural killer cells (NK cells). We studied the relationship between diet and exercise-induced increase in natural immune function. Female Sprague Dawley rats (12:12 reverse photoperiod) with access to running wheels were fed a high fat diet (HF, 62% Kcal from fat). Ten rats had access to running wheels for 6 weeks and 10 were sedentary controls. At the end of a six week period, the response to a challenge of bacterial endotoxin (lipopolysaccharide, LPS 50 ug/kg) was examined. Food intake (FI), body temperature (BT), and activity were recorded for four hours. At the end of 4 hour period, animals were sacrificed and spleens were taken for immune profile studies. During the 4 hours following LPS injection, there were no differences in FI, BT, or activity between the two groups. In the presence of a HF diet, total spleen lymphocytes were higher in sedentary animals (35.7% positive cells) as measured by flow cytometry when compared to the sedentary animals fed a normal diet (27.8% positive cells). There were no differences in the number of T helper (CD4) and T suppressor (CD8b) cells in the spleens of exercise and sedentary animals. However, in the presence of a high fat diet, exercise animals showed lower number of total lymphocytes as well as lymphocytes with cytotoxic function (CD8a). This decreased expression of cytotoxic lymphocytes in the exercised group differs from that found in previous studies. These results indicate that animals given a high fat diet, regardless of activity level, do not express the usual immune response to LPS, either behaviorally or via their immune profile.

452.7

LONG-TERM I.C.V. CORTICOTROPIN-RELEASING HORMONE INFUSION OF RATS ALTERS ENDOTOXIN-INDUCED AUTONOMIC AND NEURAL RESPONSES

A.C.E. Linthorst*, M.S. Labeur and J.M.H.M. Reul, Max Planck Institute of Psychiatry, Clinical Institute, Dept. of Neuroendocrinology, Munich, Germany.

Recently, we reported on the effects of long-term i.c.v. corticotropin-releasing hormone (CRH; 1 µg/µl/h via an Alzet minipump) infusion of male rats on the hypothalamic-pituitary-adrenocortical (HPA) axis and the immune system (Labeur et al., *Endocrinology* May 1995). A sustained HPA axis hyperactivity was observed, as indicated by increased plasma ACTH and corticosterone (CORT) levels, increased anterior pituitary POMC mRNA expression and adrenal enlargement. Moreover, the i.c.v. CRH treatment suppressed *in vitro* splenocyte activation and enhanced IL-1ß and IL-2 expression. In this study, experiments were performed to investigate whether the increased HPA axis activity and the altered cytokine expression in the CRH-infused rats has *in vivo* physiological consequences. In one set of experiments, biotelemetric transmitters were i.p. implanted to monitor body temperature and locomotion, and in another set a microdialysis probe was implanted in the hippocampus to measure serotonergic neurotransmission and free CORT levels. Compared to control animals, CRH-treated rats showed an overall increase in body temperature with a flattening of the circadian rhythm, a marked increase in locomotion and elevated free CORT levels. I.p. administration of endotoxin (lipopolysaccharide) is known to induce fever, and to increase HPA axis activity and hippocampal serotonergic neurotransmission. However, after an i.p. endotoxin injection the CRH-treated rats produced markedly reduced elevations in both body temperature and hippocampal serotonergic neurotransmission whereas the attained free CORT levels were similar. Summarizing, our results demonstrate that chronic activation of the HPA axis has profound consequences for the autonomic and neural responses to an endotoxic challenge.

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452.8

NONHYPOTENSIVE ENDOTOXEMIA INCREASES PLASMA NOREPINEPHRINE AND INDUCES FOS IN THE CENTRAL NUCLEUS OF THE AMYGDALA.

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Conscious, chronically instrumented male Sprague-Dawley rats were studied for blood pressure, heart rate and plasma norepinephrine responses to E. coli endotoxin (ETX). For 6 hours following bolus injection of ETX 10 µg/kg or 50 µg/kg intravenously, no hypotension was observed, however, there was significant tachycardia from 2 hours to 6 hours post-ETX. Plasma norepinephrine was significantly elevated after both doses of ETX, indicating prolonged sympathetic activation.

We chose 25 µg/kg, a dose within this nonhypotensive, sympathoexcitatory range to study Fos expression in the central nucleus of the amygdala (CeA). At time points 1 hour to 4 hours after ETX, rats were deeply anesthetized and perfused, with removal of the brains for Fos-ICC using standard techniques. Counts of Fos-stained nuclei were increased at 4 rostrocaudal levels of the CeA beginning at one hour and persisting for 4 hours after ETX. Fos expression in the medial amygdala was minimal after this stressor. This raises the possibility that the CeA, a limbic and autonomic related nucleus, may be involved in the behavioral and autonomic responses to mild acute endotoxemia. Supported by NR03801.

452.9

INDUCTION OF THE INFLAMMATORY CYTOKINES IL-1, IL-6 AND TNFα IN MICE FOLLOWING EXOGENOUS ADMINISTRATION OF INFLAMMATORY AGENTS. L.K. Jackson[1], R. Kulmer[1], S.W. Rogers[1,2,3], and L.C. Gabring*[1,3,4] S.L.C. VA GRECC, [1] Depts of Neurobiology and Anatomy[2], Human Molec. Biol. and Genetics[3] and Medicine[4], Univ. of Utah, S.L.C., UT.

The inflammatory cytokines IL-1, IL-6, and TNFα are expressed in the CNS and there is increasing interest in the role of these mediators in neuronal function during health and disease. We have examined the expression of these cytokines in the peripheral immune system and the CNS following intravenous administration of lipopolysaccharide (LPS). At specific times after LPS administration, mice were perfused with saline to remove contaminating blood cells and the brains were dissected into cortex, hippocampus, cerebellum, midbrain and hypothalamus. Spleens from these animals were also isolated. RNA was extracted and RT-PCR performed using cytokine specific primers. All results were quantified by measuring EtBr incorporation into PCR products as determined by fluorimagery, and normalized to β-actin expression. Injection of LPS induces elevations in the expression of cytokine mRNA which peaks at 2 hrs post LPS administration and decreases to normal levels by 6 hrs. All regions of the brain examined by RT-PCR demonstrated a similar pattern of cytokine mRNA induction. We investigated the expression of cytokine protein by immunohistochemistry. Results demonstrate that protein expression is more limited in distribution and associated with specific neuronal neurotransmitter receptors systems. Regions of the CNS that contain cytokine expressing cells include the globus pallidus, hippocampus and thalamus. Cytokine expressing cells are also present in some white matter tracts. Therefore, both glial and neuronal-like cells produce these inflammatory cytokines, and levels are sensitive to peripheral insult. *Supported by NIH grants AG04418, NS0990, and the American Federation for Aging Research.*

452.10

CYTOKINE REGULATION OF PROENKEPHALIN GENE EXPRESSION IN THE PARAVENTRICULAR NUCLEUS (PVN) S. E. Hyman, S.E. Lewis*O. Falkowski, K. Van Kuoghnet, D. Borsook.
Molecular and Developmental Neurobiology Laboratory, Mass. Gen. Hospital, Boston MA 02114

Lipopolysaccharide (LPS), the principal component of endotoxin, has profound physiological effects on the central nervous system and on neuroendocrine systems. These effects are thought to be mediated by various cytokines including interleukin-1ß (IL-1ß). We have produced transgenic mice expressing a human proenkephalin-ß-Galactosidase fusion gene which is correctly expressed and regulated by stress in the hypothalamus. Intraperitoneal (i.p.) injections of LPS (4mg/kg) produced an increase in transgene expression in the PVN but not in the supraoptic nucleus (SON). Following a single i.p. injection of LPS transgene induction is seen within 2 hrs, is maximal at 12 hrs, and decreases at 24 and 48 hrs to below basal levels. Surprisingly, transgene induction by LPS is inhibited by pretreatment with naloxone. Induction of transgene expression in the PVN also correlates with c-Fos induction following LPS. In preliminary experiments, i.p. (0.1µg in 0.2ml NS) or i.c.v. (2ng) injections of interleukin-1ß also produced a significant increase in the transgene expression. Gel-shift analysis with nuclear extracts from the hypothalamus containing the PVN shows induction of binding to putative cytokine response elements in the proenkephalin gene. Supershift analysis showed that STAT 3 binding is induced. Binding of other cytokine regulated proteins within the proenkephalin gene is being analyzed. These results suggest a model in which proenkephalin gene expression in the PVN may be regulated in part by specific cytokine response elements.

452.11

EFFECT OF β-ENDORPHIN ON ENDOTOXIN-INDUCED FORMATION OF IL-1α, IL-1β AND IL-6 IN FETAL MIXED BRAIN CELLS *in vitro* K. P. Das*, M. K. McMillian, B. C. Wilson, P. M. Hudson and J.-S. Hong, Laboratory of Environmental Neuroscience, NIEHS-NIH, Research Triangle Park, NC 27709.

Opioids have been shown to have diverse effects on the peripheral immune system, both *in vivo* and *in vitro*, but their interactions with the glial cells of the central nervous system (CNS) have not been well studied. In the present study, we have examined the effect of opioid peptide β-endorphin (61-91) (β-end) on lipopolysaccharide (LPS)-induced production and release of IL-1α, IL-1β and IL-6 from mouse embryonic mixed brain cell cultures. Two-week-old mixed embryonic brain cell cultures were treated with LPS (1 µg/ml) and β-end (10^{-14} M to 10^{-6} M) alone and/or together for 3 h, 6 h, 12 h, 24 h, and 48 h, and cytokines were measured by ELISA. None of the β-end concentrations examined significantly altered the production or release of IL-6, and the release of IL-1α and IL-1β were not detectable; LPS significantly induced both the formation and release of all three cytokines. β-endorphin (10^{-14} M to 10^{-6} M), however, suppressed the LPS-induced increases of all three cytokines in the media after 48 h of treatment. These data indicate that the opioid peptide β-end has an inhibitory role in regulating the endotoxin-induced release of inflammatory cytokines, and further suggests possible roles for opioid peptides in the regulation of neuroimmune function in the CNS.

452.12

TUMOR NECROSIS FACTOR α (TNFα) MEDIATES THE RAT HYPOTHALAMO-PITUITARY-ADRENAL (HPA) RESPONSE TO ACUTE LOCAL INFLAMMATION BY AN ACTION IN BRAIN. A.V. Turnbull* and Catherine Rivier. Clayton Fndn. Labs. for Peptide Biol., The Salk Institute, La Jolla CA 92037, USA.

Inflammatory processes have a profound impact on neuroendocrine function, an effect which is thought to be mediated by cytokines. However, the precise role of individual cytokines in different inflammatory conditions (systemic versus local and acute versus chronic), and their site of action (within the brain or periphery) remains to be established. The present study investigated the role of TNFα within the brain in activation of the HPA axis by acute local inflammation in the rat.

Injection of a small volume (50 µl/100 g body weight) of the mineral oil turpentine into the hindlimb produced an initial and brief (within the first hour) stress-induced increase in plasma adrenocorticotropin (ACTH). This was followed by a swelling of the limb, a rise in plasma interleukin-6 concentration and a secondary rise in plasma ACTH, which peaked at around 8h. Immunoneutralization of corticotropin-releasing factor or arginine vasopressin indicated that this secondary, inflammation-induced, rise in ACTH was mediated by a combination of both these hypothalamic neuropeptides.

Prior central (intracerebroventricular) administration of either a soluble TNF receptor construct (rhTNFR(p80):Fc, 1-50 µg, Immunex, Seattle) or a neutralizing rabbit anti-mouse TNFα (5 µl) antiserum, inhibited the secondary peak rise in plasma ACTH induced by local inflammation by 62-72%. In contrast, these treatments administered intravenously, at the same doses, had no significant effect on the ACTH response.

These data indicate that acute local inflammation in the rat induces activation of the HPA axis which is dependent on the action of TNFα within the brain. Whether TNFα is synthesized within the brain, or is derived from peripheral sources, remains to be determined.

452.13

INFLAMMATORY STRESS MODULATES CORTICOTROPIN-RELEASING FACTOR RECEPTOR A (CRF-RA) mRNA LEVELS IN RAT PITUITARY. J-M. Aubry *, G. Pozzoli, A.V. Turnbull, Catherine Rivier and W.W. Vale, Clayton Foundation Labs. for Peptide Biol., The Salk Institute, La Jolla CA 92037, USA.

Inflammatory stress produces profound activation of the hypothalamo-pituitary-adrenal (HPA) axis. The majority of evidence indicates that inflammatory mediators (i.e. cytokines) act at the level of the hypothalamus to stimulate the secretion of CRF, the major adrenocorticotropin (ACTH) secretagogue. However, while a number of studies have described direct pituitary actions, the effects of inflammatory stimuli on corticotrope sensitivity to CRF remain to be elucidated. The aim of this study was to determine the effects of systemic or local inflammation on the levels of mRNA for the rat pituitary CRF receptor (CRF-RA).

Both systemic (intravenous lipopolysaccharide, LPS, 5µg/kg) and local inflammation (intramuscular turpentine, 50 µl/100 g BW) increased plasma concentrations of ACTH and the cytokine, interleukin-6 (IL-6). Although systemic LPS induced much higher levels of ACTH (4 fold) and IL-6 (50 fold) than local inflammation, these responses were of shorter duration (LPS approximately 6h, turpentine>12h). RNAse protection assay analysis of total RNA isolated from whole pituitary, indicated that systemic LPS produced a decrease in CRF-RA mRNA, that was evident by 2h after injection (to 65% of controls) and more marked by 6h (to 40% of controls). Similarly, local inflammation produced a reduction in pituitary CRF-RA mRNA levels (to 45% of controls at 8h).

These data indicate that CRF receptor mRNA levels in the pituitary of the rat are markedly reduced during both systemic and local inflammation, suggesting that pituitary sensitivity to CRF may be altered by these stimuli. Whether the modulation of pituitary CRF-RA is due to direct effects of inflammatory mediators or results from changes in HPA activity (eg. increased exposure to CRF), remains to be determined.

452.14

INFLAMMATORY RESPONSES TO ADENOVIRUS VECTOR GENE THERAPY IN THE BRAIN. A.P. Byrnes*, M.J.A. Wood and H.M. Charlton. Dept. of Human Anatomy, Univ. Oxford, Oxford OX1 3QX, UK

Non-replicating viral vectors are being widely considered for gene therapy in the brain, but few studies have examined the immune response to these vectors and whether it results in any pathology. The lengthy expression that is possible from E1-deleted adenovirus in the adult brain deserves special attention, as in most other organs a T cell immune response eliminates the virus within weeks. We have used a variety of approaches to characterize the inflammation which follows an injection of adenovirus vector.

- By depleting $CD4^+$ or $CD8^+$ T cells, we have determined that adenovirus vectors in the brain induce both an early phase of inflammation that is independent of T cells, and a later phase that depends on T cells, particularly of the $CD4^+$ subset. This second phase includes extensive recruitment of leukocytes into the brain and perivascular cuffing.
- By two months after an injection of vector into the brain, inflammation has essentially ceased. If a peripheral injection of adenovirus vector is then given, a much more severe immune response resumes in the brain around the original injection site, leading to demyelination.

We conclude that the inflammatory response to adenovirus vectors, although severe, probably does not result in damage to the brain and is not sufficiently strong to eliminate the virus. However, the virus which remains in the brain is still potentially a target of the immune system, as after a second injection of virus in the periphery a strong T cell response develops which causes damage in the brain. In addition to their implications for gene therapy, these studies may form a basis for the better understanding of immune responses to persistent viruses in the brain, as well as the role of T cells in demyelinating pathology.

452.15

CRF AND IL-1 RECEPTOR UPREGULATION IN LOCALIZED INFLAMMATION. S. Mousa[1,2], M. Schäfer[1,2], W. Mitchell[2]*, C. Stein[1], [1]Dep.Anesthesiology, Johns Hopkins University, [2]NIDA/Addiction Research Center, Baltimore, MD21224.

Previously, we showed that injecting CRF or IL-1β into inflamed tissue caused local release of opioid peptides by a receptor specific mechanism, and opioids in turn produce antinociceptive effects. In the present study we examined CRF and IL-1β binding with regard to specificity, anatomical localization and quantitative differences in inflamed and non-inflamed tissue by means of in vitro receptor autoradiography. Four days after intraplantar injection of Freund's complete adjuvant, sections from subcutaneous paw tissue, popliteal lymph nodes and spleen were examined autoradiographically using ^{125}I-oCRF and ^{125}I-IL-1β as ligands. CRF and IL-1β binding sites were present on immune cells in the inflamed subcutaneous paw tissue and lymph node but were practically absent on nerves and in non-inflamed subcutaneous paw tissue. The number of CRF and IL-1β receptors, as detected by densitometry and counting of autoradiographic grains is greatly enhanced in inflamed paws and lymph node but it does change in the contralateral paw, lymph node or spleen. These findings are consistent with our in vivo studies that have shown potent antinociceptive effects of locally applied CRF or IL-1β in inflamed but not in non-inflamed tissue.

452.16

A NEW MODEL OF NEUROGENIC INFLAMMATION USING A NEUROTROPIC VIRUS. H. Guo[1], D. Goff[1], R. Schmidt[2], L. Jasmin[1]* [1]Neurosurgery & Cell Biology Georgetown Univ., Washington DC, [2]Urology, Univ. of Colorado, Denver CO.

Interstitial cystitis is a chronic painful disease of unknown etiology in which there is evidence for a neurogenic component to the inflammation. To explore this possibility we have devised a model in which infection of the spinal cord with a herpes virus, pseudorabies virus (PRV), induces an inflammatory cystitis. Injection of PRV ($2x10^5$ pfu) in a tail muscle led to retrograde infection of the spinal cord segments innervating the bladder. On day 3 post-inoculation clear signs of sacral myelitis were found without any bladder inflammation (n=12). On day 4, the animals exhibited signs of urinary retention. On day 5, they presented hematuria and a hemorrhagic, inflamed, and distended bladder. The surrounding viscera appeared normal. Animals in which PRV was injected at a site other than the tail, such as a cervical myotome (n=3) or in the bladder wall (n=3), manifested systemic viral disease after 5 days, yet their bladder remained normal. After Evans Blue injection (iv.), animals with an inflamed bladder exhibited significant ($p<0.01$) retention of the dye in the bladder wall compared to controls and short survivals. In addition to establishing this model of cystitis we studied the neural pathways involved in its induction. Intravesical application of resiniferatoxin, or lumbosacral sympathectomies prevented the appearance of the cystitis but not of the myelitis as confirmed with immunocytochemistry for PRV. Sensory denervation of the bladder by dorsal rhizotomies did not prevent the appearance of the cystitis. From these results we conclude that PRV can induce visceral inflammation after inoculation at a somatic site and that this inflammation is dependent on the sympathetic efferent pathway.

452.17

CENTRAL α-MSH EFFECTS ON TWO MODELS OF PERIPHERAL INFLAMMATION IN THE RAT. E. D. Milligan, P. G. Green, J. D. Levine and M. F. Dallman*. Dept. of Physio UCSF, San Francisco, CA., 94143.

When injected i.v.t., α-melanocyte-stimulating hormone (α-MSH), a pro-opiomelanocortin derivative, reduces both picryl-chloride-induced and intradermal cytokine-induced inflammation of mouse ears. (Lipton et al, 1991, Peptides,12:795-798) To test the effects of central α-MSH on inflammation in rats, we administered 3rd ventricular injections of α-MSH$_{(1-13)}$ at 0.1, 0.25, 0.5, 1.0, or 2.5, in 2.5μl, or 5.0μg in 5.0μl to alter xylene-induced inflammation of an ear. Anti-inflammatory effects of 3rd ventricular injections of a-MSH were measured by changes in ear thickness (with a spring-loaded micrometer) prior to and 2, 4, and 6 hours after xylene application. Results revealed a U-shaped, dose effect of α-MSH$_{(1-13)}$ on inflammation. Maximal anti-inflammatory effects were obsrved at 0.5 and 1.0μg of α-MSH. Additionally, we tested the effects of a 3rd ventricular injection of α-MSH at 0.1, 0.25, 0.5, or 1.0μg on bradykinin-induced plasma extravasation in the knee joint assessed by measurement of Evans blue dye in the fluid perfusing the rat knee joint. Central α-MSH dose-dependently inhibited bradykinin-induced plasma extravasation within 10 minutes of 3rd ventricular administration. We conclude that central α-MSH$_{(1-13)}$ has anti-inflammatory effects on two rat models of inflammation: xylene-induced inflammation of the ear and bradykinin infused into the knee joint.

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SOMATIC AND VISCERAL AFFERENTS: VISCERAL AFFERENTS

453.1

NITRIC OXIDE OF PRIMARY SENSORY ORIGIN IS A NEUROMODULATOR IN SYMPATHETIC GANGLIA AND BLOOD VESSELS. Z.L. Zheng, B. Satterfield, R.D. Dey, T.L. Anthony, R. Hendriks, D.L. Kreulen*. Depts of Physiology and Anatomy, West Virginia Univ., Morgantown, WV, 26506.

A sensory nerve-mediated vasodilatation in guinea pig inferior mesenteric artery can be attenuated by treatment of *in vitro* preparations with nitric oxide synthase inhibitors. The purpose of this study was to determine the localization of NOS in primary sensory neurons. Adult guinea pig thoracic and lumbar dorsal root ganglia (DRG) (n=2) were dissected, fixed, sectioned and stained for immunofluorescence. Using a rabbit polyclonal antibody, neuronal nitric oxide synthase-like immunoreactivity was localized in 12% of cells (38/328) of DRG. In these same ganglia 23% (78/339) of neurons stained for substance P (SP). In the inferior mesenteric ganglion (IMG) there was an extensive network of NOS-positive nerve fibers surrounding principal ganglion cells, but sympathetic neuron perikarya were not stained. To test the hypothesis that NO modulates synaptic transmission in sympathetic ganglia, synaptic potentials were recorded with intercellular microelectrodes before and after the administration of a NOS inhibitor. Nω-nitro-L-arginine methyl ester (L-NAME) (10 μM) increased the amplitude and duration of the slow EPSP (sEPSP) by 59% and 43%, respectively (n=18, $p<0.01$) and hyperpolarized the membrane of principal ganglion cells by 2-10 mV. These studies suggest that primary sensory nerves are a source of NO in sympathetic ganglia and blood vessels. They further suggest that the DRG is a source of NOS-containing fibers in IMG and mesenteric blood vessels. Support: NIH-HL 27781.

453.2

RENAL SYMPATHETIC RESPONSES TO SOMATIC AND VISCERAL STIMULATION IN SPINALLY-TRANSECTED RATS.. L.P. Schramm* and D. Chau. Dept. of Neuroscience, The Johns Hopkins University, Baltimore, MD 21205.

These experiments provide a detailed mapping of the responses of renal sympathetic nerve activity (RSA) to cutaneous stimulation and to several forms of visceral stimulation. The effects of dorsal rhizotomy on responses were also examined. Experiments were conducted on 24 male, chloralose-anesthetized, C_1-transected, paralyzed, artificially-respired, Sprague-Dawley rats. Arterial pressure was measured *via* a right femoral arterial cannula. RSA was recorded from the left renal nerves, rectified, and quantified. Rats were shaved and marked with a uniform reference grid. Two types of cutaneous stimuli, light brushing and pinching with toothed forceps, were delivered for periods of 3 seconds. Magnitudes of responses in RSA were noted for 44 stimulated sites on an identical map for each rat. Responses were considered significant if they represented a 10 % change in activity. Light brushing of all sites inhibited RSA. Pinching of the caudal left flank increased RSA. Pinching of all other sites inhibited RSA. Pressure on the renal pelvis and traction on either the rectal temperature probe or the arterial cannula produced large increases in RSA. Increases in RSA elicited from the left flank were surprisingly resistant to dorsal rhizotomy. Increases in RSA were mapped before and after concurrent section of 3 or 4 left dorsal roots. Responses were most markedly reduced by concurrent section of roots T_{13}, L_1, and L_2. However, reduced responses remained even after section of all dorsal roots between T_8 and S_4 in one rat. We conclude that in the spinally-transected rat, RSA is increased by stimulation of only a small portion of the body surface but that these portions affect the spinal cord *via* a wide range of dorsal roots and, perhaps, *via* ventral root afferents. More widely distributed visceral afferents are capable of exciting RSA. Supported in part by NIH grant HL16315.

453.3

DYNAMIC RESPONSES OF RENAL R2 CHEMORECEPTORS TO INTRAPELVIC PRESSURE INCREASES. N.G. Moss and I.V Karastoianova* Department of Physiology UNC Chapel Hill NC. 27599.

R2 chemoreceptors are sensitive to the ionic composition of intrapelvic urine and thus, indirectly, to pressure gradients across the pelvic epithelium that cause backflux of urine into the renal interstitium. These studies have investigated the dynamic components of this relationship by recording afferent renal nerve activity (ARNA) in anesthetized rats during intrapelvic pressure increases of 0.05 (slow), 0.15 (medium), 0.3 (fast) mmHg/sec with diuretic (DU) and non-diuretic (NDU) urine. In 7 rats pressure increases with NDU caused multiunit ARNA to increase in a direct, linear relationship with pressure. Rate of increase in ARNA (% of control) correlated with the rates of pressure increase (slow = 5.1% per mmHg, medium = 8.1% per mmHg, fast = 12.4% per mmHg, $p<0.001$ for all comparisons). The same preparations were not activated by backflow of isotonic saline, confirming that these were chemoreceptor responses. Pressure increases with DU were also excitatory but the ARNA responses were not different between each of the three filling rates and the response showed a pressure threshold of 5 mmHg before excitation began. Analysis of single unit activity from 17 R2 chemoreceptors in 7 rats showed a similar relationship with filling rates. However, the R2 response to pressure increases was curvilinear, suggesting that the multiunit response results from a heterogenous receptor population. Thus, renal chemoreceptor responses to intrapelvic pressure increases have a dynamic component modulated by the rate of pelvic filling and urine composition.

453.4

C-FOS IMMUNOCYTOCHEMICAL LABELING WITHIN THE RAT DORSAL HORN AFTER RENAL AFFERENT STIMULATION. G.K. Fitch, K.P. Patel and M.L. Weiss*. Dept. of Anatomy and Physiology, Kansas State University, Manhattan, KS 66506-5602

Retrograde labeling studies using the rat indicated that dorsal root ganglion cells from T_8 to L_2 innervate the left kidney. Further, electrical stimulation of renal afferents influences dorsal horn neurons in spinal segments T_{10} to L_1. To investigate the spinal neurons influenced by renal afferent stimulation, we used *c-fos* immunocytochemistry following renal afferent stimulation to indicate neuronal activation. In anesthetized rats, a left renal nerve branch was located and either unstimulated (sham) or electrically stimulated (12 volt, 2 ms pulses at 7 Hz; 30 s on / 30 s off; repeated 30 times). In rats with flank incisions, the renal nerve was sectioned or crushed and electrical stimulation was applied to the proximal end of the nerve. After the stimulation protocol, the rat survived for 1.5 hour prior to perfusion. The brain and spinal cord were harvested and histologically processed. Immunocytochemistry using antibody directed against the fos protein was performed on free-floating sections. Electrical stimulation of renal afferents produced a 64% increase in the number of labeled cells in spinal cord dorsal horns relative to sham stimulated animals. Most labeled cells were found in the left dorsal horn of spinal segments T_{11} and T_{12}. In rats with ventral midline incisions, renal afferents were activated either electrically or by selective activation of renal mechanoreceptors. The renal nerve was not sectioned or crushed before stimulation. Following stimulation in these animals, the left dorsal horn had more labeled cells than the right dorsal horn. Renal denervation greatly reduced the number of fos labeled cells in the dorsal horn after mechanoreceptor activation. Our results indicate that activation of renal afferents can induce *c-fos* expression in the dorsal horn. Sponsored by the American Heart Association, Kansas Affiliate.

453.5

ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL FEATURES OF PELVIC AFFERENTS INNERVATING THE RAT URINARY BLADDER. W. Jiang and J.F.B. Morrison. (Spon: Brain Research Association), University of Leeds, Leeds, LS2 9NQ, United Kingdom.

Recordings from 100 pelvic nerve bladder afferents were made in pentobarbitone anaesthetised rats during slow distensions with saline (controls), and using artificial urine containing high potassium, osmolar or proton concentrations, or capsaicin, for up to 6 hours. The urinary bladder was distended at 0.16ml/min for four minutes; the maximum intravesical pressure was generally below 40 mmHg. Fibres that did not respond to this distension were termed 'silent' afferent. Three functional different types of afferents were found: Aδ and C low threshold mechanoreceptors, a few high threshold mechanoreceptors and 'silent' units. 44% of units were unmyelinated. The range of thresholds of the Aδ and C units overlapped, but the mean threshold of the C fibres is greater (15.0±0.3 vs 9.48±0.5 mmHg, mean±SE); the slope of the stimulus responses curve was also less in the C-fibres. 75% of the Aδ and 86% of C-fibres can be excited by intravesical chemical stimulation and showed sensitisation: the slope of their stimulus response curves increased; and 'silent' afferents became mechanosensitive, with thresholds of 17.6±0.4 mmHg. 42% of units showed initial sensitisation and then depression with 400 to 500 mM KCl only, which is presumably due to depolarisation block of potassium. The slope of the stimulus responses curve was less in the C-fibres. 18% of the studied units did not respond to any chemical stimulus. Aδ and C-mechanosensitive afferents showed biphasic responses to the intravesical capsaicin injection. Acute capsaicin caused excitation in 80% of units and this lasted at least 1.5 hours. Desensitisation was never seen before 1.5-2.0 hours had elapsed. The majority of mechanosensory receptors in this study respond to intravesical chemical stimuli, some of which (potassium and protons) can occur within the physiological range. 'Silent' afferents develop mechanosensitivity following chemical stimulation. It is possible that some of these sensory endings are present in the bladder epithelium.

453.6

MODIFICATIONS OF MOTOR RESPONSES IN THE RAT URINARY BLADDER FOLLOWING PERIGANGLIAR INJECTION OF COLCHICINE. A. Lecci, R. Patacchini, S. Giuliani, E. Theodorsson[1] and C.A. Maggi*. Pharmacol. Res.Dept. "A. Menarini" Pharmaceuticals, Florence, Italy. and [1] Dept. of Clin. Chem. Karolinska Hosp., Stockholm, Sweden.

The aim of this study was to assess the effect of blocking the axonal transport by local injection of colchicine at pelvic ganglia level on the sensory and "efferent" function mediated by capsaicin-sensitive primary afferent neurons innervating the urinary bladder in rats. Bilateral injection of colchicine (125 nmol/ganglion) in the prostatic tissue underneath the pelvic ganglia of male rats induced a time-dependent reduction (maximal effect at 72 h, 100% of reduction) of the contraction of the rat isolated bladder induced by capsaicin (1 μM). The response to electrical field stimulation (0.1-20 Hz) was also reduced, although at a lesser extent, the direct bladder contractions induced by substance P (100 nM) or KCl (80 mM) were not affected by colchicine pretreatment. In vivo, perigangliar injection of colchicine (72 h before) increased bladder capacity, and reduced the amplitude of micturition contractions during the cystomethogram in urethane-anesthetized rats. Topical application of capsaicin (15 nmol/rat) onto the bladder or onto the stomach induced a cardiovascular pressor reflex in urethane-anesthetized, spinal cord transected rats. Colchicine pretreatment reduced by 50% the pressor response elicited by chemoceptive stimulation of the bladder but not that arising from the stomach. Colchicine pretreatment abolished capsaicin-induced release of CGRP-i.r. from the urinary bladder and time-dependently reduced bladder levels of of SP-, NKA-, CGRP-, NPY-, but not of VIP-i.r. Present findings demonstrate that blockade of axoplasmic transport completely eliminates the "efferent" but only partially affects the sensory function of capsaicin-sensitive neurons.

453.7

VAGAL AFFERENT ELECTRICAL STIMULATION PRODUCES PUPIL DILATATION IN THE RAT. Barry R. Komisaruk[1]*, Rafael Cueva-Rolon[2], Lisbeth Gomez[2], Maria Ganduglia-Pirovano[1], Giorgio Sansone[1] and Ralph Bianca[1]. Institute of Animal Behavior, Rutgers, The State University of New Jersey, Newark, NJ 07102[1] and Center for Research in Animal Reproduction (CIRA), CINVESTAV, Unidad Tlaxcala, Mexico[2].

Previous studies in this laboratory have shown that, in the rat, vaginocervical stimulation (VS) produces an immediate and marked dilatation of the pupil of the eye (PD). VS-produced PD is reduced but not abolished by a) bilateral transection of all known genito-spinal nerves (pelvic, hypogastric and pudendal) or b) complete transection of the spinal cord at the level of vertebra Thoracic 7. Subsequent bilateral subdiaphragmatic vagotomy abolished the residual VS-produced PD. Based on these findings, we hypothesized that electrical stimulation of vagus nerve afferents would produce pupil dilatation. To test this hypothesis, rats were anesthetized with pentobarbital (3.9mg/100g bw). The left vagus nerve was transected near the clavicle. The proximal (central) stump of the nerve was placed on a pair of silver wire electrodes and stimulated by 1 sec trains of 0.1 msec-duration biphasic square wave pulses at 50Hz. The pupil image (ipsilateral eye) was recorded via a close-up video camera, and pupil diameter was measured from a monitor. Pupil dilatation was directly proportional to stimulation intensity, ranging from 5.6% above baseline diameter at 1.0 V to 47% above baseline diameter at 40 V. The magnitude of the PD was calibrated against force of VS. The magnitude of PD produced by 5V or 40V electrical stimulation applied to the vagus nerve central stump was equivalent to that produced by 100g or 350g vaginocervical mechanical force, respectively. The present findings provide further evidence that vaginocervical stimulation can be conveyed directly to the brain via vagus nerve afferents, thereby bypassing the spinal cord. Support: NIH - Fogarty International Center - 1R03-TW00394 (BRK).

453.8

LOCALIZATION OF ADENOSINE (A1) RECEPTORS TO CARDIAC AFFERENT NEURONS IN ADULT RATS. H.R Middlekauff, S.A. Rivkees+ +, H.E. Raybould*, S. Sampogna, E.A. Mayer. UCLA Sch of Med, Los Angeles, California 90024, and + +Indiana University Sch of Med, Indianapolis, IN 46202.

Background: Adenosine stimulates afferent neurons in a variety of tissues, including the myocardium, producing ischemic-like pain. Unmyelinated spinal afferents mediate visceral pain, while vagal afferents play a role in pain modulation and reflex regulation. It is unknown if adenosine activates sensory afferent neurons directly, or through intermediaries.

Aim: To determine if adenosine receptors(A1R) are located on cardiac spinal and/or vagal afferent neurons.

Methods: Bilateral nodose ganglia(NG) and C7-T5 dorsal root ganglia(DRG) from 8 adult Sprague-Dawley rats (180-200g) were used in the following studies: 1) radioligand binding performed in quadruplicate with [^{3}H]DPCPX, a highly selective A1R agonist(n=5); 2) *in situ* hybridization probes using ^{35}S labeled cRNA probes(n=3); and 3) following retrograde labeling of cardiac afferent neurons with fluorogold, immunocytochemistry (ICC) with polyclonal antisera against A1R(n=3). Results: 1) Specific binding for [^{3}H]DPCPX (fmol/mg protein) was present: DRG 12.2 ± 0.07, NG 39.6 ± 1.2. 2) A1R mRNA was detectable in both NGs and DRGs. 3) Fluorogold labeled a subset of small and large nerve bodies within bilateral NGs and C7-T5 DRGs. A1R ICC labeling was found in the majority of both large and small neurons, both in fluorogold labeled and unlabeled cells.

Conclusion: A1R are present in both spinal and vagal afferent neurons, including those innervating the heart. Thus these afferents are likely to participate in the generation of ischemic pain in response to endogenously released adenosine, and may play a role in the mediation of viscero-visceral reflex responses associated with cardiac ischemia.

453.9

PRESENCE AND LOCALIZATION OF NEUROTROPHIN RECEPTOR TYROSINE KINASE (TrkA, TrkB, TrkC) mRNAs IN VISCERAL AFFERENT NEURONS OF THE NODOSE AND PETROSAL GANGLIA. H. Zhuo[1]* and C.J. Helke[1,2]. [1]Dept. of Pharmacol. & [2]Neurosci. Program, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

This study was conducted to reveal the expression of the high affinity tyrosine kinase (Trk) receptors for neurotrophins in visceral afferent neurons of the nodose and petrosal ganglia of adult and neonatal rats. We used *in situ* hybridization histochemistry with [^{35}S]-labeled oligonucleotide probes to Trk mRNAs. Neurons containing TrkA mRNA were found in the adult nodose and petrosal ganglia. About 10% of nodose ganglion neurons and 38% of petrosal ganglion neurons contained TrkA mRNA. The nodose and petrosal ganglia from 1 day old neonates also expressed TrkA mRNA. No TrkB mRNA-containing neurons were detected in the adult nodose and petrosal ganglia, whereas TrkB mRNA was detected in 1 day old neonatal nodose and petrosal ganglia. TrkC mRNA was found in about 9% of nodose ganglion neurons and 11% of petrosal ganglion neurons of adult rats. Likewise, low but detectable levels of TrkC mRNA were seen in 1 day old neonatal nodose and petrosal ganglia. These data demonstrate the presence of TrkA and TrkC in the adult nodose and petrosal ganglia and provide a substrate for the ongoing neurotrophin-induced regulation of neuronal function. The presence of TrkC in the nodose ganglion is consistent with the transport of [^{125}I]-NT-3, the neurotrophin ligand for TrkC, by vagal afferent neurons of the nodose ganglion (Helke et al., this meeting). The presence of TrkB mRNA in neonatal but not in adult nodose and petrosal ganglia suggests a developmental down-regulation of TrkB receptor expression in these placodally-derived visceral afferent neurons. (supported by NIH Grant R01 NS20991)

453.10

RETROGRADE AXONAL TRANSPORT OF NEUROTROPHIN-3 (NT-3) BY THE CERVICAL VAGUS NERVE TO THE NODOSE GANGLION IN THE RAT. C.J. Helke[1]*, K. Adryan[1], R. Curtis[2], H. Zhuo[1], and P.S. DiStefano[2]. [1]Dept. of Pharmacol., Uniformed Services Univ. of the Health Sciences, Bethesda, MD, 20814, and [2]Regeneron Pharmaceuticals, Inc., Tarrytown, NY,10591.

Placodally-derived visceral sensory neurons of the nodose ganglion in the rat are responsive to the trophic effects of NT-3 during development. However, little is known of the responsiveness of mature nodose ganglion neurons to NT-3. Because retrograde transport of a neurotrophin is thought to be predictive of neuronal responsiveness, we have studied the transport of NT-3 by neurons of the nodose ganglion which comprise the afferent vagus nerve. ^{125}I-NT-3 or ^{125}I-cytochrome C was administered to the cut-end of the cervical vagus nerve of adult rats. In competition studies, ^{125}I-NT-3 was administered with an excess of unlabeled NT-3, NGF, BDNF, or NT-4. After 17 hours, ganglia were dissected and ^{125}I was determined by gamma counting followed by emulsion autoradiography of tissue sections. ^{125}I-NT-3 was retrogradely transported from the cervical vagus nerve to neuronal perikarya in the ipsilateral nodose ganglion. ^{125}I-NT-3 labeling was not found in the ipsilateral superior cervical or contralateral nodose ganglia. ^{125}I-cytochrome C (physically similar to NT-3) was not significantly transported. The transport of ^{125}I-NT-3 to the nodose ganglion was reduced by 85% in the presence of unlabeled NT-3 and by 40% in the presence of unlabeled NGF. Unlabeled BDNF and NT-4 had no signigicant effect on the transport of ^{125}I-NT-3 to the nodose ganglion. Preliminary data also showed the transport of ^{125}I-NT-3 by the efferent vagus nerve to perikarya in the dorsal motor nucleus of the vagus and nucleus ambiguus.

These data demonstrate the receptor-mediated retrograde axonal transport of ^{125}I-NT-3 by vagal afferent neurons of the nodose ganglion. The profile of the competition studies suggests an involvement of neurotrophin receptors, TrkC and perhaps TrkA, in the transport. (Supported by NIH grant NS20991 to CJH)

453.11

CAPSAICIN-RESISTANT VAGAL AFFERENTS: MORPHOLOGICAL EVIDENCE USING DiI AND HRP TRACING. H.-R. Berthoud*, A.E. Willing, D.C. Irminger, K. Mueller, and W.L. Neuhuber, Pennington Biomedical Research Center, Louisiana State Univ., Baton Rouge, LA, 70808, USA, and Anatomy Institute, Univ. of Erlangen, D-91054 Erlangen, Germany.

In order to identify the peripheral and central projections of putative capsaicin-resistant vagal afferents we used the carbocyanine dye DiI and HRP injected into the nodose ganglia or the cut cervical vagus, respectively. In the first experiment capsaicin or vehicle was administered to neonatal Wistar rats (50 mg/kg) or young adult SD rats (3 injections over 36 h, 12.5, 30, and 75 mg/kg, i.p.), followed 10 days later by DiI or WGA-HRP injections into the left nodose ganglion. Behavioral effectiveness of capsaicin treatment was tested by means of the eye-wiping test. There was no difference in the number of DiI-labeled vagal intraganglionic laminar endings (IGLEs) in esophagus and stomach between capsaicin and vehicle treated rats, but a substantial reduction of IGLEs in the duodenum of capsaicin treated animals. In the second experiment capsaicin was administered to neonatal Wistar rats (50 mg/kg), followed 3 months later by HRP application to the central cut ends of either the left cervical vagus or the left splanchnic nerves. Following cervical vagal HRP application there was no noticeable difference in labeled perikarya of the ipsilateral nodose ganglion or afferent axon terminals throughout the NTS, between capsaicin and vehicle treated rats. In contrast, splanchnic nerve application of HRP in capsaicin pretreated rats resulted in a 60-80% reduction in labeled T9-T11 dorsal root ganglion cells and a drastic reduction of labeled afferent axon terminals in the spinal cord. These results demonstrate that unlike spinal afferents, abdominal vagal afferents are less sensitive to capsaicin, and that the capsaicin sensitive vagal afferents may be restricted to a population that innervates the small intestine but not the esophagus and stomach. Supported by NIH grant DK 47348 and DFG-SFB grant 353/B9.

453.12

DISSOCIATION OF GASTRIC PRESSURE AND TENSION RESPONSES FROM GASTRIC VAGAL AFFERENT RESPONSES TO CHOLECYSTOKININ AND NEUROMEDIN C IN RAT. G. J. Schwartz*, E.E. Ladenheim, and T.H. Moran. Dept. Psychiatry & Behav. Sci., Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

Exogenous administration of a range of brain-gut peptides, including cholecystokinin (CCK) and the mammalian bombesin-like peptide neuromedin C (NMC) have been demonstrated to suppress feeding. To evaluate the potential role for peripheral peptide elicited gastrointestinal neuromuscular events in the suppression of food intake produced by these peptides, we examined the changes in gastric pressure and gastric wall tension produced by intravenous (jugular vein, i.v.) and close celiac arterial (i.a.) infusion of a dose range of CCK and NMC (10 - 1000 pmol) in anesthetized rats. We simultaneously recorded the neurophysiological activity of gastric load-sensitive single vagal afferent fibers to evaluate the correlation between peptide-induced gastrointestinal events and the production of gastric vagal afferent signals. Both i.a. and i.v. CCK and NMC elicited dose-dependent increases in the neurophysiological discharge rates of a population of single vagal afferent fibers with gastric receptive fields. NMC also elicited dose-dependent increases in both intragastric pressure and gastric wall tension, while CCK elicited a modest reduction in intragastric pressure, and no changes in gastric tension. These data suggest that different mechanisms underlie the ability of these peptides to elicit vagal afferent signals. Supported by NIH DK 47208 and DK46448.

453.13

THE ROLE OF THE SYMPATHETIC NERVOUS SYSTEM IN CHRONIC VISCERAL PAIN. U. Wesselmann*[1], A. L. Burnett[2] and J. N. Campbell[1]. [1]Blaustein Pain Treatment Center and [2]James Buchanan Brady Urological Institute, Johns Hopkins Hospital, Baltimore, MD 21287.

Chronic visceral pain is a challenging problem because of the wide differential diagnosis and lack of successful treatment options. The reproductive organs, which have a very dense sympathetic innervation, seem to be more prone to chronic pain states than other visceral organs with a predominantly parasympathetic innervation. Chronic pain can sometimes even persist despite the surgical removal of the organ that was thought to be the source of pain (persistent pain after hysterectomy or orchiectomy). In contrast chronic visceral pain associated with organs with a predominately parasympathetic supply is usually cured when the organ is surgically removed (persistent pain after tonsillectomy or cholecystectomy is rare). We have tested the hypothesis that chronic testicular pain is sympathetically maintained. The testis was chosen as a model for visceral pain, since it is the only visceral organ that can easily be examined clinically. We treated 5 patients with chronic unilateral non-malignant testicular pain (≥ 6 mo duration) with sympathetic blockade including lumbar sympathetic blocks with local anesthetic, placebo-controlled phentolamine infusions and oral alpha-1 adrenergic antagonists. All patients had undergone a full urological exam (including ultrasound and evaluation for infection) and psychological evaluation. All patients had significant pain relief from sympathetic blockade, reducing pain from 6/10 to 1/10 on a verbal pain scale. Duration of pain relief varied between patients and depending on the modality of sympathetic blockade, ranging from 3hrs to persistent pain relief. The preliminary results support the hypothesis that the concept of sympathetically maintained pain can be extended to certain visceral chronic pain syndromes.

453.14

RESPONSE CHARACTERISTICS OF ABDOMINAL VAGAL AFFERENTS DURING ISCHEMIA

H.-L. Pan, Z.B. Zeisse, L.-X. Zhang* and J.C. Longhurst. Cardiovascular Medicine, Univ. of Calif., Davis, CA 95616

Activation of abdominal sympathetic visceral afferents elicits nociception and autonomic cardiovascular reflexes during ischemia. It is not known, however, whether and how abdominal vagal afferents respond to ischemia. Single-unit activity of abdominal vagal afferents (n=29) was recorded from decentralized right thoracic vagus nerve in anesthetized cats during 15 min of ischemia. Afferent nerve endings were located on the stomach, pancreas and duodenum with conduction velocity ranging from 0.30 to 1.15 m/sec. Occlusion of the thoracic descending aorta gradually increased the discharge activity of 5 afferents from 0.16±0.04 to 0.45±0.06 imp/sec ($p<0.05$). Discharge frequency of 14 afferents was mainly inhibited during 15 min of ischemia (0.85±0.28 to 0.13±0.04 imp/sec, $p<0.05$) although the activity displayed transient increase for the first 5 min of ischemia in 4/14 afferents. In the remaining 10 afferents, the discharge frequency was not altered significantly during ischemia (0.12±0.05 to 0.13±0.04 imp/sec). Intra-arterial injection (1 μg/kg) but not serosal application (10 μg/ml) of bradykinin activated 19 of 29 afferents from 0.58±0.08 to 1.72±0.23 imp/sec ($p<0.05$). These data indicate that although most abdominal vagal afferents lack the capacity to encode ischemic event, a subgroup of afferents are capable of conveying ischemic information to the central nervous system.

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453 15

CENTRAL ACTION OF TRH ACTIVATES CAPSAICIN-SENSITIVE SPLANCHNIC AFFERENTS WITH RECEPTIVE FIELDS IN THE STOMACH. T.J.O-Lee, J.Y.Wei,& Y.Taché*. CURE/Gastroenteric Biol. Ctr., VA Wadsworth Med. Ctr. & Dept. of Med. & Brain Res. Inst., UCLA, Los Angeles, CA 90073.

It has been demonstrated that the gastric hyperemic response to central vagal activation induced by intracisternal TRH analog RX-77368 is mediated by local effector function of capsaicin-sensitive afferent fibers releasing calcitonin-gene related peptide (CGRP)(Am J Physiol 267:G1041-G1049, 1994). In this study, we investigated the way low doses of TRH analog (1.5 ng / rat) injected into the cisterna magna altered the afferent activities of the celiac branch of the left splanchnic nerve. The celiac nerve is approached by laparotomy By first locating the super-renal ganglia, the celiac branch can be identified with little difficulty Unit action potentials of the nerve filament were recorded with bipolar platinum electrodes (dia. 30 um). Single unit analysis was done by separating each unit with a window discriminator based on the amplitude and wave-form of each spike. Nine units from 3 separate animals have thus far been analysed. Seven of the 9 units exhibited spontaneous firing activities, and among these, significant increases in activity (287±49%, mean±SE; $P<0.02$, unpaired Student's t-test) were observed after ic injection of TRH. The results were compared against the activity change after ic injection of saline (136±21%). The 2 units that had no spontaneous activity were unaffected by ic saline but were activated by ic TRH (firing at 20-50 pulses/min after injection). To ensure that the units investigated originated from the stomach, we perfused capsaicin intraluminally (640 nmol/ml, 0.69-0.84 ml/min) for 15-20 min. in each experiment. The activity of all nine units increased during capsaicin perfusion (144±15%). In addition to the perfusion, we also distended the stomach with 5 ml of saline. All nine units also responded to stomach distension with an increase in activity (202±19%). These data suggest that splanchnic afferents with receptive fields in the stomach are activated by central administration of TRH analog. The pathway for this activation is likely to involve gastric-vagal efferents, but the exact mechanism remains to be further investigated.

453 16

NEURAL PATHWAYS INVOLVED IN EMESIS IN *SUNCUS MURINUS*. N. Matsuki,* M. Saito and H. Saito. Department of Chemical Pharmacology Faculty of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113, JAPAN.

To investigate the neural pathways that cause emesis, expression of c-Fos protein was investigated immunohistochemically in *Suncus murinus*. Cisplatin or motion was used as an emetic stimulus. Cisplatin enhanced the expression of c-Fos-like immunoreactivity (FLI) strongly in the nucleus tractus solitarius (NTS) and the dorsal motor nucleus of the vagus (DMV) and slightly in the area postrema (AP). FLI was not detected in other brain regions. Pretreatment of the animals with a 5-HT_3 receptor antagonist, tropisetron, abolished the emesis caused by cisplatin completely and reduced FLI in the NTS and the DMV strongly Abdominal vagotomy also abolished the emesis completely but suppressed FLI partially Administration of cisplatin into mice did not cause emesis nor retch but FLI was observed in AP and NTS. The motion caused FLI in the NTS and DMV but not in AP Pretreatment of animals with a 5-HT_{1A} receptor agonist, 8-OH-DPAT abolished motion-induced emesis and partially reduced FLI in NTS and DMV These results suggest that NTS and DMV are part of "vomiting center" in *Suncus murinus* because they were similarly activated by two completely different emetogenic stimuli. Cisplatin may have a direct effect on NTS and DMV through 5-HT3 receptors but the stimulation is either not strong enough to cause emesis or independent from emetogenic effect.

453 17

PERITONEAL IRRITATION-INDUCED ILEUS IN RATS INVOLVEMENT OF CRF PATHWAYS B.Bonaz N.Cristina and C.Feuerstein INSERM-LAPSEN U318 38043 Grenoble cedex 09 France

Peritoneal irritation induced by acetic acid (AA) is a noxious visceral stimulus resulting in digestive ileus in rats central injection of the CRF antagonist reverses this ileus (1) Aims to study in rat brain 1) the effect of AA on the expression of Fos mRNA, 2) the localization of this transcript in CRF perikarya of the paraventricular nucleus (PVN) of the hypothalamus Methods male SD rats received an ip injection of either AA (0 6 vol% 10 ml/kg) or saline (controls) Animals were perfused (4% paraformaldehyde-borax) 1h 2h 3h 4h and 6h after ip injections and brains processed for *in situ* hybridization (ISH) using ^{35}S riboprobes Colocalization of the transcript within CRF immunoreactive neurons of the PVN was determined using a combination of immunocytochemistry and ISH. Results AA induced Fos mRNA expression in the NTS and ventrolateral medulla parabrachial nucleus locus coeruleus cerebral cortex PVN (mainly parvocellular part) and supraoptic nucleus of the hypothalamus This expression peaked 1h after the injection of AA, decreased at 2h and totally vanished 6h after injection In the parvo PVN CRF-immunoreactive neurons expressed the Fos transcript Conclusions AA induces Fos expression in nuclei (PVN locus coeruleus NTS) controlling digestive motility in rats In the parvo-PVN CRF immunoreactive neurons expressed Fos mRNA thus confirming the involvement of CRF pathways in peritoneal irritation-induced ileus

(1)Rivière et al J Pharmacol Exp Ther 1995 (in press)

453 18

TEMPERATURE AND ANION SPECIES MODIFY THE CHORDA TYMPANI NERVE RESPONSE TO SALT STIMULATION IN RATS. R. F. LUNDY, JR.* and R. J. CONTRERAS, Program in Neuroscience, Florida State Univ., Tallahassee, FL 32306-1051.

The goal of the present study was to extend the work of previous research on the neural mechanism(s) of salt taste using a novel stimulus delivery system. We investigated the effects of tongue adaptation temperature (25° vs. 35° C) on the summated responses of the whole chorda tympani nerve to a broad range of salts mixed with and without 100 μM amiloride hydrochloride in male Sprague-Dawley rats using a novel stimulus delivery system. The stimuli consisted of 500 mM concentrations of NaCl, Na_2SO_4, sodium acetate (NaAc), KCl, K_2SO_4, potassium acetate (KAc), NH_4Cl, $(NH_4)_2SO_4$, ammonium acetate (NH_4Ac), and ChCl. Each stimulus was presented twice: first by itself for 45 s (protocol A), and second with the first 15 s by itself, the next 15 s mixed with amiloride, and the last 15 s by itself again with uninterrupted flow (protocol B). In the absence of amiloride, the phasic response magnitudes of the chorda tympani nerve to all stimuli were smaller at 25° than at 35° C; except for KCl and KAc. Similarly, the tonic response magnitudes to the same stimuli, except NaCl, were also smaller at 25° than at 35 °C. Amiloride suppressed the chorda tympani nerve responses to all stimuli so that NaAc>Na_2SO_4=KAc= NaCl>K_2SO_4=KCl=ChCl>NH_4Ac= $(NH_4)_2SO_4$=NH_4Cl. However, the degree of amiloride suppression for NaCl, Na_2SO_4, and the ammonium salts was greater at 25° than at 35° C. Amiloride completely suppressed the chorda tympani nerve responses to NaAc at 25° and 35° C. Substituting SO_4 for Cl in sodium salts, and Ac for Cl in both sodium and potassium salts increased amiloride suppression. Taken altogether, the findings suggest that: (1) the degree of amiloride suppression of the chorda tympani nerve to all salt stimuli is a function of lingual mucosal temperature and/or anion species; and (2) amiloride's ability to block ion channels is either not as specific as previously suspected or many taste stimuli express various affinities for amiloride-sensitive Na^+ channels.

453.19

GFAP-IMMUNOREACTIVITY IN PULP CELLS, PRETERMINAL AXOPLASM, AND SCHWANN CELLS OF RAT AND MONKEY DENTAL TISSUE. M.R. Byers*, T. Maeda, F.F. Solca. Anesthesiology and Biochemistry Univ Washington; Oral Anatomy Niigata University Japan

Patch clamp studies have recently identified potassium and sodium currents in cultured human pulp cells as well as immunoreactivity (IR) for substance P and glial fibrillary acidic protein (GFAP) (Davidson et al., J. Dent. Res. 73:121 '94). Our study was done to identify GFAP-IR cells in jaw tissue of young and old rats and monkeys using light and electron microscopy and western blot techniques. Rats (aged 1 wk to 1 yr) and monkeys (1 & 6 yr) were perfusion fixed; jaw tissues were decalcified and analyzed by standard ABC immuno techniques using polyclonal anti-GFAP and monoclonal anti neurofilament. Western blot demonstrated that there was no cross-reactivity of the GFAP antibodies with neurofilament protein. GFAP-IR was found in pulp cells of immature teeth, but not in adults; and odontoblasts were never labeled. Preterminal axoplasm of dental and gingival nerves had intense GFAP-IR, associated with axonal cytoskeletal filaments. Schwann cell label was variable: some pulpal Schwann cells had GFAP-IR while others did not; periodontal Ruffini terminal Schwann cells were not labeled. GFAP-IR was weak or absent in trigeminal ganglion cell bodies, but intense in perivascular fibers in the adjacent fascia. We conclude that GFAP-IR intermediate filaments characterize preterminal axoplasm of some types of sensory nerve fiber, some Schwann cells and immature non-odontoblast pulp cells. Supported by NIH DE05159.

454.1

RECORDINGS OF AFFERENT AND EFFERENT C-FIBRE ACTIVITY FOLLOWING CUTANEOUS ARGON LASER STIMULATION IN MAN B. Olausson[1], J. O. Skarphedinsson[2], M. Elam[1], Gunnar Wallin[1] & B. C. Shyu[3]*, [1]) Dept. Clin. Neurophysiol, Sahlgren Univ. Hosp., S-414 45 Göteborg, Sweden, [2]) Dept. Physiol Univ of Island, [3])Academia Sinica, Taipei, ROC

Stimulation of the skin in man by high intensity carbon dioxide laser can induce pain sensations which has been correlated to evoked C-fibre activity recorded in the peripheral nerve. The present study aimed to investigate the reaction pattern in cutaneous C-fibre afferents and reflex sympathetic activity following cutaneous argon laser stimulation in man. Microneurographic nerve recordings were performed in the peroneal nerve in healthy volunteers (n=11). Single C-fibre afferents were identified. In the same recording sites efferent multiunit thin fibre activity were simultaneously recorded. Sympathetic effector organ responses were monitored with laser doppler flowmetry and skin resistance recordings. The pain perception was measured with a visual analogue scale. Mechano-heat sensitive nociceptive C-fibre units were activated by the argon laser. Linear relations were found between the subjective pain rating, the intensity of the laser stimulation and the number of action potentials following laser stimulation, respectively. The latency shift in the electrically evoked activity was related to the number of action potentials evoked by, and the time elapsed, since the laser stimulation. The temporal profile of the reflex sympathetic nervous responses suggest laser induced activity also in A-delta afferents. Cutaneous argon laser stimulation induced selective activity in polymodal nociceptive afferent C-fibres. The degree of activation was linked to the psycophysical pain report. The nociceptive stimulation induced a reflex activity in the skin sympathetic fibres suggesting laser induced activity in A-delta afferents.

454.2

ACTIVATION OF MENINGEAL AFFERENTS IN THE RAT: RESPONSES OF TRIGEMINAL BRAIN STEM NEURONS AND DURAL ARTERIAL FLOW K. Meßlinger*, A. Ebersberger, M. Pawlak, K. Schepelmann, R.F. Schmidt
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Severe headaches such as migraine pain result from a massive activation of nociceptive afferents in meningeal structures, where neurogenic inflammatory processes may play a significant role. The noxious stimuli initially involved are unknown. A preparation was developed in the rat in which the activation of meningeal afferents could be examined by two methods: electrophysiological recordings from trigeminal brain stem neurons and monitoring of dural blood flow changes indicating neurogenic inflammation.

The dura mater encephali was exposed by removing the parietal bone up to the sagittal superior sinus. Meningeal afferents were stimulated with electrical pulses using bipolar electrodes lowered on the dura. Chemical stimuli were applied topically or by injection through a catheter into the sagittal sinus. Extracellular recordings were made from neurons in the subnucleus caudalis of the spinal trigeminal tract. The blood flow was monitored with a laser Doppler flowmeter around branches of the medial meningeal artery.

Neurons in deeper laminae of the nucleus caudalis driven by meningeal afferents were identified by electrical stimulation and by probing the receptive fields on the dura. Most of these units had convergent input from the cornea and/or from the periorbital skin. A proportion of them could be activated by injection of bradykinin, prostaglandin E_2, and a combination of several inflammatory mediators into the sagittal sinus. The dural arterial flow was locally increased by series of electrical stimuli which were able to activate dural afferents. These increases in flow depended on the polarity, the strength and the frequency of stimuli and could be abolished by local anesthetics.

We conclude that local electrical stimulation of dural structures activates meningeal afferents and causes neurogenic inflammatory responses. These afferents project to trigeminal brain stem neurons and may be activated by inflammatory mediators accumulated in the intracranial venous compartment. The preparation presented here may be a valuable model for studying the processes which are involved in the activation of meningeal afferents and the pathophysiological changes that lead to headaches.

454.3

ELECTROPHYSIOLOGICAL EVIDENCE THAT MENINGEAL AFFERENTS ARE NOCICEPTIVE. Geoffrey Bove*, Michael Moskowitz.
Massachusetts General Hospital, CNY 149-6403, Charlestown, MA 02129.

The meninges possess a rich supply of small diameter afferent fibers that are presumed to be nociceptive. Using classical teased fiber recordings, we have recorded from single axons of the guinea pig nasociliary nerve innervating the dura mater covering the frontal lobe and olfactory bulb. The fibers were characterized according to their conduction velocity (CV) and response to mechanical stimulation with sharp forceps or sharpened 0.5 mm pin. All mechanically sensitive fibers had CVs of <1 m/s, indicating that they were unmyelinated (c- fiber). The receptive fields were punctate and occasionally multiple, located in close relation to either blood vessels or the ethmoidal nerve. The discharges were slowly or non-adapting during the stimulus, and stopped abruptly when the stimulus was removed. Sustained discharge rates were as high as 40 Hz, with instantaneous frequencies as high as 100 Hz, and roughly paralleled the mechanical stimulus intensity. No units had ongoing discharge when isolated, but one did develop activity after repeated mechanical stimulation (0.5 Hz). The mechanical threshold, CVs, and absence of background discharge are similar to those reported for mechanically sensitive nociceptors from cutaneous and deep tissues.

These data demonstrate that the meninges are innervated by fibers that share properties with nociceptors from other tissues. The receptive field locations correlate with data collected from human volunteers. These nociceptors are likely to be involved in the transmission of intracranial pain, including migraine.

(Supported by NIAMSD ARO8239 and NINDS NS 21558)

454.4

PROTONS ALTER INTRACELLULAR Ca2+ IN A SUBPOPULATION OF CULTURED TRIGEMINAL GANGLION NEURONS. X.-S. Zhang and B. P. Bryant*, Monell Chemical Senses Center, Philadelphia PA, 19104

Psychophysical studies demonstrate that a subcutaneous pH of 6.2 is sufficient to cause pain in humans and that the perception of pain increases with increasing hydrogen ion concentration (Lindahl,1962). Single-unit recordings from mammalian nerves show that protons activate a subpopulation of afferents innervating skin and cornea(Steen et al., 1992; Belmonte et al.,1991). In single trigeminal ganglion (TG)neurons, inward currents activated by protons at pH 6 share similarities with capsaicin(CAP)-activated inward currents(Liu and Simon,1994). To further elucidate the cellular mechanisms by which protons and CAP are transduced to cause pain and irritation we have examined the effect of these stimuli on intracellular Ca^{2+}.

In cultured neonatal rat TG neurons, 60% of the neurons are sensitive to CAP, and a subpopulation of the CAP-sensitive neurons expressed CGRP using a cobalt uptake assay and immunocytochemical technique. Proton-sensitive neurons were not detected. This suggested that CAP and proton sensitivity may be mediated by different mechanisms or that the cobalt assay was not suitable. Using the Ca2+-sensitive indicator FURA-2 to measure intracellular [Ca2+] in TG neurons, we have found that 0.25uM CAP elevated the concentration of intracellular free Ca2+ in 62% of cultured neonatal TG neurons. 20% of the neurons responded only to low pH. Protons (pH<6.6) caused a decrease or increase of intracellular [Ca2+] in different neurons. The percentage of CAP-sensitive neurons which responded to protons with a decrease in intracellular [Ca2+] was concentration dependent in the range of pH6.5 to pH5. All neurons which responded to protons with an increase in intracellular [Ca2+] were also sensitive to 0.25uM CAP. The percentage of neurons which responded to protons with an increase in intracellular [Ca2+] was constant across the range of pH6.5 to pH5. While this heterogeneity may reflect acid-sensitive processes on the cell body, it may also reflect the presence of an additional proton sensitive transduction mechanism that is independent from CAP sensitivity. Supported by DC01921 and Kirin Brewery Co. Ltd.

454.5

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF TRIGEMINAL GANGLION CELLS THAT INNERVATE INTRACRANIAL DURAL VENOUS SINUSES IN THE RAT. A.M. Strassman*, S.A. Raymond and R. Burstein. Dept. Anesthes., Beth Israel Hosp. and Harvard Med. Sch., Boston, MA 02115.

Major intracranial blood vessels of the dura and pia receive an afferent innervation from the trigeminal nerve which has been postulated to play a critical role in the mediation of vascular headache syndromes such as migraine. Although the trigeminal afferents that supply this innervation have been the subject of intense study by anatomical and pharmacological approaches, direct information on their physiological response properties is lacking. In order to examine more directly the physiological properties that underly sensory signalling in this pathway, single unit extracellular recording techniques were used to identify and characterize cells in the trigeminal ganglion that innervate dural blood vessels in urethane-anesthetized rats. Such cells were initially identified by their constant latency response (<.1msec jitter) to single shock electrical stimuli (0.1-0.5 msec pulses) delivered to the dural surface overlying the ipsilateral transverse sinus. Response latencies were 0.8 - 13 msec, indicative of conduction velocities of approximately 1.2 - 19 m/sec. Dural stimulation sites of lowest threshold (typically 20-100 uA) were always on the ipsilateral transverse sinus or sinus confluens and were surrounded by dural sites that exhibited higher thresholds or were ineffective (>5 mA). A subset of neurons exhibited mechanical sensitivity. Some neurons could be activated by light stroking within a restricted field overlying the transverse sinus, whereas others were activated only by more intense mechanical probing or traction. Further characterization of the neurons' response properties, including possible chemosensitivity, will be important for understanding their role in cranial vascular sensation. (Supported in part by the National Headache Foundation, NINDS (NIH), and Beth Israel Hospital Dept. Anesthesia.)

454.6

MODULATION OF TRIGEMINAL SENSITIVITY TO IRRITANTS T.K. Baumann*[1,2] and M.E. Martenson[1]
Division of Neurosurgery[1] & Department of Pharmacology[2], Oregon Health Sciences University, Portland, OR 97201

Both trigeminal and spinal ganglion neurons show a strong potentiation of responses to the irritant capsaicin in an acidic environment. The present study revealed that there is also a strong interaction between protons and piperine, another vanilloid irritant. We studied the mechanism of the interaction between protons and the vanilloids. Whole-cell patch clamp recordings were performed on cultured adult rat TG neurons clamped near their resting membrane potential (-60 mV). Piperine (10 μM) caused a sustained inward current associated with an increase in membrane conductance. When protons and piperine were co-applied, the membrane currents evoked in piperine-sensitive TG neurons far exceeded the algebraic sum of the responses to the two stimuli applied in isolation. Capsazepine blocked the response of TG neurons to piperine (or capsaicin) at both physiological and acidic pH. In the presence of capsazepine, responses to the mixture of a vanilloid and protons resembled the response to the low pH stimulus applied alone. These findings suggest that protons either increase the availability or affinity of the vanilloid receptor, change the kinetics of the receptor/channel complex or carry a substantial current. (Supported by the National Headache Foundation)

454.7

LECTIN STAINING OF UNMYELINATED AXONS IN VARIOUS SKIN REGIONS OF MALE RATS. J.C. Petruska*, W.J. Streit, and R.D. Johnson. Depts. of Neurosci. & Physiol. Sci., Univ. of Florida, Gainesville, FL 32610.

Characterization of various plant lectins has revealed specific binding to distinct neuronal populations. In peripheral nerves, it has been shown by others that the lectin *Griffonia* (or *Bandeiraea*) *simplicifolia* I-B_4 nearly exclusively labels unmyelinated visceral and somatic afferent fibers and their cell bodies of origin, but also labels myelinated gustatory afferents and a small percentage of sympathetic postganglionic cell bodies. The DRG cells giving rise to unmyelinated peripheral and central processes that are positive for the GS-I-B_4 lectin are nearly all fluoride-resistant acid phosphatase (FRAP, a major sensory neuron marker) positive and also include most C-fibers that are negative for the known neuropeptides (e.g. CGRP, SP, etc.). The purpose of the present study was to anatomically characterize the GS-I-B_4 positive fibers in various types of skin. The specificity of GS-I-B_4 in frozen and paraffin sections was examined in the glabrous and hairy skin of the foot, hairy skin of the scrotum, and the mucocutaneous skin surrounding the external urethral orifice. Both Remak bundles present in small dermal nerve branches and single unmyelinated fibers were clearly stained. Lectin- positive axons coursed through the subepithelial tissue, and in some cases, amongst the epithelial cells. Serial section analysis of GS-I-B_4 and CGRP-IR upheld the findings in peripheral nerves and ganglia that the two markers minimally colocalize. Use of the GS-I-B_4 isolectin in conjunction with markers for other neuronal populations (i.e. autonomic and peptidergic fibers) may enable the specific visualization of C-fiber afferents in various skin targets. Such specific visualization in the target tissue of this population will enable more detailed study of cutaneous C-fiber function, morphology, and plasticity.

454.8

SEPARATE AND OVERLAPPING POPULATIONS OF LUMBAR DORSAL ROOT GANGLION CELLS ARE REVEALED BY RETROGRADE TRANSPORT OF A MIXTURE OF TWO LECTINS. H. Wang, P. Shortland, H. Johnsson* & C. Molander. Dept. of Neuroscience, Karolinska Institutet, S-171 77 Stockholm, Sweden.

The plant lectins soybean agglutinin (SBA) and Griffonia simplicifolia (B4) exibit specific binding to terminal α-D-galactose groups that are expressed on subsets of small sized dorsal root ganglia (DRG) cells (Plenderleith et al., '90; Wang et al., '94). Binding studies indicate that SBA & B4 label the same cell populations (Streit et al., '86) but a review of the literature suggests that the 2 lectins may react differently to peripheral nerve injury. As a first step in identifying the responses of these lectins to injury, the distribution & possible colocalisation of these 2 markers were examined in the L4-5 DRGs in normal adult rats. A mixture of 2-5% B4 & SBA was injected into the sciatic nerve and 3 days later the relevant DRGs and spinal cord segments processed using double labelling immunofluorescent techniques. In the DRGs, cell profiles with a visible nucleus that were immunofluorescent for only SBA or B4 and for those that were double labeled were counted. Preliminary results indicate that approximately twice as many cell profiles were labelled by SBA compared to B4. Of the SBA population, 20.3% were also B4 positive while 36.5% of the B4 population were also SBA immunoreactive. B4 and SBA labelled terminals in the dorsal horn were predominately located in lamina IIi. The present results indicate that SBA and B4 lectins label separate and partially overlapping populations of C fibers and may provide a useful tool for studying the effects of peripheral nerve injury on different subpopulations of C-fibers. Supported by Åke Wibergs stiftelse.

454.9

DESENSITIZATION OF THE CAPSAICIN-ACTIVATED CURRENT IN RAT DORSAL ROOT GANGLION NEURONS. P.A. Koplas*, G.S. Oxford, and R.L. Rosenberg. Curriculum in Neurobiology, University of North Carolina, Chapel Hill, NC 27599.

Capsaicin (8-methyl N-vanillyl 6-nonenamide) has proven to be a valuable tool in the study of primary sensory afferents. This pungent component of the *Capsicum* pepper family activates a non-selective cation channel in nociceptive neurons. The whole-cell patch clamp technique was used to investigate the desensitization of capsaicin-activated currents in cultured neonatal rat dorsal root ganglion (DRG) neurons. Ion substitution experiments demonstrated calcium permeation through the capsaicin-activated channels, with a calculated P_{Ca}/P_{Na} of 4.0. Barium is less permeant in comparison to calcium, and magnesium supports relatively little current through these channels. The following two types of desensitization behavior were examined: (1) **acute desensitization**, the current inactivation occuring during a prolonged capsaicin exposure; and, (2) **tachyphylaxis**, the reduction in the amplitude of current responses for repeated capsaicin applications. Both categories of desensitization were dependent on the external calcium concentration and degree of internal calcium chelation, and insensitive to changes in capsaicin concentration. Barium supported both acute desensitization and tachyphylaxis, but to a lesser extent than that observed for calcium. The manipulation of intracellular nucleotides indicated acute desensitization was not dependent on ATP and GTP, whereas the degree of tachyphylaxis was enhanced by the exclusion of ATP from the internal solution. Overall, these results provide a detailed characterization of the regulation of capsaicin responses by desensitization processes in cultured DRG neurons.

454.10

CHARACTERIZATION OF CAPSAICIN RESPONSES IN INTACT RAT DORSAL ROOT GANGLION NEURONS. G.S. Oxford*, P.A. Koplas,and R.L. Rosenberg. Curriculum in Neurobiology, University of North Carolina, Chapel Hill, NC 27599.

Capsaicin is the pungent component of the *Capsicum* pepper family which activates a non-selective cation channel in nociceptive neurons. The two techniques of intracellular calcium imaging and nystatin perforated-patch clamp recording were used to characterize capsaicin responses in intact cultured neonatal rat dorsal root ganglion (DRG) neurons. For the calcium imaging experiments, DRG neurons were loaded with the fluorescent dye fluo-3 AM (5 μM). Capsaicin (0.03-1 μM) caused an increase in the internal calcium signal, which was dependent on the presence of external calcium. The calcium signals produced by capsaicin and caffeine (10 mM) were additive, suggesting that capsaicin stimulation does not directly lead to calcium release from internal caffeine-sensitive stores. The addition of external cadmium to block voltage-gated calcium channels did not prevent the increase in internal calcium, suggesting that calcium can enter the neurons through capsaicin-gated channels. In the nystatin perforated-patch experiments, the desensitization of capsaicin responses was not significantly different from the whole-cell behavior, indicating that desensitization processes are not compromised by whole-cell dialysis of the intracellular environment. (Supported by NIH NS18788 and HL49449).

454.11

PHOSPHOLIPASE A_2 SENSITIVITY OF THE DORSAL ROOT AND DORSAL ROOT GANGLION. AC OZAKTAY*, S KALLAKURI, JM CAVANAUGH. Bioengineering Center, Wayne State University, Detroit, MI 48202, U.S.A.

The aim of this study was to investigate the electrophysiological effects of phospholipase A_2 (PLA_2) applied to lumbar dorsal roots and dorsal root ganglia (DRG). **METHODS:** Rabbits (3-4 kg, male) were anesthetized and an L5-L7 laminectomy was performed. Recordings were made from split L6 or L7 dorsal roots using dual bipolar electrodes. Latency between the electrodes was used to determine conduction velocity (CV). PLA_2 (150-450 U, *naja naja* venom, Sigma) in a buffered carrier solution (100 μl, pH 7.5) was then applied to the dorsal roots and DRG. The spontaneous discharge rates (SDRs) were recorded on analog tape for later computer analysis. **RESULTS:** *Multi-unit:* SDR decreased within 10 min for 6 of 10 PLA_2 applications. In 2 applications (200 U of PLA_2), SDR showed either frequent bursting or a steady increase over 20 min. *Single units:* The most prominent findings were in the *silent units*. They (n=8) were activated after PLA_2 application and had a peak SDR after 15-20 min. *Group II units* (n=3) disappeared within 10 min. In one of the 4 *group III units* SDR increased and stayed stable over 25 min while the other 3 showed a decrease within 10 min of PLA_2 application. These results suggest chemosensitivity of dorsal roots and DRG to PLA_2. Supported by NIH Grant AR41739 (JMC).

454.12

BRADYKININ SENSITIZES RAT CUTANEOUS C-FIBRE POLYMODAL NOCICEPTORS TO INTERLEUKIN-2. H.A. Martin*. Div. of Neurobiology, University School of Medicine, Newcastle upon Tyne NE2 4HH, U.K.

We have recently reported that interleukin-2 (IL-2) activates a third of rat cutaneous polymodal nociceptors (Martin & Murphy, *Arch. Physiol. Biochem.* **103**, 1995). These nociceptors were subsequently activated by histamine, bradykinin (BK) and topical acetic acid (AA), respectively in this order. However, the cross-reactivity between agents was not assessed due to experimental limitation. Curiously, we found in preliminary studies that units tested with BK (75ng/3μl) responded better to subsequent IL-2 (0.06U/3μl). This observation needed further investigation.

Nociceptors were isolated in an *in vivo* rat saphenous nerve preparation (Martin *et al.*, *Neurosci.* **22**, 1987) and characterized by their responsiveness to mechanical, thermal heat and chemical stimuli (rat IL-2: 1.2 U/3μl; BK: 75 ng/3μl; AA; Sigma). IL-2 and BK were injected (3μl) into individual receptive fields. Two series of experiments were conducted: 1°) Two IL-2 injections preceded BK. Then, two other IL-2 injections followed BK; 2°) BK was injected first and two IL-2 injections followed later. Injections were separated by 10 min when units were silent. Data were analyzed using Spike2 and statistical significance was assessed in a paired-sample *t*-test. Mean response of the first IL-2 injection equals 100% (reference).

In the first series of experiments, mean response to the 2nd IL-2 (prior to BK) decreased to 55% of the reference level. After BK, mean response to 3rd and 4th IL-2 increased to 155% of reference ($p < 0.02$). In the second series, vigorous IL-2 responses were sustained without tachyphylaxis.

In conclusion, BK sensitizes cutaneous polymodal nociceptors to IL-2, which may explain the occurrence of pruritus in inflamed skin.

454.13

SPONTANEOUS FIRING IN ISOLOATED RAT DORSAL ROOT GANGLION NEURONS FROM RATS WITH A PAINFUL NEUROPATHY. R. E. Study*, M.G. Kral. Pain Research Group, Dept. of Anesthesia, Brigham & Women's Hospital, Boston,MA,02115

Neuropathic pain from nerve injury has been associated with spontaneous action potential(AP) activity in primary afferents, which may be involved in the induction and maintenance of this condition. We show here that such spontaneous activity is maintained in DRG neuron somata after isolation and AP threshold is lower.

A neuropathic pain state was induced in male Sprague-Dawley rats by chronic constriction injury(CCI), applying four loose ligatures to the sciatic nerve as described by Bennett and Xie(*Pain* 33:87-107, 1988). DRG of L4 and L5 were removed from anesthetized animals 3-21 days postop. Neurons were dissociated enzymatically and cells were then plated to culture dishes and used within 6 hours. Recordings were done using whole cell patch clamp under current clamp conditions.

Results show a 20% incidence of spontaneous activity in CCI neurons in comparison to a 3% incidence in controls. This difference is significant($P<0.01$ with Fischers exact test). These results are maintained over the several hours of the cell prep. Spontaneously firing cells exhibited two types of electrical activity: a regular rapid firing and random firing of single or short bursts of 2-4 APs. Firing was often triggered by spontaneous membrane potential fluctuations. The AP threshold for spontaneously active cells was -34.5 ± 2.7mV compared to -18.7 ± 1.5 mV for quiescent CCI neurons and -20.5 ± 2.2mV for control cells. The threshold for spontaneously active cells is significantly lower in comparison to both quiescent CCI and control cells ($P<0.001$ with students t-test). The basis of this lower threshold is unknown, but it may be the cause of the higher incidence of spontaneous firing in these neurons.

454.14

THE ROLE OF NON-SPECIFIC CATION CHANNELS IN COLD TRANSDUCTION

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The molecular and cellular processes involved in cold transduction remain unknown due to the small size, and restricted accessibility of cold fiber terminals. The goal of this study was to investigate the role of Na^+, K^+, Ca^{2+}, non-specific cation channels, as well as Na-K ATPase, in cold transduction. Single unit extracellular recordings were obtained from 59 corneal cold nociceptors in 26 rabbits. The adapting temperature was 35°C in all experiments. Thermal responsiveness for each fiber was characterized with a -5°C temperature ramp (-1°C/sec). Pharmacological channel blockers (uM), diltiazem (10), 4-aminopyridine (10), quinine (100), glibenclamide (10), TEA (20), Cs+ (2), ouabain (10), Ni/Cd (50 & 200), quinidine (100), apamin (10), were then applied to the terminal field of the recorded cold receptor. Neural activity was characterized by 5 parameters: tonic firing rate, cold evoked maximum action potential (AP) frequency, evoked adapted AP frequency, and rate of neural activation and adaptation. Our previous work identified the rate of neural activation as being the best reflection of the dynamic cold transduction process. The dynamic cold response was attenuated by all compounds that block nonspecific cation channels (diltiazem, 4-AP, and quinine). Tonic cold fiber activity was increased by all compounds tested and known to cause neural depolarization (TEA, Cs^+, ouabain, and glibenclamide). Nonspecific depression of all cold fiber activity was seen when using a Na^+ channel blocking concentration of Ni/Cd and quinidine. Ni/Cd (50 uM), which blocks all voltage dependent Ca^{2+} channels, and apamin, had no effect upon cold fiber activity. The results of this study implicate a non-specific cation channel in the transduction process of dynamic cooling.

Work supported by the NIH NS28646 and the Sid W. Richardson Foundation.

454.15

PSYCHOPHYSICAL MEASURES OF COLD PAIN SENSATION IN HUMANS: COMPARISON WITH EVOKED RESPONSES OF PRIMARY AFFERENT NOCICEPTORS. D.A. Simone*[1], J. Li[1], E.R. Stevens[1], B. Allen[1] and K.C. Kajander[2]. Depts. of [1]Psychiatry and [2]Oral Science, University of Minnesota, Minneapolis MN 55455.

Psychophysical characteristics and the underlying neural mechanisms of pain sensation evoked by cold temperatures below 0° C have not been thoroughly investigated. In the present study, comparisons were made between psychophysical measures of cold pain sensation in humans and evoked activity of primary afferent nociceptors in monkeys. Six subjects judged the magnitude and quality of cold pain sensation evoked by stimulus temperatures between 20° C and -12° C. Stimuli were delivered in descending order of 2° increments to the volar forearm via a 1 cm^2 Peltier stimulator. Each stimulus was of 5 sec duration and applied every 3 min. Thresholds for cold pain were > 10° C for 5 of 6 subjects. Using regression analysis, in 4 of 6 subjects, pain produced by stimuli above -2° C increased at a significantly slower rate that the pain evoked by temperatures below -2° C. The quality of cold sensation changed dramatically at temperatures below 0° C, from one of dull aching to one of sharp, pricking pain. In correlative electrophysiological studies, thresholds of all C nociceptors ($n=20$) were $\geq$ -2° C, whereas thresholds of 12 of 24 Aδ nociceptors were $\leq$ -2° C. These data suggest that C and some Aδ nociceptors contribute to cold pain sensation at stimulus temperatures above -2° C. The sharp pricking sensation evoked by stimulus temperatures below -2° C is likely to be mediated by the recruitment of additional Aδ nociceptors.

454.16

ACTIVITY-DEPENDENT VARIATIONS IN CONDUCTION VELOCITY OF A-DELTA FIBERS OF RAT SCIATIC NERVE S.C. Nam[1*], C.K. Won[2], M.Y. Park[2], H.S. Park[2], and H.C. Shin[2] Dept. of Oral Physiology, Coll. of Dentistry, Seoul National Univ., Seoul & Dept. of Physiology[2], Coll. of Med., Hallym Univ. Chunchon, Korea

Previously, we have demonstrated different profiles of the activity-dependent changes in conduction velocity (CV) of C-fibers of rat sciatic nerve. In the present study, changes of the CV and subsequent conduction block (CB) were characterized following impulse activity (0.5, 1.0, 5.0, 10.0, 25.0, 50.0, 100.0 and 200.0 Hz, 1 min) in single A-delta fibers (n=19, range of resting CVs: 2.15 - 18.64 m/sec, at 0.5 Hz) of isolated rat sciatic nerves. A-delta fibers which had similar resting CVs often exhibited quite different profiles of the activity-dependent latency change and/or CB following impulses. Although A-delta fibers showed only small (1.17 ± 0.5%) decrease of CV at the lowest activity rate (1.0 Hz), they exhibited much extensive increase of latencies at higher activity states (10.0 Hz: 11.54 ± 2.9%, 25.0 Hz: 28.03 ± 6.1%, 50.0 Hz: 22.81 ± 4.8%, 100.0 Hz: 23.38 ± 5.0%). The frequency of CB increased dramatically as the activity rate rose (5.0 Hz: 10.5%, 10.0 Hz: 21.1%, 25.0 Hz: 31.6%, 50.0 Hz: 36.8%, 100.0 Hz: 68.4% and 200 Hz: 89.5%). Five of 19 fibers showed intermittant CB prior to the occurrence of the complete block, whereas 14 of 19 fibers exhibited direct CB without showing intermittant firing activities. These results imply underlying variation among A-delta fibers in the activity-dependent excitability changes, especially in the buildup and recovery of the hypoexcitable phases (supported by KOSEF 94-0403-15-02-3).

454.17

MECHANICAL RECEPTIVE FIELD STRUCTURE FOR CUTANEOUS C- AND A-FIBER NOCICEPTORS IN THE MONKEY DETERMINED WITH GRADED INTENSITY STIMULI. R.M.Slugg*, R.A.Meyer, and J.N.Campbell. School of Medicine, Johns Hopkins Univ., Balto., MD 21287

Receptive field (RF) maps have traditionally been determined with hand-held von Frey probes. With use of a new, computer-controlled mechanical stimulator system, we are now able to obtain high-resolution, quantitative maps of nociceptor response at different stimulus intensities. Standard teased fiber techniques were used to record from 18 C-fiber and 7 A-fiber nociceptors that innervate hairy skin in the anesthetized monkey. Force-controlled stimuli (2-64 gm, 1 s) were delivered to the skin via thin (100 μm) blade-shaped probes. The stimuli were delivered in 100 μm increments along a line through the middle of the RF and orthogonal to the blade. Blade-shaped probes were chosen to activate the whole RF in one dimension (along the length of the blade) and to permit high spatial resolution maps in the other dimension (perpendicular to the blade). This allows functional mapping of two dimensional structures from data obtained in one dimension. The one-dimensional RF maps were obtained by plotting the response as a function of probe location. Various RF architectures were observed including 1) multiple, fine, punctate structures with dimensions less than 200 μm wide, 2) several broad, rounded structures with dimensions greater than 500 μm, and 3) combinations of these. As stimulus intensity increased, the response at the peaks increased, the punctate structures became broader, and the valleys between adjacent peaks became less distinct. The RF architecture was reproducible with repeated mapping. This technique will allow investigations of alterations in RF architecture that follow cutaneous injury.

454.18

PLASMA EXTRAVASATION IS REDUCED ON THE PLANTAR SURFACE OF THE RAT HIND PAW FOLLOWING LIGATION OF THE L5 AND L6 SPINAL NERVES Z. Ali[1], M. Ringkamp[2], R. A. Meyer[1,3]* and S. N. Raja[2] Dept. of Neurosurgery[1], Anesthesiology[2], Applied Physics Lab.[3], Johns Hopkins University, Baltimore, MD 21287

Ligation of spinal nerves L5 & L6 leads to behavioral signs of hyperalgesia on the plantar surface of the rat hind paw. In this study plasma extravasation was used to examine C-fiber innervation of the paw following spinal nerve ligation. Spinal nerves L5 and L6 were ligated in 8 male Sprague-Dawley rats. Three weeks after surgery the rats were anaesthetized with pentobarbital, paralysed and artificially ventilated. Both sciatic nerves were exposed and cut at the sciatic notch. An intravenous bolus of Evans blue (50 mg/kg) was administered 5 min before both sciatic nerves were stimulated (80 V, 1 ms, 5 Hz) for 10 min. The rats were perfused and fixed with formalin. Plasma extravasation was markedly reduced in the paw ipsilateral to the nerve ligations when compared to the equivalent paw of 8 concurrent, non-lesioned control rats. Within the L5 dermatome which covers the lateral aspect of the foot, extravasation was almost abolished on both dorsal and plantar surfaces. Surprisingly, extravasation also appeared to be abolished on the plantar surface in the region of the footpads, an area predominantly innervated by the non-lesioned, L4 spinal nerve. In contrast, plasma extravasation in the L4 distribution on the dorsum of the foot was not decreased. The decreased extravasation near the footpads may be explained by: 1) a change in function of nociceptors in the L4 spinal nerve, 2) a substantial denervation of the footpad region by the L5 lesion, or 3) an enhanced vasoconstriction associated with stimulation of sympathetic fibers in the sciatic nerve.

454.19

TIME COURSE OF FORMALIN-INDUCED RESPONSE OF C-FIBERS IN THE SAPHENOUS NERVE OF RAT. W.D. McCall, Jr.*, Kimberly D. Tanner, and Jon D. Levine SUNY Buffalo & UCSF.

While the formalin test is a widely used behavioral model of tonic chemogenic pain and is well characterized, little is known about the response of peripheral afferent fibers to formalin.

Single C-fiber primary afferents were isolated from the saphenous nerve of pentobarbital-anesthetized rats. C-fibers were identified by having conduction velocities less than 2 m/s and a positive collision test. Formalin (2.5 %, 50 µl) was injected in the vicinity of the fiber's mechanical receptive field. Activity of the fiber was recorded from 10 minutes prior to the injection to 60 minutes after the injection. Action potentials were counted in 3-minute bins.

Following formalin injection, the average number of spikes per bin in C-fibers (n=26) showed two peaks. The first peak was 70.5 ± 28.5 (mean ± s.e.m.) spikes per bin at 3 min, which declined to pre-injection levels at 9 to 12 min, then rose gradually to a second peak of 39.0 ± 23.1 at 48 min. The average of 8 saline-injected C-fibers showed no significant increase of activity.

The time course of the response to formalin of C-fiber afferents is similar to that of the behavioral response, is not explained by the injection of vehicle, and suggests that C-fiber activity could contribute to the behavioral response in phase 2 as well as phase 1 of the formalin test.

SOMATIC AND VISCERAL AFFERENTS: MECHANORECEPTORS

455.1

MECHANICAL STATES ENCODED BY JOINT CAPSULE AFFERENTS. P. S. Khalsa*, and P. Grigg, Phys. Dept., Univ. Mass. Med. Sch., Worcester, MA 01655

Joint capsule afferents from the cat knee posterior capsule were studied for their sensitivity to in-plane stretch. The posterior joint capsule was excised from the knee and subjected to stretching *in vitro*. Forces were applied through tabs along the edges (3 tabs / edge) of the capsule (Fig. 1), using an apparatus that allowed for independent control of each load on each tab. By appropriately loading the tissue margins, it was possible to establish states of uniaxial and biaxial tension, as well as shear. Plane stresses were calculated from the loads along the tissue margins. Strains were calculated from deformations of the capsule measured by tracking markers on its surface. Full characterization of tissue stresses and strains made it possible to determine strain energy density and the magnitudes of other coordinate invariant mechanical states. Single afferents were recorded from filaments of the posterior articular nerve. All afferents had conduction velocities in the group 2 or group 3 range. Individual afferents exhibited pronounced directional sensitivity. There was no overall preferred orientation. Linear multiple regression of the combined stress and strain components on the neuronal response revealed high correlation (R = 0.84), indicating that the measured mechanical states strongly determine neuronal response. Correlation analysis between neuronal discharge and individual stress or strain components revealed no consistent relationship between neuronal response and any single tensor component. However, there was a much stronger relationship between neuronal discharge and stress variables than with strain variables. The strongest relationship was observed between neuronal response and the first invariant of the stress tensor. Supported by NIH grants NS-10783, 5-F32-AR08225, and FCER grant 92-6-2.

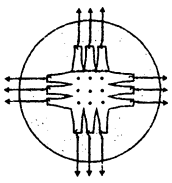

Figure 1

455.2

MECHANICAL TUNING OF THE PACINIAN CORPUSCLE'S ACCESSORY CAPSULE AND ITS RELATIONSHIP TO TRANSDUCTION MECHANISMS. B. Pietras* and S.J. Bolanowski, Institute for Sensory Research and Department of Bioengineering and Neuroscience, Syracuse University, Syracuse, New York 13244.

The magnitude of receptor potentials recorded from isolated Pacinian corpuscles in response to sinusoidal vibrations display a U-shaped frequency response curve, centered around 300 Hz. The low frequency portion of this curve can be partially explained by the mechanical properties of the accessory capsule, while the high-frequency portion of the curve it thought to be due to transduction channel kinetics. To assess the mechanical contribution of the accessory capsule to the Pacinian corpuscle's frequency response, individual Pacinian corpuscles were mechanically stimulated with a cylindrical probe (diameter ≈ 250 µm) statically, and with sinusoidal vibrations ranging from 1 to 900 Hz, allowing for video analysis of the lamellar motion in response to the mechanical stimuli. It was found that the lamellae motion had a band-pass characteristic whereby the peak-to-peak change of the interlamellar spacing as a function of frequency displayed a U-shaped function analogous to the physiological frequency response. Thus the mechanical properties of the accessory capsule contribute not only to the low-frequency portion of the physiological frequency response, but also to the high-frequency portion. It is possible that the Pacinian corpuscle's reduced sensitivity at high frquency is due to the mechanics of the capsule and not transduction channel kinetics. Work supported by NSF, IBN-9211561.

455.3

QUANTITATIVE CHARACTERIZATION OF RAPIDLY ADAPTING CORNEAL MECHANO-RECPETORS.

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Measurement of force thresholds in intact vertebrate mechanoreceptors is complicated by their surrounding tissue medium. This situation is greatly simplified in the cornea where the mechanoreceptors are embedded in a homogenous, avascular non-keratinized epithelium. Single unit extracellular recordings were obtained from 7 corneal mechano-sensitive nociceptors from 5 rabbits. A newly developed polysilicon, piezo resistive, force sensing microprobe was used to determine the sensitivity of these fibers. The microprobe (25 uM width) was force-feedback closed-loop controlled and allowed independent control of the duration, force, and rate of force of the stimulation probe. Cornea mechano-sensitive fibers displayed characteristics common with other rapidly adapting mechano-receptors. The fibers are normally silent, but in response to a rapidly applied force will display a dynamic response followed by a tonic response which lasts up to 4 sec. In general, the greater the magnitude and rate of change of the stimulating force the greater the number of evoked action potentials, and the longer the duration of the sustained tonic response. Short intertrial intervals (< 20 sec) with repeat stimulation leads to a decrement in response. Ascending force trials, over the range of 0 - 1.25 mN, in steps of .05 mN (100 mN/sec, 4 sec duration and 20 sec inter trial interval), were used to determine that the response threshold of these mechanoreceptors was 0.1 mN +/- .09 mN. This threshold is significantly lower than previously reported for any population of cutaneous mechanosensitive fibers, including the cornea, which was previously estimated with von Frey Hairs to be 0.64 to 1.2 mN. An "off response" appeared in some mechanoreceptors after the sustained application of forces > 1mN. This corneal mechanoreceptor preparation will allow further quantitative studies of mechanical transduction mechanisms.

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455.4

PERIPHERAL NEURAL REPRESENTATION OF THE SHAPES AND ORIENTATIONS OF THREE-DIMENSIONAL OBJECTS STROKED ACROSS THE MONKEY FINGERPAD R.H. LaMotte*, C. Lu and M.A. Srinivasan, Dept. of Anesthesiology, Yale University School of Medicine, New Haven, CT 06510 and Research Laboratory of Electronics, MIT, Cambridge, MA 02139

The neural representation of object shape and orientation was studied in responses of rapidly and slowly adapting mechanoreceptive primary afferent fibers (SAs and RAs) to ellipsoidally shaped objects stroked across the fingerpad of the anesthetized monkey. Each ellipsoid, 0.5 mm high and mounted on a flat plate, had a radius of 5 mm on the major axis and 1, 3 or 5 mm on the minor. A servocontrolled stimulator stroked the object back and forth across the skin in the horizontal plane (x,y) along a series of laterally shifted parallel linear trajectories while maintaining a contact force of 5, 10 or 20 g-wt. Orientation of the object and direction and velocity of stroking were varied. 3-D "Spatial event plots" (SEPs) of impulses/0.2 mm bin plotted as height over object position (x,y) were interpreted as instantaneous, spatially distributed responses of a population of fibers. The contour of the base of the SEPs of RAs and SAs encoded the 2-D outline of the object in contact with the skin. The orientation of the outline, determined by a principal component analysis, was closely related to the physical orientation of the object on the skin.

An average of 5 central, vertical cross-sections through the SEP parallel to the minor axes of the ellipsoids revealed a contour that was related non-isomorphically to object shape. Peak height of this contour (maximum spatial discharge rate) increased and width of its base (extent of receptor activity in the skin) decreased with increasing object eccentricity for both RAs and SAs. Peak height also changed with changes in contact force, object orientation, and stroke velocity and direction. When peak heights were normalized, object curvature was correlated with the rate of decline of the falling phase in the response profiles for 11 of 16 SAs, but not for the 11 RAs tested. The spatial response measures of shape were more resistant than the intensive measures (discharge rate) to changes in contact force and the direction and velocity of stroking. Supported by NIH grant NS15888 and ONR grant N00014-92-J-1814.

455.5

PREDICTING THE RESPONSE OF A POPULATION OF MECHANORECEPTORS USING 3D FINITE ELEMENT MODELS OF PRIMATE FINGER TIPS.
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Geometrically accurate 3-dimensional finite element models of human and monkey finger tips were developed based on data obtained using a video-microscopy system. The differences in the mechanical properties of various layers of the skin, the adipose tissues and the bone was accounted for by modeling the fingertip in layers such that each layer could be assigned distinct material properties. To accurately predict the experimentally observed *in vivo* surface deformations of human and monkey finger tips under a line load, at least three distinct layers were found to be necessary. The epidermis and dermis were represented by a stiff outer layer, the adipose tissue was modeled by a relatively compliant intermediate layer, and the bone was modeled as the rigid innermost layer. This model also predicted very well the experimentally observed *in vivo* surface deformations of human finger tips under cylindrical indentors of various radii.

The stresses and strains at typical receptor locations calculated from the models were correlated with electrophysiologically recorded responses of slowly adapting (SA-I) afferents in monkeys to indentations by rectangular bars, cylinders, and sinusoidal step shapes. The strain energy density and the maximum compressive strain at the receptor locations were found to be directly related to the experimentally recorded neural response. Thus, the predictions of the finite element models matched the data from both biomechanical and neurophysiological experiments. The spatial response profile, obtained from the neural data recorded from single afferent nerve fibers, was found to be significantly different from the population response predicted by the model, owing to the differences in the location of the receptors in the population. These empirically validated models can also be used for hypothesizing how the CNS might infer object shape from tactile neural input. (Supported by NIH Grant NS33778, ONR URI Grant N00014-92-J-1814, and Pittsburgh Supercomputing Center Grant MSS940007P)

455.6

SPATIAL SUMMATION OF THRESHOLDS FOR PERCEIVED PRESSURE, SHARPNESS, AND MECHANICALLY EVOKED CUTANEOUS PAIN.
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Psychophysically, spatial summation can be defined as a decrease in threshold accompanying an increased area of stimulation. The present study examined to what extent different mechanically evoked percepts (pressure, sharpness, and pain) demonstrate spatial summation. Various probes were used to apply prescribed forces to the dorsal surface of the digits of 19 healthy subjects. Some tests used a single probe (circular contact area of 0.05 or 0.1mm^2), while other tests used two probes of the same size, separated by 5mm. This distance provided for stimulation of two largely separate sets of receptors, yet was perceived as a single stimulation site.

The three perceptual qualities showed differing degrees of spatial summation: sharpness averaged 18% summation, pain averaged 46%, while pressure averaged 76%. The small degree of spatial summation for sharpness threshold is consistent with the theory that perceived sharpness is based on near threshold activity of a small number of nociceptors. The modest amount of spatial summation for pain suggests that distinctly suprathreshold activation of nociceptors is required for mechanically evoked pain perception. However, spatial summation for nociceptors appears to be less than that for slowly adapting mechanoreceptors, as evidenced by the greater spatial summation observed for pressure vs. pain thresholds. (Supported by NIH grant NS-28559).

455.7

IDENTIFYING SENSORY RECEPTORS FROM SOMAL SPIKES USING NEURAL NETS AND DISCRIMINATE ANALYSIS.
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Retrospective analysis of somal electrophysiology from intracellularly recorded, physiologically identified cat DRG somata demonstrates that both neural nets and discriminate analysis (DA) provide adequate tools for specifying peripheral receptor type from measurable parameters of individual somal spikes. Specifically, somata could be identified as innervating muscle spindles, hairs or high threshold mechanoreceptors (HTMRs). 156 units were selected and analyzed from archived, parametized DC records. No units were excluded as outliers. Indeed, original data have not been examined for this study; rather, data were culled from a database for all units with spikes ≥60mV initial rising phase and entries for at least 6 of the following 12 parameters: spike duration, risetime, falltime, half amplitude duration, half amplitude after hyperpolarization duration, spike amplitude, overshoot amplitude, after hyperpolarization amplitude, resting membrane potential, peripheral fiber conduction velocity (PFCV), convexity of shoulder on spike depolarization phase, and convexity of shoulder on repolarization phase.

Conventional analysis of these same spike parameters showed statistical differences among afferent types (ANOVA, corrections for multiple comparisons, Rose 1986) but was of little predictive value. Our back propagation neural net with 12 input nodes, 10 nodes in one hidden layer and 4 output nodes, and trained with 1% random noise added to each input during training, readily reached our criterion of 100% correct identification using a pseudo jack-knife procedure. Moreover, hairs and HTMRs could be segregated into Down, G1 & G2, and Aβ, Aδ & C, respectively, even when PFCV was omitted. DA did as well, but also indicated that almost equal results could be obtained with but 4 parameters. DA has the additional feature of providing the relative value/contribution of each parameter in the analysis.

Coupling these mathematical tools with computer controlled data acquisition should greatly improve efficiency of animal use and investigators.

455.8

MECHANORECEPTOR ACTIVITY RECORDED FROM THE HUMAN LINGUAL NERVE. M. Trulsson; G. Essick*, S. Vines and J. Zuniga. Dental Research Center, Univ. of North Carolina, Chapel Hill, NC 27599-7455.

The human tongue is exquisitely sensitive to tactile stimulation. Very little, however, is known about the peripheral neural mechanisms underlying lingual sensation. The present study sought to explore the mechanoreceptive innervation of the tip of the tongue. The lingual nerve was approached intraorally using a microneurographic technique similar to that employed to study the human inferior alveolar nerve (Trulsson et al., *J Physiol* 447:373-389, 1992). Tactile stimulation of the lingual mucosa was provided by blunt probes and von Frey hairs mounted to a force transducer. To date, 6 healthy young adult subjects have been studied.

The innervation territories of 9 nerve fascicles were mapped. The fields always included the tip of the tongue and extended at least 3 cm posteriorally. No field extended more than 1-2 mm across the midline.

Twelve single mechanoreceptive afferents were isolated and classified. Each exhibited an extremely small, well defined receptive field (RF; median = 2 mm^2; range = 1 - 20 mm^2) and responded to very low forces (median threshold = 0.19 mN; range = 0.03 - 2 mN). Seven of the 12 afferents adapted rapidly to suprathreshold forces applied to the RF. Responses at both the onset and offset of sustained indentation were observed. Since no afferent was sensitive to tapping stimuli applied remote to the RF, all seven resembled the fast adapting type I afferents supplying the glabrous skin of the human hand. Five of the 12 afferents adapted slowly to suprathreshold forces applied to the RF. Two of these afferents exhibited irregular responses to sustained indentation and were particularly sensitive to changes in deformation of the mucosa. Three afferents exhibited highly regular responses and were spontaneously active. As such, these two groups correspond to the type I and II slowly adapting afferents of the hand. Supported by the Swedish Medical Research Council (grants no. 8667, 11087) and NIH (grants no. DE10141, DE07509).

455.9

RESPONSIVENESS OF MESENCEPHALIC TRIGEMINAL NUCLEUS NEURONS TO EXCITATORY AMINO ACIDS.
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The central location of the mesencephalic trigeminal nucleus (MeV) makes the cell somas of the primary sensory afferents that compose this nucleus accessible targets for modulation of sensory input by fibres projecting from other parts of the central nervous system. In fact, modulation of the activity of MeV neurons has been reported in response to fictive mastication, although the modulatory mechanism was undetermined (Kolta et al. (1990) J. Neurophysiol. 64, 1067-1076). Immunocytochemistry studies have revealed a prominent innervation of MeV cell bodies by nerve terminals containing a variety of putative neurotransmitters including, but not limited to, histamine, dopamine, serotonin, noradrenaline, and adenosine. However, when tested electrophysiologically none of these putative neurotransmitters have elicited changes in the activity of MeV cells.

We have performed intracellular recordings from MeV neurons in a rat brain slice preparation to determine if exogenously applied excitatory amino acids (EAAs) can alter the membrane properties of these cells. The recorded neurons were found to exhibit glutamate responsiveness that was composed of both an N-methyl-D-aspartate (NMDA) component and a non-NMDA component. The NMDA-mediated response consisted of a slowly rising depolarization that frequently reached threshold for action potential generation. Furthermore, this response displayed typical Mg^{2+} sensitivity and could be abolished by the potent and selective NMDA antagonist 3-((R)-2-carboxypiperazin-4-yl)-propyl-1-phosphonate (CPP). The non-NMDA component of the glutamate response could be antagonized by 6-cyano-7-nitroqinoxaline-2,3-dione (CNQX). Kainate (KA) application caused a slow depolarizing response that was CNQX sensitive suggesting that the non-NMDA component of the glutamate response is , at least in part, mediated by the KA subtype of glutamate receptor. This report documents for the first time responsiveness of MeV neurons to EAAs and may provide insight into the mechanisms responsible for modulation of their activity. (Supported by MRC Canada)

456.1

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

We have shown in rats that lesions of Vc, an important brainstem relay of orofacial nociceptive information, interfere with the increases of electromyographic (EMG) activity in digastric (DIG) and masseter (MASS) muscles reflexly evoked by injection of mustard oil into the temporomandibular joint region. This study was initiated to test whether microstimulation of Vc produces excitatory effects on DIG and MASS. In 8 male Sprague-Dawley rats (250-350 g) anaesthetized with halothane/N_2O/O_2, microstimulation (1-ms duration train of three 50-μs cathodal pulses; <1 mA; train frequency 0.3 Hz) was applied via a microelectrode (0.2-3 MΩ at 1kHz) introduced into the exposed caudal brainstem at 4 rostrocaudal planes (0.5 mm rostral, and 1.0, 2.5 and 4.0 mm caudal to obex); stimuli were delivered at different depths within medial and lateral Vc and adjacent reticular formation. EMG activity was recorded from ipsilateral DIG and MASS for measurement of incidence, threshold and latency of evoked EMG activity. EMG activity in DIG and MASS could be readily evoked in all animals , with the lowest threshold sites (mean$\pm$S.D., 153.1$\pm$63.1 μA for DIG and 157.8$\pm$59.0 μA for MASS) being superficial loci within the medial portion of Vc in the most caudal plane. The threshold for evoking EMG activity in both DIG and MASS gradually decreased from rostral to caudal planes and was significantly lower (i) in the most caudal plane than the more rostral planes (Repeated-measures ANOVA, $p<0.001$), (ii) in medial Vc than lateral Vc or reticular formation ($p<0.01$), and (iii) in superficial layers than deep layers of Vc ($p<0.001$). The shortest latency of EMG activity evoked by 3xT stimulation was also significantly lower in the most caudal plane (4.43$\pm$0.81 ms for DIG and 4.8$\pm$0.89 ms for MASS) than the most rostral plane (7.18$\pm$2.68 ms for DIG and 8.28$\pm$3.31 ms for MASS, $p<0.05$). These findings indicate that Vc may produce excitatory effects on jaw muscles, and the latency values suggest that these effects involve multisynaptic or slowly conducting pathways from Vc to DIG and MASS motoneurons. Supported by Canadian MRC MT12286.

456.2

DYNORPHIN-IMMUNOREACTIVITY IS INCREASED IN THE SPINAL TRIGEMINAL NUCLEUS IN AN ANIMAL MODEL OF OROFACIAL INFLAMMATION. Y.-O. Lee[1]* and A. J. Beitz[2]. Graduate Programs in Oral Biology[1] and Neuroscience[2], University of Minnesota, St. Paul, MN 55108.

We have utilized an animal model of orofacial inflammation (Haas *et al.*, 1992) to assess possible changes in dynorphin immunostaining in the spinal trigeminal nucleus (STN). Inflammation of the temporomandibular joint (TMJ) was induced by paraperiosteal injection of mustard oil (30 μl of 20 % allyl isothiocyanate in mineral oil; Aldrich Chemicals Co.; Milwaukee, WI) into the periarticular TMJ tissue of the anesthetized rat.

Twelve animals were randomly assigned into one of the following three groups after initial baseline testing for mechanical hyperalgesia using von Frey hairs (Vos *et al.*, 1994): 1) mustard oil (MO) injection group, 2) saline (SAL) injection group, and 3) a control group not subjected to TMJ injection. Animals were tested for mechanical hyperalgesia at one, three, five, and seven days post-injection. Following behavioral testing on day seven, animals were perfused transcardially with 4 % paraformaldehyde and subsequently processed for immunocytochemical staining using an antiserum against dynorphin A(1-17) (Peninsula; Belmont, CA).

Animals with MO injection into TMJ demonstrated behavioral signs of mechanical hyperalgesia at all post-injection testing timepoints as determined by larger difference scores compared to the SAL-injected group or the control group. A difference score was obtained through subtraction of the contralateral response score (non-injected side) from the ipsilateral response score (injected side). In the MO injection group, there was a significant increase in the number of dynorphin-immunoreactive (DYN-IR) cell bodies in the ipsilateral subnucleus caudalis of the STN where primary afferents from the inflamed TMJs presumably terminate. These data suggest that dynorphin-containing neurons in the STN play a role in the response of this nucleus to TMJ inflammation. Possible changes in DYN-IR fibers are currently being investigated using a computerized image analysis system. (Supported by NIH grant DE06682)

456.3

ASCENDING NOCICEPTIVE CONTROL SYSTEM IS MEDIATED IN THE ROSTRAL VENTRAL MEDULLA RW Gear* and JD Levine, Depts. of Stomatology and Oral and Maxillofacial Surgery, Univ. of Calif., San Francisco

We have previously shown that lumbar intrathecal (i.t.) administration of the μ-receptor selective opioid DAMGO in the rat attenuates (i.e., is antinociceptive to) the trigeminal jaw-opening reflex (JOR) and, further, that naloxone microinjected into the nucleus accumbens blocks attenuation of the JOR by i.t. administered DAMGO. To explain these results, we proposed the existence of an ascending spino-supraspinal nociceptive control system that is mediated by an opioid link in the nucleus accumbens (J Neurosci 15(1995):3154-61). Since naloxone microinjected into either the periaqueductal gray (PAG) or the rostral ventral medulla (RVM) does not block the effect of i.t. DAMGO on the JOR, opioidergic circuitry in these sites does not appear not to be involved in this proposed ascending pathway. In contrast, we now report the $GABA_A$ agonist, muscimol (20 ng), microinjected into the RVM completely blocks the attenuation of the JOR by i.t. DAMGO. Also, suppression of the JOR by DAMGO (60 ng) microinjected in the PAG is antagonized by naloxone (2 μg) microinjected in the RVM, confirming previous reports that activation of a descending nociceptive control system by opioids injected into the PAG is mediated, at least in part, by opioidergic circuitry in the RVM. These results suggest that nonopioidergic circuitry, possibly subject to GABA inhibition, in RVM is required to mediate the antinociceptive effect of i.t. DAMGO on the JOR and that the RVM is an important site in the ascending nociceptive control system. Furthermore, since opioidergic circuits in the RVM are implicated in antinociception produced by DAMGO microinjected in the PAG but not by DAMGO administered i.t., the classic descending nociceptive control system appears to be mediated by different supraspinal circuitry than the proposed ascending nociceptive control system.

456.4

IS THE PARABRACHIAL NUCLEUS ESSENTIAL FOR REFLEX AUTONOMIC RESPONSES ELICITED BY NOXIOUS ELECTRICAL STIMULATION OF TOOTH DENTIN?

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Noxious stimulation of trigeminal sensory nerves induces reflex changes in arterial blood pressure and heart rate. Previous studies in this laboratory determined that nociceptive neurons in the spinal trigeminal nucleus have dense projections to the lateral parabrachial nucleus and moderate to light projections to the ventral lateral medulla and the A5 region. However, the relative importance of trigeminal projections to the lateral parabrachial nucleus and the other brain stem autonomic cell groups, in the autonomic reflex response, remains to be determined.

In one series of experiments, arterial blood pressure was monitored during noxious electrical stimulation of the rat mandibular incisor before and after infracollicular and mid-pons coronal knife cuts. Infracollicular knife cuts had little or no effect on the pressor responses to tooth stimulation whereas the mid-pons knife cuts abolished the pressor responses. In all cases, the mid-pons knife cuts were near the caudal end of the facial nucleus. In a second series of experiments, injections (300 nl) of 2% lidocaine or 10mM cobaltous chloride into the A5 region completely blocked the pressor response. In contrast, injections of lidocaine or cobalt into the lateral parabrachial region produced only slight attenuation of the pressor responses elicited by noxious electrical stimulation of the tooth dentin. The results suggest that reflex autonomic changes in blood pressure induced by noxious stimulation of tooth dentin are mediated, predominately, in the A5 region and to a lesser extent in the lateral parabrachial nucleus. Supported by the New Brunswick Heart and Stroke Foundation.

456.5

NEUROPEPTIDES IN SPINAL PATHWAYS TO THE RAT INTERNAL LATERAL PARABRACHIAL NUCLEUS. K. Feil*& H. Herbert, Dept. Animal Physiol., Univ. Tübingen, Auf der Morgenstelle 28, D-72076 Tübingen, Germany.

The parabrachial complex receives topographically organized somatosensory inputs from the spinal cord and the spinal trigeminal nuclei (Sp5). In the present study we analyzed the afferents to the internal lateral (il) PB in more detail by using retrograde tracing with Fluoro-Gold (FG) combined with fluorescence immunocytochemistry for vasoactive intestinal polypeptide (VIP) and substance P (SP).

Following FG injections into the PBil retrogradely labeled neurons were observed in the lateral spinal nucleus (LSN) and the lateral reticulated area (LRA). The majority was present in the LSN of cervical and lumbo-sacral spinal segments, whereas in the thoracic spinal cord only few labeled neurons were seen. Retrogradely labeled neurons that were also immunoreactive (ir) for **VIP** were predominantly found in the **LSN** of **lumbo-sacral** segments while in the LSN of upper spinal segments only few double labeled neurons were found. In the LRA we observed in all spinal segments only occassionally VIP-ir neurons. In contrast, neurons double labeled for **SP** were almost exclusively present in the **LSN** of **upper cervical** spinal segments while the LSN of thoracic and lumbo-sacral segments was largely devoid of double labeled cells. Sections through the parabrachial complex stained for VIP and SP also showed a differential pattern of staining in the PBil. VIP-ir fibers were predominantly found in a bowl-shaped area bordering the PBil ventrally, while SP-ir fibers were found within the PBil proper. A similar pattern of termination of spinal fibers in the PBil was already previously observed.

Our data indicate that the projections from the LSN to the PBil consist of two different pathways: a VIP-ergic pathway which originates in the lumbo-sacral LSN and terminates in the ventral aspects of the PBil and a SP-ergic pathway which originates in the upper cervical LSN and terminates in the PBil proper. It is conceivable that they represent two functionally different routes which are coded by two different neuropeptides. (Supported by DFG He 1842/3-2)

456.6

THALAMIC PROJECTIONS FROM THE MEDULLARY SUBNUCLEUS RETICULARIS DORSALIS (SRD) OF THE RAT STUDIED WITH THE PHA-L METHOD. L. Villanueva, J. F. Bernard and D. Le Bars (SPON: European Neuroscience Association). INSERM, U-161, 2, rue d' Alésia, Paris, (France).

SRD is a caudal area within the brainstem that processes specifically cutaneous and visceral nociceptive information from the whole body. Their neurones are exclusively activated by Aδ- and C- fibre inputs and encode cutaneous and visceral stimuli within the noxious range. We examined the distribution and organization of thalamic projections from the SRD following injections of Phaseolus Vulgaris Leucoagglutinin (PHA-L).

Male Sprague-Dawley rats were anaesthetized with chloral hydrate and PHA-L (5%) was electrophoretically injected into the SRD. Two weeks later, the rats were perfused transcardially, the brain was removed and cut in serial sections which were reacted with PHA-L antibody and ABC complex.

SRD projections were observed in several thalamic nuclei, and terminal labelling was almost exclusively contralateral. In the ventral complex, dense labelled terminals were found in the ventromedial and ventrolateral nuclei. In the intralaminar complex, terminal labelling was observed in the parafascicular and central lateral nuclei. Labelling was also found in the midline reuniens nucleus. The highest density of labelling was observed in the ventromedial nucleus, throughout all its rostrocaudal extent.

Together with our previous electrophysiological and anatomical data, this study suggests that the SRD provides a link in spino-reticulo-thalamic pathways implicated in the processing of pain.

456.7

DIRECT PROJECTION OF TRIGEMINAL BRAINSTEM NEURONS TO THE HYPOTHALAMUS OF THE RAT. A. Malick* and R. Burstein. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

Somatosensory stimulation of structures innervated by the trigeminal nerve is capable of inducing changes in the activity of hypothalamic neurons associated with neuroendocrine and autonomic functions. To understand how such sensory signals reach these neurons, we examined the possibility that cells in the different nuclei of the trigeminal brainstem nuclear complex (TBNC) and upper cervical spinal cord (C1-2) project directly to different hypothalamic areas.

In three groups of rats the retrograde tracer Fluoro-Gold was injected unilaterally into the rostral hypothalamus, caudal hypothalamus, or entire hypothalamus. One week later the brainstem and upper cervical segments were processed, and the TBNC and dorsal horn of C1-2 examined for labelled cells. Following unilateral injections of the entire hypothalamus 941 neurons were counted bilaterally (66% contra) within SpVp (3%), SpVo (5%), SpVi (6%), SpVi/Vc (13%), SpVc (28%), and the dorsal horn of C1 (24%), and C2 (20%). Following injections centered in the rostral and caudal hypothalamus, 856 and 398 neurons were found in the examined trigeminal areas, respectively. In these cases, labelled cells were also distributed bilaterally throughout the length of the TBNC and C1-2. The majority of neurons found in SpVc and C1-2 dorsal horn of all three groups were located within laminae I-II and V, areas known to participate in nociceptive transmission.

The findings of this study suggest that neurons located throughout the TBNC and dorsal horn of C1-2 project directly to the rostral and caudal hypothalamus. These cells may participate in relaying somatosensory information from trigeminal primary afferents to hypothalamic regions involved in autonomic and neuroendocrine regulation. Supported by NIDR grant # 1R29DE1090401A1.

456.8

CORTICAL PROJECTIONS OF VMpo, A SPECIFIC PAIN AND TEMPERATURE RELAY IN PRIMATE THALAMUS. A.D. (Bud) Craig*, K. Krout, and E.-T. Zhang. Div. of Neurobiol., Barrow Neurol. Inst., Phoenix, AZ.

Anatomical and physiological work in the macaque has demonstrated that VMpo is a cyto- and chemo-architectonically distinct nucleus that receives dense, topographic lamina I spino- and trigemino-thalamic input and contains a concentration of nociceptive- and thermoreceptive-specific neurons; further, VMpo in the human thalamus is located in the region associated with pain and temperature sensation by stimulation, lesion and recording data (Craig et al., 1994, Nature 372:770). We have used multiple anterograde tracers to examine the cortical projections of VMpo in the cynomolgus macaque.

Localization of VMpo in 7 anesthetized monkeys was based on recordings from topographically organized single units and clusters of nociceptive- and thermoreceptive-specific neurons. Iontophoretic or hydraulic injections of labeled dextrans (biotin or fluorescent red or green) or PHA-L were made with micropipettes. Animals survived 3-6 wks. Dense, topographic terminal labeling observed in the dorsal part of the anterior insula that extended beyond the fundus of the limiting sulcus, anterior to SII, was ascribed to VMpo projections. Light labeling was also found in SII, and a small, dense burst occurred in the fundus of the central sulcus in most cases. Adjacent regions of posterior thalamus appear to project strongly to SII, Ri, lateral area 6, and the granular insula, consistent with earlier studies by others.

These observations indicate that the primate anterior insula receives input associated with specific pain and temperature sensation. This provides a structural basis for the strong activation of this region by noxious and thermal stimuli in human PET studies, and it is consistent with the limbic afferent role proposed for lamina I. (Supported by NS 25616)

456.9

Changes in Regional Cerebral Blood Flow Associated with Painful Peripheral Mononeuropathy in the Rat. Thomas Morrow, Pamela Paulson and Kenneth Casey Depts of Neurology, Physiology, Univ of Michigan and VAMC, Ann Arbor, MI 48105

Chronic constriction injury (CCI) produced by loose ligation of the common sciatic nerve in the rat is a widely used model of chronic neuropathic pain (CNP). Extensive investigations with this model have demonstrated the occurrence of spontaneous pain, thermal hyperalgesia and mechanical allodynia. These alterations in pain perception are thought to reflect a functional reorganization in CNS structures forming a neural network for pain, which may be responsible for the development and maintainence of CNP. We quantified changes in forebrain activation by injecting ^{99m}Tc-HMPAO to measure regional cerebral blood flow (rCBF) in rats. rCBF was measured in 4 male S-D rats 2-4 weeks after CCI, the period when CNP is reported to be maximum. Under halothane, a 24-ga catheter was placed in the tail vein. Subjects were then placed in soft restraint and allowed to recover from anesthesia for 45 minutes. 10 mCi of ^{99m}Tc-HMPAO was injected through the catheter. Five minutes later the rats were overdosed with chloral hydrate and the brain removed. Twenty micron frozen sections were cut and mounted. Standard autoradiographic images of brain sections were then analyzed for relative optical density. Regions of interest were sampled and statistically analyzed (t-tests) for differences. In the absence of overt stimulation, we found increased rCBF within structures known to be part of the forebrain network for pain. These structures include: cingulate cortex, somatosensory cortex and lateral thalamus. In addition, although not yet statistically significant because of small sample size, the anterior dorsal thalamus also showed a tendency toward increased activation bilaterally during CNP. This thalamic nucleus has connections to the limbic system, including connections to the cingulate cortex. The findings that forebrain patterns of rCBF change in association with the behavioral signs of CNP provide new insight into the central mechanisms of CNP.

456.10

REDUCTION OF AUTOTOMY BEHAVIOUR FOLLOWING PERIPHERAL NEURECTOMY BY A SINGLE INJECTION OF BUPIVICAINE INTO THE CINGULUM BUNDLE OF RATS Jane E. Magnusson* and Anthony L. Vaccarino. Department of Psychology, University of New Orleans, LA 70148.

Peripheral neurectomy in rats is followed by autotomy of the denervated zone, which is assumed to represent an index of pain or dysesthesia. We previously demonstrated that periodic anesthetic blockade of the cingulum bundle reduces the amount of autotomy following peripheral neurectomy. In the present study, we examined whether a single injection of a local anesthetic (bupivicaine) into the cingulum given immediately prior to or shortly after peripheral neurectomy could reduce autotomy behaviour. Under sodium pentobarbital anesthesia, male Long-Evans rats were implanted bilaterally with cannulae aimed at the anterior cingulum bundle. Following a 7-14 day recovery period, the rats were anesthetized and the sciatic and saphenous nerves were sectioned free, ligated and cut. Starting immediately prior to or 20 minutes after nerve section, rats were infused bilaterally into the cingulum bundle with 1 ul of 0.75% bupivicaine or saline over a 2-min period. Beginning the day after nerve section the rats were examined daily for signs of autotomy. Anesthetic blockade of the cingulum bundle at the time of nerve section, or shortly after nerve section, was found to delay the onset and reduce the overall degree of autotomy behaviour. These results demonstrate that a single injection of bupivicaine reduces autotomy behaviour for periods that far exceed the duration of the anesthetic block. This research was supported by a Louisiana Education Quality Support Fund (LEQSF).

456.11

RESPONSES OF PRIMATE NOCICEPTIVE SI NEURONS TO INTRADERMAL CAPSAICIN INJECTIONS. D.K. Douglass*, M. Sholas, R. Dubner, D.A. Thomas and D.R. Kenshalo, Jr. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

In humans intradermal injection of capsaicin produces burning pain, cutaneous hyperalgesia and allodynia. In the present study we examined the changes in response characteristics of nociceptive neurons in the primary somatosensory cortex (SI) after an intradermal injection of capsaicin. The monkeys were anesthetized with alpha-chloralose and supplemented by Nembutal. A craniotomy was performed over the foot or hand representation and single units were isolated using tungsten microelectrodes. Each unit with slowly adapting responses to mechanical stimulation was tested for responses to a brush, pressure and pinch stimulus and the size of the receptive field determined. Neurons that responded maximally to the pinch stimulus received an intradermal injection of capsaicin (200 µg). The receptive field size and the responses to the graded series of mechanical stimuli were then determined at 5, 15 and 25 min. post-injection. After the injection, the discharge rate of all neurons increased dramatically and remained higher than the pre-injection discharge rate for an average of 75 s. Capsaicin injected into the receptive field produced an expansion in receptive field size in 22 of 30 nociceptive neurons. The expansion (10-1500%) was present within 5 min. of the injection and persisted for at least 25 min. The responses to the series of mechanical stimuli also increased after capsaicin injection with the largest relative increase to the pressure stimulus. These results indicate that nociceptive neurons in the primate SI cortex respond to intradermal injection of capsaicin and that tonic activity in nociceptive pathways can alter receptive field size and responses to mechanical stimulation. We conclude that many of the receptive field changes seen in nociceptive SI neurons can account for the burning pain, cutaneous hyperalgesia and allodynia in humans after injection of capsaicin.

456.12

SI RECEPTIVE FIELD CHANGES PRODUCED BY INTRADERMAL INJECTION OF CAPSAICIN ARE REVERSED BY MK-801, A NMDA RECEPTOR ANTAGONIST. M. Sholas*, D. K. Douglass, R. Dubner, and D.R. Kenshalo, Jr. Neurobiology & Anesthesiology Branch, NIDR, NIH, Bethesda, MD. 20892.

Intradermal injection of capsaicin produces burning pain and mechanical hyperalgesia in humans. In this study, we attempted to determine if NMDA receptors were involved in the expansion of the receptive field (RF), and in the increase in responses to mechanical stimulation that is likely involved in these sensations. The monkeys were anesthetized and a craniotomy was performed over SI. Tungsten microelectrodes were used to record from single units responding preferentially to noxious stimulation of glaborous skin. Once isolated, a graded series of mechanical stimuli -- brush (camel hair brush), pressure (large arterial clamp), and pinch (small arterial clamp) -- was delivered to the RF. Capsaicin (200 µg) was intradermally injected, and the RF was again characterized at five minutes post injection. Finally, the animal received an IV injection of the NMDA channel blocker, MK-801 (0.1 mg/kg), and the neuronal response properties were re-evaluated 25-45 minutes after capsaicin injection.

Ten of twelve units included in the study showed a RF expansion after capsaicin, which averaged 381% (± 139) of the original size. Baseline activity of the units increased from 11 (±1.48) to 34 (±7.50) Hz. Responses to all mechanical stimuli increased. Once expanded, IV injection of MK-801 reduced the average RF of the ten cells that responded to capsaicin to an area only 253% (± 102) of the original area. Baseline activity decreased after MK-801 to 17 (±2.79) Hz, which was similar to control values. After MK-801, neuronal responses to the series of graded mechanical stimuli were decreased as compared to capsaicin, and were equivalent to control responses. Two cells which did not respond to capsaicin were not affected by injection of MK-801. The findings suggest that NMDA-receptors are involved in the hyperexcitability of SI neurons caused by intradermal capsaicin.

456.13

CHEMICAL LESIONS IN THE STRIATUM INHIBIT NOCICEPTIVE REFLEXES IN RATS. N.E. Saadé, S.A. Shbeir, S.J. Jabbur and S.F. Atweh*. Fac. of Medicine, American Univ. of Beirut - Lebanon.

Previous work from our laboratory has shown that chemical lesions of the neostriatum inhibited deafferentation pain in rats by reducing the incidence and delaying the onset of autotomy following leg denervation (Saadé et al. Society for Neuroscience, 1994: 20, 1573). The aim of this study was to investigate whether similar chemical lesions affect the latencies of acute pain tests such as the tail immersion (TI) and the hot plate (HP) tests.

Two groups of rats were subjected to TI and HP tests for two weeks prior to injections in the left striatum of either 9µl of saline (sham group, n=7) or of 2µg of kainic acid (striatum-lesioned group, n=12). After 3 weeks of testing, both groups received similar injections of saline and kainic acid in their right striatum and were subjected to pain tests for another period of three weeks. Sham injected group did not show any significant changes in the latencies of both pain tests. Unilateral lesion of striatum produced a 22% and 33% increase of TF and HP latencies, respectively; While bilateral lesions of striatum increased the latencies of TF and HP tests by 50% and 93% respectively. These results suggest that the basal ganglia play a role in processing nociceptive information and in modulating pain related reflexes even at spinal level.

(Supported by a grant from the Diana Tamari Sabbagh Fund and LNRC).

456.14

RESPONSES OF NEURONS IN THE CAUDAL INTRALAMINAR THALAMIC COMPLEX OF THE RAT TO STIMULATION OF THE UTERUS, CERVIX, COLON AND SKIN. G. Guilbaud*, K.J. Berkley, J.-M. Benoist and M. Gautron. Unité de Recherches de Physiopharmacologie du Système Nerveux, INSERM U. 161, 2 rue d'Alésia, 75014 Paris, France.

Neurons in the lateral thalamus in and near the ventrobasal complex have recently been shown by several groups to respond to both skin and visceral stimulation. Given that neurons in lateral and medial thalamus have long been thought to subserve different aspects of pain sensation, the present study examined responses of neurons in the caudal intralaminar complex (IL) to skin and visceral stimuli so that their response characteristics could be compared with those of lateral thalamic neurons to identical stimuli studied earlier (Berkley, et al, *J. Neurophysiol.*, 69, 1993). In 12 adult rats anesthetized with halothane (2-2.5%) and nitrous oxide (2/3) in oxygen (1/3), responses of 35 caudal IL neurons were tested to mechanical stimulation of skin (brush, pressure, noxious pinch) and 4 pelvic visceral stimuli (noxious distension of uterine horn and vaginal canal, gentle disdenstion of colon and probing cervix). As previous studies of IL in males and other species have shown, many neurons (24/35) responded to frankly noxious stimulation of skin in several body regions; only 3 of them responded to gentle skin stimulation. Here it was also found that some neurons (16/24) additionally responded to one or more visceral stimuli, while 4/35 responded only to a visceral stimulus. The most effective visceral stimuli were the noxious ones. Thus, in contrast with neurons in lateral thalamus that exhibit a variety of response characteristics to both noxious and nonnoxious somatic and visceral stimuli, neurons in medial thalamus appear to be signalling information mainly when intense somatic and visceral stimuli are frankly above the noxious threshold, providing further support for the concept that pain-related functions of these two regions differ.

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456.15

SPINOHYPOTHALAMIC TRACT (SHT) NEURONS IN THE CERVICAL ENLARGEMENT OF RATS: LOCATIONS OF ANTIDROMICALLY IDENTIFIED AXONS IN THE CONTRALATERAL BRAINS. E. Kostarczyk*, X. Zhang and G.J. Giesler, Jr., Dept. of Cell Biol. & Neuroanat., Univ. of Minnesota, Minneapolis, MN 55455.

Many nociceptive spinal neurons in rats project directly to hypothalamus. In this study, the course of ascending SHT axons and the possible existence of branches from them were examined in the medulla, pons and midbrain. Ninety-eight neurons were antidromically activated from the contralateral hypothalamus. Their axons were antidromically activated at 568 low threshold points at multiple anterior-posterior levels of the contralateral brain and rostral spinal cord. In caudal segment C1, axons ascended in the ventral lateral funiculus. As the axons projected rostrally, they shifted into the dorsal lateral funiculus of rostral C1. In the caudal medulla, parent SHT axons ascended near ventrolateral reticular n and n ambiguus. Evidence for numerous branches from SHT axons was seen in the medulla. These branches appeared to be located in n. ambiguus (11 branches), medullary reticular field (19), parvocellular n (6), gigantocellular (4) reticular n, cuneate n (4) and n solitary tract (3). In the rostral medulla, SHT parent axons were concentrated near the lateral paragigantocellular n. In the pons, parent SHT axons were located within and surrounding the facial n and branches of the SHT axons were noted in the pontine reticular formation (3). In the caudal midbrain, parent SHT axons coursed dorsally adjacent to the lateral lemniscus. They then traveled toward the thalamus in the brachium of inferior colliculus. SHT parent axons ascended through the posterior nucleus of thalamus, medial to the medial geniculate n. These axons then entered supraoptic decussation and coursed toward the hypothalamus. Each of 45 tested neurons responded preferentially or specifically (38%) to noxious mechanical stimuli. These studies provide evidence that SHT axons may provide nociceptive information to a variety of brainstem nuclei. Supported by NS25932.

PAIN MODULATION: PHARMACOLOGY—OPIOIDS I

457.1

OPIOIDS MODULATE NMDA-EVOKED RESPONSES OF NEURONS IN THE SUPERFICIAL AND THE DEEPER DORSAL HORN OF THE MEDULLA. K.M. Zhang, X-M. Wang, A.M. Peterson, S. Bhattacharjee, and S.S. Mokha*. Dept. of Physiology, Meharry Medical College, Nashville, TN 37208.

We have previously reported, based on systemic studies, that multiple opioid receptors (µ, δ and κ) modulate nociceptive and non-nociceptive inputs in the superficial and the deeper dorsal horn of the medulla (trigeminal nucleus caudalis). The effects of iontophoretically applied receptor-selective opioid agonists have not been investigated previously either on the natural stimuli-evoked or the N-methyl-d-aspartate (NMDA)-evoked responses of physiologically characterized neurons in the superficial and the deeper dorsal horn of the medulla. The present study was, therefore, designed to investigate the role of *mu* (DAMGO) and *delta* (DPDPE) opioid receptor selective agonists in modulating the NMDA-evoked responses of neurons in the trigeminal nucleus caudalis. Extracellular single unit recordings were made with the central barrel of a seven barreled microelectrode in rats anesthetized with urethane (1.5 g/kg, i.p.). The other barrels contained freshly made solutions of [D-Ala^2,N-Me-Phe^4 ,Gly^5-ol]enkephalin (DAMGO, 25 mM, pH 4.5), [D-Pen^{2,5}]enkephalin (DPDPE, 10 mM, pH 4.5), NMDA (50 mM, pH 8.2), DL-2-Amino-5-Phosphonovaleric acid (AP-5, 50 mM, pH 8.0), naloxone hydrochloride (50 mM, pH 4.5), and NaCl for current balancing. Neurons were characterized nociceptive specific (NS), wide dynamic range (WDR), low threshold (LTH) using natural stimuli (brush, pressure, pinch, squeeze, heat). Opioids were ejected with positive current whereas NMDA and AP-5 were ejected with negative current. DAMGO (40-120 nA) and DPDPE (40-80 nA) primarily produced reduction in the NMDA (15-90 nA, cycle) evoked responses of NS, WDR and LTH neurons in the superficial and the deeper dorsal horn of the medulla. However, excitatory or biphasic effects were also observed. It is concluded that mu and delta opioid receptor selective agonists produce modulation of the NMDA-evoked responses of nociceptive and non-nociceptive neurons in the medullary dorsal horn.

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457.2

TOLERANCE, CROSS-TOLERANCE AND DEPENDENCE FOR µ-OPIOID AND A_1-ADENOSINE PERIPHERAL ANTINOCICEPTION IN THE RAT. K. O. Aley, P. G. Green* and J. D. Levine
Depts. of Medicine and Oral Surgery, and Division of Neurosciences, UCSF, Box 0452A, San Francisco, CA 94143.

The selective µ-opioid agonist, DAMGO, or the selective A_1-adenosine agonist CPA, when co-injected intradermally with prostaglandin E_2 (PGE_2) into the rat hind paw, dose-dependently inhibited PGE_2-induced mechanical hyperalgesia in the Randall-Selitto paw-withdrawal test in the rat. Repeated (3 hourly) intradermal injections of DAMGO or CPA produced tolerance to their antinociceptive effects. Furthermore, repeated (3 hourly) intradermal injections of DAMGO produced cross-tolerance to the antinociceptive effect of CPA, and vice versa. The demonstration of cross-tolerance between the peripheral antinociceptive effects of DAMGO and CPA supports the suggestion that both DAMGO and CPA produce antinociception by acting on the same cell, presumably the primary afferent nociceptor, and that tolerance requires changes subsequent to activation of µ-opioid and A_1-adenosine receptors. When injected alone in normal rats, the opioid antagonist naloxone had no effect on paw-withdrawal threshold, but it produced withdrawal hyperalgesia in DAMGO-tolerant rats. This naloxone-induced hyperalgesia was reversed when naloxone was co-injected with DAMGO, but not when it was co-injected with CPA. Similarly, the A_1-adenosine antagonist, PACPX, produced hyperalgesia in CPA tolerant paws, which was prevented by a co-injection of CPA with PACPX, but not when co-injected with DAMGO. Naloxone elicited withdrawal responses (reduction in paw-withdrawal threshold) in CPA as well as DAMGO tolerant paws and PACPX elicited withdrawal responses in DAMGO as well as CPA tolerant paws. These observations suggest that dependence to µ-opioid and A_1-adenosine agonists involves changes mediated by a common second messenger system.

457.3

ACUTE OPIOID TOLERANCE IN MOUSE SPINAL CORD: INVOLVEMENT OF NMDA RECEPTORS AND CONTRAST WITH ALPHA-2 ADRENERGIC RECEPTORS. C.A. Fairbanks*[1], K.F. Kitto[1], H.H. Wu[3], G.L. Wilcox[1,2]. [1]Department of Pharmacology, [2]Graduate Program in Neuroscience, [3]Dept. of Cell Biology and Neuroanatomy. University of Minnesota, Minneapolis, MN, U.S.A.

Both NMDA and α_2 adrenergic receptors have been implicated in spinal opioid antinociception and the development of opioid tolerance. The present experiments sought to test whether acute morphine tolerance shares the dependence on NMDA receptors previously shown with chronic tolerance and whether acute or chronic opioid tolerance generalizes to the α_{2A} receptor.

Acute tolerance to morphine was induced in male ICR mice (15-20g) by intrathecal (i.t.) injection of morphine (20 nmol). Chronic tolerance to β-endorphin (previously defined as μ opioid tolerance) was induced by i.t. implantation of 10^5 AtT20 cells. Selective action of α_{2A} receptors was accomplished by a administration of a mixture of α_2 adrenergic agonist UK14801 (3, 10, 30 nmol) and α_{2C} antagonist prazosin (1μM) was used to selectively activate a_{2A} receptors. Antinociception was detected via the hot water (55° C) tail flick test.

I Coinjection (i.t.) of the NMDA antagonist MK801 (1μg) with morphine (20 nmol) prevented acute tolerance to morphine measured 6 h later.II Acute tolerance to morphine was not accompanied by cross tolerance to UK; in fact, it appeared to enhance the efficacy of UK + prazosin. III Chronic opioid tolerance (AtT20 cell implanted mice) did not alter the UK dose response curve.

The evidence presented here supports a role for NMDA receptors in the development of acute opioid tolerance extending other studies with repeated morphine injection. In agreement with other studies in rat, chronic opioid tolerance is not accompanied by cross tolerance at α_2 adrenergic receptors and may, in fact, enhance α_{2A} mediated spinal antinociception. (Supported by NIH/K02-DA-00145 & NIH/R01-DA-04274)

457.4

TAIL-FLICK: TEST INTERVAL CAN MODIFY THE DEVELOPMENT OF BOTH SENSITIZATION AND MORPHINE ANALGESIA.
R.J. Brennan, J.P. O'Brien, P.J. DeMarco, A.E. Baldwin, & J.T. Cannon*. Dept. of Psychology & Neuroscience Program, University of Scranton, Scranton, PA 18510-4596.

The tail-flick response to heating is one of the most widely used indices of nociception in rodents. Several lines of evidence, however, suggest that this test may possess some undesirable characteristics. For example, it may be altered both qualitatively and quantitatively by variations in heat intensity (e.g., Cannon et al., 1990; Kelly, 1983; Ness & Gebhart, 1986). It also interacts with tail temperature (e.g., Hole & Tjølsen, 1990). In anesthetized animals, inter-flick interval (IFI, 1 vs. 10 min) can affect the degree to which latencies decrease across the first 10-20 min of testing (O'Brien et al., 1994). Using a 1 min IFI, Baldwin et al. (1992) found that exposing rats to a single prolonged tail-flick stimulus can produce long lasting reductions of flick latencies. The first study reported here compares the degree of sensitization that a single suprathreshold stimulus produces in animals tested at either 1 or 10 min IFIs. The second study examines the analgesic effects of morphine in animals tested at these IFIs.

In the first study, 48 pentobarbital-anesthetized male albino rats (8/group) were tested at either a 1 or 10 min IFI, with half of the animals receiving a single prolonged trial (10 sec, approximately 100% above baseline latencies) after 10 min of baseline testing. Baseline latencies were shorter with a 1 min IFI. The prolonged trial produced clear sensitization only in animals tested at the 1 min IFI, with decreases in latencies being seen at all tail spots tested, but the greatest decrease evidenced at the spot that received suprathreshold stimulation.

In the second study, 64 pentobarbital-anesthetized male albino rats (8/group) were tested at either 1 or 10 min IFI. After 10 min of baseline testing, animals received an injection of either saline or morphine (2.5, 5, or 10 mg/kg, s.c.). When comparing 1 and 10 min IFIs: shorter latencies were exhibited by saline injected animals tested with a 1 min IFI; maximal levels of analgesia were comparable for each of the morphine doses; and analgesia developed significantly more slowly in animals that were injected with 5 mg/kg of morphine and tested at the 10 min IFI. Tail temperature was a significant covariate for several analyses; it does not, however, explain the IFI effect in the 5 mg/kg morphine condition.

457.5

DIFFERENTIAL EFFECTS OF MORPHINE ON DESCENDING CONTROLS MODULATING THE SENSORY AND MOTOR FACETS OF A NOCICEPTIVE SPINAL REFLEX. D. Bouhassira, D. Chitour, F. Guirimand and D. Le Bars (SPON ; Eur. Neurosci. Ass.) INSERM U-161, 2, rue d'Alésia 75014 Paris, France.

Intravenous morphine exhibits a dose-dependent biphasic effect on electromyographic (EMG) recordings of a nociceptive flexion reflex in the anaesthetised rat : low (< 2 mg/kg) and higher doses elicit facilitatory and depressive effect respectively. The former, a priori paradoxical, effect could be due to an action on the motor limb of the reflex arc, either directly at the spinal level or, indirectly via an action at supraspinal sites, on descending controls.

We investigated in anaesthetised rats (halothane 1 %) the effects of 0.5 mg/kg i.v. morphine on both a nociceptive flexion reflex and the responses of dorsal horn convergent neurones. These effects were compared in sham-operated and rats with lesion of the nucleus raphe magnus (NRM), a supraspinal site for morphine action and a source of descending controls modulating the spinal transmission of nociceptive signals at both sensory and motor levels.

EMG recordings of a C-fibre reflex elicited by electrical stimulation of the sural nerve territory were made from the ipsilateral biceps femoris muscle. Convergent neurones with excitatory receptive field in the territory of the sural nerve were simultaneously recorded from the ipsilateral dorsal horn. Recruitment curves for C-fibre evoked reflexes and neuronal responses were built by varying the stimulus intensity from 0 to 40 mA, before and 20 minutes after morphine. In sham-operated animals, morphine had no significant effect on the threshold but facilitated (> 150%), in a naloxone-reversible fashion, the reflex elicited by suprathreshold stimuli (>3 X threshold). The facilitations disappeared in animals with NRM lesions. No significant effects of morphine were observed on the C-fibre evoked responses of convergent neurones in either group of animals.

These results revealed a differential effect of morphine on sensory and motor facets of a nociceptive reflex. Facilitations of the EMG responses elicited by low systemic doses of morphine were probably related to an action on descending controls of the motor limb of the reflex arc through the NRM.

457.6

INTRAPLANTAR MORPHINE DEPRESSES SPINAL c-FOS EXPRESSION INDUCED DURING INFLAMMATORY PAIN STATES IN THE RAT P. Honoré*, J. Buritova, Y. Chapman and J.-M. Besson, INSERM U.161, 75014 Paris, France.

This study evaluated the effect of pharmacological modulations of peripheral μ-opioid receptors on Fos-like immunoreactivity (FLI) in rat lumbar spinal cord neurons induced 1h30 and 3h after intraplantar carrageenin injection.

One and half hours (h.) after intraplantar injection of carrageenin (6 mg/150 μl), in the hind paw, FLI neurons (76±2 neurons/section) were observed in the dorsal horn, predominantly in the superficial laminae (74±2%), of the L4 -5 segments of the spinal cord. Three hours after carrageenin injection considerably more FLI neurons were observed in the dorsal horn of the spinal cord (241±8 neurons/section), at this time point both superficial (I-II) and deep laminae (V-VI) were densely labelled (45±2% and 37±1% of carrageenin induced FLI neurons, respectively).

Co-administration of intraplantar morphine (10, 25 or 50μg/50μl of saline), dose-dependently (r^2=0.366, $p<0.05$) decreased the number of superficial FLI neurons induced at 1h30 after intraplantar carrageenin, without influencing the peripheral inflammation. The effects of the highest dose of morphine (45±4% reduction, $p<0.01$) were significantly blocked by co-administered intraplantar methiodine naloxone (20μg/50μl of saline), which injected alone had no effect. The highest dose of morphine (50μg), injected intravenously, did not influence either spinal c-Fos expression or peripheral inflammation. In addition, co-administration of intraplantar morphine (10, 25 or 50μg/50μl of saline), dose-dependently (r^2=0.705, $p<0.0001$) decreased the total number of FLI neurons induced at 3h after intraplantar carrageenin and reduced the peripheral inflammation. The highest concentration of morphine significantly reduced both the total expression of c-Fos (37±3%, $p<0.0001$, reduction of control) and the enhanced paw and ankle diameter (50±2% reduction, $p<0.0001$, 70±1%, $p<0.0001$, reduction of control, respectively). These effects were blocked by a co-administration of intraplantar methiodine naloxone.

These results demonstrate that intraplantar morphine reduces peripheral inflammation and the subsequent spinal expression of c-Fos, thus illustrating the role of peripheral opioid receptors during inflammatory nociception.

457.7

RELATIONSHIP BETWEEN NEUROENDOCRINE ACTIVITY, PAIN, STRESS, AND THE DEVELOPMENT OF MORPHINE TOLERANCE: A STRAIN COMPARISON. Leland C. Couret, Jr. and Anthony L. Vaccarino* Department of Psychology, University of New Orleans, LA 70148.

We previously reported that morphine fails to produce analgesic tolerance and dependence when administered in the presence of formalin-induced pain. The hypothalamic-pituitary-adrenal (HPA) axis is known to respond to stressful stimuli, including pain. To examine whether the blockade of tolerance by pain is related to HPA activity, we assessed the development of tolerance to morphine analgesia in an inbred strain of rats that lack typical stress-induced HPA responses (Lewis strain). Female Lewis rats were injected with morphine (20 mg/kg) or saline for four consecutive days in the presence or absence of formalin-induced pain. The analgesic effect of test doses of morphine (5, 10 or 20 mg/kg) was then measured in the tail-flick test 24 h after tolerance induction. Female Fischer rats, which show typical stress-induced HPA responses, were used for comparison. Analgesic tolerance was produced in both strains when morphine was delivered in the absence of pain. However, the presence of pain during tolerance induction prevented the development of analgesic tolerance in Fischer rats, but not Lewis rats. The differential effects of pain on the development of morphine tolerance are suggested to be related to genetically determined differences in stress-induced HPA activity. This research was supported by a LSU Neuroscience Incentive Grant and a UNO Research Council Grant.

457.8

LOSS OF EFFICACY AND POTENCY OF SPINAL MORPHINE TO INHIBIT THE TAIL-FLICK REFLEX IN RATS WITH A NERVE CONSTRICTION INJURY. Michael H. Ossipov, Yvan Lopez, Michael L. Nichols, Di Bian and Frank Porreca*. Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724.

Nerve constriction injury produces thermal hyperalgesia and mechanical allodynia analogous to clinical conditions of neuropathic pain. However, the effect of neuropathic injury on acute nociception has not been studied. Nerve constriction injury was produced by unilateral ligation of the L5/L6 spinal roots of the sciatic nerve of male Sprague-Dawley rats. Intrathecal (*i.th.*) catheters were inserted for spinal drug administration. Response to acute nociception was measured by determining the tail flick (TF) latency to immersion of the tail into a 55ºC water bath before (control) and after (treatment) *i.th.* morphine administration. A cut-off latency of 15 sec was employed. Data were converted to % maximal possible effect (%MPE) by the equation: 100 x ((test latency - control latency)/(15 - control latency)). In sham-operated rats, morphine produced dose-dependent antinociception with a maximal effect of 100% MPE at 60 μg; the A_{50} (95% C.L.) was 22 (17 - 30) μg. In nerve-ligated rats, morphine also produced dose-dependent increase in TF latency, but with a maximal effect of only 60 ± 17% obtained at 100 μg; the calculated A_{50} (95% C.L.) from the lower portion of the dose-effect curve was 82 (46 - 146) μg, representing at least a 4-fold decrease in potency compared to controls. Antinociception in both sham and nerve-injured animals was readily reversed by naloxone (5 mg/kg, *i.p.*). These data indicate that nerve constriction injury substantially reduces the potency and efficacy of *i.th.* morphine in affecting acute nociceptive input. Conceivably, degeneration of primary afferents subsequent to nerve constriction injury results in a loss of μ opioid receptors in the dorsal horn. The data also imply that a major part of normally observed morphine antinociception is related to activity at presynaptic opioid receptors.

457.9

"REKINDLING" EFFECT OF INTRATHECAL INJECTION OF NALOXONE ON JAW MUSCLE ACTIVITY EVOKED BY MUSTARD OIL APPLICATION TO TEMPOROMANDIBULAR JOINT IN RAT: POSSIBLE INVOLVEMENT OF TRIGEMINAL SUBNUCLEUS CAUDALIS (V_C). K.Seo, B.J.Sessle, D.A.Haas* and J.W.Hu, Faculty of Dentistry, University of Toronto, M5G 1G6, Canada

We have recently reported that systemic administration of the opioid antagonist naloxone can induce a recurrence ("rekindling") of increased electromyographic (EMG) activity evoked in jaw muscles by injection of the C-fibre excitant and inflammatory irritant mustard oil (MO) into the temporomandibular joint (TMJ) region of rats. A likely site of opioid action is V_C which is integrally involved in orofacial nociceptive transmission. This possibility was tested in 24 male rats anesthetized with halothane/N_2O/O_2 by injecting naloxone intrathecally (i.t.) via a cannula (PE-10) introduced into the brainstem subarachnoid space and placed near the brainstem surface overlying the left (ipsilateral, IP) V_C. EMG activity was recorded bilaterally from digastric (DIG) and masseter (MASS) muscles, and MO (20%, 20μl) was injected into the IP-TMJ region. Naloxone (1μg, 10μg, 30μg) or saline (as vehicle control) was applied (10μl, i.t.) 30 minutes after MO application by which time the MO-evoked activity had subsided. Naloxone induced a significant dose-dependent increase in EMG activity in IP-MASS, IP-DIG and contralateral (CL)-DIG ($p<0.05$ ANOVA), but not in CL-MASS ($p>0.05$). The change in peak EMG activity was dose-dependent for IP- and CL-DIG ($p<0.05$), but not for either IP- or CL-MASS ($p>0.05$). The duration of the naloxone-induced "rekindling" effect was dose-dependent for IP-DIG and IP-MASS ($p<0.05$), but not for either CL-DIG or CL-MASS ($p>0.05$); the latency of the "rekindling" effect also did not appear to be affected by the different dose levels. These results suggest that V_C, or an adjacent brainstem region, may be a site of action of the opioid "rekindling" effect on MO-evoked jaw muscle activities. Supported by Can. MRC MT-12286 and NIH DE-09559.

457.10

MORPHINE SENSITIVITY OF SPINAL- AND SUPRASPINAL-MEDIATED NOCICEPTIVE RESPONSES USING A NOVEL FOCAL ELECTRICAL STIMULATION PARADIGM. M. Vincler★, W. Maixner★, C. J. Vierck†, and A. R. Light†*. Departments of Pharmacology★ and Physiology†, University of North Carolina, Chapel Hill, NC 27599-7545.

The antinociceptive effects of morphine in rats have been established using a variety of techniques utilizing different nociceptive stimuli such as heat, electric shock, and algesic chemicals. Among these stimuli, electric shock has been one of the most poorly characterized. We have developed a technique which allows us to use focal electrical stimulation of the hindpaw and measure both spinal-mediated and supraspinal-mediated responses. This was accomplished by suspending the rat and attaching two small electrodes to the hindpaws. The hindlimbs were tethered to a force transducer allowing the measurement of a spinal-mediated response (hindlimb reflex). The right forepaw rested on a lever which, when pressed, terminated the shock and allowed the measurement of a supraspinal-mediated response (latency). Using this paradigm, we examined the effects of morphine (0.3, 1.0, 3.0, and 10.0 mg/kg) on bar press latency and hindlimb reflex force at various intensities of electric shock (0.05 - 0.8 mA). The 2 lower doses of morphine did not alter bar press latency or hindlimb reflex at any of the stimulus intensities tested. Morphine at 3.0 mg/kg increased bar press latency for stimulus intensities ranging from 0.05-0.6 mA, but had no effect at 0.7 and 0.8 mA. Hindlimb reflex force, however, was attenuated from 0.2-0.8 mA. Morphine at 10.0 mg/kg increased bar press latency and attenuated hindlimb reflex force at all stimulus intensities. These data suggest that focal electrical stimulation can be used to test the antinociceptive effects of opioids and that morphine is more effective at attenuating spinal-mediated nociceptive responses than supraspinal-mediated responses in this paradigm. *Supported by NIDA grant DA04420 (A.R.L.) and NIDA grant DA07244 (L.A. Dykstra).*

457.11

A NON-PEPTIDE NEUROTENSIN ANTAGONIST (SR48692) POTENTIATES THE ANTINOCICEPTIVE ACTION OF MORPHINE IN RATS. Smith, D.J.*, Smith, D.L., Monroe, P.J., Urban, M.O. and Gully, D. Dept. Anesth. WVU, Morgantown WV 26506, and Sanofi Recherche, Toulouse Cedex, France.

Neurotensin's neuronal projections from the periaqueductal gray (PAG) to the rostroventral medial medulla (RVM) function in a pain facilitory fashion following the microinjection of an antinociceptive dose of morphine into the PAG of rats (Neurosci. Lett. 174: 21, 1994). Microinjection of an antagonistic dose of the peptidergic neurotensin partial agonist D-trp-11-neurotensin into the RVM potentiates the antinociceptive response to PAG morphine (6nmol). Similarly, the microinjection of the non-peptide neurotensin antagonist SR48692 into the RVM enhances PAG morphine (Can. J. Physiol. Pharmacol. 72 (S1): 414, 1994). Since the non-peptide neurotensin antagonist penetrates the blood-brain-barrier, the current study was undertaken to determine if systemically administered SR48692 would enhance the antinociceptive action (i.e. heat-induced tail flick test; TFL) of morphine in rats. In this regard, i.p. administered SR48692 caused a marked potentiation of the response to PAG morphine (6 nmol), essentially doubling the magnitude and duration of the opioid's antinociceptive effect. The dose response relationship for the antagonist was bell-shaped with 0.1 and 0.3 mg/kg (i.p.) producing the effect, while higher doses were ineffective. Furthermore, systemically administered SR48692 produced a distinct potentiation of the action of systemically administered morphine (2 mg/kg, i.p.). Again the magnitude and duration of the response to the opioid was doubled, with the dose response relationship for the effect of SR48692 being bell-shaped (effective doses range, 0.03-0.3 mg/kg, i.p.). These data suggest that the novel non-peptide neurotensin antagonist, SR48692, may be useful as an adjunct to analgesic therapy to enhance narcotic efficacy.

457.12

STUDIES OF OPIOID AND ALPHA2 ANALGESIA AFTER SPINAL ADMINISTRATION IN AMPHIBIANS. C. W. Stevens* and G.M. Brenner Oklahoma State University, College of Osteopathic Medicine, Tulsa, OK 74107-1898.

Spinal administration of opioid and alpha-adrenergic agents in mammals produces potent and dose-dependent analgesic effects. To further characterize spinal sites of analgesic action in non-mammalian vertebrates, we used an alternative model for assessing analgesia in the Northern grass frog, *Rana pipiens*. Pain thresholds were estimated using the acetic acid test. Log-spaced doses of opioid agonists (*mu:* dermorphin, DAMGO, fentanyl, morphine; *delta:* DSLET, DADLE, DPDPE, deltorphin; and *kappa:* CI977, bremazocine, U50488, nalorphine) or alpha-adrenergic (dexmedetomidine, epinephrine, norepinephrine, clonidine) agonists were administered intraspinally at the level of the lumbar spinal cord using a hand-held microsyringe. All agents produced a dose-dependent analgesia which lasted at least four hours. ED50 values for the 12 opioids ranged from 0.04 nmol/frog for dermorphin to 43.1 nmol/frog for nalorphine. ED50 values for the alpha-adrenergic agents ranged from 6.4 nmol/frog for dexmedetomidine to 248 nmol/frog for clonidine. These data suggest that *mu*, *delta*, and *kappa* opioid receptors, as well as *alpha*$_2$-adrenergic receptors, mediate analgesia in the frog spinal cord. Supported by the Whitehall Foundation and NIH grant DA07326.

457.13

EFFECTS OF MORPHINE OR NERVE INJURY ON MECHANICAL, THERMAL AND CHEMICAL RESPONSE THRESHOLDS IN FROGS. S. Willenbring* and C.W.Stevens. OSU-Coll.Osteo.Med., Tulsa, OK 74107

Hind paw withdrawal responses to mechanical and thermal stimuli serve as quantifiable methods of assessing hypersensitivity following peripheral nerve injury in rodent models of neuropathic pain. The acetic acid test (AAT) provides a similar morphine-sensitive test in amphibians, although it has thus far been used only in studies of acute pain. We evaluated mechanical and thermal responses and their sensitivity to systemic morphine administration in the northern grass frog, *Rana pipiens* with comparison to AAT. Frogs were randomly divided into 3 groups, which received systemic (sc) injections of saline (control, n=12) or morphine at 100 (n=10) or 300 (n=10) nmol/g. Mechanical, thermal and AAT response thresholds were measured at 1, 2 and 3 hours post-injection and compared to prior baseline values. Morphine produced dose-dependent elevations in all three tests ($p<.05$, 100 nmol; $p<.01$, 300 nmol). Additionally, we evaluated changes in mechanical, thermal and AAT responses following sciatic nerve injury in a separate group of frogs. Following the establishment of baseline responses, frogs were randomly divided into 3 groups, which were subsequently subjected to chronic constriction injury (CCI; n=13), sciatic cryoneurolysis (SCN; n=13) or sham surgery (control; n=6). Responses were then measured 3 times per week for 4 weeks. Preliminary results indicate a significant decrease in acetic acid ($p<.01$) and mechanical ($p<.05$) response thresholds following either lesion at 2 weeks post-injury. These results suggest a potential usefulness of the amphibian for future investigations of the neural and pharmacological mechanisms of acute and neuropathic pain states. (support: NIH 1-F32-NS09732-01)

457.14

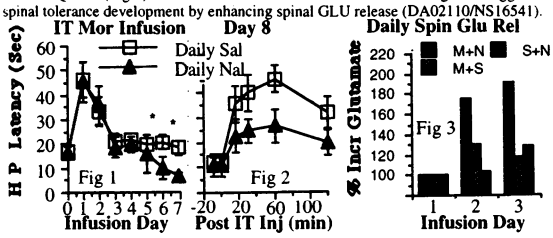
THE NEURONAL PATHWAYS THAT MEDIATE THE ANTINOCICEPTION PRODUCED BY MICROINJECTION OF MORPHINE IN THE RAT PERIAQUEDUCTAL GRAY. Herbert K. Proudfit* and Fang Fang, Dept. of Pharmacology, Univ. of Illinois at Chicago, Chicago, IL 60612.

Experiments were done to determine the neurotransmitters involved in mediating the antinociception produced by microinjecting morphine in the periaqueductal gray (PAG). The antinociceptive effects of morphine microinjected into the ventrolateral PAG were determined using both the tail flick and the foot withdrawal responses to noxious radiant heat in lightly anesthetized rats. The increase in the foot withdrawal response latency produced by morphine was reversed by intrathecal injection the cholinergic muscarinic antagonist atropine, but was not affected by the α_2-adrenoceptor antagonist yohimbine, the serotonergic receptor antagonist methysergide, or the opioid receptor antagonist naloxone. In contrast, the increase in the tail flick response latency produced by morphine was reduced by either yohimbine, methysergide or atropine. In summary, α_2-adrenoceptors, serotonergic, and cholinergic muscarinic appear to mediate the antinociception produced by morphine using the tail flick test, but only cholinergic muscarinic receptors appear to modulate the antinociceptive effects of morphine using the foot withdrawal response. These results indicate that the nociception produced by noxious heating of the feet is modulated by different neuronal systems than that produced by noxious heating of the tail. This work was supported by grant DA03980 from the National Institute on Drug Abuse.

457.15

EFFECT OF TRANSIENT ANTAGONISM ON SPINAL MORPHINE TOLERANCE AND SPINAL RELEASE OF GLUTAMATE. Takae Ibuki*, Stuart A. Dunbar and Tony L. Yaksh. Dept. of Anesthesiol, Univ. of Ca San Diego, La Jolla, CA 92093

To confirm whether tolerance development depends on duration of receptor occupancy or is influenced by receptor onset and offset, rats were implanted with intrathecal (IT) catheters connected to osmotic minipumps to receive morphine (MOR 20 nmol/hr) or saline (SAL) infusions for 7 days. Rats were tested daily on hot plate (HP) and then daily injected with naloxone (NAL 0.6 mg/kg) or SAL subcutaneously (SQ). On day 7 infusion was terminated and on day 8 HP response to a single IT probe-bolus of MOR (60 nmol) was observed. In separate rats a spinal loop dialysis catheter was also placed to measure glu (GLU) release before and after daily SQ NAL injection in MOR or SAL-infused rats. ITMOR infusion evoked a maximum increase on HP which diminished over ensuing days (Fig 1). ITSAL was without effect. Day 8 response to IT-probe MOR was ITSAL+SQSAL=ITSAL+SQNAL>ITMOR+SQSAL>ITMOR+SQNAL (Fig 2 shows ITMOR-infused rats results). Daily resting spinal GLU release increased modestly in rats with ITMOR and was significantly elevated in rats receiving IT MOR+SQNAL (Fig 3). Results indicate that transient naloxone antagonism aggravates spinal tolerance development by enhancing spinal GLU release (DA02110/NS16541).



457.16

MORPHINE ANALGESIA OF THERMAL HYPERALGESIA, TOLERANCE AND PHYSICAL DEPENDENCE. M.Backonja*, M.F.Fabbrocini, and C.V.Levenick. Dept. of Neurology, Univ. of Wisconsin, Madison, WI, 53792.

Thermal hyperalgesia, a sign of neuropathic pain, can be effectively relieved with an opioid analgesic such as morphine, in a dose dependent fashion without the loss of analgesic effect, i.e. without the development of tolerance, as demonstrated in our earlier study. In this study the form of tolerance, the response to an acute single dose of morphine in animals chronically treated with morphine, and physical dependence precipitated with administration of naloxone were investigated. Thermal hyperalgesia was induced with loose ligation of the sciatic nerve, as per Bennett and Xie (1988), and a control group of rats received sham surgery. Morphine in doses of 20mg/kg/day was continuously delivered for seven days via osmotic pumps (Alza) and produced analgesia in ligated and sham-operated rats. Control groups of ligated and sham-operated rats were treated with placebo, received normal saline via osmotic pumps. Foot withdrawal latencies from radiant heat, as per Hargreaves et al (1988), were obtained by examiners who were blinded as to what treatment animals received. Tolerance was tested on the last day of morphine infusion with administration of a single 5mg/kg dose of morphine to all rats. Physical dependence signs was precipitated with administration of 1mg/kg of naloxone. A single dose of 5mg/kg of morphine produced the greatest analgesia in ligated rats treated with saline, modest analgesia in ligated rats treated with morphine and sham rats treated with saline, and no effect on sham rats treated with morphine, suggesting tolerance only in the latter group. Physical dependence induced naloxone precipitated signs of withdrawal in all rats but signs were more pronounced in rats chronically treated with morphine. In conclusion, this study demonstrates that tolerance to an opioid in the case of neuropathic, measured by a couple of methods, is not an inevitable outcome.

PAIN MODULATION: PHARMACOLOGY—OPIOIDS II

458.1

BIOCHEMICAL AND PHARMACOLOGICAL CHARACTERIZATION OF MULTIPLE ß-ENDORPHINERGIC SYSTEMS IN THE RAT PAG: IDENTIFICATION OF FACILITORY AS WELL AS INHIBITORY NOCICEPTIVE MECHANISMS P.J. Monroe,* A.A. Hawranko, D.L. Smith, and D.J. Smith. Depts. of Anesthesiology & Pharm./Tox., WV Univ., Morgantown, WV 26506-9134

The administration of ß-endorphin and morphine into the periaqueductal gray (PAG) of rats results in profound antinociception. We have previously demonstrated that in addition to activating neuronal mechanisms common to both agonists, ß-endorphin interacts with unique mechanisms which can be discriminated based on their differential sensitivities to the opioid antagonists CTP (D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2) and naltrexone (Smith et al., Reg. Peptides 50S; 199, 1994). Consistent with these results, [^{125}I]-ß-endorphin was shown in the current study to label a population of sites which (compared to those labelled by [^{3}H]-morphine) displayed a significantly higher affinity for CTP. In addition, a naltrexone insensitive binding component was identified in the [^{125}I]-ß-endorphin, but not [^{3}H]-morphine assays. Furthermore, comparable competitor affinities were determined across assays, suggesting an interaction of the two radioligands with common PAG sites. A naltrexone insensitive component to ß-endorphin antinociception was also identified in studies which evaluated the ability of the antagonist to shift the ß-endorphin dose response curve. Interestingly, the ability of low doses of CTP and naltrexone to inhibit increasing doses of ß-endorphin was described by a U shaped dose effect curve. The response to low (0.1 and 0.3 nmol) and high (10 nmol), but not intermediate (1 and 3 nmol) doses of ß-endorphin were antagonized by a 1 pmol dose of CTP, or a 30 pmol dose of naltrexone. As there was no evidence for allosteric interactions between [^{125}I]-ß-endorphin binding sites in the PAG, these results suggest that ß-endorphin may be activating pain facilitory mechanisms which counterbalance the overall antinociceptive effect of the peptide. Moreover, these pain facilitory mechanisms appear to be involved in the modulation of antinociceptive responses in animals exposed to stress. (see accompanying abstract by Hawranko et al.).

458.2

PAIN FACILITORY AND INHIBITORY SYSTEMS ARE ACTIVATED BY ß-ENDORPHIN: STRESS ENHANCES FACILITATION. A.A. Hawranko*, P.J. Monroe, and D.J. Smith Depts. of Anes. & Pharm., WVU., Morgantown, WV 26506

Opioids produce pain facilitation, as well as pain inhibition (see Parvini *et al.*, JPET. 265: 286, 1993). We have previously shown that repetitive exposure of rats to the noxious heat associated with hot-plate testing (52.5°C) causes stress induced analgesia (i.e. a 1.5 - 2 sec increase in tail flick latency, TFL), and an alteration in the ability to pharmacologically distinguish neuronal processes involved in ß-endorphin's antinociceptive actions in the periaqueductal gray (PAG) region of the midbrain (Hawranko *et al.*, Brain Res. 667:283, 1994). Currently, we report that occurring concomitantly with the stress are a reduction in the antinociceptive potency of ß-endorphin, and the demonstration of a pain facilitory role for the peptide at doses lower than those associated with antinociception. That is, when stereotaxically microinjected into the PAG of awake male rats not exposed to hot plate testing, ß-endorphin produces a dose dependent (0.1 - 10 nmol) increase in TFL. Multiple neuronal mechanisms account for this response, one of which is naltrexone resistant (Smith *et al.*, Reg. Peptides 50S:99, 1994). However, the antinociceptive mechanisms appear to change with hot plate stress. The maximal antinociception resulting from PAG microinjections of ß-endorphin is sharply reduced from 70% in the nonexposed rats to 15% above baseline in the hot plate exposed rats, and can be completely blocked by naltrexone. Moreover, a naltrexone sensitive pain facilitation (about 2 sec reduction in latency) is observed with doses of ß-endorphin (0.03 nmol) which were inactive in the non stressed rat. This facilitation is blocked by low doses of the opioid antagonist CTP, resulting in a marked enhancement of ß-endorphin antinociception to levels greater than those observed in nonstressed rats. Thus, opioid pain facilitation occurs at least in part via spinopetal neuronal processes originating in the PAG. In addition, facilitation of pain responses may be a more prominent effect of opioids under some environmental conditions.

458.3

SPINAL ANTINOCICEPTION FOLLOWING STIMULATION OF THE AMYGDALA DEPENDS ON OPIOID RECEPTORS IN THE VENTRAL PERIAQUEDUCTAL GRAY. S. A. Tershner* and F. J. Helmstetter. Department of Psychology, University of Wisconsin, Milwaukee, WI 53201.

The presence of certain environmental stressors activates a decending antinociceptive neural system which includes the amygdala, periaqueductal gray (PAG), and rostroventral medulla (RVM). Lesions of each of these brain sites as well as the application of *mu* opioid receptor antagonists to the PAG have been shown to be effective in blocking stress-induced hypoalgesia in behaving rats. Recent studies conducted in our laboratory have shown that microinjection of *mu* receptor agonists into the anterior basolateral nucleus of the amygdala (BLA) will inhibit the tail flick (TF) reflex evoked by radiant heat in barbiturate anesthetized rats, and this effect is blocked by lesions of the PAG. The purpose of this experiment was to determine if the antinociception following forebrain opioid injection is expressed through a second opioid synapse located in the PAG. Rats maintained under constant barbiturate infusion were implanted with cannulae aimed at the BLA and ventrolateral PAG. Radiant heat TF latency was recorded at 2 min intervals. As we have previously shown, DAMGO (0.5 µg x 2) applied to the BLA inhibited TF. This inhibition of TF was prevented by pretreatment of the vPAG with naltrexone (NTX, 5.0 µg). However, NTX injections in the vPAG made after DAMGO treatment did not reverse the effect. Pretreatment with the selective *mu* antagonist CTOP (0.05 µg) in vPAG was also effective supporting a role for *mu* opioids in this structure. Unlike CTOP, the putative epsilon receptor antagonist beta-endorphin$_{1\text{-}27}$ (6.0 µg) failed to block TF inhibition. These findings together with other recent data suggest that *mu* opioid receptors located within multiple CNS structures from the telencephalon to the dorsal horn may be important for antinociception. Our ability to activate PAG neurons presumably via an intact functional subset of their forebrain inputs will allow for more detailed study of the physiology of this structure.

458.4

DYNORPHIN (1-8) MODULATES THE BASAL AND MECHANICALLY EVOKED RELEASE OF SUBSTANCE P IN THE DORSAL HORN OF THE RAT. V.Zachariou* and B.D. Goldstein. Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta GA 30912.

Substance P (SP) is an 11 amino-acid peptide found in high concentrations in the dorsal horn of the spinal cord, primarily in the terminals of the unmyelinated primary afferent fibers. Evidence suggests that SP is a modulator of nociceptive information. Dynorphin (1-8) is an opioid peptide also found in the superficial laminae of the dorsal horn, which possesses higher selectivity for the κ-receptor. Previous studies in this laboratory showed that dynorphin (1-8) prevented the increase in the release of SP like-immunoreactivity (SPLI) during the application of a noxious thermal stimulus. The aim of this study was to investigate the effect of dynorphin (1-8) on the release of SPLI during the application of a noxious mechanical stimulus. A push-pull cannula was stereotaxically introduced into the superficial dorsal horn in decerebrate/spinal transected rats and the dorsal horn was perfused with artificial CSF. Noxious mechanical stimuli were applied to different areas of the ipsilateral hind paw and lower limb. The perfusate was collected and assayed for SPLI using RIA. Dynorphin (1-8) applied to the dorsal horn (1µM) prevented the mechanically evoked release of SPLI. The effect of dynorphin (1-8) was blocked by the κ-opioid receptor antagonist, nor-BNI. The effect of dynorphin (1-8) on the release of SPLI during the application of the mechanical stimulus was similar to that seen during the application of a thermal stimulus. Nor-BNI alone increased the basal release of SPLI, in agreement with previous studies. However, unlike the prolonged increase in the release of SPLI in the presence of nor-BNI following the thermal stimulus, the release of the peptide following the mechanical stimulus returned to baseline almost immediately. The above data suggest that the κ-receptor differentially regulates the release of SPLI following thermal or mechanical stimuli.

458.5

EFFECTS OF SNX-111, A HIGH-AFFINITY N-TYPE VOLTAGE-SENSITIVE CALCIUM CHANNEL ANTAGONIST ON MORPHINE-INDUCED RESPIRATORY DEPRESSION IN RATS. R.R. Dean*, S. S. Bowersox, and R.R. Luther. NEUREX Corporation, Menlo Park, CA 94025.

The influence of SNX-111 (synthetic ω-conopeptide MVIIA, a high-affinity antagonist of N-type voltage-sensitive calcium channels) on respiratory depression induced by morphine sulfate was studied in awake, male Sprague Dawley rats. Using whole-body plethysmography, respiratory minute-volume responses to breathing air containing 10% CO_2 were measured before, and 30, 60, 90, and 120 minutes after administering a combination of morphine sulfate (10 mg/kg or 30 mg/kg) or saline subcutaneously and SNX-111 (0.1 μg) or saline intrathecally. Intrathecal bolus injections of SNX-111 both attenuated and shortened the duration of respiratory depression induced by 10 mg/kg morphine but had little or no effect on respiratory depression induced by 30 mg/kg morphine sulfate. SNX-111 did not affect respiratory minute-volume responses to CO_2 inhalation when it was administered after subcutaneous injections of saline. These findings indicate that intrathecal administration of SNX-111 does not depress respiration nor does-it potentiate the respiratory depressant actions of morphine sulfate in rats.

458.6

EFFECTS OF KAPPA-OPIOID RECEPTOR AGONISTS ON MECHANOSENSITIVE PELVIC NERVE AFFERENT FIBERS INNERVATING COLON OF RAT. X. Su*, J. N. Sengupta and G. F. Gebhart. The University of Iowa, Department of Pharmacology, Bowen Science Building, Iowa City, Iowa 52242

Pelvic nerve afferent fibers from the colon travel primarily in the S1 dorsal roots. In the present study, 177 fibers in the S1 dorsal root were identified by electrical stimulation of the pelvic nerve; 53 (30%) responded to noxious colorectal distension (CRD; 80mmHg). Stimulus-response functions of 23 fibers were tested to graded CRD (5 to 100 mmHg, 30 sec.) and eight were tested after colonic instillation of 5% acetic acid (n=3) or 5% mustard oil (n=5). The effects of U50,488, U69,593, U62,066, and ICI204,488 were tested on responses of 31 fibers (15 untreated and 16 treated) to noxious CRD (80 mmHg, 30sec., every 4 min.). Of 15 fibers studied from untreated colon, 13 were inhibited by U50,488 (n=5), U69,593 (n=4), or U62,066 (n=4) in a dose-related manner. The ED50s for U50,488, U69,593, and U62,066 were 8.1±0.8, 8.4±2.2, and 5.7±1.2 mg/kg, respectively. ICI204,488, a peripheral κ-opioid receptor agonist, had no effect on two fibers studied to date. Responses of 16 fibers pretreated with acetic acid or mustard oil were tested with U50,488 (n=9), U69,593 (n=3), or U62,066 (n=4). There was no apparent difference in the inhibitory effect on these compounds on untreated and treated colon. The results suggest that κ-opioid agonists inhibit the responses of pelvic nerve afferents by activition of peripheral κ-opioid receptors.

458.7

EFFECT OF U50,488 ON MECHANOSENSITIVE AFFERENT FIBERS IN THE PELVIC NERVE INNERVATING THE URINARY BLADDER OF RAT. J. N. Sengupta*, X. Su and G. F. Gebhart. The University of Iowa, Department of Pharmacology, Bowen Science Building, Iowa City, Iowa 52242

Pelvic afferent fibers innervating the lower urinary tract travel in L6 and S1 dorsal roots. In the present study, 306 afferent fibers in the decentralized L6 dorsal root were identified by pelvic nerve stimulation; 163 (53.27%) responded to noxious urinary bladder distension (UBD; 80mmHg). Fifty-seven fibers were unmyelinated C-fibers (mean: 2.03±0.04 m/s) and 106 were myelinated Aδ-fibers (mean: 8.31±0.05 m/s). Stimulus-response functions of 13 fibers were tested to graded UBD (5 to 100 mmHg, 30 sec.) and seven of them were also tested after instillation of 0.5 ml of 30% xylene in silicone oil into the bladder. The effect of U50,488, a κ-opioid receptor agonist, was tested on responses to noxious UBD (80mmHg, 30 sec, every 4 min). U50,488 produced dose-dependent inhibition of responses to noxious UBD. The mean inhibition produced by U50,488 (16mg/kg) was to 41.2±2.2% (n=5) and 33.4±5.9% (n=9) of control from untreated and xylene-treated bladders, respectively. There was no apparent difference in the dose-response functions of U50,488 between afferents studied from untreated and treated bladder. The inhibition of responses of fibers by U50,488H suggests an activition of peripheral κ-opioid receptors.

458.8

κ-OPIOID RECEPTOR AGONISTS ATTENUATE RESPONSES TO COLORECTAL DISTENSION IN RATS WITH NORMAL OR INFLAMED COLONS. M.B.Burton*, and G.F.Gebhart. University of Iowa, Iowa City, IA, 52246.

Recently, κ-opioid receptor agonists have been reported to significantly attenuate responses of pelvic nerve afferents to noxious colorectal distension (CRD) (see Xu *et al.*, and Sengupta *et al.*, this meeting). The aim of this study was to evaluate the effects of the κ-opioid receptor agonists U-50,488, U-69,593, and ICI-204,448 on pseudaffective (visceromotor and pressor) responses to CRD in awake, unrestrained rats. The effects of these drugs were studied in rats with normal and with inflamed colons.

Rats with externalized femoral arterial and venous catheters and EMG electrodes (peritoneal musculature) were given 1 ml of either saline or 5% acetic acid (HAc) intracolonically 6 hours prior to testing. CRD was administered as a staircase function (0-80 mmHg) over 70s at 4 min intervals. Drugs were administered iv. using a cumulative dose regimen. All three agonists dose-dependently attenuated the pseudaffective responses to CRD in rats with normal or with inflamed colons. U-50,488 U-69,593 and ICI-204,448 all produced rightward shifts of the stimulus-response curve and significantly increased the extrapolated response thresholds. The slopes of these curves were decreased after U-50,488 and U-69,593, but not after ICI-204,448. At doses producing near maximal antinociceptive effects, U-69,593 and U-50,488 (but not ICI-204,448) also produced agitation.

These results indicate: first, that κ-opioid agonists can attenuate responses to noxious visceral stimuli; second, the actions of these agonists are, at least in part, mediated through peripheral receptors; and third, κ-opioid agonists may be useful in the treatment of visceral pain.

458.9

INTRATHECAL DELTORPHIN OR DPDPE PRODUCES ANTINOCICEPTION, BUT DOES NOT SUPPRESS FOS-LIKE IMMUNOREACTIVITY (FLI) IN THE RAT SPINAL CORD. D.L. Hammond*[1], H. Wang[2], N. Nakashima[2] and A.I. Basbaum[2], [1]Dept. of Anes. & Crit. Care, Univ. of Chicago, Chicago, IL 60637 and [2]Dept. of Anat. and Physiol., Keck Cntr. for Integr. Neurosci., UCSF, San Francisco, CA 94143

This study examined the ability of i.t. administration of the δ-1 receptor agonist DPDPE or the δ-2 receptor agonist [D-Ala[2], Glu[4]]deltorphin (DELT) to inhibit the behavioral response and the expression of FLI in the spinal cord induced by i.pl. injection of formalin in the rat. Five days after implantation of i.t. catheters, male S-D rats were i.t.-injected with either vehicle, DPDPE (10-60 μg), DELT (3-30 μg), or the μ receptor agonist DAMGO (0.3 μg). Ten min later, 100 μl of 5% formalin was injected i.pl. in one hindpaw and the number of flinches that occurred in the next hr was counted. The rats were then perfused with fixative and the spinal cord processed for FLI by standard immunocytochemical methods. Although DPDPE and DELT each dose-dependently suppressed formalin-evoked pain behaviors, FLI was not reduced by DELT and was only marginally reduced in the deep dorsal horn and ventral horn by the highest dose of DPDPE. However, FLI was nearly completely inhibited by an equiantinociceptive dose of DAMGO. In the absence of formalin, FLI-neurons were not evident in vehicle-, DPDPE or DELT-treated rats. These data suggest that (1) the production of antinociception by δ opioid receptor agonists is independent of the suppression of FLI in the spinal cord and (2) the intracellular mechanisms responsible for δ-mediated opioid antinociception differ from those responsible for μ-mediated opioid antinociception. Supported by DA06736 and DA08377.

458.10

ANTINOCICEPTIVE EFFECT OF KAPPA- AND MU-OPIOID AGONISTS ON THE CARDIOVASCULAR RESPONSE INDUCED BY VAGINAL DISTENSION L. Diop*, N. Friese, S.G. Dahl and P.J.M. Rivière. Dept. of Gastroenterology, Institut de Recherche Jouveinal, 3-9, rue de la Loge, 94265 Fresnes, France.

Both uterine and vaginal distensions increase spinal and supraspinal neuronal activity and induce an escape behavior in response to this painful stimulus. The cardiovascular reflex (CVR) response has been widely used to measure the intensity of pain induced by distension of visceral hollow organs. The present study used the CVR response to evaluate the antinociceptive activity of μ- and κ-opioid agonists against pain induced by vaginal distension (VD) in anaesthetized rats.

The CVR responses induced by VD were measured in pentobarbital (60 mg/kg i.p.) anaesthetized female rats. VD, by the inflation of a 1 cm length latex balloon placed in the vagina with 1.5 mL of water for 1 min, were performed every 5 min.

VD induced a reproducible hypotensive response (27.5 ± 1.6 mm Hg). Morphine (0.03 - 1 mg/kg i.v.) and U-50,488H (0.08 - 1.6 mg/kg i.v.) produced a dose-related inhibition of the CVR induced by VD. ED_{50} were 0.16 and 0.49 mg/kg i.v. respectively. Morphine (0.3 - 10 μg/rat) administered i.c.v. displayed an antinociceptive effect (81.4 ± 7.9 % at 0.3 μg/rat) whereas U-50,488H (30 - 300 μg/rat) was inactive by this route. A low dose of naloxone (30 μg/kg i.v.) blocked the response to morphine but not to U-50,488H. The κ-opioid antagonist, nor-BNI (10 mg/kg s.c.) abolished the response to U-50,488H but not to morphine.

This demonstrates that CVR response is a reliable parameter to assess the antinociceptive activity of opioids in distension-induced vaginal pain and that the κ-opioid agonist, U-50,488H, blocks vaginal nociception by acting on peripheral κ-opioid receptors. In contrast, both central and peripheral μ-opioid receptors may be involved in morphine-induced antinociception. To our knowledge, this is the first demonstration of a κ-opioid receptor mediated antinociceptive effect in VD.

458.11

EFFECT OF FENTANYL AND NALOXONE ON THALAMICALLY INDUCED PAIN IN HUMANS. A.L. Velasco*, F. Brito, M. Gallegos, F. Jiménez, F. Velasco and M. Velasco. Unit of Neurology and Neurosurgery, General Hospital of Mexico, S.S. and UMR Neurophysiolosy, National Medical Center, IMSS, Mexico City 73032

This work was performed on epileptic patients with intractable generalized tonic clinic seizures and chronically implanted electrodes in the Centro Median Thalamic Nuclei (CM)as a neuroaugmentative procedure for seizure control. As a collateral observation, we found that acute unilateral electrical stimulation of CM (10 sec trains of rectangular square pulses, frequency 60/sec, duration 1.0 msec and threshold intensity=1200-2000μA) in its more medial and basal portion close to nucleus parafasciculares (coordinates: AP=10, lat=2/10 and *H=+1/10 of the AC-PC line length) produced an* intense pain response on the contralateral face neck and arm characterized by long lasting (9-13 min) EEG desynchronization, tachicardia and hypertonia of the chin muscles.

In these patients, single 5.0 μg/kg i.v. injections of fentanyl completely blocked the threshold to pain response and was increased from 1200-2000μA to 5000μA. This effect was reversed by naloxone (3.0μg/kg) which was administered 30 min after fentanyl.

In contrast, no changes in threshold and intensity of the pain response were observed after comparative injections of saline solution.

These data suggest that fentanyl (opiod antagonist) is able to interfere with pain at the level of the thalamus in addition to its well known effect at the spinal cord.

Partially supported by the National Council of Science and Technology contract F348-A9301

458.12

Opioid peptide gene delivery to mouse sensory neurons using herpes virus vectors. G. Davar*, B.P. Vos, R. Day, W. Dong, D. Coen, L. Fang, W.R. Bebrin. Dept. of Anesthesia, Brigham and Women's Hosp., Dept. of Biol. Chem. and Molec. Pharm., Harvard Medical School, Boston, MA 02115 USA, and Clin. Research Institute of Montreal, Montreal, Quebec, Canada.

Herpes simplex viruses are potentially useful vectors for the delivery of genes encoding opioid peptides to sensory neurons in vivo. To explore the potential of such vectors, a herpes simplex virus (HSV) recombinant, *tk*LTRdynB (see Davar et al., '93), containing the full length coding sequence for rat prodynorphin inserted into the herpes simplex thymidine kinase gene and downstream of the MoMLV-LTR promoter, was inoculated onto the mouse cornea. Evidence of prodynorphin gene expression was observed in rostromedial trigeminal ganglion neurons 4 and 8 days following inoculation by in situ hybridization. Markedly greater numbers of prodynorphin-labeled neurons were observed in *tk*LTRdynB-infected animals than were observed in control virus- or mock-infected animals. Prodynorphin expression occurred only in neurons that expressed HSV latency-associated transcripts (LAT), a *marker of HSV infection.*

In a separate preliminary experiment, *tk*LTRdynB was *inoculated onto the* skin of mice in the periocular region. Low intensity mechanical stimulation of this area 3 days after infection produced a higher frequency of directed face wiping than was observed after infection with a similarly-constructed lacZ-containing TK^- virus (Wilcoxon Signed Rank Test, $p < .05$).

These data are evidence that the gene for prodynorphin can be delivered to and expressed in mouse sensory neurons in vivo using an HSV vector. Our working hypothesis is that release of dynorphin peptides from HSV-infected sensory neurons can contribute to changes in the behavioral response to mechanical stimulation.

458.13

OPIOID INVOLVEMENT IN ELECTROMYOGRAPHIC (EMG) RESPONSES INDUCED BY INJECTION OF INFLAMMATORY IRRITANT INTO DEEP NECK TISSUES. J.W. Hu*, I. Tatourian and H. Vernon. Faculty of Dentistry, University of Toronto, and Canadian Memorial Chiropractic College, Toronto, Ontario, Canada.

We previously demonstrated that injection of the inflammatory irritant and C-fiber excitant mustard oil (MO, 20%, 20 μL) into the C2 paravertebral region produces an EMG activity increase in various neck and jaw muscles (Hu et al, Pain 1993;55:234). To determine if endogenous opioids are involved in these MO-induced EMG responses, experiments were performed in 23 anesthetized (halothane/N_2O/O_2) rats in which bipolar EMG electrodes were inserted into bilateral deep neck muscles, ipsilateral trapezius muscle and ipsilateral masseter muscle. The opiate antagonist naloxone (0.6 mg/kg, n=7; 1.2 mg/kg, n=7; 2.5 mg/kg, n=9) administered (i.v.) 30 minutes after MO injection (or after the EMG response level had returned to baseline level) produced dose-dependent EMG activity increases in all 4 muscles. The two higher doses of naloxone produced higher incidences (>50% vs <15% for the lowest dose) of EMG activity increases at a relative constant latency (13.3 ± 5.7 min, mean ± S.D.) and duration (12.6 ± 5.8 min); repeated doses of naloxone provoked higher incidences and larger EMG responses than the initial naloxone administration. The MO-induced EMG activity increase is consistent with the concept of "central sensitization", and the ability of the opioid antagonist naloxone to "rekindle" this increase is consistent with our other findings (Yu et al, J. Neurophysiol. 1994;72;1430) of the involvement of endogenous opioids in modulatory mechanisms underlying central sensitization. Supported by the Foundation for Chiropractic Education and Research (94-03-08).

PAIN MODULATION: PHARMACOLOGY—ALLODYNIA

459.1

MEXILETINE INHIBITS INTRATHECAL STRYCHNINE-INDUCED ALLODYNIA AND NOXIOUS PAW PINCH IN THE RAT. S.E. Sherman*, C.W.Loomis and E.M. Hodge. School of Pharmacy & Division of Basic Medical Sciences, Memorial University, St. John's, Newfoundland, Canada A1B 3V6

The blockade of spinal glycine receptors with intrathecal (i.t.) strychnine (STR) produces a reversible, segmentally localized allodynia-like state in the rat. The purpose of this study was to investigate the effect of mexiletine, a sodium channel blocker used in the management of clinical allodynia, on STR-induced allodynia, and to compare its effect on mechanical nociception. Male, Sprague-Dawley rats, fitted with chronic i.t. catheters, were lightly-anesthetized with urethane. Stimulus-evoked changes in blood pressure and heart rate were recorded from the left carotid artery and cortical electroencephalographic (EEG) activity was continuously monitored using subdermal needle electrodes. After i.t. STR (40 μg), normally innocuous hair deflection (HD) elicited a marked increase in mean arterial pressure and heart rate, an abrupt motor withdrawal response, and desynchronization of the EEG, comparable to those observed with calibrated, bilateral noxious hind-paw pinch (without STR). Mexiletine (5-30 mg/kg i.v.), given 5 min before i.t. STR, dose dependently inhibited the pressor, heart rate and motor withdrawal responses evoked by HD; these effects were significantly different from control at 15 and 30 mg/kg. A similar mexiletine dose-response curve was observed against noxious hind-paw pinch. The percent synchrony in the EEG was significantly increased with i.v. mexiletine at 30 mg/kg, suggesting the inhibition at this dose was a non-specific effect. In conclusion, systemic mexiletine attenuates the cardiovascular and motor withdrawal responses evoked by HD in the presence of i.t. STR, and by noxious mechanical stimulation (without STR), with comparable potency. These results are consistent with the effect of mexiletine in clinical allodynia and in a focal spinal ischemia model of allodynia. (Supported by MRC of Canada)

459.2

DYNORPHIN AND NMDA RECEPTOR-MEDIATED GLUTAMATE IN ALLODYNIA DEVELOPMENT, R. Wagner* and J.A. DeLeo, Dept. of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH, 03755.

Spinal glutamate, via the NMDA receptor, and the *kappa* opioid dynorphin are implicated in the development of neuropathic pain following peripheral nerve injury. In addition, dynorphin has nonopioid functions that involve the NMDA receptor. Sciatic cryoneurolysis (SCN) is a complete but transient injury to the sciatic nerve that produces mechanical allodynia at 21-28 days. Since allodynia appears later in SCN than in other neuropathy models, we questioned if it was NMDA receptor-dependent. We also explored an interrelationship of spinal dynorphin and glutamate following SCN using quantitative immunohistochemistry. Rats received 0.25 mg/kg MK-801 i.p. twice daily from 0-7 or 0-21 days post-SCN to preemptively block the NMDA receptor. In different rats, an antibody to dynorphin (1-13) was given i.p. at 16.6 mg/kg twice daily from 14-21 days post-SCN. Allodynia outcome at 42 days was assessed using von Frey hairs, and dynorphin (DYN) and glutamate (GLUT) immunoreactivity was quantitated at 42 days in laminae II-III by measuring proportional area stained and relative optical density, respectively. Only 0-7 day MK-801 increased mechanical thresholds (mean ± S.E.M., 7g ± 1.2) from saline controls (3.9g ± 0.6) by Students t-test [$p<0.05$]. However, this did not prevent allodynia since baseline thresholds were 12 or 15g. However, pharmacological treatments did alter resulting DYN and GLUT. Dynorphin antibody increased GLUT as compared with saline controls (807.2 ± 3.61 vs. 779.6 ± 8.3) [$p<0.01$]. MK-801 from 0-21 days decreased DYN compared to controls, but not significantly. This study suggests that allodynia development following SCN involves only an early and limited NMDA receptor contribution, and that decreasing available dynorphin had significant and lasting effects on spinal glutamate expression.

459.3

PHARMACOLOGY OF TACTILE ALLODYNIA IN THE STREPTOZOTOCIN DIABETIC RAT

S. R. Chaplan[1]*, N.A. Calcutt[2], and T.L. Yaksh[1], Depts. of Anesthesiology[1] and Pathology[2], UCSD, 9500 Gilman Drive, La Jolla CA 92093.

Tactile allodynia (light mechanical stimulus perceived as painful) is a prominent abnormality in painful diabetic neuropathy; investigations in rat models of diabetes to date have focused on pressure hyperalgesia and thermal stimuli. We have recently reported the finding of hyperglycemia-related tactile allodynia in the streptozotocin (STZ) diabetic rat, which may be prevented by systemic insulin treatment. In this study we report investigations on the spinal pharmacology of the allodynia. Adult female Sprague Dawley rats were rendered diabetic with a single dose of STZ, 50 mg/kg , i.p. At 3 weeks of diabetes, lumbar intrathecal catheters were implanted. At 4 to 6 weeks of diabetes, paw withdrawal thresholds (PWT) to graded von Frey hairs were measured in awake, unrestrained rats before and after spinal drug applications. Acute treatments with intrathecal dexmedetomidine (alpha-2 agonist), AP5 (NMDA antagonist), CP96345 (NK1 antagonist) and morphine (μ agonist) normalized PWT in a dose-response fashion; ketorolac (COX inhibitor) also had significant efficacy. These results strongly suggest the presence of synaptic facilitation at the spinal level in this model. However, the spinal pharmacology of this diabetic allodynia is not identical to other models of allodynia or spinal facilitation. This model promises to be useful in the study of hyperglycemia-related abnormalities of afferent processing in diabetes, and in the search for treatments for painful diabetic neuropathy in humans. *(Support : NIH, RSDSA)*

459.4

CHARACTERIZATION OF THE ANTIALLODYNIC EFFICACY OF MORPHINE IN A MODEL OF NEUROPATHIC PAIN IN RATS. D. Bian, M.L. Nichols, M.H. Ossipov, *R.B. Raffa* and F. Porreca. Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724 and *Drug Discovery Research, R.W. Johnson, Pharmaceutical Research Institute, Spring House, PA 19477.

Neuropathic pains have often been classified as opioid-resistant. The present studies have investigated systemic (intraperitoneal; *i.p.*), intrathecal (*i.th.*) and intracerebroventricular (*i.c.v.*) antiallodynic actions of morphine in a nerve constriction model (L5-L6) of neuropathic pain in rats. Allodynia was assessed by determining the response threshold to stimulation with von Frey filaments. Morphine administered *i.p.* (3-30 mg/kg) or *i.c.v.* (1-10 μg) produced dose-dependent antiallodynic effects which were readily antagonized by naloxone (5 mg/kg, *i.p.* at -10 min). The *i.p.* and *i.c.v.* morphine antiallodynic A_{50} values (and 95% confidence limits) were 6.4 (5.3-7.6) mg/kg and 2.2 (1.0-4.6) μg, respectively. Morphine administered *i.th.* (3-100 μg) did not produce any antiallodynic actions. These data suggest that the failures of morphine to produce antiallodynic effects when administered *i.th.* in this model may be due, in part, to a decrease in available spinal μ opioid receptors which may result from degeneration of primary afferent fibers after nerve constriction injury. In contrast, the antiallodynic actions of *i.p.* or *i.c.v.* morphine appear to depend on supraspinal activation of opioid (μ?) receptors and subsequent activation of descending modulatory systems. The inconsistent data seen clinically with morphine in neuropathic pains may be related to the lack of supraspinal/spinal synergy that is normally associated with morphine efficacy in conditions of acute pain.

459.5

MODULATION OF SPINAL OPIOID EFFICACY IN A MODEL OF NEUROPATHIC PAIN IN RATS. M.L. Nichols, D. Bian, M.H. Ossipov, E.D. French* and F. Porreca. Dept. Pharmacology, Univ. of Arizona HSC, Tucson, AZ 85724

Neuropathic pains have often been classified as opioid resistant. The present studies have investigated the antiallodynic actions of intrathecal (*i.th.*) opioids in a nerve constriction model of neuropathic pain in rats. Morphine, at doses up to 300 nmol (100 μg), was ineffective at altering allodynia. In contrast, [D-Ala^2, NMPhe^4, Gly-ol]enkephalin (DAMGO) produced dose-related antiallodynic actions with an A_{50} value (95% C.L.) of 1.2 (0.8-1.8) nmol. Furthermore, [D-Ala^2, Glu^4]deltorphin (DELT) was able to produce a significant antiallodynic effect (~70% of the maximum) at 300 nmol; the A_{50}(95% C.L.) for DELT was 111 (91-136) nmol. Co-administration of morphine (90 nmol) with an ineffective dose of DELT (10 nmol) was able to produce a significant, long-lasting antiallodynic action; this apparently synergistic effect was antagonized by *i.th.* naltrindole (NTI) at a dose selective for opioid δ receptors (67 nmol). Blockade of cholecystokinin-B (CCK_B) receptors with L365,260 (6.3 pmol, *i.th.*) did not produce any effects alone; however, coadministration of morphine (90 nmol) with L365,260 resulted in significant antiallodynic actions (~65% of maximum); this effect was also antagonized by a δ-receptor selective dose of *i.th.* NTI. Both an opioid δ agonist, and a CCK_B antagonist, though ineffective alone, increased morphine efficacy in an NTI-sensitive fashion. These findings suggest that blockade of CCK_B receptors may enhance the availability of endogenous enkephalins which increase morphine efficacy via a μ-δ interaction. Additionally, the finding that DAMGO, but not morphine, was able to attenuate allodynia suggest that the actions of μ opioids in this model may be related to efficacy and that morphine may be unable to produce antiallodynic effects in this model due, in part, to a reduction in available μ receptors which may occur as a result of presumed degeneration of primary afferents following nerve constriction injury. The data suggest that the use of high efficacy μ opioids may be more effective in the treatment of allodynia associated with neuropathies and that CCK_B antagonists, and eventually, opioid δ agonists, may be useful adjuncts.

459.6

NEW PAIN MODEL INDUCED BY A MONOCLONAL ANTIBODY, CHEMOTHERAPEUTIC AGENT. L.S. Sorkin*, R. Slart, T.L. Yaksh and A.L. Yu, Depts of Anesthesiology and Pediatrics, UCSD, La Jolla, CA. 92093

Systemic administration of an antibody against the GD2 ganglioside is a promising treatment for neuroblastoma. A serious side effect is the development of an opiate resistant pain state. We sought to determine if antibody administration to rats would also lead to a quantifiable pain state, thus producing a model for developing better pain management.

Halothane anesthetized rats (Sprague-Dawley 300-350 g male) were implanted with jugular catheters and allowed to recover for 3 hrs. After acclimation to a test chamber, withdrawal threshold (WT) to mechanical stimulation was measured with von Frey hairs applied to the hindpaws. An index of whole body touch evoked agitation was also obtained. Following baseline measurements, saline, 0.1, 1.0, 3.0 or 10 mg/kg of antibody was administered through the catheter. Behavioral responses were measured every 15 min for the next 3 hours. The experimenter was blinded to the syringe contents. Another group of rats given 1.0 mg/kg antibody were monitored for blood pressure (BP) and heart rate (HR).

Saline and 0.1 mg/kg antibody both initiated a small drop in WT (allodynia). WT returned to baseline for the controls and was maintained for the experimental group. All 3 higher doses of antibody caused a large, precipitous drop in WT for the entire observation period. Touch evoked agitation was produced by all doses of antibody. Resting BP and HR following 1.0 mg/kg antibody increased (mean change 14 mm Hg and 30 beats/min, respectively).

These results indicate that systemic administration of GD2 antibody in the rat reliably produces mechanical allodynia and may serve as a model for assessing the pharmacology of this clinically relevant pain state.

459.7

INVOLVEMENT OF α_2-ADRENOCEPTORS IN THE PERIAQUEDUCTAL GRAY-INDUCED INHIBITION OF DORSAL HORN CELL ACTIVITY IN RATS. Y.B.PENG*, Q.LIN, W.D.WILLIS. Dept. of Anatomy and Neuroscience, The University of Texas Medical Branch, Galveston, Texas 77555-1069

Serotonergic, opioid, and noradrenergic systems are considered the major descending analgesic systems. The relationship between stimulating PAG and the likely effect on the noradrenergic system is of great interest. Brain stem cell groups containing noradrenergic neurons that project to the spinal cord include the locus coeruleus, subcoeruleus, Kölliker-Fuse nucleus, parabrachial nuclei and the more caudal A5 cell group. A projection from the PAG to the locus coeruleus and parabrachial region has been reported. Kwiat et al demonstrated that there are noradrenergic neurons that send axon branches both to the spinal cord and to the PAG. It is possible that electrical stimulation of the PAG might activate bulbospinal noradrenergic controls via antidromic activation of the noradrenergic axons which project to the PAG, and subsequent orthodromic activation of the spinally projecting collaterals of the noradrenergic cells in A5, locus coeruleus, subcoeruleus and A7/Kölliker-Fuse nucleus. The current experiment was designed to study the effect of α_2-adrenoceptors on spinal dorsal horn cells on PAG-induced inhibition. Male Sprague-Dawley rats (270-360g) were used in the study. A microdialysis fiber was introduced into the dorsal horn of the lumbar enlargement for drug administration. An electrode was placed in the vicinity of the microdialysis fiber for extracellular single unit recording. When stimulating the PAG, responses to mechanical stimuli applied to the skin (brush, press, and pinch) were inhibited. When a α_2-adrenoceptor antagonist, idazoxan (1 mM), was administered, the background activity and the responses of the cell to brush, press, and pinch stimuli increased. Interestingly, the PAG-induced inhibition to mechanical stimuli was reduced by idazoxan. This result suggests an involvement of α_2-adrenoceptors in PAG-induced inhibition of dorsal horn cell activity. This study was supported by NIH Grants NS09743 and NS11255.

459.8

PROTEIN KINASE C INFLUENCES THE EFFECTIVENESS OF PERIAQUEDUCTAL GRAY-INDUCED INHIBITION OF PRIMATE SPINOTHALAMIC TRACT NEURONS BY DESENSITIZING SPINAL GLYCINE AND GABA RECEPTORS. Q. Lin*, Y.B. Peng and W.D. Willis. Dept. of Anatomy and Neurosciences, UTMB, Galveston, TX 77555-1069.

Previously we demonstrated that spinal glycinergic and GABAergic interneurons are involved in the inhibition of primate spinothalamic tract (STT) neurons by stimulation in the periaqueductal gray (PAG). In this study, we examined the changes in the inhibition mediated by glycine and GABA that occur when STT cells were sensitized by intradermal (i.d.) injection of capsaicin and the involvement of protein kinase C (PKC) in this process. Recordings were made from STT cells in anesthetized monkeys (*M fascicularis*) using a multi-barreled microelectrode. Responses of STT neurons to innocuous and noxious mechanical stimuli applied to the skin were inhibited by iontophoretic application of glycine, GABA and muscimol and by stimulation in PAG. Sensitization of responses of STT cells to mechanical stimuli was accompanied by a reduction in glycine and PAG inhibition, but GABA inhibition was only attenuated in some cells, and muscimol inhibition was unchanged or increased. Similar results were observed when PKC activator, phorbol ester (12-*O*-tetra-decanoylphorbol-13-acetate) was infused within the spinal dorsal horn by microdialysis. Conversely, spinal cord administration of the selective PKC inhibitor, NPC-15437, prevented the capsaicin-induced sensitization and glycine, GABA and PAG inhibition of STT cells. Our results provide evidence that i.d. injection of capsaicin may result in the desensitization of glycine and perhaps $GABA_B$, but not $GABA_A$, receptors in the spinal cord, and this reduces the effectiveness of PAG inhibition. These events are likely to contribute to allodynia and hyperalgesia seen after i.d. injection of capsaicin, and PKC may mediate the desensitization of some inhibitory amino acid receptors. (Supported by NIH grants NS 09743 and NS11255).

459.9

EXCITATORY AMINO ACIDS (EAA) IN THE PERIPHERY CONTRIBUTE TO THE DEVELOPMENT OF ALLODYNIA AND THERMAL HYPERALGESIA IN ARTHRITIC RATS. K.N. Westlund*, N.B. Lawand, and W.D. Willis Dept. of Anatomy and Neurosciences, Univ. of Texas Med. Branch, Galveston, TX 77555.

EAA receptors are thought to mediate fast excitatory synaptic transmission in the mammalian CNS. However, EAAs may have specific, receptor mediated functions in the periphery also. The aim of the present study is to investigate the role of peripherally released EAAs in the development of hyperalgesia and allodynia. Inflammation was induced in one group of rats by injection of kaolin/carrageenan into the knee joint. With a CMA-20 microdialysis probe inserted in the knee joint cavity, it was determined that glutamate, aspartate and arginine concentrations were increased with inflammation. A second group of rats was injected with L-aspartate (ASP), L-glutamate (GLU) and arginine (ARG) either alone or in different combinations. Paw withdrawal latencies (PWL) to radiant heat and withdrawal thresholds (WT) for innocuous mechanical stimuli were measured before and after intraarticular administration of the AAs. When administered alone, the AAs did not have any effect on withdrawal from noxious and innocuous stimuli. However, when combinations of ASP/GLU, ASP/ARG or ASP/GLU/ARG were applied, a significant decrease in PWL and WT was observed as compared to baseline. Therefore, hyperalgesia could be induced by intraarticular injection of combinations of AAs, although the joint did not become inflamed. NMDA (AP7) and non-NMDA (CNQX) receptor antagonists applied intraarticularly in arthritic animals as well as rats injected with ASP/GLU/ARG reversed the mechanical allodynia and thermal hyperalgesia. Our data suggest that EAAs injected or released in the synovial fluid may act on glutamate receptors in the knee joint capsule to produce mechanical allodynia and thermal hyperalgesia but not inflammation. (Supported by NS32778, NS11255, and NS09743.)

PHOTORECEPTORS AND RPE

460.1

LIGHT-MEDIATED RETINOIC ACID PRODUCTION. E. Wagner, P. McCaffery, J. Mey and U. C. Dräger*, E.K. Shriver Center, Waltham, MA 02254 and Harvard Medical School, Boston MA.

The transcriptional activator retinoic acid (RA) is generated from retinaldehyde by retinaldehyde dehydrogenases. In most tissues we find a quantitative correlation between levels of retinaldehyde dehydrogenases and levels of endogenous RA, but the postnatal eye shows an anomaly: while under dark-adapted conditions the two parameters correlate well, illumination of the eye results in a disproportional increase in RA synthesis. Appearance of this light-induced RA production in the developing eye coincides with maturation of rhodopsin, indicating that it is due to all-*trans* retinaldehyde, released from bleached rhodopsin, becoming accessible to the dehydrogenases. Although the main fate of released all-*trans* retinaldehyde is capture by retinoid binding proteins for regeneration of 11-*cis* retinaldehyde, bright light is likely to transiently saturate the visual recycling mechanism. As the vertebrate eye contains high levels of cytosolic retinaldehyde dehydrogenases, and as the oxidation of retinaldehyde to RA is an irreversible reaction, RA production is probably an unavoidable by-product of bright illumination, providing a mechanism by which light can directly influence gene transcription. Transcription of several ocular proteins is known to respond to light, including arrestin. We find that RA injection into mice causes a dose-dependent increase in arrestin immunoreactivity in the outer nuclear layer. Light-mediated RA production due to precursor release from bleached rhodopsin provides a plausible and general mechanism for light effects in the vertebrate eye which cannot be explained by electric activity. Supported by R01 EY03819 and a gift from Johnson & Johnson.

460.2

LIGHT-INDUCED FOS EXPRESSION IN THE VISUAL SYSTEM OF THE *RD* MOUSE. C.M.Araki[1], N.M.Hossokawa[2], L.R.G.Britto[2], and D.E.Hamassaki-Britto*[1]. Depts. [1]Histology and Embryology and [2]Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo (SP), 05508-900, Brazil.

Several mutations in the gene encoding the rod cGMP-phosphodiesterase during early development are responsible for the degeneration of photoreceptors in the *rd* mouse. This study was undertaken to evaluate the possibility that the remaining retinal cells and cells in central visual areas could be activated by visual stimuli. Normal (C57BL/6) and *rd* (C3H) adult mice were dark-adapted, and just before the visual stimulation an occluder was placed over one of the eyes (control). The visual stimulus consisted of flashes or diffuse light that were presented for 1-1.5 h to the opened eye. An antibody against the Fos protein (Oncogene) was used with the avidin-biotin-peroxidase method to determine the cells that were activated using this protocol. Fos-positive cells were observed in the inner third of the inner nuclear layer and in the ganglion cell layer of both normal and *rd* mice. Only a few Fos-positive cells were seen in control retinae of either normal or *rd* mice. Fos-positive cells were also observed in the superficial layers of the contralateral superior colliculus and ventral lateral geniculate nucleus of both strains of mice, whereas the correspondent ipsilateral regions contained a very small number of stained cells. Taken together with previous behavioral data indicative of residual vision in *rd* mouse, our data suggest that the *rd* mouse visual system retains some functional characteristics.
Supported by: FAPESP and CNPq (Brazil).

460.3

PHOTORECEPTOR DEVELOPMENT IN CULTURE. ARNOLD G. HYNDMAN*. DEPARTMENT OF BIOLOGICAL SCIENCES, RUTGERS UNIV., PISCATAWAY, NJ 08855

EXTRINSIC FACTORS IN THE NEURAL RETINA ARE BELIEVED TO PLAY AN IMPORTANT ROLE IN DETERMINING WHETHER PRECURSORS WILL DIFFERENTIATE INTO EITHER NEURONS OR PHOTORECEPTORS. IN ORDER TO FURTHER EXAMINE THIS PHENOMENON, CULTURES FROM EMBRYONIC DAY 6 CHICK NEURAL RETINAE WERE DISSECTED, DISSOCIATED AND CULTURED IN CONDITIONS KNOWN TO YIELD A HIGH PERCENTAGE OF PHOTORECEPTORS. FOR THE FIRST TWO DAYS IN VITRO CULTURE MEDIA WAS SUPPLEMENTED WITH 5% FETAL BOVINE SERUM (FBS). AT THE END OF THIS PERIOD, SOME CULTURES WERE PLACED IN A SERUM-FREE MEDIUM SUPPLEMENTED WITH ONLY CATALASE AND INSULIN (CI). ALL CULTURES WERE EXAMINED AT 3 DAYS IN VITRO. CELL SURVIVAL WAS 50% GREATER IN CI CULTURES COMPARED TO FBS CULTURES. THIS INCREASE WAS DUE TO AN INCREASE IN THE NUMBER OF PHOTORECEPTORS PRESENT. THE ADDITION OF TRANSFERRIN, BASIC FIBROBLAST GROWTH FACTOR AND TAURINE TO CI CULTURES RESULTED IN SURVIVAL OF NEURONS AND PHOTORECEPTORS SIMILAR THAT OF CULTURES SUPPLEMENTED WITH CI ALONE. PHOTORECEPTORS (PHR) WERE CLASSIFIED BASED ON THEIR IN VITRO MORPHOLOGY. PHR WITH A SMALL FILOPODIA (A RUDIMENTARY OUTER SEGMENT?) AND LITTLE NEURITE DEVELOPMENT WAS CALLED TYPE 1. TYPE 2 PHR WAS SIMILAR, HOWEVER, THERE WAS NOTABLY MORE NEURITE DEVELOPMENT ORIGINATING FROM THE FILOPODIA AND FROM THE OPPOSITE END OF THE CELL. TYPE 3 PHR LACKED A FILOPODIA, BUT THERE WERE NEURITES PRESENT AT EACH END OF THE CELL. FBS CULTURES CONTAINED 60% TYPE 1 PHR, 36% TYPE 2 PHR AND 4% TYPE 3 PHR. CI CULTURES CONTAINED 49% TYPE 1 PHR, 48% TYPE 2 PHR AND 3% PHR. FROM THESE STUDIES, IT CAN BE CONCLUDED THAT FACTORS IN SERUM CAN INFLUENCE THE DIFFERENTIATION AND/OR SURVIVAL OF PHOTORECEPTOR CELLS OR THEIR PRECURSORS. TRANSFERRIN, BASIC FIBROBLAST GROWTH FACTOR AND TAURINE WHICH MAY HAVE IMPORTANT ROLES IN PHOTORECEPTOR DEVELOPMENT DID NOT AFFECT THESE INITIAL STAGES OF SURVIVAL OR DIFFERENTIATION.

460.4

SPECIFIC PHAGOCYTOSIS TRIGGERS EXPRESSION OF IMMEDIATE EARLY TRANSCRIPTION FACTORS IN RETINAL PIGMENT EPITHELIUM (RPE) CELLS. A.V. Ershov, W.J. Lukiw, D.M. Linn*, N.G. Bazan, Eye and Neuroscience Center, LSU Medical Center, New Orleans, LA.

Phagocytosis of used tips of rod outer segments (ROS) by RPE cells is vitally important for maintaining structural and functional integrity of the retina. Phagocytosis deficiency in dystrophic RCS-*p*$^+$ rat RPE cells leads to degeneration of the retina and complete blindness. We studied the expression of immediate early gene mRNAs and their gene products during the phagocytosis of ROS by normal Long Evans (LE) and RCS-*rdy*$^+$*p*$^+$ rats, and by dystrophic RCS-*p*$^+$ rat RPE cells in primary cell culture, using Northern blot analysis and electrophoretic mobility shift assay (EMSA). Northern analysis revealed that the levels of c-FOS, TIS1 and ZIF268 mRNA were rapidly, strongly and transiently increased in normal RPE cells during ROS phagocytosis, but not during phagocytosis of non-specific latex particles. No increase in the gene expression was found in RCS-*p*$^+$ dystrophic RPE cells challenged with ROS. As shown by EMSA, a prominent short-term increase in the intensity of gel shifted band was detected using nuclear protein extract derived from ROS-challenged normal LE RPE cells and ZIF268 or AP-2 consensus oligonucleotides. No such increase was detected when using NFKB consensus oligonucleotide. At any time point, neither expression of COX2 mRNA was detected by Northern analysis, nor any shifted band was revealed by EMSA using COX2 promoter region DNA. As a positive control, COX2 mRNA band could be revealed on Northern blots after treatment of LE RPE cells with TPA or PDGF. The results suggest that in RPE cells ROS-specific phagocytosis is accompanied by a short-term over-expression of immediate early genes, coding transcription factors, as well as their gene products. The specific pattern of the expression of these transcription factors would then be able to further modify the expression of other genes in a gene cascade. Supported by NIH grant EY05121.

460.5

ULTRAVIOLET LIGHT INDUCES FOS PROTEIN EXPRESSION IN THE RAT VISUAL SYSTEM. B. Robinson* and S. Amir, Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Quebec, Canada.

Electrophysiological evidence that the spectral sensitivity of the retina in rodents extends into the ultraviolet range and behavioral evidence that rodents can detect ultraviolet wavelengths suggests that ultraviolet light plays a role in vision. Little is known, however, about the effect of ultraviolet light on the rodent visual system. Here we used immunostaining for the cellular phosphoprotein Fos to examine the effect of ultraviolet light exposure on patterns of neuronal activation in rat brain. Nighttime exposure to diffuse monochromatic ultraviolet light (1-30 min, λ max 360 nm; half bandwidth, 8.8 nm; irradiance at eye level, 10 $\mu W/cm^2$) induced extensive Fos expression in visual cortex and lateral geniculate complex. Within the lateral geniculate, ultraviolet light induced Fos expression in the ventrolateral, but not dorsolateral, nucleus and in the intergeniculate leaflet. The effect of ultraviolet light on Fos expression was blocked by either bilateral orbital enucleation or bilateral optic nerve transection, and it appeared to be mediated by a mechanism bearing characteristics of a specific ultraviolet receptor. This is the first demonstration that ultraviolet stimuli detected by a receptor in the retina can gain access to, and stimulates neuronal activity in, brain centers involved in visual perception (visual cortex) and luminance detection (ventral lateral geniculate nucleus and intergeniculate leaflet).

460.6

LOCALIZATION OF *DROSOPHILA* RETINOID BINDING PROTEIN IN THE COMPOUND EYE. K. Shim[1], J. Polizzi[1], C. F. Thomas[2], B. N. Wiggert[3], G. Kutty[3], T. Duncan[3], K. Kutty[3], and W.S. Stark*[1], [1]Biology Dept., Saint Louis Uni. (SLU), St. Louis, MO 63103, [2]Integrated Micros. Resource (IMR), Univ. of Wisconsin, Madison, WI 53706, [3]Nat'l Eye Institute, 9000 Rockville Pike, Bethesda, MD 20892.

Carotenoid deprivation decreases proteins relevant to *Drosophila* vision (Western analyses, R.D.Lee, MS Thesis, SLU Biology, 1994). We used antibodies and FITC conjugated anti-IgG secondaries to localize proteins using fluorescence and confocal microscopes. An antibody to Rh1 (R1-6 opsin), made in collaboration with S. J. Fliesler, Ophthalmology, SLU, helped us to optimize our methodology. DRBP (*Drosophila* retinoid binding protein, Duncan et al., 1994 *Arch. Biochem. Biophys. 312*:158-166. 1994) may be a *Drosophila* homologue of vertebrate IRBP (interphotoreceptor RBP) well-known as a transporter of retinoids between retinal pigment epithelium (RPE) and photoreceptors. DRBP is expressed specifically in cone (Semper) cells, four per ommatidium, between photoreceptor cells and the dioptric pseudocone with processes extending to basement membrane. Deprived flies have lower staining, consistent with Western analyses (Lee). Pre-immune staining gave the expected result of a negative control. The deprivation result suggests that DRBP is relevant to retinoid handling. Localization implies that cone cells may be analogous to vertebrate RPE in cell to cell retinoid transport. Earlier, in *Calliphora*, vitamin A and photoisomerase were localized to tissue near the cornea (Schwemer, in *Facets of Vision* Stavenga & Hardie eds. Berlin, Springer, 112-133, 1989), consistent with our finding. Support: NIH grants RO1 EY07192 to WSS & DRR-570 at the IMR.

460.7

RET 1, A *CIS*-ACTING ELEMENT THAT IS SUFFICIENT TO DIRECT OPSIN EXPRESSION IN ROD PHOTORECEPTORS J. Martinez, X. Yu, L. Leconte, C.J. Barnstable*, Neuroscience Program, Yale University, New Haven, CT.

RET 1 is a binding site for retinal nuclear proteins located at -136 to -110 bp in the rat opsin promoter (Morabito et al., J Biol. Chem. 266:9667, 1991). A similar sequence is found in the upstream regions of many other photoreceptor genes. Alignment of these sequences has allowed the definition of a 7 base consensus sequence, CAATTAG, which exhibits the binding activity of the full length RET 1 element. A 40 kD protein that binds to RET 1 has been purified over 2 X 10^5 to apparent homogeneity. The function of RET 1 has been tested in two sets of transgenic mice. In one set, a mutant RET 1 in an otherwise normal rat opsin promoter failed to drive expression of a *lacZ* marker gene in 8 of 10 lines. In 2 lines very faint expression in photoreceptors was detected. In the second set, RET 1 elements alone were used to direct expression of *lacZ*. Expression of *lacZ* was detected in 9 out of 12 lines. In all 9 lines expression was found in photoreceptors. In 2 lines expression was also detected in the ganglion cell layer and the ciliary epithelium. In 3 lines a characteristic pattern of expression was found in the embryo CNS in addition to the normal retinal expression. The results suggest that interaction of a 40 kD protein with RET 1 is both necessary and sufficient to drive gene expression in rod photoreceptors. Further, our results indicate that RET 1-like elements may also be important in the developing nervous system. Supported by NS 20483 and the RP foundation.

460.8

SIMULTANEOUS DETECTION OF MULTIPLE OPSIN mRNAs IN GOLDFISH RETINA WITH NONISOTOPIC IN SITU HYBRIDIZATION. L.K. Barthel and P.A. Raymond* Dept. of Anat. & Cell Bio., University of Michigan, Ann Arbor, MI 48109.

It is generally believed that individual photoreceptor cells express a single opsin gene, and the spectral absorption properties of the resultant visual pigment establishes the spectral identity of that cell. Recent evidence from microspectrophotometric measurements in some species of fishes and from immunocytochemical analyses of developing cones in rodents has suggested that individual photoreceptors might express a combination of opsin genes or might switch from one opsin to another during development. In tetraploid species, such as goldfish, it is likely that each of the opsin genes is duplicated, but it is not yet known whether multiple genes are expressed in each photoreceptor of a given spectral subtype or whether the spectral subclasses are heterogeneous.

We sought to explore techniques to allow the simultaneous detection of more than one opsin mRNA in the goldfish retina. cRNA probes labeled with either digoxigenin-UTP (DIG-RNA) or fluorescein-UTP (FL-RNA) were generated by in vitro transcription from goldfish opsin cDNA clones. The probes were combined, hybridized to retinal tissues, and detected with antibodies to digoxigenin and fluorescein. Alkaline phosphatase (AP) and horseradish peroxidase (HRP) enzymatic histochemistry was used to detect the antibodies. One successful combination was DIG-RNA probes to goldfish blue cone opsin visualized with HRP/DAB combined with FL-RNA probes to goldfish red cone opsins revealed by AP/Vector Blue. The brown HRP reaction product was confined to short single cones, and the blue AP reaction product was only in long single and double cones with no overlap in signals, consistent with the known spectral identities of goldfish cones. Further experiments will use additional combinations of opsin probes, in both mature and developing retina, with special attention to temporal order and patterning at onset of expression and to photoreceptors that might express more than one opsin. Supported by NIH EY 04318 and NSF IBN 9222046.

460.9

CLONING OF A NOVEL EXTRARETINAL OPSIN FROM CATFISH BARBEL. Seth Blackshaw , Yonatan H. Grad, and Solomon H. Snyder* Department of Neuroscience, Johns Hopkins University School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205.

Extraretinal and extrapineal photosensitivity has been implicated in contributing to camoflauge and circadian processes in a variety of lower vertebrates, including fish, amphibians, and reptiles. Very little, however, is known about the molecular mechanism of this photosensitivity, and it is unclear whether and receptor-mediated mechanisms are involved . In the course of attempting to clone G-protein coupled taste receptors from channel catfish, we obtained a PCR product encoding a novel opsin from barbel cDNA. A putative full-length genomic clone showed roughly 40% amino acid identity to both chicken pinopsin and fish red and green cone pigments over the region excluding the amino and carboxy terminal domains, in addition to exhibiting an intron-exon structure not seen in any other vertebrate photopigment. All residues implicated in directly contacting retinal via site-directed mutagenesis of rhodopsin are conserved. Northern analysis showed two transcripts in ventral skin, but not in retina or barbel RNA. Attempts to localize transcript via in situ hybridization, and attempts to reconstitute spectrally active protein are underway, and will be discussed.

460.10

ISOLATION AND CHARACTERISATION OF VISUAL PIGMENT GENES FROM STOMATOPOD CRUSTACEA. A.J.H. Brown, N.J. Marshall, M.F. Land and J.F. Burke* Sussex Centre for Neuroscience, School of Biological Sciences, University of Sussex, Brighton BN1 9QG, UK.

Colour vision in many species is based upon the differential light absorbtion by visual pigments. Microspectrophotometric and electrophysiological data suggest the retina of stomatopod crustacea contains up to 16 different photoreceptor cell types including 6 UV receptors covering the spectral range from 325nm to 700nm.

We have employed RT-PCR techniques to obtain two different full length cDNA clones from the mantis shrimp, *Gonodactylus oerstedii*. Comparison of deduced amino acid sequences has revealed 70% and 60% sequence identity to Mantid and Crayfish opsins.

Northern blot and *in situ* hybridisation techniques were used to study mRNA expression within the retina. Preliminary data suggest a localisation of one sequence within the proximal row 2 photoreceptor cell of the enlarged retinal midband region, absorbing maximally at 525nm.

In order to explain the number of visual pigments required to produce the electrophysiological recordings obtained, we have undertaken PCR amplification of the *G. oerstedii* genomic DNA with the same primers as used in RT-PCR. These data and Southern blot hybridisations suggest that at least one of these genes may be present in multiple copies that differ in the organisation of the intron/exon boundaries. This leaves open the possibility that multiple visual pigments could arise by alternative splicing.

This work is supported by a BBSRC grant to Prof. M O'Shea.

460.11

RETINAL TOPOGRAPHY AND PUTATIVE ULTRAVIOLET-SENSITIVE CONES IN ADULT, SPAWNING SALMONID FISHES C.W. Hawryshyn*, L. Beaudet and I. Novales Flamarique. Dept. Biology, U. Victoria, P.O Box 1700, Victoria, B.C. Canada V8W 2Y2

Ultraviolet-sensitive cones (UV cones) disappear from the retina during the course of ontogeny in several species of salmonid fishes. In juveniles, the putative UV cones occupy the corner position within the retinal cone square mosaic and are thus referred to as corner cones. In this project, we used conventional histological techniques to study the distribution, densities and dimensions of the different cone types in the retinae of wild adult, spawning fishes from four species of salmonids (*Oncorhynchus nerka, O. keta, O. tschawytscha* and *O. mykiss*). Two areas of higher cone density were located in the ventral and the dorso-nasal retina. In contrast to previous hypotheses, corner cones were present over extensive areas. In all species studied, corner cones were found in the temporal retina, dorsally and ventrally. The distribution of these cones was not associated with the regions of higher cone densities. We also found some areas where the cone arrangement did not allow the corner cones to be identified. In these cases, however, single/double cone ratios were consistent with those found in areas containing corner cones. Experimental work has indicated the possibility of UV cones reintegrating the retina subsequent to their loss (Browman and Hawryshyn, 1994, Vision Research, 11:1397-1406). Our results are consistent with these findings. Finally, the location of the putative UV cones with respect to the zones of higher photoreceptor density indicates that they are probably not used for visual tasks requiring higher visual acuity. Research supported by NSERC operating grant to CWH, FCAR, NSERC and the University of Victoria scholarships to LB and INF

460.12

PHOTORECEPTOR MOSAIC AND ESTIMATE OF VISUAL ACUITY IN THE CEBUS MONKEY. Andrade da Costa, B.L.S. and J.N. Hokoç*. Lab. Neurobiologia da Retina, IBCCF°/UFRJ-RJ 21949-900, Brazil.

The retinal photoreceptor mosaic was studied in the diurnal New World primate *Cebus apella*. Parameters such as the spatial density of cones and rods, the geometry of the sampling array, the cone inner segment diameter and the inter-cone spacing were analyzed. Video-enhanced differential interference contrast optics (Curcio et al ,1987) were used to analyze retinas of six adults animals. The total number of cones and rods estimated was 3.8 ± 0.07 and 48 ± 0.10 millions, respectively. Peak fovea cone density averages 152,023 cones/mm^2 and decreases about 20-40 fold towards the periphery. The cone density increases in the retina's far periphery, adjacent to *ora serrata*, with a more pronounced peak in the nasal quadrant. The cone density is asymmetrical along the horizontal meridian but not along the vertical meridian. The highest rod densities are located along an elliptical ring at eccentricities between 3.5 to 5.0 mm of the *foveola*. Asymmetries in this density distribution were observed along the vertical and horizontal meridians. The cone center-to-center spacing obtained from the nearest-neighbor analysis reveals a minimal spacing at the *foveola* ranging of 2.2 to 3.5μm. Assuming a RMF= 197.3 μm/degree and a hexagonal array, the Nyquist limit estimated in the foveola ranged from the 30.0 to 56.2 cycles/ degree, decreasing about 2.5 X by 2 degrees of eccentricity. Compared to Old World primates the optical parameters of the *Cebus*' eye are scaled relative to *Macaca fascicularis*. However, in despite of differences in the resolution grain and in the strategies for capturing light, the majority of qualitative and quantitative aspects of the photoreceptor mosaic is more similar to that described in human. Supported by: FINEP,CNPq, CAPES, CEPEG/UFRJ.

460.13

CALCIUM IS A REQUISITE FOR RHABDOM SHEDDING UNDER NATURAL LIGHTING IN *LIMULUS* R.N. Jinks* and S.C. Chamberlain. Department of Bioengineering & Neuroscience and Institute for Sensory Research, Syracuse University, Syracuse, NY 13244.

Photoreceptors of the lateral eye of the horseshoe crab, *Limulus*, shed their photosensitive membrane at dawn each morning under natural lighting. Protein kinase C (PKC) activators (phorbol esters and diacylglycerol (DAG) analogs), injected intraretinally, initiate rhabdom shedding in the absence of a light trigger. Inositol trisphosphate (IP_3), when injected simultaneously with DAG analog, decreases the concentration of DAG analog required for the triggering of rhabdom shedding by 10 times, implicating a Ca^{2+}-dependent PKC isozyme in the cascade that communicates dawn to the intracellular machinery responsible for the breakdown of the rhabdom. Immunocytochemical evidence suggests a PKC βII-like isozyme. DAG and IP_3 are produced naturally in the lateral eye in response to light (*Nature* **311**: 160). If the PKC isozyme that mediates light-triggered rhabdom turnover is Ca^{2+}-sensitive, then chelating the intracellular free Ca^{2+} in the photoreceptor cells of the intact eye at dawn should inhibit activation of the PKC and thereby inhibit rhabdom shedding.

Adult male *Limulus*, maintained under natural lighting, were placed in complete darkness at sunset. One eye of each animal was injected intraretinally the following morning in darkness with 2.0 ml of 500 μM BAPTA-AM, a cell-permeant Ca^{2+} chelator, in Ca^{2+}-free *Limulus* Ringer's. The other eye served as an untreated internal control. Retinas incubated with BAPTA-AM in the dark for 30-45 min prior to exposure to direct sunlight for 15 min displayed a marked decrease in rhabdom shedding (8.3% of the ommatidia shed their rhabdoms) in comparison with the control retinas in which >90% of the ommatidia shed. Eyes were fixed in direct sunlight 15 min after light onset by intraretinal injection of our standard EM fix. Rhabdom shedding in the BAPTA-AM loaded retinas was limited to fewer than 5 photoreceptors within each of the 8.3% of the ommatidia that displayed lamellar whorls of shed membrane along the internal margin of the rhabdom.

Inhibition of natural light-triggered rhabdom shedding by buffering the first morning light-induced rise in intracellular Ca^{2+} implies that shedding requires Ca^{2+}. Either the Ca^{2+}-sensitive PKC presumed to mediate light-triggered shedding was not activated under these conditions, or a separate, unknown Ca^{2+}-sensitive mechanism exists downstream from PKC in this cascade. We are currently developing PKC assays to investigate both of these possibilities.

Supported by NIH EY-03446 and Syracuse University.

460.14

USE OF A RETROVIRAL VECTOR WITH AN INTERNAL OPSIN PROMOTER TO DIRECT GENE EXPRESSION TO RETINAL PHOTORECEPTOR CELLS. M. Kido, K.A. Rich, G. Yang, E. Barron, D.B. Kohn[1], M.R. Al-Ubaidi[1], and J.C. Blanks*. Doheny Eye Institute and Depts. Ophthalmology and [1]Pediatrics, USC School of Medicine, Los Angeles, CA and [1]Dept. of Ophthalmology, University of Illinois, Chicago, IL.

Viral-mediated gene transfer holds promise as a potential treatment for inherited retinal degeneration. We have evaluated the potential of a retroviral vector with an internal opsin promoter fragment to direct gene expression to retinal photoreceptor (PR) cells. Two recombinant retroviral vectors were prepared, each carrying a 1.4 kb fragment of the mouse opsin promoter, which was placed downstream from the neo^R gene in the MMuLV-based vector G1Na. One vector contained the bacterial lacZ gene, and the other the cDNA for mouse rod PR phosphodiesterase (PDE) β-subunit, each linked to the opsin promoter fragment. These vectors were tested for their ability to direct gene expression after infection of 3T3 cells, or of neonatal mouse retinal cells and explants in primary culture. Both lacZ and PDE β-subunit mRNA were expressed only at low levels in 3T3 fibroblasts. Northern analysis indicated that these were LTR-driven transcripts. Infection of neonatal mouse retinal cells or explants with BAG retrovirus, in which lacZ is driven by the viral LTR, resulted in expression in many cell types, while in contrast the opsin-lacZ retrovirus mediated the transfer and expression of the lacZ reporter gene specifically to PR cells. We are now evaluating the potential therapeutic effect of the opsin-PDE retrovirus following subretinal injections in newborn *rd* mice. In conclusion, the internal opsin promoter fragment appears capable of selectively directing gene expression to PR cells after retroviral-mediated gene transfer and may also allow for developmentally-appropriate timing of expression of PR-specific genes following gene therapy *in vivo*.

(Supported by EY03040 and EY03042, RPB and the Hoover Foundation).

AUDITORY SYSTEMS: CENTRAL PHYSIOLOGY V

461.1

Corticothalamic inputs to the rat medial geniculate body. **Ed Bartlett* and Phil Smith, Dept. of Anatomy and Neurosciences Training Program. University of Wisconsin Medical School. Madison, WI, 53706.**

The auditory thalamus or medial geniculate body (MGB) receives a strong corticothalamic input. In visual and somatosensory systems, corticothalamic inputs arise from layer VI and V pyramidal cells which give rise, respectively, to small terminals on distal dendrites of thalamocortical cells and large glomerular-like terminals on primary dendrites in specific areas of "extralemniscal" thalamus. Our *in vivo* gross injections of neurobiotin in auditory cortex confirmed preliminary data (Welker and Rouiller, '91) that corticothalamic axons projecting to MGB give rise to small or large swellings. E.M. analysis showed that the small swellings are terminals on small dendritic profiles while the large swellings are glomerular, terminating on a large dendrite and its specializations. To determine the effects of these corticogeniculate inputs we have begun to record intracellularly from rat MGB cells in an *in vitro* slice preparation using neurobiotin-filled sharp glass electrodes. In dorsal MGB cells, single shocks to corticothalamic afferents often caused a short latency (2-4 ms) epsp, followed in some cases by a long ipsp (50-600 ms). High shock strengths generated a second, slower epsp component. Simultaneous bath application of DNQX and APV abolished the epsp while DNQX or APV alone reduced the first and second components of the epsp, respectively. Bicuculline did not block the ipsp. Repetitive shocking of the corticothalamic input resulted in a prolonged depolarization lasting 2-5 s after stimulus offset which persisted in DNQX, APV, or both drugs simultaneously and could not be duplicated by repetitive brief depolarizing current pulses. Thus, corticothalamic inputs can activate rapidly acting ionotropic non-NMDA and NMDA receptors as well as an additional long lasting response. We are presently testing whether this lasting response occurs due to activation of postsynaptic metabotropic glutamate receptors at the small, layer VI synapses.

Supported by NSF grant BNS-8901993 and NIH grant RO1-DC01999 to P. Smith.

461.2

THE CONTROL OF MEMBRANE POTENTIAL AND MODE OF SYNAPTIC TRANSMISSION IN AUDITORY THALAMUS. B. Hu*, V. Senatorov, D. Mooney, and A. Miller. Loeb Research Institute, Ottawa Civic Hospital, Ottawa, ON, Canada, K1Y 4E9.

The primary and nonprimary auditory thalami display distinct modes of synaptic transmission *in vivo*. Neurons in the latter pathway are characterized by low-excitability, a slow synaptic response and a low responding speed (below 10 Hz). Here, we propose a theoretical model to account for this phenomenon

We used the Mullins-Noda and Morton equations to model the resting membrane potential of medial geniculate body (MGB) neurons. Four families of membrane conductances were considered: the Na,K-ATPase, the voltage-dependent inward rectifying cation channel (I_h), the muscarinc-gated potassium conductance and the leak potassium conductance. The K^+ and Na^+ membrane permeabilities/ conductances were based on experimental data obtained *in vitro* (see this meeting). In this model, the "resting" membrane potential of nonprimary MGB cells was found to be below -70mV, 5-10mV more negative than in the primary pathway. This moderate membrane hyperpolarization changed the mode of synaptic activity in nonprimary cells in three ways. 1) It significantly reduced spontaneous or random spiking of the cell, thereby creating a low-excitability/low-noise state. 2) It allowed compounded EPSPs to activate an intrinsic, low-threshold calcium conductance (or LTS). This EPSP-LTS coupling gave rise to a slow, ramp-like membrane depolarization and a delayed bursting discharge. 3) It limited the responding rate of the cell to below 5 responses/s due partly to the LTS refractoriness. These features are comparable to the response properties of nonprimary thalamic neurons observed *in vivo* and may represent a non-stochastic mode of synaptic transmission for sensory event detection and integration. *Supported by MRC of Canada*

461.3

DIFFERENTIAL RESTING MEMBRANE POTENTIAL REGULATION IN PRIMARY AND NONPRIMARY AUDITORY THALAMUS: INTRINSIC MECHANISM MEDIATED BY NA,K-ATPase. V.Senatorov* and Bin Hu. Loeb Research Institute, Ottawa Civic Hospital, Ottawa, ON, Canada, K1Y 4E9.

Primary (ventral) and nonprimary (dorsal) thalamic relay neurons of the rat medial geniculate body (MGB) exhibit a different average resting membrane potential *in vitro*, with the latter being 5-10 mV more hyperpolarized. To explore the underlying mechanism, we examined the role of Na,K-ATPase. Whole-cell patch clamp recordings were obtained from MGB neurons in explants from adult rats at 32°C. Bath application of Na,K-ATPase inhibitors strophantidin or dihydroouabain at concentrations of 30-200 μM for 10s produced a transient, dose-dependent inward current in 80% of cells (I_{peak}= 20-100 pA) or, under current clamp, a 2-10 mV membrane depolarization. The drug-evoked inward current displayed no clear voltage-dependence and was not abolished by various blockers acting on voltage-activated K^+ or Na^+ channels. A similar membrane inward current was induced after pump activity was inhibited using dialysis of the cell with pipette solution containing zero-[Na^+] or following removal of extracellular K^+. The average peak current induced by pump inhibitors was ~50% larger in the nonprimary MGB cells than that recorded in primary MGB neurons. Neurons exhibiting a larger pump current usually had a more negative resting membrane potential (the difference ranged from 5 to 10 mV).

Incubation of enzymatically dissociated MGB neurons with monoclonal antibodies against α1, α2 or α3 pump subunits revealed strong immunoreactivity for only the α3 subunit. Overall immunoreactivity was significantly stronger in dorsal MGB neurons than in their ventral counterparts. It appears, therefore, that a gradient of Na,K-ATPase expression may be partly responsible for the resting membrane potential gradient between ventral and dorsal MGB pathways. *Supported by MRC of Canada*

461.4

DIFFERENTIAL RESTING MEMBRANE POTENTIAL REGULATION IN PRIMARY AND NONPRIMARY AUDITORY THALAMUS: EXTRINSIC MECHANISM MEDIATED BY MUSCARINIC RECEPTOR D. M. Mooney* and Bin Hu. Loeb Research Institute, Ottawa Civic Hospital, Ottawa, ON, Canada, K1Y 4E9.

To determine the physiological role of cholinergic reticular input to primary (MGv) and nonprimary (MGd) auditory thalamus, we examined the membrane effect of (±)muscarine in vitro. Bath application at concentrations of 20 to 90 μM for 20 sec produced membrane depolarization in MGv neurons (15/20). In contrast, application of muscarine to MGd cells at the same concentrations caused a membrane hyperpolarization (12/13). The membrane depolarization response in MGv cells was associated with an average 21% reduction in membrane conductance (n=12), whereas the membrane hyperpolarization in MGd cells was accompanied by an average 15% increase in membrane conductance (n=11). Both muscarinic membrane depolarization and hyperpolarization were unaffected following blockade of synaptic transmission by TTX; they were reversibly blocked by pirenzepine (1 to 10 μM) when moderate concentrations (10 to 30 μM) of muscarine were applied. Preliminary evidence suggests that the hyperpolarizing effect was mediated by the opening of a membrane potassium conductance. The effects of muscarine on MGv/d neurons were further examined through extracellular recordings of synaptically-evoked responses. In MGv, the burst synaptic response elicited by stimulation of collicular input was largely abolished by muscarine (22/32). In contrast, bursting synaptic response in the majority of MGd cells remained unblocked (21/32). Hence, muscarinic receptor activation seems capable of regulating the resting membrane potential and the firing mode of thalamic relay neurons in a pathway-specific manner. *Supported by MRC of Canada.*

461.5

ELEVATION SENSITIVITY OF SINGLE NEURONS IN THE MEDIAL GENICULATE BODY (MGB) OF THE CAT. P.Poirier*, F.K.Samson and T.J.Imig. Dept. of Physiology, Kansas Univ. Med. Ctr., Kansas City, KS, 66103.

Elevation tuning of high-frequency MGB neurons (N=70; BF:4-37kHz) that derive their azimuth sensitivity from monaural spectral (MD) or binaural disparity (BD) cues was studied in barbiturate-anesthetized cats. Noise bursts that varied in azimuth, elevation and SPL were presented throughout the frontal field, and spatial receptive fields (SRFs; N=48) were defined as areas in which responses exceeded 50% of maximum. An elevation function, averaged over SPL, was obtained for each cell at a preferred azimuth (response≥ 75% at 0° elevation), and units with modulation ≥75% were classified as elevation sensitive. MD and BD cells differed in SRF location, shape, size and in elevation sensitivity. Most MD cells had focal SRFs that were restricted in azimuth and elevation. Almost all MD SRFs became smaller with increasing SPL. A great majority of MD cells was elevation sensitive, and as a group MD cells were more narrowly tuned than BD cells. The elevation tuning of MD cells was similar under monaural and binaural conditions showing that it derived from monaural cues. SRF size was significantly smaller for MD than for BD cells. BD cells comprised three sub-groups. SRFs of BD-EI cells covered one hemifield, typically the contralateral one. BD-FAC cells, characterized by binaural facilitation, had SRFs that were located either on the midline (BD-MID) or lateral to it (BD-LF). SRF size was significantly greater for BD-LF than for BD-EI or BD-MID groups (BD-LF>BD-EI>BD-MID). In contrast to MD cells, SRF size of most BD units expanded or was unaffected by increasing SPL. The vast majority of BD cells was elevation insensitive, and the sensitive ones were broadly tuned. These results indicate that MD cells are more sensitive and more narrowly tuned to elevation than BD cells, and that they derive elevation tuning from monaural spectral cues (supported by FCAR, FRSQ and NIDCD #DC00173).

461.6

EFFECT OF BASELINE LEVEL ON SENSITIVITY TO RATE OF RISE OF MEDIAL GENICULATE NEURONS IN THE SQUIRREL MONKEY. J.F Olsen*, Lab. Neurophysiology, NIMH, Poolesville, MD 20837.

The responses of most neurons in the medial geniculate (MGN) to a broadband noise burst are determined by rate of rise (peak amplitude/rise time; Olsen, *Soc. Neurosci. Abstr. 20*: 321, 1994). This study examines the effect of baseline level on the responses of MGN neurons to linear amplitude increments of a broadband noise. Baseline level (0-73 dB SPL), step amplitude (6-24 dB re. baseline), and step rise time (3-96 ms) were varied independently. Extracellular recordings were made from 42 MGN neurons in halothane-anesthetized monkeys. For most neurons, response probability increased as a linear function of the step's rate of rise, and was independent of peak level (baseline level + step amplitude). Responses were 100% modulated by rate of rise, regardless of baseline amplitude. At low baseline amplitudes (generally below 40 dB SPL), there was little effect of baseline amplitude on threshold rate of rise. At higher baseline amplitudes, threshold rate of rise increased at least 1 dB per dB increase in baseline level. The rate of rise at saturation also increased, such that the range of rates over which neural responses were modulated increased linearly with baseline amplitude. Thus, MGN neurons signal amplitude increments over a wide range of baseline levels with no loss of responsiveness. The data suggest that baseline level is represented by an adaptation level in these neurons.

461.7

PERIODICITY PITCH AND PARTY EFFECT: TEMPORAL PROCESSING, INTRINSIC OSCILLATIONS AND BINDING IN THE AUDITORY SYSTEM G. Langner* Zoological Institute of THD, 64287 Darmstadt, Germany

An important clue for detection of acoustic signals in noisy environments (e.g. cocktail parties) is periodicity. Signals with the same periodicity have the same pitch, independent of waveform and spectral content. Broadband signals are typical for human and animal communication and are decomposed by the cochlear frequency filter in different components. The resulting binding problem is comparable to that in visual perception. The present paper provides evidence for the hypothesis that the same temporal processing-synchronized neuronal representations, intrinsic oscillations and neural integration delays followed by coincidence mechanisms- underlies pitch perception and auditory binding. Due to their oscillatory and integrative membrane properties, chopper and pauser neurons in the cochlear nucleus provide synchronized and delayed responses to periodic envelopes. Their convergence on coincidence neurons in the auditory midbrain transforms temporal into rate information. Low frequency neurons were found which may contribute to binding by integrating over a broad frequency range. The transformation of the temporal into a rate code is supplemented by a transformation into a spatial code with the result that periodicity information is represented at the level of the midbrain orthogonal to the tonotopic map. Since *temporal* information about high modulation frequencies is essentially lost in the midbrain, the auditory cortex is provided predominantly with *spatial* information. Correspondingly, frequency and periodicity pitch are represented in orthogonal maps in the forebrain of mynah bird. Furthermore, magnetoencephalographic investigations provided evidence for an orthogonal representation of pitch and frequency information in the auditory cortex of human subjects.

461.8

MICROSTIMULATION OF AUDITORY CORTEX FOR A POTENTIAL NEUROPROSTHETIC USING A 100-ELECTRODE ARRAY. Patrick J. Rousche*, Craig T. Nordhausen, and Richard A. Normann, Moran Laboratories, Department of Bioengineering, University of Utah, Salt Lake City, Utah, 84112, USA

We propose that a cortical auditory prosthetic could be based upon intracortical microstimulation of auditory cortex using an array of penetrating electrodes. This approach hinges upon 3 key issues: tonotopicity, electrical activation, and chronic threshold stability. To address these issues, we have conducted neural recording and stimulation experiments using the Utah Intracortical Electrode Array in the feline primary auditory cortex (AI).

The Utah array consists of 100, 1 mm platinum-tipped electrodes positioned in a 10x10 matrix, with an interelectrode spacing of 400 um. This array can be used acutely, for recording from all one hundred electrode sites or chronically, where 10 electrodes are accessed through a percutaneous connector.

Tonotopicity: Acute recording studies with this array have verified the existence of a tonotopic map in cat AI over an investigated frequency range of 5-35 Khz. Characteristic frequencies were recorded from an average of 85 of the 100 electrode sites in each of 4 preparations. Electrical activation: Cats implanted in AI with a chronic electrode array were trained to identify the presence or absence of auditory stimuli. Trials were then introduced in which AI was electrically stimulated in the absence of external auditory stimuli. Minimum current levels required to evoke an auditory detection response ranged from 20-120 uamps (biphasic, cathodic first, current pulses of width 150 usec delivered at 250 Hz for .6 sec, total charge from 3-18 nC). Chronic stability: Behavioral responses to stimulation were obtained up to 8 weeks post-implant. However, threshold current and thus charge levels were not constant from electrode to electrode or from week to week. If chronic stability can be assured with electrical activation through tonotopically placed electrodes, then a cortical auditory prosthesis may be possible.

461.9

THE FUNCTIONAL ORGANIZATION OF THE BARN OWL AUDITORY FOREBRAIN (FIELD L). Y.E. Cohen* and E.I. Knudsen. Dept. Neurobiology, Stanford Univ. School of Med. Stanford, CA 94305.

The barn owl forebrain is critical for certain aspects of sound localization behavior. We are examining how auditory spatial information is organized in the primary forebrain auditory area, Field L, the avian analogue of mammalian auditory cortex. In particular, we are interested in determining whether regions of Field L are specialized to process binaural localization cues, such as interaural phase differences (IPD) and interaural level differences (ILD).

Neuronal activity in Field L was recorded extracellularly during dichotic presentation of digitally synthesized noise or tone bursts. Field L contains at least two functionally distinct regions: (1) a tonotopic region located rostrolaterally and (2) a non-tonotopic "belt" region which adjoins the tonotopic region at its caudal and medial borders. The tonotopic region was distinguished by a caudorostral progression of frequency tuning, sharp tuning for frequencies above 3 kHz, and unit tuning for both ILD and IPD. In contrast, units in the non-tonotopic belt region often had complex frequency tuning and were tuned very rarely for both ILD and IPD.

The auditory properties of the tonotopic region suggest that it is specialized for processing spatial information. The properties of units in the belt region suggest that it may process non-spatial features of the auditory scene such as vocalizations.

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461.10

DOES BINAURAL INTERACTION INCREASE FREQUENCY SELECTIVITY IN GUINEA PIG AUDITORY CORTEX ?

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The relation between the tonotopic arrangements and the binaurality in the guinea pig auditory cortex AI was investigated by using optical recording with a voltage sensitive dye (RH795). Spatio-temporal neural response pattern to acoustic stimuli of tone bursts of 4 kHz and 8 kHz with different sound intensities on each ear were measured in AI of the anaesthetized animal and compared. That is, topographic imaging of neural response to interaural intensity differences (IIDs) was evaluated in relation with the tonotopical arrangements in AI of the left hemisphere of the animal. The tonotopic arrangements appear in the form of island-like clusters that become larger with higher peak amplitude with increasing sound pressure level (SPL), but stable regarding the peak amplitude position (as discussed before at the previous this meetings). These clusters of the responses were weakened, contracted and delayed for ipsilaterally dominant stimuli, the SPL at the ipsilateral ear being stronger than that at the contralateral ear compared to the response to the same SPL as both ears. On the other hand, the responses for contralaterally dominant stimuli showed shorter latencies but similar amplitude as the control with similarly equal SPL. The distribution of the time delay of the correlation functions between the responses for the contralaterally dominant stimuli or of the ipsilaterally dominant stimuli and the responses for the binaural equal intensity stimuli at 128 recording pixels showed significantly larger in the rim of the tonotopical cluster. Considering the longer delay time of the neural responses to contralateral stimulation as compared to ipsilateral stimulation and predominant inhibitory inputs from ipsilateral ear, the results indicate that the topographic organization of neural sensitivity to IIDs account for increased tuning sensitivity for coding of sound frequencies. Thus, another important aspect related to frequency tuning of the binaural interaction of the primary auditory cortex in the guinea pig is demonstrated.

461.11

ELECTRICAL STIMULATION OF AI MODULATES NEURONAL RESPONSES TO PURE-TONES IN THE CAT MEDIAL GENICULATE BODY J. He[1]*, T. Futami[1], T. Hashikawa[1], E. G. Jones[1,2]. [1]Lab. for Neural Systems, Frontier Research Program, The Institute of Physical and Chemical Research (RIKEN), Saitama 351-01, Japan and [2]Dept. of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

The corticothalamic projection has been suggested to modulate information processing in the thalamus. We studied the effect of corticofugal modulation of neuronal responses to sound stimuli in the medial geniculate body (MGB) by electrical activation of the primary auditory cortex. Single- and multi-unit responses to pure-tones were recorded in the ventral nucleus of the MGB in sodium pentobarbital anesthetized cats. Pulse burst electrical stimulation was applied to different sites around a single isofrequency band or to different isofrequency bands in AI. Tuning curves in firing rate at varied sound intensity were recorded before and during AI stimulation. The corticofugal connection between AI stimulation sites and the recording sites was confirmed histologically in anterograde tracer experiments.

Stimulation of the cortex enhanced the responses of most MGB neurons at the same best-frequency site to their best frequency and sharpened their tuning curves in some cases. Stimulation of different iso-frequency bands inhibited the neuronal responses to their best-frequencies and shifted the tuning curves of some MGB neurons. The corticofugal modulation effect was stronger to low-intensity sound stimuli than to high-intensity sound stimuli.

The results suggest the presence of a variety of modulations of responses in thalamic neurons by the corticothalamic fiber system.

461.12

MONAURAL AUDITORY DEPRIVATION FROM ONSET OF HEARING AFFECTS DEVELOPMENT OF AUDITORY CORTICAL FIELDS IN THE MONGOLIAN GERBIL *(MERIONES UNGUICULATUS)*

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In auditory cortex (AC) of the Mongolian Gerbil the primary auditory field (AI), the anterior auditory field (AAF) and additional activity in an intermediate zone (IZ) between AI and AAF could be distinguished at P 15 using the fluoro-2-deoxyglucose (2DG) mapping technique. Tonotopic activity in AI and AAF appeared at P 19. Here, we investigated the affects of preauditory induction of peripheral hearing loss on the development of activity pattern from P 19 to P 27. We prevented development and opening of the left external ear canal surgically under anesthesia at P 9. Auditory deprivation could be maintained for life or reversed by surgical opening of an artificial external ear canal.

2DG-Experiments were performed at P 19 and P 27 in alert animals deprivated since P 9. After injection of 9 µCi ^{14}C-fluoro-2-deoxyglucose i. p., gerbils were stimulated for 45 minutes with tones continuously alternating between 1 and 2 kHz (250 ms each frequency, 70 dB SPL free field). The left external ear canal was either closed (END), or it was quickly reconstructed under anesthesia 15 minutes before the 2DG injection, allowing air transduction during acoustical stimulation (REC). Control groups consisted of gerbils which received foam ear plugs in the left external ear canal 15 minutes before 2DG injection (PLU) and animals with normal hearing abilities (NOR).

At P 19, monaurally deprived animals (END, REC) showed no obvious asymmetry in metabolic activity of AI and AAF but a frequent lack of IZ in the right AC. Left and right secondary auditory fields showed no or low activity. Monaural deprivation may thus retard the functional maturation of some cortical fields in both hemispheres. Effects of monaural deprivation on the contralateral (right) AC could be detected in END and REC at P 27. Right AI showed increased metabolic activity as well as expansion of its rostrocaudal extent. Metabolic activity in IZ could be identified in the left AC but was missing in the right AC. PLU animals in contrast showed more or less reduced activity in right AC.

Obviously, imbalance of auditory input, due to preauditory monaural deprivation, interferes with normal development of auditory cortex. Low metabolic activity in secondary auditory fields at P 19 may indicate a role in processing of binaural input. Increased activity in AI and absence of activity in IZ contralateral to the deprived ear at P 27 may be due to desturbed balance of excitatory and inhibitory mechanisms after a critical period.

461.13

REPRESENTATION BY SPIKE COINCIDENCE IN PRIMARY SENSORY CORTEX. R.C. deCharms, C.E. Schreiner* and M.M. Merzenich, Keck Center for Integrative Neuroscience, UCSF, San Francisco, CA 94143.

These experiments investigated the hypothesis that the coincident firing of large populations of cortical neurons may constitute a fundamental element of primary cortical stimulus representation, particularly relevant during continuous stimuli. Neurons in the primary cortical areas AI, SI and VI respond in a predominantly phasic fashion, which is strikingly mismatched to the ongoing percepts evoked by continuous stimuli. We therefore explored whether coincidence-based coding is being employed, and whether it can be clearly disassociated from firing rate coding using these stimuli.

We recorded single or multiple supragranular neurons from groups of 2-6 sites at once (at .1-.5mm separations) in the primary auditory cortex of three pentobarbital anesthetized common marmoset monkeys, using conventional microelectrode recording methods and spike sorting.

The mean firing rate time course of many locations is nearly identical following a brief (50msec) tone burst as during a four second continuous tone; there is typically little or no information about the ongoing stimulus present in individual firing rates during the tonic phase of the stimulus. However, spikes from pairs of locations with no tonic firing rate change often very reliably indicate the presence of a stimulus during this time by the degree of spike coincidence from the two locations. Modulation of spike coordination during the tonic phase of continuous stimuli satisfies a number of additional criteria of a representational system: 1) it is stimulus specific and frequency tuned for each pair of locations; 2) it can increase or decrease from background depending upon the stimulus presented; 3) it can follow the time course of long stimuli when individual firing rate does not; and 4) it forms an organized topographic map across the cortical surface. (Funded by NIH grant NS-10414; NSF Graduate Fellowship).

462.1

FUNCTIONAL ACITIVATION OF AUDITORY CORTEX: A PET STUDY IN JAPANESE MACAQUE. T. Hashikawa[1]*, J. He[1], T. Kakiuchi[2], H. Tsukada[2,3], M. Molinari[1], and E. G. Jones[1,4]. [1]Lab. for Neural Systems, RIKEN, Wako, Japan, [2]Centr. Res. Lab., Hamamatsu Photonics, Shizuoka, Japan, [3]Subtemtomole Biorecog. Proj., JRDC, Osaka, Japan, and [4]Dept. of Anat. and Neurobiol., Univ. of California, Irvine, CA 92717.

The primary auditory cortex of Japanese monkey is located in the center of the supratemporal plane of the superior temporal gyrus, being coincident with an annectant gyrus typical of this species, and characterized by dense immunostaining for a calcium binding protein, parvalbumin (Hashikawa et al., 1994, Soc. Neurosci. Abstr., 20). To evaluate its functional state in vivo, tonal stimuli were applied to anesthetized monkeys and the activated regions were examined by positron emission tomography (PET) using $H_2{}^{15}O$ to measure regional cerebral blood flow. In comparison with activities in the control state (white noise stimulation), pure tone stimulation activated multiple cortical regions, including those on the supratemporal plane, the posterodorsal inferotemporal cortex, the posterior lip of the lunate sulcus, and the dorsal prefrontal cortex. The central core of the activated region varied when a coo sound, a species-specific communication sound, was used as a stimulation. Coo stimulation produced activation sites in peripheral regions of the supratemporal plane, e.g., the anterior lip of the superior temporal sulcus and the medial wall of the annectant gyrus, as well as in the central portion of the plane. In contrast, reversed (nonsense) coo sounds evoked stronger activation in the insular and ventral prefrontal cortex than on the supratemporal plane.

The present results confirm the anatomically defined auditory regions on the supratemporal plane and suggest that extensive cortical regions are involved in auditory information processing having potentially differentiated functions.

462.2

A COMPARISON OF EVOKED POTENTIALS AND HIGH FREQUENCY (GAMMA-BAND) OSCILLATING POTENTIALS IN RAT AUDITORY CORTEX D.S. Barth* and M.N. Franowicz Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

Middle latency auditory evoked potentials (MAEP) and steady-state (40-Hz) evoked potentials, as well as spontaneous and click-evoked gamma-band oscillations, were recorded from 15 lightly anesthetized rats using an 8x8 electrode epipial array covering auditory cortex and adjacent areas to determine and compare the spatiotemporal distributions of these four phenomena.

Results indicate that both the MAEP complex, and the steady-state 40-Hz response with its associated slow potential, are highly stereotyped in lightly anesthetized rodent cortex. Their spatiotemporal distributions are probably determined in large part by asynchronous activation of parallel thalamocortical projection systems.

No direct link was found between either the MAEP or the steady-state 40-Hz response to spontaneous or evoked gamma-band oscillations in auditory cortex. A notable spatiotemporal variability of gamma oscillations, with rapid spread within areas 41, 36 and 20, suggests a neural generator within the cortex independent of direct thalamocortical pacemaker influence. This hypothesis is further supported by the observation that neither the spatial distribution nor the phase of ongoing gamma oscillations were influenced by auditory stimulation. However, post-inhibitory enhancement of gamma oscillations following auditory stimulation may indicate a role in auditory information processing that is not reflected in either a stimulus specific spatial distribution or phase relationship.

462.3

A NEUROPHYSIOLOGIC CORRELATE OF COMODULATION MASKING RELEASE (CMR). C. King, T. Nicol, T. Carrell, T. McGee and N. Kraus*, Auditory Neuroscience Lab., Northwestern University, Evanston, IL 60202.

Hall et al. (JASA 76:50-56, 1984) first demonstrated an increase in the release from masking (i.e. better threshold) for a signal centered in amplitude modulated (AM) noise as the noise bandwidth is widened. This increase was seen even when the bandwidth was wider than the critical band for the signal. This effect has been termed comodulation masking release (CMR) because the key element for obtaining the release from masking is the correlated or coherent AM rates of noisebands. It is thought that the auditory system can use CMR to improve the detectability of signals in noisy listening situations.

There have been numerous human psychophysical experiments examining the nature of CMR, but to date, nothing has been published regarding the possible underlying neurophysiology. The purpose of this study is to demonstrate a neurophysiologic correlate in a guinea pig model to the behavioral psychophysical measures of CMR, using the mismatch negativity (MMN), an auditory evoked potential. The MMN is seen as a negativity in the response to an infrequent stimulus presented in a train of frequent stimuli (an oddball paradigm).

In this experiment, two 75 dB, 400 Hz-wide noisebands centered at 2 kHz and 2.7 kHz were comodulated at 30 Hz for the infrequent stimulus in the oddball paradigm. For the frequent stimulus, the noiseband centered at 2 kHz was modulated at 30 Hz and the noiseband centered at 2.7 kHz was modulated at 51 Hz (conflicting modulation). In an experimental condition, a 2 kHz tone was added to both the frequent (conflicting modulation) and the infrequent (comodulation) stimuli. The 2 kHz tone was set at threshold for the conflicting modulated stimulus. (This threshold was determined earlier using human subjects in a psychophysical task.) In a control condition, no tone was present in order to demonstrate that any differences seen in the experimental condition were due to CMR and not to differences in the comodulated and conflicting modulated noisebands themselves.

Epidural electrodes were placed on anesthetized albino guinea pigs over the primary auditory cortex and over the posterior midline. Previous work in our lab has shown that the posterior midline is the best site for recording the MMN in guinea pigs. Results revealed a mismatch negativity at the midline recording site for the experimental condition, but not for the control condition. Therefore, differences seen in the experimental condition appear to represent a neurophysiologic reflection of CMR.

462.4

THE P13 MIDDLE LATENCY AUDITORY EVOKED POTENTIAL IN A RAT MODEL OF ANXIETY USING CHRONIC CORTICOSTERONE INJECTION. H. Miyazato, R.D. Skinner* and E. Garcia-Rill. Dept. of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

The human P1 (or P50) middle latency auditory evoked potential, which is characterized by: 1) sleep state dependence, 2) rapid habituation, and 3) blockade by scopolamine, is a useful tool in assessing psychiatric disorders. Post-traumatic stress disorder (PTSD) patients have markedly decreased habituation of the P1 potential (Gillette, et al., 1995). Also, preliminary results suggest a decrease in locus coeruleus (LC) cell number in post-mortem human brains from anxiety disorder patients (Freeman, et al., 1993). Because of the link between PTSD and stress, we studied the effects of chronic corticosterone injection on the P13 potential, which has been demonstrated to be a rat model of the human P1 potential (Miyazato, et al., Brain Res. Bull. 37:247-255, 1995).

Under anesthesia, adult male Sprague-Dawley rats were implanted with epidural screws at the vertex (Vx) and the auditory cortex (ACx). Three weeks after surgery, rats were injected (s.c.) daily with corticosterone 5mg (CORT) or vehicle (CTL) and recordings were performed weekly using a paired click paradigm (ISI=0.25 and 1.0sec, 0.03Hz stimulation rate). Results showed that CORT markedly decreased habituation of the Vx P13 potential. After four weeks of injections, there was no significant difference between CORT and CTL rats at an ISI of 0.25sec. However, at an ISI of 1.0sec, CORT animals showed a 101±15% amplitude in the P13 potential following the second of two click stimuli, compared to a 25±19% amplitude in CTL animals ($p<0.0001$). After nine weeks of injections, CORT animals showed 95±21% (vs CTL 6±8%, $p<0.0001$) and 114±21% (vs CTL 22±13%, $p<0.0001$) amplitude at ISIs of 0.25 and 1.0sec, respectively. In contrast to the dramatic change in the P13 potential, the ACx Pa potential showed no changes after CORT.

These preliminary results suggest that cholinergic mesopontine neurons (which participate in the manifestation of the P13 potential) undergo decreased habituation following chronic corticosterone injection, possibly due to decreased inhibitory LC input. Supported by USPHS Grant NS20246.

462.5

SPATIO-TEMPORAL ANALYSIS, NEOCORTICAL EEGs AND BEHAVIORAL ANALYSIS OF CONDITIONED RABBITS. J.L. Chin, D.R. Graham-Squire, S.N. Truong, W.J. Freeman, and E. Marg*. Dep't of Molecular and Cell Biology, School of Optometry, University of California, Berkeley CA 94720.

Rabbits with 8x8 epidural arrays (0.5-0.8 mm spacing) on the auditory cortex were conditioned to respond to stimuli in a classical aversive paradigm. Stimuli consisted of short tones of various frequencies. 64 EEG traces were recorded during a 6 second period. Respiratory waves were simultaneously recorded using a pneumograph belt placed around the trunk. Changes in respiration were used as the conditioned response to the conditioned stimulus. Patterns of behavioral responses were followed and probabilities of a response occurring were calculated to determine whether habituation to the stimulus was achieved. The EEG traces were subsequently classified into groups based on stimulus type and order of presentation. Root mean square amplitude was calculated to represent each spatial pattern by a 64x1 column vector. Euclidean distance in 64 space was used to classify spatial patterns of those groupings. We conclude that distinct spatial patterns existed for each presented stimuli. Comparisons of control periods within sessions did not separate but between sessions, control periods displayed sustained differences across days, indicating long term changes. Analysis of behavioral responses yielded a gradual decrease in the response probability as stimuli presentations continued. We wish to determine whether spatial patterns change with habituation and whether changes in behavioral responses reflect prior changes in the cortex.

462.6

CHRONIC MICROELECTRODE INVESTIGATIONS OF HUMAN AUDITORY CORTEX. M. Howard*, I. Volkov, M. Ollendieck, H. Damasio, P. Abbas, C. Brown, B. Gantz. Univ. of Iowa Sch. of Medicine, Iowa City, IA 52242.

A new method has been developed which enables investigators to chronically record individual neuron activity from MRI defined anatomical locations in the human brain. Hybrid depth electrodes (HDE) are stereotactically placed in patients requiring depth electrode (DE) evaluation as part of their treatment for medically intractable epilepsy. Each HDE has standard clinical recording sites and eight pairs of high impedance bipolar microelectrode recording sites positioned along the shaft. Using a HDE does not increase risk to the patient. To date, eleven HDEs have been placed in three patients. Single unit activity was recorded from each of the microelectrode recording contacts for periods ranging from 5 to 14 days. Post-implantation stereotaxic imaging identified recording site locations.

One HDE was positioned along the length of a patient's right transverse temporal (Heschl's) gyrus (HG). Evoked potentials (EP) and single-unit recordings were obtained as clicks and tone bursts were presented to the left ear. EPs demonstrated an initial cortical response 15 ms following onset of sound stimuli, and the magnitude of the EPs were frequency dependent. A tonotopic pattern was observed with best frequencies systematically increasing as more medial-caudal recording sites were sampled. Single units from multiple recording sites along the HDE shaft displayed frequency dependent responses to tones. Responses were predominantly tonic in nature and single unit best frequencies displayed a tonotopic pattern similar to that observed with EPs. These results unambiguously demonstrate a tonotopic organization within human auditory cortex similar to that reported in monkey (Merzenich and Brugge, 1973). This new method appears suitable for future investigates of more complex issues related to auditory cortex plasticity.

462.7

A COMPARISON OF LAMINAR EVOKED POTENTIALS, STEADY-STATE (40 HZ) EVOKED POTENTIALS AND HIGH FREQUENCY (GAMMA-BAND) OSCILLATING POTENTIALS IN RAT AUDITORY CORTEX. B. BRETT* AND D.S. BARTH
Dept.of Psychology, University of Colorado, Boulder, Co. 80309

Mid-latency auditory evoked potentials (MAEPs), steady state (40-Hz) evoked potentials, and spontaneous gamma-band oscillating potentials were recorded from lightly anesthetized rats using a 16 channel laminar electrode array positioned in area 41 to compare the spatiotemporal characteristics of these potentials in the cortical lamina.

Current source density (CSD) and principle component analysis (PCA) of MAEPs recorded in the cortical lamina provide evidence of dynamic interactions between supragranular and infragranular neuronal subpopulations in primary auditory cortex. Analysis of steady-state 40 Hz evoked potentials indicates that these potentials exhibit dynamic interactions similar to those of MAEPs, suggesting that the same populations of cells which produce MAEPs also produce steady-state 40 Hz evoked potentials. Analysis of spontaneous gamma-band oscillations indicates that these potentials display different spatiotemporal characteristics.

We propose that MAEPs and steady-state 40 Hz evoked potentials result primarily from the asynchronous activation of parallel thalamocortical projections from the medial geniculate (MG), while spontaneous gamma-band oscillations have a more complex neurogenesis which may involve different thalamocortical and intracortical circuitry.

462.8

AUDITORY MISMATCH NEGATIVITY EVOKED RESPONSES HAVE BOTH TEMPORAL AND FRONTAL GENERATORS. R.E. Greenblatt, D. Voreades Source/Signal Imaging, San Diego CA 92102, H. Gould, Memphis Univ. Memphis TN 38105 and D.F. Rose*, LeBonheur Children's Medical Center and Univ. Tenn., Memphis TN 38105.

Mismatch negativity (MMN) is observed in scalp electrical recordings in response to a rare deviant tone following a sequence of standard tones, and is thought to be an electrical correlate of auditory novelty detection. In order to estimate the cortical generators for this and other components of the auditory evoked potential, we have obtained 64 channel electrical recordings from 10 normal adult subjects for whom volumetric magnetic resonance images were also available.

For each subject, tone burst stimuli sequences were presented unilaterally with identical standard tones, and deviant tones (10%) which varied either in duration, intensity, or pitch. Data were averaged off-line to produce artifact free estimates of the evoked responses to the standard and deviant tones. The MMN signal was then obtained as the difference of the deviant response less the standard response. Source estimates were obtained from the voltage data using generalized linear inverse techniques with Laplacian regularization. Head shape information was obtained from digitized electrode locations and used as the basis for a three shell forward solution.

Source estimates from individuals show some variation, but certain general features are apparent. Sources derived from the N1 peak have a contralateral maximum in a location consistent with a superior temporal generator. Additional, secondary, N1 generators may also be inferred. The MMN signal appears to have at least three generators, one in contralateral (left) temporal cortex (at a similar location to the principal N1 cortical source), a second which is in right frontal cortex, and a third near ipsilateral (right) temporal cortex.

462.9

THE DECOMPOSITION OF EVOKED SCALP POTENTIAL DATA INTO PARAMETRIC EQUIVALENT DIPOLE COMPONENTS: APPLICATION TO THE AUDITORY P50. H. L. Gillary*. Department of Physiology, University of Hawaii, Honolulu, HI 96822.

A procedure has been developed to represent multi-channel averaged evoked scalp electrical potential data in terms of potentials generated on a homogeneous spherical volume conductor by overlapping parametric spatio-temporal equivalent dipole current sources designed to facilitate inferences about real intracranial sources. For the simplest (8 parameter) dipole component (DC), the magnitude function of time is Gaussian and the location and orientation constant over time; added parameters can allow rotation and a more flexible magnitude function. The data are first segmented at time points of minimum variance and constrained DCs successively fitted to them using a simplex algorithm to minimize residual variance. Ultimately each data segment is represented as the sum of a few principal DCs with maxima within the segment, the tails of DCs in adjacent segments, and a residual.

The procedure has been applied to segments of 14-channel band-pass filtered auditory evoked potential data that include the P50 wave, obtained using a protocol in which two clicks 500 ms apart were presented every 7-8 s. The respective responses to the two clicks could differ markedly in topography. For example, for one subject the P50 segment to click 1 was well explained primarily by one principal DC whereas that to click 2 required two quite dissimilar principal DCs (one of which, although smaller in amplitude, showed marked similarity to the major DC for click 1) and displayed a much larger residual that resisted further DC decomposition. The procedure, readily modified with regard to head and current source model and minimization algorithm, shows promise as a general means to summarize and explore scalp potential dynamics, and to identify components that correlate with specific clinical conditions.

462.10

COMPARISON OF RELATIVE AND ABSOLUTE SOUND LOCALIZATION ABILITY IN NORMAL HUMAN SUBJECTS. S.D.R.R. Makhamra, D.C. Guard, P. Geiger, and G.H. Recanzone* Center for Neuroscience and Dept. Neurobiol. Physiol. & Beha-., University of California at Davis, Davis, CA 95616.

Sound localization paradigms have been used by others to either define the minimum audible angle (relative localization), or measure the subject's judgments of sound source locations (absolute localization). Comparisons between these paradigms are difficult due to differences in acoustic stimuli, subjects, etc. In this study, the same apparatus and stimuli were used in order to directly compare performance between absolute and relative localization paradigms in the same subjects.

All experiments were performed in a darkened sound booth on four normal hearing subjects (aged 20-32 yr.). Tonal (1 kHz and 4 kHz) or gaussian noise stimuli were presented for 200 msec (5 msec rise/fall) at 30 +/- 2 dB re threshold for that subject. Stimuli were presented from 8 degrees to the left, to 56 degrees to the right along the horizontal meridian. For the relative localization task, stimuli were sequentially presented 3-9 times from a single speaker every 800 msec, then the same acoustic stimulus was presented at one of 14 other possible locations. For the absolute localization task, a single stimulus was presented from one of 15 possible locations, and the subjects were required to face that speaker location.

Minimum audible angle (MAA) measurements were smallest for noise (2-3 deg) and 1 kHz tones (10-15 deg) with little effect of eccentricity of the starting speaker. MAAs for 4 kHz tones were larger (15-30 deg) and increased with increasing eccentricity. Absolute localization judgments ranged over 20 deg for noise, 20-50 degrees for 1 kHz tones, and 30-60 degrees for 4 kHz tones. These ranges increased with increasing eccentricity for both 1 kHz and 4 kHz tones, but not noise.

These data indicate that subjects are better at determining that a stimulus has changed location than at determining the precise location of the stimulus in space, and suggests that absolute localization tasks may provide a better metric in comparisons of perceptual acuity with spatial receptive fields recorded from neurons in the central auditory nervous system.

462.11

BINAURAL AND MONAURAL SOUND LOCALIZATION IN ACALLOSAL HUMAN LISTENERS. N. Lessard, F. Leporé*, P. Poirier, J. Villemagne and M. Lassonde. Groupe de Recherche en Neuropsychologie Expérimentale, Univ. de Montréal, Canada.

In order to elucidate the callosal involvement in sound localization in free-field space, the present study examined response accuracy to monaurally and binaurally presented auditory targets in acallosal subjects (Ss). In these Ss, binaural cues to localization (ΔI, ΔT) are integrated at the cortical and subcortical level without the additional support of the telencephalic commissure. Because acallosal Ss developed with this reduced source of binaural activation of cortical cells, moreover, they might have perfected their ability to use monaural cues to sound localization. This hypothesis was tested by examining localization performance using monaural stimulation. Three Ss with callosal agenesis and 14 controls were asked to localize broad band noise bursts (BBN) of fixed intensity in the horizontal plane in an anechoic chamber. BBN were delivered randomly through 16 loudspeakers, which were mounted at 10° intervals on a calibrated perimeter frame. Two conditions were tested: i) localization of a fixed-sound source; ii) localization of the beginning and the end of a simulated moving sound. Listeners had to report the apparent stimulus location by pointing with the index finger the position on the sound perimeter. The results indicated that there was a small tendency for the acallosal Ss to be somewhat less accurate than the controls in binaural localization. However, under monaural testing conditions, the acallosals performed significantly better than the controls. The latter data suggest that because of their lack of corpus callosum, acallosal Ss compensate for their reduced acces to cortical binaural cues by making greater use of monaural cues.

462.12

SPATIAL AND TEMPORAL CHANGES OF THE MAGNETIC FIELD PRODUCED BY THE CEREBRAL CORTEX DURING 40 HZ AUDITORY EXCITATION K.H. Knuth, J.Dh. Broadhurst Dept. of Physics, Univ. of Minnesota, Minneapolis MN 55455, B.J. Schwartz, C.C. Gallen MEG Laboratory, Scripps Research Institute, La Jolla, CA 92037

* The magnetic fields from steady-state auditory responses driven by clicks at approximately 40 Hz were examined using a 37-channel biomagnetometer. The magnetic fields were found to be periodic in time, oscillating at the stimulation frequency. Initially in the cycle, the magnetic fields exhibited a bipolar form consistent with a single current dipole. At longer post-stimulus latencies, however, this bipolar form dissipated leaving a multipole field. Examination of the differences between the phases of the response detected in the individual sensors showed that a stationary equivalent current dipole is not an accurate model of the neural magnetic field generators. However, the location of the active tissue was found to change in a coherent manner.

462.13

NEUROMAGNETIC FIELD RESPONSES TO CHANGES OF INTERAURAL TIME DIFFERENCE IN THE HUMAN AUDITORY CORTEX. K. Mori[1]*, S. Imaizumi[1], S. Kiritani[1] and M. Yumoto[2]. [1]Research Institute of Logopedics and Phoniatrics, and [2]University of Tokyo Hospital. Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113 Japan.

Representation of external space in the human auditory cortex still remains elusive, in spite of evoked response studies to interaural time differences (ITD) by electroencephalography (EEG) (Picton *et al.*,1991), as well as by magnetoencephalography (MEG) (McEvoy *et al.*, 1993). We recorded neuromagnetic responses from the left and right temporal cortices using a 37-channel magnetogradiometer (BTi) in 6 normal-hearing adult volunteers. Use of continuous, binaurally-correlated gaussian noise stimuli and selective averaging eliminated components unrelated to ITD shifts from the recording. ITD was changed between 0 and 400 μs, with the steps either 200 or 400 μs. Amplitude peaks of the root mean square (RMS) of the evoked magnetic fields were observed typically at or around 80, 140, and 240 ms latencies, replicating previous EEG results. ITD shifts representing virtual sound movements to the contralateral direction evoked larger responses than those to the ipsilateral to the recorded hemisphere, although the movements in the far ipsilateral virtual field evoked smaller responses. The evoked magnetic field waveshapes of responses to 0–>200 and 200–>400 μs ITD transitions were more similar than any other pair of responses, like those to 200–>0 and 200–>400 μs or 0–>200 and 400–>200 μs transitions, suggesting that the relative sound movement rather than movement origin or destination is the primary contributor to the MEG response, which in turn may correspond to the psychophysical lower threshold for sound movement detection than absolute localization errors. However, since estimated current dipole positions for the responses to 0–>200 and 200–>400 μs transitions of ITD were slightly offset by several millimeters from each other, depending on subjects and recorded sides, absolute places in virtual space may also be mapped in the temporal auditory cortex.

462.14

EFFECTS OF PSYCHOPHYSICAL TRAINING ON THE ENTRAINMENT OF PRIMARY AUDITORY CORTICAL NEURONS TO AMPLITUDE MODULATED TONES. R. Beitel*, C. Schreiner, X. Wang, S. Cheung, W. Jenkins and M. Merzenich. Keck Center, UCSF, San Francisco, CA 94143.

We have tested the hypothesis that the entrained response of neurons in the primary auditory cortex (A1) can be modified by reward-contingent stimulation with amplitude modulated (AM) tones over the range of temporal modulation rates that occur naturally in the vocalizations of an adult primate. Owl monkeys were trained to report a change in the modulation rate of a 1.0 kHz tonal carrier when the rate changed from a standard frequency (4Hz or 10Hz) to higher, comparison frequencies. Following training, the monkeys were anesthesized with pentobarbital sodium, and the 1.0 kHz isofrequency region of A1 mapped with tungsten microelectrodes. Free-field signals were either sequential AM tones (standard-comparison) that simulated the stimuli used in psychophysical training, or simple (2Hz-96Hz) AM tones. Digitized multiunit neuronal responses were analyzed as periodic functions of the AM signals (events/cycle) to derive temporal modulation transfer functions (tMTFs). Based on a preliminary analysis of data, results indicate that: 1) psychophysical thresholds for detection were 3 or 4 Hz above the standard AM rates used in training; 2) tMTFs were bandpass in form with a median best modulation frequency significantly higher than those reported by other investigators for untrained cats and monkeys; and 3) relative to the entrained response to the standard AM tone, the response entrained to the comparison AM tone was smaller when the modulation rate was below psychophysical threshold and larger when the modulation rate was above psychophysical threshold. The results suggest that behavioral performance is reflected in the entrainment of neuronal responses in A1, which is modified by training. (Supported by NIH Grant NS-10414 and ONR.)

462.15

AUDITORY SALTATION: HEARING BACKWARDS IN TIME. R. Hari*. Low Temperature Laboratory, Helsinki University of Technology, 02150 Espoo, Finland.

Sensory integration and backwards masking studies indicate that the nervous system collects information for 100–200 ms before conscious perception takes place. The present study shows similar sluggishness in an experiment of directional hearing in which the subjects mislocalized sounds which followed each other in rapid succession.

Fourteen healthy adults listened to trains of 8 binaural clicks, 4 left-ear leading (interaural difference 0.8 ms) followed by 4 right-ear-leading. The clicks were separated by 30–500 ms in different sequences. At ISIs of less than 120 ms the subjects perceived illusorily that the clicks jumped in equidistant steps from left to right. The perception was symmetrical without any bias towards the more recent stimuli. Monaural clicks, left-sided followed by right-sided ones, did not produce a similar illusion. Since impulses activating overlapping neuronal populations in close succession, as the binaural clicks evidently did, have a high probability of arising from the same source, the occurrence of the illusion for the binaural but not for the (ecologically nonvalid) monaural trains may reflect neuronal constraints of interpretative brain processes.

These data suggest that the brain integrates sound locations for 100–150 ms and that the conscious perception may be delayed by about 500 ms.

Supported by the Academy of Finland.

462.16

AUDITORY EVOKED FIELDS TO ATTENDED AND NON-ATTENDED ILLUSORY SOUND MOTION. L. McEvoy* and J.P. Mäkelä. Low Temperature Lab. Helsinki Univ of Tech. 02150 Espoo, Finland.

Auditory motion can be simulated by presenting binaural sounds with time-varying interaural intensity differences. The illusion of motion in 4 directions was produced by varying a 6 dB intensity difference between the two ears in the middle of a 600ms, 1 kHz tone. To examine human cortical responses to this correlate of auditory motion, magnetic fields were recorded from 7 subjects with a 122-channel whole-head magnetometer while subjects ignored or attended to the stimuli. The stimuli elicited clear responses in auditory cortices of both hemispheres, with strongest responses occurring about 100 ms after stimulus and transition onsets. A sustained field was also present throughout the tone. Over each hemisphere, the largest transition responses occurred to stimuli that appeared to move into the contralateral field and the smallest to stimuli that appeared to move away from the subject. The variation of response amplitude with simulated motion suggests that the responses are mediated by directionally selective cells in human auditory cortex. Attention to the stimuli elicited activity in frontal, parietal, and midline regions, as well as enhancing the sustained response from auditory areas of supratemporal cortex.

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462.17

THE RESPONSES OF LEFT AND RIGHT HESCHL'S GYRI TO THE FORMANT TRANSITIONS OF AUDITORY STIMULI: A PET STUDY. Belin P., Zilbovicius M.*, Fontaine A., Garnier M., d'Alessandro C., Chantome M., Frouin V., Mangin J.F., Zouaoui A., Dormont D., Masure M.C., Samson S., Marsault C., Neel F., Samson Y. CEA-SHFJ, and LIMSI, Orsay, UCV Salpêtrière, Paris.

The perception of consonants depends on brief formant transitions (FT) of auditory stimuli, which in turn may be selectively processed by left hemisphere cortical regions (Schwartz & Tallal, Science 1980). Here, we used H2O15 PET stimulation studies to investigate the responses of left and right primary auditory cortices (Heschl's gyri) induced by brief auditory stimuli with and without formant transitions. rCBF was measured in 6 right-handed normal volunteers, at rest and in five passive listening conditions: 1) Consonant-vowel-consonant (CVC)-like stimuli with short FT (C:40ms, V:200ms) 2) CVC-like stimuli with long FT (C:200ms, V:200ms) FT, 3) Vowel-like stimuli (V: 200ms) 4) Short-duration 750hz bursts (40ms), and 5) Long-duration 750hz bursts (200 ms). Heschl's gyri were localized on individual coregistered PET-MR images.

The short FT stimulus significantly activated both left (10.5±7%) and right (7.7±7%) Heschl's gyri, as well as the long FT stimulus (L:10.1±4% vs R:11.6±6%). The differences between left-right activations, and short-long FT were not significant. The vowel-like stimulus produces an activation that was significant in the right Heschl's gyrus (5.6±4%, $p<.02$), but not in the left (4.9±5%, ns). Bursts also activated both Heschl's gyri. Yet, Heschl's activation was significantly higher for FT stimuli (10±6%), than for vowel-like stimuli (5.2±4%) or bursts (5.8±5%).

In summary, as expected, Heschl's gyri are more sensitive to formant transitions than to steady-state stimuli, but we found no evidence for asymmetrical processing at this early cortical level. (supported in part by a DRC grant of AP-HP)

462.18

A SELF-ORGANIZING MODEL OF AUDITORY CORTICAL TOPOGRAPHY: EMERGENCE OF FUNCTIONAL SEGREGATION DIVERSITY. K.Sameshima*[1] and C.E.Schreiner[2]. [1]School of Medicine Univ. São Paulo, 01246-903 São Paulo SP, Brazil. [2]Coleman Laboratory, UCSF, San Francisco, CA 94143-0732.

The primary auditory cortex (AI) of cats, ferrets, and monkeys shows a number of systematic topographic segregations for different sound parameters along the isofrequency dimension, such as bandwidth, threshold, latency etc. We investigated the emergence of topographic organizations in the auditory cortex using a self-organizing single-compartmental neural network model with normalizing Hebbian pre- and post-synaptic cells activity dependent plasticity rule. Our model consists of five layers representing one cochlear (excitatory), two subcortical (excitatory and inhibitory) and two cortical (excitatory and inhibitory) levels. The model incorporates a novel feature, namely an ontogenetically predetermined increasing spread of subcortico-cortical connections in the isofrequency domain. The network was trained with sets of randomly or sequentially ordered inputs with different temporal patterns. Integration among two or more tone stimuli occurs if inputs generate temporo-spatially coincident activation. After training sessions, keeping synaptic weights constant, cortical excitatory cell responses were measured by applying single tones or tone sequences. In general, the ventral portion of the isofrequency dimension emerged as sharply tuned and the dorsal-half as broadly tuned. Occasionally, the center of the isofrequency domain was the most sharply tuned region. For nets trained with randomly sequenced tones with short interstimulus intervals, stronger responses were found in the ventral half. Thus, the model provides a variety of specific topographic patterns that can be quite similar to those observed in physiological experiments. A variety of topographic functional segregation patterns can emerge by manipulating the input stimulus patterns. (Supported by HCFMUSP, FAPESP 91/2182-8, CNPq 301059/94-2 and ONR Grant N00014-91-J-1317)

462.19

COMPUTATIONAL PRINCIPLES OF MULTISENSORY INTEGRATION: STUDIES OF ADAPTATION TO NOVEL VISUO-AUDITORY REMAPPINGS. Z. Ghahramani*, D.M. Wolpert & M.I. Jordan. Department of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

To explore the computational principles of human multisensory integration we have examined subjects' localization pointing errors in the azimuth to randomly interleaved visual, auditory and combined visuo-auditory targets. In a baseline study the visual and auditory stimuli coincided both temporally and spatially. In two remapping studies the normal relationship between the visual and auditory spatial maps was altered. In the first remapping study, a 15° bias was introduced between the maps by offsetting the relative locations of auditory and visual targets during combined presentation. In a second study, variance was introduced by adding zero-mean, 10° s.d. Gaussian noise between the location of the auditory and visual stimuli. Results on the baseline bias and variance of localization responses and the adaptation induced by the two perturbation paradigms are discussed in the context of cooperative (e.g. minimum variance) and competitive (e.g. winner-take-all) statistically-based fusion models.

462.20

PARALLEL HABITUATION AND SENSITIZATION OF AUDITORY EVOKED RESPONSES IN CHILDREN J. Karhu*, E. Herrgård, A. Pääkkönen, and J. Partanen, Kuopio University Hospital, Kuopio, Finland.

Developmental changes modulate auditory event-related potentials (ERPs). Latency decreases with age may reflect increased efficiency in receiving networks, and changes in topographic distribution may indicate changes in the mode of auditory processing. In adults, detection of any sound activates auditory cortices and frontal areas at 100 ms (N100). A novel sound elicits N100, which coincides in time with vertex response belonging to automatic orienting reaction.

We compared the auditory processing during the detection of novel input and subsequent repetitions of stimuli in 45 school-aged children (age 9 years) and in 20 adults (mean age 28 years; range 23–49 years). Subjects received trains of identical tones separated by 12 s silence, which exceeds the putative duration of sensory (echoic) memory. Evoked electric signals were monitored continuously from 21 scalp electrodes and analysed off-line.

In both children and adults, a prominent 100 ms response at vertex followed the first tone in train, and decreased to half in size after the second tone. In children, the amplitude of a 150-180 ms response over temporal auditory areas diminished similarly. In parallel, a frontal–central response at 260–300 ms enhanced markedly.

ERPs over temporal areas were significantly larger in children than in adults but habituated similarly as the vertex response suggesting that wider auditory networks activate automatically during orientation to novel input. Furthermore, sensitization of a temporally and topograhically separate neuronal population to repeated input was obvious. We hypothetize that this dual behaviour reflects building of neural representations in networks instrumental in elementary auditory perception and may reveal plastic capacity in developing brain.

OLFACTORY SENSES: ACCESSORY OLFACTORY SYSTEM

463.1

REPLACEMENT OF SENSORY CELLS IN VOMERONASAL EPITHELIUM OF ADULT HAMSTER AFTER NERVE TRANSECTION. M. Ichikawa, T. Osada, R. M. Costanzo*. Tokyo Metropolitan Institute for Neuroscience, Tokyo, Japan and Virginia Commonwealth University, Medical College of Virginia, Richmond, VA 23298-0551.

Sensory cells in the vomeronasal epithelium are replaced following experimentally induced degeneration. Transection of vomeronasal nerves results in degeneration of sensory cells followed by replacement of the receptor cell population. A quantitative analysis was made to determine the time course and degree of cell replacement in the vomeronasal epithelium following unilateral section of vomeronasal nerves in adult hamsters. Histological measurements of the number of sensory cells and epithelial thickness were made for up to 50 days postoperatively. Results for each experimental animal were expressed as a percentage of the contralateral control side. There was gradual degeneration of sensory cells, the number ranging from 60-100% on day 4 and 30-40% by day 7. During days 7-15 sensory cell replacement was observed. Cell number increased and reached a level of 80% by day 21. Epithelial thickness decreased to 70% during the degeneration period and did not return to control levels by day 21. This study confirms that vomeronasal sensory cells are capable of replacement following degeneration. However, the observed recovery did not reach control levels. Assessment of the functional capacity of replacement cells requires further study.

463.2

DIFFERENTIAL LOCALIZATION OF NADPH-DIAPHORASE ACTIVITY IN THE MOUSE VOMERONASAL SYSTEM M. Halpern* and C. Jia, Program in Neural and Behavioral Science, SUNY Hlth Sci. Ctr. at Brooklyn, 450 Clarkson Ave., Brooklyn, NY 11203.

The anterior and posterior portions of the accessory olfactory bulb (AOB) of some mammals differentially express a number of cell markers including the lectin VVA, olfactory marker protein (OMP), G proteins and NADPH-diaphorase activity. In the opossum AOB all of these markers are differentially expressed, whereas in the rat AOB the VVA lectin and G proteins only are differentially expressed and OMP and NADPH-diaphorase activity are homogeneously expressed. The distribution of NADPH-diaphorase activity was examined in the vomeronasal system of the mouse using a direct histochemical staining technique. In the AOB, the somata and neuropil in the granule cell layer and neuropil in the mitral-tufted cell layer were labeled. In the nerve-glomerular layers, only the anterior half was labeled. The boundary between the labeled anterior half and unlabeled posterior half of the nerve-glomerular layers was very sharp. In the sensory epithelium of the vomeronasal organ, the receptor neurons located in the middle layer were more darkly labeled than receptor neurons more basally situated. This finding is consistent with our previous observation in opossums suggesting that the receptor neurons in the middle layer of the sensory epithelium project to the anterior part of the AOB. Since NADPH-diaphorase activity indicates the presence of enzymes that require NADPH as a cofactor, this finding also suggests that the receptor neurons in the middle layer that project to the anterior part of the AOB may be functionally different from the receptor neurons in the basal layers.

463.3

DIFFERENTIAL EXPRESSION OF G PROTEINS IN SUBCLASSES OF VOMERONASAL RECEPTOR NEURONS AND THEIR SEPARATE TERMINATIONS IN THE ACCESSORY OLFACTORY BULB OF THE RAT AND MOUSE. C. Jia* and M. Halpern, Program in Neural and Behavioral Sci., SUNY Hlth. Sci. Ctr. at Brooklyn, 450 Clarkson Ave., Brooklyn, NY 11203.

It has previously been reported that the G proteins are differentially expressed in the nerve-glomerular layers of the accessory olfactory bulb (AOB) of rats (Shinohara et al. J.Neurosci.,1992). In order to understand the possible significance of the heterogeneity in the AOB, the AOB and vomeronasal (VN) organ of rats and mice were stained using antibodies to G proteins (anti-Gαi2 and anti-Gαo). In the nerve-glomerular layers of the AOB of rats and mice, anti-Gαi2 stains only the anterior half, and anti-Gαo stains only the posterior half. Since the nerve-glomerular layers contain axons and the terminals of VN receptor neurons, cross sections of the VN organ were processed for staining with the same antibodies. Anti-Gαi2 stains the neurons in the middle layers (middle 1/3) of the VN epithelium and anti-Gαo stains neurons in the deep layers (basal 1/3) of the VN epithelium. Staining is located in dendrites, the microvillar surface, cell bodies and axon bundles. The axon bundles stained by anti-Gαi2 can be followed from the VN organ to the anterior half of the nerve-glomerular layers of the AOB, while the axons stained by anti-Gαo can be followed from VN organ to the posterior half of the nerve-glomerular layers. The axons stained by the two antibodies are intermingled near the VN organ but gradually sort out toward the AOB. These results suggest that (1) there are two populations of mature receptor neurons in the VN organ, one expressing Gαi2 and the other expressing Gαo; (2) the Gαi2-expressing receptor neurons in the middle layers of the VN epithelium project to the anterior half of the AOB, and Gαo-expressing receptor neurons in the deep layers of the VN epithelium project to the posterior half of the AOB; and (3) this pattern of projections may be a common feature of organization in the mammalian VN system since similar results have been observed in the opossum.

463.4

PHYSIOLOGICAL PROPERTIES OF VOMERONASAL RECEPTOR CELLS IN AQUATIC SALAMANDERS (*AMBYSTOMA*). H.L. Eisthen*, R.J. Delay, and V.E. Dionne. Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543.

The vomeronasal system is an accessory olfactory system that originated in tetrapods; the sensory epithelium is innervated by terminal nerve fibers that contain gonadotropin releasing hormone (GnRH). The function of the vomeronasal system in aquatic amphibians is not known. As a first step toward understanding the modulation of vomeronasal receptor cell activity by odorants and by GnRH, we are characterizing the conductances present in these cells. Using a slice preparation, whole-cell recordings were made from vomeronasal receptor cells in two subspecies of tiger salamanders (*A. tigrinum mavortium* and *A. t. mexicanum*). Application of tetrodotoxin revealed that a large portion of the inward current was carried by Na^+. The contribution of Na^+ varied among cells, ranging from 60 to 100% of the inward current (n = 10). The remainder of the inward current was carried by Ca^{2+}, as demonstrated by the use of Ca^{2+} substitutes (Ba^{2+}) and blockers (Co^{2+}, Cd^{2+}). The magnitude of the outward current was greatly reduced in the presence of Cs^+, a K^+ channel blocker (n = 62). Furthermore, extracellular application of Ba^{2+} reduced the outward current in the presence of Cs^+, suggesting the additional presence of both Ca^{2+}-dependent and -independent outward currents (n = 23). We are currently examining the contribution of Cl^- and a nonselective cation conductance to both the voltage-activated and Ca^{2+}-dependent components of the outward current in these cells.

463.5

EVIDENCE FOR DIFFERENT MECHANISMS OF SENSORY TRANSDUCTION IN THE VOMERONASAL ORGAN AND OLFACTORY EPITHELIUM.

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The olfactory system of mammals is divided into two parts: the main olfactory system and the accessory olfactory system. The sensory neurons of the accessory system are located in the neuroepithelium of the vomeronasal organ (VNO), a tubular structure that lies beneath the nasal cavity and opens into it. Previous studies indicate that the VNO is involved in the sensing of pheromones that regulate sexual and social behaviors and endocrine function. At present, nothing is known about the mechanisms underlying the transduction of pheromonal signals in the VNO. One important issue is whether sensory transduction in the VNO proceeds via a G protein coupled mechanism as it does in the olfactory epithelium (OE) of the nasal cavity. To address this question, we have isolated cDNAs encoding G protein α subunits expressed in the VNO and have examined their patterns of expression. Our results indicate that Gαolf, which is highly expressed in OE neurons and is thought to mediate sensory transduction in these cells, is not expressed in VNO neurons. Instead, we observe high level expression of genes encoding two other Gα subunits, Gαo and Gαi2 in VNO neurons. Immunostaining reveals that Gαo and Gαi proteins are present at the microvillar surface of the VNO neuroepithelium, the expected location of molecules involved in sensory transduction. Our results indicate that there are two distinct subpopulations of VNO neurons, one of which expresses high levels of Gαo and the other high levels of Gαi2, and that these two subpopulations of cells are located in different parts of the VNO epithelium. These findings suggest that: 1) VNO neurons transduce signals via mechanisms different from those used by neurons in the OE, 2) sensory transduction in the VNO proceeds via mechanisms involving G proteins, and 3) there are two different types of VNO neurons that have distinct locations in the neuroepithelium and use different G proteins to transduce sensory signals.

463.6

MORPHOLOGY OF SYNAPTIC PLASTICITY INDUCED BY URINARY STIMULATION IN ACCESSORY OLFACTORY BULB.

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We have studied whether natural stimuli through sensory systems to which animals are exposed affect synaptic structure of the vomeronasal system. This system, consisting of the vomeronasal organ, the accessory olfactory bulb (AOB) and the higher vomeronasal centers receiving afferents from the AOB, is known to play a critical role in the perception and processing of conspecific chemical signals (pheromones) in mammals. The axons of vomeronasal sensory cells make synapses on the dendrites of mitral/tufted cells in the glomeruli of the AOB. The effects of urinary stimuli on synapses were examined in the AOB of adult male golden hamsters. At 30 days of age, male littermates were divided to three groups and exposed to one of three materials: 1) the female hamster urine, 2) the female rat urine, 3) and the control (distilled water), each animal was reared independently. After two weeks, the AOBs of animals from each group were prepared for electron microscopic examination. The length of synaptic active zone and the length of synaptic apposition zone in the glomeruli of AOB were measured: the synaptic active zone was characterized by the postsynaptic thickenings and the synaptic apposition zone was characterized by the close apposition area between pre and postsynaptic membranes. The length of synaptic active zone was longer in both the hamster urine group and the rat urine group compared with the control group. The length of synaptic apposition zone was longer in the control group compared with the rat urine group, there was no difference between hamster urine group and control group. These results demonstrate that exposure to the female urine induces morphological changes of synapses in the AOB of adult male hamster.

463.7

APHRODISIN IMMUNOREACTIVE EPITHELIAL CELLS ARE ASSOCIATED WITH NERVE TERMINALS IN THE REPRODUCTIVE TRACT OF THE FEMALE HAMSTER.

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Aphrodisin, a 17 kD protein purified from hamster vaginal discharge, elicits copulatory behavior and increases c-fos immunoreactivity in the accessory olfactory bulb of the male hamster. In this study, we localized the protein in the female hamster reproductive tract with the ABC-DAB immunolabeling technique using aphrodisin antiserum. Aphrodisin immunoreactivity was found in the oviduct and uterus. In the oviduct, the aphrodisin containing cells were located at the epithelium. The cells were mostly goblet in shape with a constricted base. Their slender basal ends reached to the basal laminae with either a bulbar or bifurcated ending. Nerve terminals with synaptic profiles were observed to contact these cells. In the uterus, immunoreactivity was located in the epithelial cells and uterine glands. In the estrous animal, the endometrium exhibited strong aphrodisin immunoreactivity, but the shedding process caused the immunopositive cells to be unrecognizable. These results indicate that the secretory cells of the oviductal and uterine epithelium, and uterine glands are the main source of aphrodisin, and may be neurally regulated.

Supported by PHS grant NS12344.

OLFACTORY SENSES: OLFACTORY BULB

464.1

SPATIO-TEMPORAL DISTRIBUTION OF Ca^{2+} TRANSIENTS AND LATE DEPOLARIZATIONS IN SALAMANDER MITRAL/TUFTED (M/T) CELLS

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Relative long lasting depolarizations at glomerular and subglomerular levels of the olfactory bulb has been associated to Ca^{2+} currents. Using voltage sensitive dyes, optical signals evoked either by electrical or odor stimulations consist on slow components which have been related to dendritic Ca^{2+} transients. In M/T intracellular recordings, conditions which reduce inhibitory actions can unmask late depolarizations at the soma probably also related to Ca^{2+} transients. In the present study, calcium-sensitive probes were used to determine the role of Ca^{2+} transients in these long lasting dendritic depolarizations. Experiments were conducted in isolated epithelium-bulb preparations. Horizontal slices of the bulb with the olfactory nerve fibers attached to the epithelium were put in a split-bath tissue chamber. In single M/T cells injected with non-permeant forms of FURA-2, FLUO-3 or CALCIUM GREEN, olfactory nerve electrical stimulation gave rise to relatively long-lasting Ca^{2+} transients distributed predominantly across the dendritic arborization. Odor stimuli also evoked Ca^{2+} transients on M/T dendrites. In both cases, Ca^{2+} transients were non-homogeneously distributed throughout M/T dendrites, suggesting the activation of multifocal dendritic electrogenic zones. The spatio-temporal distribution of these Ca^{2+} transients depended on (a) the sites of activity at the epithelium and (b) the activation of inhibitory circuits. Blockade of inhibitory feedbacks using either bicuculline and/or phaclofen increased the magnitude, duration and spatial distribution of these Ca^{2+} transients. These conditions also facilitated the propagation of these transients to the soma level. These data support the notion that the activation of Ca^{2+} transients regulate the spatio-temporal distribution of the activity in M/T dendrites and might have implications in the generation of long lasting excitability changes that occur in M/T cells either following odor or electric-elicited activity. Supported by NIH Grant RO1-DC01804-04 and Dept. Anat.& Cell Biol., SUNY, Brooklyn.

464.2

SPATIO-TEMPORAL DYNAMICS OF OSCILLATORY ACTIVITY IN FROG OLFACTORY BULB

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Odor stimulation of the nasal epithelium evokes an oscillatory field potential (FP) in olfactory bulbs (OBs) of virtually all vertebrates. Odor-evoked responses at 8-12 Hz (T=21°) as well as broad-band spontaneous activity (peak ≈2-4 Hz) are known, distinctive features of FPs from frog OB. We recorded odor-evoked and spontaneous activity *in vivo* using the voltage-sensitive dye RH795 and linear arrays of 2-7 photodiodes or a high speed CCD camera to image the ventral surface of OBs (frogs are pithed, paralyzed and perfused with high osmolarity ringer to reduce movement artifacts). Power spectra of simultaneous FP and optical measurements from 100x200 μm spots are essentially equivalent during spontaneous and odor-evoked activity. Multi-site optical measurements indicate odor-evoked oscillations are spatially coherent, typically with no phase shifts along the rostral-caudal axis of the OB, although in some instances shifts of up to π were seen. During spontaneous activity spatial coherence is less than that during odor-evoked activity and exhibits phase jitter, i.e., irregular waves whose direction of propagation along the rostral-caudal axis fluctuates. Interestingly, after odor stimulation the frequency of oscillations returns to its spontaneous range while the spatial coherence remains high for many seconds. Intracellular recordings from mitral cells in an *in vitro* nose-forebrain preparation reveal that most mitral cells show odor-evoked membrane potential oscillations synchronized to the FP but there is considerable variation between cells in the extent of firing during each depolarizing phase of the oscillation. NSERC OGP0121698

464.3

LOCALIZATION OF THE GLUTAMATE-RECEPTOR SUBUNITS NMDAR1 AND GLUR1 IN THE RAT OLFACTORY BULB.

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Recent immunocytochemical and electrophysiological data indicate that glutamate may be the neurotransmitter released by olfactory nerve fibers. In the present study, we have investigated the expression and localization of two glutamate-receptor subunits (NMDAR1 and GluR1) in the rat olfactory bulb (OB) using Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) and immunocytochemistry.

Semiquantitative RT-PCR experiments showed that both the GluR1 and the NMDAR1 mRNAs are abundantly expressed and that the NMDAR1-a splicing variant approximately doubles the NMDAR1-b mRNA. NMDAR1-immunoreactivity was present in mitral and tufted cells, as well as in interneurons (granular and periglomerular cells). In the olfactory glomeruli, a punctate immunoreactivity was observed. The AMPA-binding subunit GluR1 was expressed by short-axon cells, by mitral/tufted cells, and by neurons located in the periglomerular region. The external plexiform layer and the glomeruli were also immunoreactive. Electron-microscopic immunocytochemistry showed that both NMDAR1 and GluR1 can be present at the synapses established by olfactory nerve terminals with the dendrites of mitral/tufted and periglomerular cells. However, only a few of these synapses were labelled. NMDAR1 was also present at some dendro-dendritic synapses between mitral/tufted and periglomerular cells, and GluR1 was found in glial cell processes.

These results are consistent with the assumption that glutamate is a neurotransmitter released by olfactory nerve fibers, and indicate a high heterogeneity in the expression of glutamate receptors by the postsynaptic neurons. (Study supported by M.U.R.S.T. 40% and 60%, C.N.R.).

464.4

ANALYSIS OF EXCITATORY SYNAPTIC TRANSMISSION IN RAT OLFACTORY BULB SLICES.

W. Chen*, K. Kato and G.M. Shepherd. Section of Neurobiology and Neurosurgery, Yale University School of Medicine, New Haven, CT 06510

Most of our knowledge of synaptic transmission in olfactory bulb has been obtained from the in vitro turtle preparation. Here we extend the analysis by developing an in vitro slice preparation of rat olfactory bulb.

Slices were cut either horizontally or parasagitally from olfactory bulbs of 20-40 day old rats. Both whole-cell and sharp electrode intracellular recordings were obtained from cells in the mitral cell layer, some of which were identified as mitral cells by biocytin staining. Excitatory synaptic responses to ONL stimulation have been evoked in 22 of 31 recorded cells. These neurons generally responded with a long lasting depolarization (ranging from 200 ms to 5 s). Bath application of 20 µM bicuculline methiodide (n=3) had little effect on this depolarization, excluding the possible synaptic contribution of other bulbar neurons to this depolarization. The ONL-evoked EPSPs in mitral cells were completely blocked by 20 µM CNQX plus 100 µM APV (n=6), suggesting that excitatory amino acids are the main transmitter of rat olfactory axon terminals in the glomeruli, consistent with previous findings in the turtle (Berkowicz et al, 1994). LOT stimulation evoked antidromic impulses, accompanied in 9 cells by a depolarizing wave lasting up to 150 msec, resembling the self-excitatory depolarization described by Nicoll and Jahr (1982) in the turtle. Supported by grants from NIDCD and NIMH, NASA & NIDCD (Human Brain Project)

464.5

INTRINSIC FIRING PROPERTIES OF OLFACTORY BULB NEURONS IN AN IN VITRO BRAIN SLICE PREPARATION IN RAT.

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Understanding the dynamics of a neural network such as the mammalian olfactory bulb (OB) requires a precise knowledge of the intrinsic properties of its neuronal components. We have undertaken experiments to describe the intrinsic firing properties of OB neurons. Using *in vitro* brain slice techniques, we performed intra- and extracellular recordings of the neuronal spontaneous activity and response to electrical stimulation of the olfactory nerve (ON) of relay cells and interneurons. Recordings were also performed in the presence of blockers for most excitatory and inhibitory synapses known in the OB, in order to artificially disconnect the neurons from the rest of the network.

In this preparation OB neurons were found to have high levels of spontaneous activity. Based on the pattern of their spontaneous firing, neurons can be classified into three distinct categories: intermittent firing (IM), sustained regular (SR) and fast bursting (FB) neurons. The IM neurons are found in the mitral and external plexiform layers. These neurons fire every 6-10 sec for a duration of 2-3 sec at average firing rates of 5-50 Hz. They are often responsive to ON stimulation. Synaptic blockage induce a tremendous increase in the firing rate. The SR neurons are found in all OB layers except the glomerular layer (GL). They fire at constant rates in the range of 5 to 20 Hz. Finally, the FB neurons, found exclusively in the GL, fire bursts of 4-7 spikes (at 140-180 Hz) every 350-1000 ms. The FB neurons spontaneously synchronize their bursting activity resulting in deflections of the local field potential (LFP). Occasionally, the LFP acquires an oscillatory rhythm. These results may be important to validate realistic models of the OB neurons, and guide the steps toward building a network model of the OB.

464.6

EFFICACY OF DENDRODENDRITIC INTERACTIONS MODULATED BY VAGINOCERVICAL STIMULATION IN THE RAT OLFACTORY BULB.

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Vaginocervical stimulation at parturition plays a critical role in the induction of maternal behavior in rats, because mothers show maternal behavior immediately after parturition although they found pup odors aversive before parturition. We thus examined if vaginocervical stimulation (VCS) influences the dendrodendritic inhibition from granule cells to mitral cells in the rat olfactory bulb (OB). VCS was carried out using a rubber balloon which was inserted into the vagina and was inflated to increase intravaginal pressure to 100 mmHg for 2.5 min. The dendrodendritic inhibition does not change during VCS, but it is significantly enhanced immediately after the stimulation. Infusion of timolol (20mM, 1.0 µl), a β-blocker, into the OB blocked the VCS effect, but phentolamine (20 mM, 1.0 µl), an α-blocker, did not. These results suggest that the somatosensory stimulation to the vagina modulates the dendrodendritic inhibition from the granule to mitral cells in OB through β-receptors.

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464.7

INCREASED EXPRESSION OF THE BCL-2 PROTO-ONCOGENE IN THE DEVELOPING OLFACTORY BULB AFTER OLFACTORY DEPRIVATION.

J. Najbauer, E. Ibrahim and M. Leon*. Dept. of Psychobiology, University of California, Irvine, CA 92717-4550

The Bcl-2 gene is a key regulator of cell survival in the developing nervous system. The olfactory bulbs are a prominent site of Bcl-2 expression, with highest levels in the mitral and glomerular layers. We showed earlier that apoptosis accompanies the normal development of the olfactory bulb and that olfactory experience can modulate the number of cells that undergo apoptosis in the bulb. Here we determined whether olfactory deprivation can affect the levels of the Bcl-2 mRNA in the olfactory bulb. The external nares of rat pups were unilaterally occluded on postnatal day (PND) 2. Littermates with nonoccluded nares were used as controls. At PND 15, coronal sections from the bulbs were subjected to in situ hybridization assays for Bcl-2 mRNA. Naris closure resulted in overall increased expression of Bcl-2 in the occluded bulb when compared to the contralateral bulb ($p<0.05$, paired t-test, n=10 animals). The individual layers of the lateral portion of the occluded bulb showed increased expression of Bcl-2 (glomerular layer, $p<0.05$; external plexiform, $p<0.05$; mitral, $p<0.01$, granule, $p<0.05$), while in the medial portion, the external plexiform layer had increased levels of Bcl-2 ($p<0.001$). There were no significant differences detected in any of the bulb layers in the unoperated control group. These results indicate that olfactory experience can modulate the level of Bcl-2 expression in the olfactory bulb, and thus an increase in Bcl-2 may allow many cells to survive after olfactory deprivation. Funded by grant MH48950 from NIMH.

464.8

LOCUS COERULEUS LESIONS INCREASE DENSITY OF ß-ADRENERGIC RECEPTORS IN THE MAIN OLFACTORY BULB OF YOUNG RATS.

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Beta adrenergic receptors are located in nearly all bulb lamina, with a particularly high density of beta-1 and beta-2 adrenoceptors present in the glomerular layer. Early olfactory experiences that increase norepinephrine levels in the bulb decrease the density of both beta-1 and beta-2 adrenoceptors, as well as the number of high density beta-2 foci in the glomerular layer of the bulb. It therefore seems possible that changes in norepinephrine levels can affect the density of beta adrenoceptors in the bulb. To test this hypothesis, we performed unilateral lesions of the locus coeruleus on PND 4, and examined the density of beta-1 and beta-2 adrenergic receptors in the main olfactory bulb on PND 19 using [^{125}I]-iodopindolol receptor autoradiography. Beta-1 and beta-2 receptors were visualized using the specific receptor subtype antagonists ICI 118,551 and ICI 89,406, respectively. Locus coeruleus deafferentation resulted in a statistically significant increase in the density of beta adrenergic receptors in the ipsilateral bulb compared to the contralateral bulb. Both beta-1 and beta-2 adrenoceptor subtypes increased in density with locus coeruleus lesion, although the number of glomerular-layer, high density beta-2 foci was not significantly different between the two bulbs. Thus, changes in the level of norepinephrine in the olfactory bulb can regulate the density of beta adrenoceptors in the bulb.

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464.9

QUANTITATIVE 2-DIMENSIONAL MAPS OF GLOMERULAR 2-DEOXYGLUCOSE UPTAKE REVEAL DISTINCT RESPONSES TO CLOSELY RELATED ODORANTS. B.A. Johnson* and M. Leon. Department of Psychobiology, University of California, Irvine, CA 92717.

Recent receptor-based models of olfactory coding predict that each odor should evoke a unique pattern of glomerular activation in the bulb. Also, the overlap between patterns evoked by related odorants should be in proportion to their chemical similarities. In order to explore these and other questions regarding olfactory processing, we have mappped glomerular uptake of [^{14}C]2-deoxyglucose in rat pups exposed to odors of the major components of peppermint: L-menthol, L-menthone, and L-menthyl acetate (4 pups/odor). Quantitative 2-D maps of relative uptake were generated by taking discrete readings at 10° increments around autoradiographic images of coronal sections collected throughout the rostral-caudal extent of the bulb. After correcting for bulb size, the resultant arrays for left and right bulbs of each brain were averaged (~2500 data points/bulb). Different odors were compared by using statistical analyses of cell arrays. We found that each odor induces multiple areas of contiguous cells showing significantly increased uptake, compared to pups not exposed to an odorant. Coherent areas of decreased uptake also occur. When the minty odors are compared, 92-97% of the glomerular layer gives a similar response. Despite the widespread similarities, each odor produces areas of uptake that are significantly different from the other two odors. Menthol yields 2 unique areas of increased uptake; menthone gives 2 unique areas of increased uptake as well as 2 unique areas of decreased uptake; and menthyl acetate yields 1 unique area of increased and 1 of decreased uptake. These data are consistent with receptor-based, combinatorial models of odor coding. They also provide a means to relate chemical features of odorants to spatial patterns of olfactory bulb responses. Funded by grant HD24236 from NICHD.

464.10

SYNAPTOPHYSIN-LIKE IMMUNOREACTIVITY IN THE RAT OLFACTORY BULB AFTER RESTRICTED EARLY OLFACTORY EXPERIENCE. K. Ninomiya-Tsuboi, B.A. Johnson, C.C. Woo, J. Leon* and M. Leon, Dept. of Psychobiology, Univ. of California, Irvine, CA 92717

Synaptophysin is a synaptic vesicle protein that provides a marker of synapse distribution in the brain. Intensely stained particles initially are distributed evenly throughout olfactory bulb glomeruli, although beginning around PND 12, such particles tend to be located along the glomerular perimeter (Johnson et al. '93). To determine if this developmental pattern is affected by early olfactory experience, PND 19 bulbs were reacted with an antibody to synaptophysin after naris closure on PND 1. In spite of the negative impact of this unilateral olfactory restriction on laminar size, no difference was detected in synaptophysin immunoreactivity in any of the laminar measurements (glomeruli, external plexiform layer, internal plexiform layer or granule cell layer). Although the staining measured across entire glomeruli does not change with odor restriction, the distribution of immunoreactivity within individual glomeruli is affected by naris closure. Glomeruli of the deprived bulbs are stained homogeneously, while glomeruli of both contralateral and unoperated bulbs are stained more darkly along the perimeter than the glomerular core. Further study showed that the difference in staining between the olfactory nerve/glomerular border and glomerular core is significantly less in deprived bulbs than in either contralateral glomeruli or glomeruli from unoperated animals. These results indicate that early olfactory experience affects the developmental change in the distribution of synaptophysin immunoreactivity within glomeruli.
Funded by grant HD 24236 from NICHD.

464.11

SUBCOMPARTMENTAL ORGANIZATION OF THE RAT OLFACTORY BULB GLOMERULUS. Charles A. Greer*, Hanna Kim and Kenneth Chiu. Sections of Neurosurgery and Neurobiology, Yale University School of Medicine, New Haven, CT 06510.

Olfactory receptor cell (ORC) axons terminate in the glomerular neuropil of the olfactory bulb where they make synaptic connections with target dendrites of mitral, tufted and periglomerular neurons. We have investigated the organization of the glomerular neuropil using antibodies or the lipophilic dye DiI to label constituents for analyses using confocal microscopy. In parallel, we have used electron microscopy to assess the distribution of synaptic appositions within the glomerulus. Sprague-Dawley rats, 30-50 days postnatal, were perfused with 4% paraformaldehyde and processed for immunocytochemistry of olfactory marker protein (OMP) and MAP-2 or crystals of DiI were implanted into the olfactory nerve for tract tracing. Equivalent rats were perfused with 4% paraformaldehyde and 1% glutaraldehyde and processed for routine transmission electron microscopy. ORC axons stained with DiI occupied restricted regions within the glomeruli. OMP staining confirmed that observation. Double labeling for OMP and MAP-2 revealed a distinct interdigitation of axonal and dendritic processes within glomeruli. OMP staining, though present throughout the glomerulus, was strongest in the shell or outermost portion, an impression also gained from the DiI stained ORC axons. MAP-2 staining was less extensive and not apparent in the glomerular shell. Reconstructions of the area occupied by ORC axons versus dendrites from EM montages revealed continuous islands of axons within the glomerulus, accounting for 28.24% +/- 1.34 of the total area, with bundles of dendritic processes interspersed. The distribution of synapses within the glomerulus further suggested the segregation of axo- and dendrodendritic interactions. The results support the hypothesis of a subcompartmental organization of the olfactory bulb glomerular neuropil.
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464.12

Evidence for a stereotyped and highly organized spatial map of sensory input in the olfactory bulb.
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The odorant receptor gene family contains ~1000 members, each of which is expressed by only a small fraction of olfactory sensory neurons. Neurons that express the same odorant receptor gene are confined to one of four distinct odorant receptor expression zones in the olfactory epithelium. However, within that zone, those neurons appear to be randomly distributed, such that each zone is a mosaic of sensory neurons expressing different receptor types. Thus there may be an initial broad organization of sensory information in the nose into zones, but information within every zone is still highly distributed. We have found that odorant receptor mRNAs are present in the axons of olfactory sensory neurons. This has allowed us to examine the patterns of synapses formed in the olfactory bulb by neurons that express the same odorant receptor gene. Using in situ hybridization with specific odorant receptor probes, we find high concentrations of individual receptor mRNAs within small subsets of glomeruli in the olfactory bulb. Different odorant receptor probes hybridize to different glomeruli. Each odorant receptor probe that we have examined hybridizes to only 2-5 of the ~2000 glomeruli in each mouse olfactory bulb. The glomeruli are located at two distinct sites in each olfactory bulb, one lateral and the other medial. Furthermore, they appear to be located at approximately the same place in each olfactory bulb and in the bulbs of different animals. Our results suggest that in the olfactory bulb, sensory information that is highly distributed within a single zone in the nose is transformed into a highly organized and spatially stereotyped information map.

464.13

REGULATION OF c-FOS mRNA AND PROTEIN EXPRESSION IN OLFACTORY BULBS FROM UNILATERALLY ODOR-DEPRIVED, ADULT MICE. B.K.Jin*, L.Franzen and H.Baker. Cornell Univ. Med. Coll. at The Burke Med. Res. Inst., White Plains, NY 10605.

Odorant deprivation, produced by unilateral naris closure, profoundly reduces tyrosine hydroxylase (TH) expression within intrinsic olfactory bulb dopamine neurons. An AP-1 site, present in the TH gene, interacts with the product of the immediate early gene (IEG), c-fos, regulated by neuronal activity. To test the hypothesis that odorant stimulation and deprivation modify c-fos expression in TH neurons, adult CD-1 mice were subjected to unilateral naris closure. After two months, naris closed and control mice were exposed to either clean air for 60 min or clean air for 30 min followed by 30 min of alternating exposure to 10% isoamyl acetate (1 min) and air (4 min). TH mRNA and immunoreactivity decreased in the olfactory bulb ipsilateral to closure as previously observed. A parallel reduction occurred in c-fos mRNA and Fos-like immunoreactivity (FLI) in the glomerular as well as mitral and granule cell layers in olfactory bulbs ipsilateral to closure. Odor stimulation induced a short-lived increase in c-fos mRNA and FLI in olfactory bulbs contralateral to closure. The increase in Fos expression was region-specific in the glomerular layer but diffuse in mitral and granule cell layers. Interestingly, odor stimulation also induced strong c-fos expression in the mitral and granule cell layers and sparsely within limited periglomerular regions of olfactory bulbs ipsilateral to closure. These results suggest that c-fos expression, as in other systems, is an indicator of neuronal activity. Odor induced expression in mitral and granule cell layers may reflect increased centrifugal activity. The altered periglomerular cell Fos expression supports a role for this early gene in regulation of the TH gene. Supported by AG09686.

464.14

ALTERED PLASTICITY OF TYROSINE HYDROXYLASE EXPRESSION IN OLFACTORY BULB OF OLD MICE. [1]H. Baker*, [1]L. Franzen, [2]M. Grillo and [2]F.L. Margolis. [1]Cornell Univ. Med. Coll., Burke Med. Res. Inst., White Plains, NY 10605 and [2]Roche Inst. Mol. Biol., Nutley, NJ 07110.

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the catecholamine biosynthetic pathway. In contrast to other brain regions, we demonstrated that age-related losses in TH expression do not occur in periglomerular neurons intrinsic to the rodent olfactory bulb. We also showed that deafferentation produced in young mice by intranasal irrigation with Triton X-100 results in marked, but reversible, reductions in TH expression suggesting that the deficits are not associated with cell death. The current studies tested the hypothesis that the ability to re-express TH would be compromised in old animals. Both young (6 month) and old (20-21 month) C57BL/6JNIA mice were intranasally irrigated with either Triton X-100 in saline (100µl), to produce a reversible deafferentation, or with saline alone (control). TH expression was examined biochemically, immunocytochemically and by *in situ* hybridization. At both 10 and 30 days post-irrigation, TH expression was dramatically reduced (to about 30% of control levels) in mice of both ages. At 42 days post-treatment, TH activity recovered to 60% and 44% of control (saline) values in young and old mice, respectively. In replicate experiments at 60 days post-treatment, TH activity returned to 65% and 87% of control levels in young mice but to only 48% and 55% of control values in old mice. Olfactory marker protein levels, an indicator of reinnervation, showed similar reductions to 55% and 41% in young and old mice, respectively. These data suggest that plasticity of reinnervation and the consequent induction of TH expression is compromised in the olfactory system of old mice. Supported in part by AG09686.

464.15

DECREASED NGF AND INCREASED NGF-RECEPTOR LEVELS IN OLFACTORY BULBS OF DEVELOPING HYPOTHYROID RATS AND REVERSAL OF THIS RESPONSE BY RECOVERY. T. J. Sendera* and E. Meisami (Dept. of Physiology & Biophysics, Univ. of Illinois, Urbana, IL 61801).

Effects of early thyroid deficiency [propylthiouracil (PTU) in litter's drinking water (1g/L)] and recovery from this condition on NGF levels and expression of low-affinity-NGF-receptor (p^{75NGFR}=NGF-R) in rat olfactory bulbs (OB) were studied using an ELISA assay for NGF and immunocytochemistry for NGF-R. We have shown previously that neonatal hypothyroidism results in up to 70% deficit in total number of olfactory receptor neurons (ORNs) and a 24% loss of mitral cells. Withdrawal of PTU results in complete recovery of ORN number and OB volume. In 25- & 50-day-old hypothyroid rats, NGF levels per OB were reduced by >50%. Withdrawal of PTU doubled NGF levels in hypothyroid OBs- although recovery was incomplete by day 50. In normal OB, NGF-R expression was moderate, patchy and limited to glomeruli. NGF-R response to hypothyroidism was opposite to NGF, i.e., widespread and markedly elevated in OB glomeruli of hypothyroid rats. Upon recovery, expression returned to low, i.e., normal levels. Results suggest that thyroid hormones regulate NGF and NGF-R levels in OB: in hypothyroidism, NGF is reduced while NGF-R is up-regulated. Thyroidal normalization restores NGF and NGF-R levels. Changes in NGF and NGF–R may also follow altered proliferation and maturation of ORNs (Paternostro & Meisami, *Dev. Brain Res*, 76:151–161,'93; *ibid* 83:151-162,'94).
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464.16

IN VIVO VOLUMETRIC QUANTIFICATION OF NEURAL DEGENERATION IN RAT OLFACTORY BULB USING MAGNETIC RESONANCE IMAGING (MRI) . Saied Aghai, Esmail Meisami*, and Paul C. Lauterbur (Dept. Physiol & Biophys. & Biomedical Magnetic Resonance Lab., Univ. Illinois, Urbana, IL 61801)

We used MRI to delineate laminar organization of olfactory bulb (OB) and convolutions of olfactory conchae and to see whether atrophic responses of OB to olfactory nerve degeneration in developing rats can be visualized *in vivo*. 1% zinc sulfate was irrigated intranasally in neonatal rats resulting in destruction of olfactory epithelium. Rats were anesthetized with ketamine, xylazine and acepromazine cocktail and placed in especially constructed plastic cylindrical chambers wound with an Alderman-Grant rf coil 6.3 cm in diameter. Using a 4.7T/33 cm bore magnet and a SISCO imaging spectrometer, OB and nasal cavities were imaged using moderately T_2 weighed images. Imaging data sets were processed and displayed using the NCSA supercomputers and*Viewit* visualization software. MRI images of olfactory cavities revealed highly intricate details of conchae from coronal 0.3 mm thick images. Images of normal OB revealed 4 zones corresponding to olfactory nerve-glomerular, external plexiform, internal granular and ventricular-ependymal layers. Thickness of these layers from MRI images could be measured. Denervation studies indicated a reduction in size and volume of OB, accompanied with about 20-30% reductions in thickness of superficial OB layers. Thus MRI imaging allows us to visualize *in vivo* not only the laminar details of OB but also to quantify the effects of degeneration of olfactory nerves in the developing OB.*Supp: NSF DIR 89-20133COOP, NIH PHS 5 P41 RR05964, Servants United Foundation, UIUC Research Funds.*

464.17

THE OLFACTORY SYSTEM IN GANGLIOSIDE STORAGE DISEASE CATS. E. E. Morrison* and J. C. Dennis. Department of Anatomy and Histology, College of Veterinary Medicine, Auburn University, AL 36849-5518.

We examined the olfactory epithelium and bulb in cats carrying the neuronal storage disease GM1 gangliosidosis in cats. Gangliosides are glycosphingolipids located mainly on the outer lipid bilayer of the neuroplemma and are thought to be involved in differentiation and synaptogenesis. Gangliosidosis is a metabolic disorder affecting normal mechanisms of surface membrane production and regulation and can result in the appearance of aberrant neuronal plasticity, e.g. meganeurites. Ten matched pairs of control and mutant cats (provided by Dr. H. Baker) were euthanized and the nasal cavity and olfactory bulb immersion fixed for light and electron microscopic examination. The olfactory neuroepithelium appeared normal by LM inspection. Slides immunostained for olfactory marker protein, (OMP) a characteristic biochemical marker for mature olfactory neurons, showed mutant ORN's capable of expressing OMP. TEM examination showed ORN's cell bodies to contain large numbers of storage vesicles. Olfactory bulb mitral and tufted cells appeared abnormal by LM. Cell bodies were swollen and contained vacuoles. At the TEM level, the most conspicous feature was the large number of storage vesicles within the cell body and dendritic processes. Where Golgi impregnation was successful, mitral and tufted cells showed no apparent development of meganneurite. These results indicate that the olfactory system of animals with gangliosidosis show characteristic storage disease pathology but not aberrant neuronal plasticity. Olfactory receptor neurons show cellular pathology but still express the biochemical marker olfactory marker protein.
Supported by grant #DC01532 (EEM) and Scott-Ritchey Research Center, Auburn University.

OLFACTORY SENSES: OLFACTORY CORTEX

465.1

INHIBITION OF OLFACTORY BULB MITRAL CELLS BY STIMULATION OF PIRIFORM CORTEX ASSOCIATION FIBERS IS ENHANCED FOLLOWING LTP. J. S. Stripling*, J. L. Cauthron, and M. P. Galupo. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Stimulation of association fibers in the piriform cortex (PC) evokes a late negative wave in the granule cell layer of the olfactory bulb (OB) which appears to represent synaptic excitation of granule cells by PC association fibers. We have previously demonstrated that this negative wave undergoes long-term potentiation (LTP) following high-frequency stimulation of PC association fibers, and have proposed that this potentiation is due to LTP at synapses between cortical association fibers and OB granule cells. Because granule cells inhibit mitral cells, it follows that mitral cells should be inhibited by stimulation of PC association fibers, and that this inhibition should be enhanced following LTP. The present study was designed to test this hypothesis. LTP was induced in male Long-Evans rats by daily high-frequency stimulation of PC association fibers. Extracellular recordings of individual mitral cells were then made under urethane anesthesia (1.4 g/kg). Inhibition of mitral cell firing by stimulation of the lateral olfactory tract (LOT) and the PC association fiber system was monitored before and after LTP was reinstated by high-frequency stimulation of PC association fibers. Prior to reinstatement of LTP, stimulation of either the LOT or the PC association fiber system produced a brief inhibition of mitral cell firing. Following reinstatement of LTP, mitral cell inhibition by PC association fiber stimulation was increased in duration (typically two-fold or more), while inhibition by LOT stimulation was unaffected. This supports the conclusion that PC pyramidal cells exert substantial inhibitory control over mitral cells, and that the strength of this control can be altered by LTP at pyramidal cell synapses on granule cells. These observations emphasize the potential importance of feed-back inhibition from the PC to the OB, and its modification by experience, in olfactory processing. Supported by NIH grant DC02271 and the Marie Wilson Howells Fund.

465.2

ALL-OR-NONE THRESHOLD FOR EXPRESSION OF LTP IN THE OLFACTORY BULB AND PIRIFORM CORTEX: MODULATION BY BEHAVIORAL STATE. M. P. Galupo* and J. S. Stripling. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Previous research in this laboratory has documented the long-term potentiation (LTP) of late components in potentials evoked in the olfactory bulb (OB) and piriform cortex (PC) by stimulation of PC association fibers. The present research investigated the expression of this potentiation at near-threshold current intensities. LTP was induced in male Long-Evans rats by daily high-frequency stimulation (thirty 10-pulse trains at 100 Hz) of PC association fibers. Input/output testing was conducted both before and after daily high-frequency stimulation to determine the stimulation threshold for expression of the potentiated component of the evoked potential. In addition, behavioral effects on threshold were explored by conducting separate input/output tests during slow-wave sleep, awake immobility, and locomotion. Results indicate that the expression of LTP has a sharp current threshold, and that LTP expression occurs in an all-or-none fashion with near-threshold stimulation. Threshold is reduced following daily high-frequency stimulation and drops progressively across days as LTP develops. In addition, threshold is dependent upon behavioral state: it is lowest during slow-wave sleep, highest during locomotion, and has an intermediate value during awake immobility. These results demonstrate that the expression of this form of LTP in the OB and PC is powerfully modulated by behavioral state. Furthermore, the characteristics of LTP expression (all-or-none expression at threshold with a long latency) suggest that LTP expression is dependent upon reverberating positive feedback within a synaptically activated network.

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465.3

IS NOREPINEPHRINE DEPENDENT ON GABA IN ATTENUATING PYRAMIDAL POSTSYNAPTIC POTENTIALS IN THE PIRIFORM CORTEX?
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It has been shown that norepinephrine (NE) decreases the amplitude of postsynaptic potentials (PSPs) of pyramidal cells located in layer III of the rat piriform cortex. The focus of this study was to ascertain whether this decrease is mediated by NE acting directly on the pyramidal cells, or by NE acting indirectly through GABAergic interneurons. Coronal brain slices 400 microns thick were taken from male adult rats (n=3), and perfused with ACSF. Stimulating and recording electrodes were placed approximately 1mm apart in layer III. Stimulation was applied for 100 μsec at a constant current necessary to elicit a half-maximal PSP response. After recording a stable baseline for one hour, NE (31μM) was applied to the slice for 30 minutes and then washed out. NE caused a significant decrease in the amplitude of the PSP. Approximately an hour after restoration of baseline values, the $GABA_A$ antagonist bicucullin (30μM) was added to the ACSF medium, causing a dramatic increase in the amplitude of the PSP. An hour after recording a new baseline, NE (31μM) was reapplied, again decreasing the amplitude of the PSP. After 30 minutes the NE was washed out, returning the PSP amplitude to the bicucullin baseline level. The PSP response to NE in the presence of bicucullin suggests that NE's effect is not solely mediated by GABAergic interneurons. However, the response to NE in the presence of bicucullin was 60$\pm$3% smaller than that observed in the absence of bicucullin, suggesting that the GABAergic interneurons do play a role in the ability of NE to attenuate pyramidal PSPs in the piriform cortex. Supported by USDA NRI 94-37200-0655.

465.4

GABAergic NEURONS IN THE MOLECULAR LAYER OF PIRIFORM (OLFACTORY) CORTEX HAVE LAMINA-SPECIFIC AXONAL ARBORS.
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Previous studies in the piriform cortex have provided evidence that, as in the hippocampus, different forms of inhibition are concentrated in dendritic and somatic regions of pyramidal cells. $GABA_B$-mediated inhibition and the newly-distinguished slow $GABA_A$-mediated IPSC ($GABA_{A,slow}$; R Pearce, Neuron 10:189) are concentrated in apical dendrites, whereas cell bodies receive a higher proportion of $GABA_{A,fast}$ (A Kapur et al, Soc Neurosci Abs 19:1521). Physiological evidence for laminar specificity in axonal arbors (A Kapur et al, Soc Neurosci Abs 19:1521) suggests that these different inhibitory processes are mediated, at least in part, by different interneurons. The present study is the first step characterizing the populations of neurons that mediate different forms of inhibition in the piriform cortex. Goals are to understand the roles of inhibition in the regulation of NMDA-dependent synaptic plasticity (ED Kanter and LB Haberly, J Neurosci 13:2477; Kapur et al 20:652), integrative processes, and the prevention of epileptic bursting.

Cells were studied in layer I (molecular layer) where immunocytochemical studies have shown that most or all neurons are GABAergic (Haberly et al, JCN 26:269). Recordings were made with whole-cell patch pipettes placed under direct vision on cell bodies in slices from 1-3 week old rats. Cells were stained by diffusion of neurobiotin from the patch solution. Afferent and association fibers were stimulated with microelectrodes. This stimulation evoked prominent EPSPs, but weak IPSPs from all cells. Dendrites from all cells were varicose and sparsely spiny, but varied in form from vertically oriented arbors extending over the full depth of layer I to predominantly horizontal arbors that were restricted in laminar extent. Three distinctive axon branching patterns have been distinguished thus far: one that arborizes extensively in layer Ia (depth of afferent inputs to distal apical dendrites), one with extensive branching in the deep part of layer Ib (depth of association fiber inputs onto proximal apical dendrites), and one with axons that descend and arborize in layer II at the depth of pyramidal cell somata. The cells with arbors in deep Ib were distinctive physiologically by virtue of a rapid adaptation that limited depolarization-induced discharge to a single spike. Supported by NS19865 from NINDS.

465.5

THE EFFECTS OF SYNAPTIC TIMING IN A REALISTIC MODEL OF A LAYER II PIRIFORM PYRAMIDAL CELL.
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We constructed a compartmental model of a layer II pyramidal cell from piriform cortex based on the morphology of a stained cell from rat and using only membrane and synaptic currents known or suggested to exist in this cell type. Whole-cell recordings were made on a population of pyramidal cells in order to obtain experimental constraints for the passive properties of the neuron. Passive and active behavior (in the form of F/I curves) were used to match the model to experimental data.

In these simulations we were interested in exploring the consequences of different spatial and temporal synaptic patterns on the output of this type of neuron. Different synaptic pathways are known to terminate in different layers of the piriform cortex (afferent input in layer Ia; associative excitatory input in layer Ib; feedback inhibition in layer II). This was modeled by distributing synaptic inputs along the apical dendritic tree according to these data.

We varied the timing of synaptic input in layer Ib with respect to input arriving in layer Ia and found that EPSPs summed linearly when membrane resistance was at values based on passive *in vitro* experiments; however, when membrane resistance was lowered to simulate background synaptic activity, EPSPs did not summate linearly, and the peak of the summed EPSP shifted in time. Shifting the timing of inhibition in layer II with respect to EPSPs arriving in layer Ia, we found that as much as 40% of the inhibition was due to shunting even at depolarized resting potentials (-55mV). Peaks of summed responses were relatively stationary.

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465.6

INFORMATION PROCESSING IN THE RAT PIRIFORM CORTEX: INFLUENCE OF EXPERIENCE ON THE DISTRIBUTION OF ACTIVITY.
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The piriform cortex (PC) is a brain area which has a potential role in olfactory learning. The current view of the neural basis of learning suggests that learning is coded by changes in neural network. Thus, we used the optical recording method which enables one to record neural activity simultaneously over the entire PC. Therefore, the effects of experience could be assessed on the distribution of activity optimizing the possibility of characterizing local changes in the neural circuitry. Rats were given olfactory experience via electrical stimulation of the olfactory bulb which has a massive projection to the PC. Moreover, this technique provides precise control over odor stimulation during both experience and testing.

Four electrodes were implanted in the olfactory bulb which were used to mimic odor stimulation. In a daily training paradigm, rats were trained to discriminate between spatially distinct electrical stimulation delivered to one olfactory bulb. Control rats received olfactory bulb electrical stimulation without discrimination training. The discrimination was acquired within 5-7 days. Following acquisition, piriform cortex responses to olfactory bulb electrical stimulation were mapped using optical recording with a voltage-sensitive dye. Each of the four olfactory bulb electrodes were individually electrically stimulated (single shock, 200 µs, 0.05-1 mA), and the whole PC activity mapped using two recordings along the antero-posterior axis. The data indicate that the response of the PC to olfactory bulb stimulation was altered by olfactory experience. Specifically, a late wave component of the PC response to electrical stimulation of the olfactory bulb seems to be more frequent in rats that had received the olfactory experience. Further analysis of the data will determine whether this effect is localized to a specific area of the PC.

465.7

A NEW ANATOMICALLY AND PHYSIOLOGICALLY DISTINCT SUBDIVISION OF THE PIRIFORM (OLFACTORY) CORTEX.
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Current source-density analysis of 40-60 Hz oscillations in piriform cortex (PC) has revealed that each cycle contains a monosynaptic afferent-evoked EPSC followed by a disynaptic EPSC (KL Ketchum and LB Haberly, J Neurosci 13:3980). These 2 EPSCs are generated in adjacent distal and middle segments, respectively, of pyramidal cell apical dendrites at a fixed temporal relationship in all parts of the cortex. The demonstration of "associative LTP" between these 2 sets of synapses (ED Kanter and LB Haberly. J Neurosci 13:2477) suggests that the spatial and temporal juxtaposition during fast oscillations may play a role in learning-related adjustment of synaptic efficacies. Based on modeling analysis of propagation patterns it has been postulated that the disynaptic EPSC is mediated by cells in the ventral part of the anterior piriform cortex (APC) (KL Ketchum and LB Haberly, J Neurophysiol 69:261).

This hypothesis was tested by comparing projection patterns of pyramidal cells in dorsal and ventral parts of the APC using small extracellular injections of the anterogradely transported tracer, PHA-L. The results showed that a ventral region, consisting of a narrow strip of cortex deep to the medial half of the lateral olfactory tract (LOT) projects exclusively to a thin zone at the superficial margin of layer Ib (depth of mid-apical dendrites) where the disynaptic component is generated in oscillatory responses. From single small injections the projection is heavy throughout the full extent of the PC and all other olfactory areas. By contrast, the projection from the remainder of the APC is similar to that from the posterior PC (M Luskin and JL Price, J Comp Neur 216:292): it is concentrated in the deep part of layer Ib and in layer III of the posterior PC, with a much weaker projection to rostral regions of the PC and other olfactory areas. Physiological study of pyramidal cells deep to the LOT revealed additional distinctive features of this region: activation by afferent fibers occurs at lower thresholds and at shorter latencies with a greater degree of synchrony than in other parts of the PC (KL Ketchum, A Kapur and LB Haberly, unpublished). Based on the occurrence of the disynaptic EPSC at fixed latency relative to the afferent input in low amplitude oscillations, these features support the conclusion from the anatomical findings that this EPSC is mediated by the newly-distinguished ventral strip in the APC. Supported by NS19865 from NINDS.

465.8

REAFFERENCE AND RHINENCEPHALIC MARKERS DURING OLFACTORY PERCEPTION IN RATS.
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Reafference refers to the ability of a sensory system to expect input and carry out action based on the difference between expected and actual input. Simultaneous bipolar field potential recordings from the olfactory bulb (OB), prepiriform cortex (PPC), entorhinal cortex (EC), and dentate gyrus (DG) in rats during performance of an olfactory recognition operant task suggest a generalized model of reafference in olfactory perception: 1) "afference": a primary burst in the gamma band (40-120 Hz) is passed from the OB to PPC and to the limbic areas, the EC and DG (Kay and Freeman, Society for Neuroscience Abstracts, 1994); 2) "preafference" (similar to "efference copy"): before odor recognition a signal in the beta band (15-35 Hz) is passed from the EC to the OB; 3) "post-afference" (similar to "reafference"): after a primary burst in the OB during odor recognition a secondary nearly periodic burst is originated in the PPC at 3/4 to 5/6 the dominant frequency of the OB oscillation and is passed back to the bulb and inwardly to the limbic areas. This burst is correlated with the behavioral motor response (bar press). The afferent burst rides the crest of the low frequency oscillation (3-12 Hz) correlated with the inhalation phase of the respiratory cycle in the OB, while the post-afferent burst, which is often 3-4 times the amplitude of the afferent burst, occurs in the troughs of the respiratory wave. (Supported by NIMH MH06686 and ONR N00014-93-1-0938 and N00014-93-1-1951.)

466.1

CALRETININ GENE EXPRESSION IN THE HUMAN THALAMUS. A. Parent*, F. Cicchetti and P. V. Gould, Neurobiology Res. Ctr. and Dept. of Pathol, Enfant-Jésus Hospital, Québec, Canada, G1J 1Z4.

Previous studies from this laboratory have provided evidence for the existence of calretinin (CR) in human basal ganglia. In order to study the localization and levels of expression of this calcium-binding protein in the human thalamus, we undertook an *in situ* hybridization study on formalin-fixed postmortem material from normal individuals. The brain slabs that were used contained a large portion of the diencephalon and parts of the basal ganglia. The slabs were cut with a freezing microtome into 50 µm-thick sections and processed for X-ray film radioautography and emulsion radioautography. The presence and levels of CR mRNA were detected with an antisense ^{35}S-labeled riboprobe synthesized by in vitro transcription of cDNA clones encoding for human CR. A sense riboprobe served as negative control. As visualized on X-ray film radioautographs, high concentrations of CR gene transcripts occurred in several thalamic nuclei, including the reticular nucleus, the centromedial nucleus, the paracentral nucleus, and several midline nuclei, as well as a number of hypothalamic nuclei. In the reticular thalamic nucleus, neurons expressing CR mRNA were few in number and formed typical clusters scattered throughout the rostrocaudal extent of the nucleus. Observations of emulsion radioautographs revealed that each neuron in these reticular nucleus clusters was heavily labeled. In contrast to the reticular nucleus, virtually all neurons in the intralaminar and midline nuclei expressed high levels of CR mRNA. When examined on X-ray films, these heavily labeled neurons formed a very prominent rim around the poorly labeled mediodorsal nucleus. This zone of intense labeling was continuous ventrally with the hypothalamus, whose neurons also contain high concentrations of CR gene transcripts. These results reveal that CR mRNA is expressed by specific thalamic nuclei in humans, particularly those nuclei projecting heavily to the basal ganglia, and that the presence of CR gene transcripts can easily be detected on formalin-fixed sections of the human brain.

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466.2

NEURONS CONTAINING CALRETININ AND EXPRESSING SUBSTANCE P (NK1) RECEPTORS IN THE STRIATUM OF NORMAL AND HUNTINGTON'S DISEASE PATIENTS. F. Cicchetti*, P. V. Gould and A. Parent, Neurobiology Res. Ctr. and Dept. of Pathol., Enfant-Jésus Hospital, Québec, Canada, G1J 1Z4.

Single immunohistochemical studies of the striatum in normal individuals have revealed the presence of both large and medium aspiny neurons enriched with the calcium-binding protein calretinin (CR). The medium CR neurons outnumbered the large CR neurons throughout the striatum, but the former were more abundant in the caudate nucleus whereas the latter predominated in the putamen. In addition, medium and large aspiny neurons displaying immunoreactivity for substance P (NK1) receptors were also detected in the human striatum. In contrast to CR neurons, however, the proportion of the two types of SPR neurons was about the same in the putamen and in the caudate nucleus. Double-immunostaining experiments have also shown that a significant proportion of the large CR neurons and a small contingent of medium CR neurons displayed SPR immunoreactivity. Furthermore, somatostatin was found to be colocalized in a subset of medium SPR neurons. Thus, the medium SPR neurons appear to correspond, at least in part, to the well characterized somatostatinergic striatal interneurons. On the basis of their morphological features and patterns of distribution, the large SPR and CR neurons are believed to represent subsets of the large cholinergic striatal interneurons. These normative data have allowed us to examine the status of the chemospecific striatal neurons in various neurodegenerative diseases. In the present study, analysis of the striatum in advance cases of Huntington's chorea have revealed that both CR and SPR immunoreactivities were unaltered, despite the marked atrophy of both caudate nucleus and putamen that characterizes this neurodegenerative disease. These findings provide the first evidence for the existence of CR and SPR neurons in the human striatum and indicate that these chemospecific neurons are selectively spared in Huntington's disease.

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466.3

CALRETININ IN THE PRIMATE THALAMUS IS MAINLY LOCATED IN LIMBIC-ASSOCIATED TERRITORIES. M.-C. Asselin*, M. Fortin and A. Parent, Centre de recherche en Neurobiologie, Fac. of Med., Univ. Laval, Québec (Qc.), Canada, G1K 7P4.

A comparative immunohistochemical study of the distribution of the calcium-binding protein calretinin (CR) was undertaken in the thalamus of squirrel monkeys (*Saimiri sciureus*) and humans. In the anterior division of the thalamus, a moderate to dense immunostaining is observed in most of the nuclei, represented by the anterior ventral (AV), anterior dorsal (AD) and anterior medial (AM) nuclei. Exceptions reside in the human AD, where CR fibers are more densely stained and in the absence of a human AM nucleus. CR immunoreactivity (CR-IR) in the mediodorsal nucleus (MD) has an arrangement individual to human and non human primates. The human MD displays a weakly stained CR neuropil, and a few densely immunoreactive cell bodies concentrated in central and ventral regions of the nucleus rostrocaudally. In monkeys, moderately stained cells and fibers are concentrated in the central part of the MD where they form a patchy arrangement. In more caudal regions, these patches of immunoreactive perikarya and fibers extend to more lateral and medial areas. The CR cells found in the monkey MD are of small size and their distribution seem to be confined to the parvicellular division, the other parts of the MD being totally devoid of immunoreactive cell bodies. In both humans and monkeys, a much weaker CR-IR is seen in the ventral and posterior nuclear groups, as well as the centre médian of the paralaminar division. Features observed in both species include a very dense and uniform immunoreactivity in the midline nuclear group and its peripheral subdivisions, especially the anterior ventral and some members of the lateral group (Pt, CSL). Other common characteristics include heterogeneous distributions in the paracentral, central lateral with very few labeled cell bodies compared to the whole population. The parafascicular nucleus harbor a group of CR cells confined to its medial portion in both monkeys and humans. The main pattern of CR-IR displayed in both human and monkey seems to be closely related to the thalamic divisions that receive major inputs from CR-positive neurons in limbic structures such as the amygdala and the ventral striatum. The present data reveal that CR is a useful chemical marker for parts of the thalamus related to limbic function. [Supported by FRSQ and MRC].

466.4

IN VIVO, IN VITRO AND COMPUTATIONAL ANALYSIS OF DENDRITIC CURRENTS IN THALAMIC RETICULAR NEURONS.

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Thalamic reticular (RE) neurons are involved in the genesis of synchronized thalamocortical oscillations, which depend in part on their complex bursting properties. We have investigated the intrinsic bursting properties of RE cells using computational models based on morphological and electrophysiological data. Simulations of a reconstructed RE cell were compared directly to the recordings from the same cell, which allowed precise values for the passive parameters to be obtained. Voltage-clamp data were obtained on the low-threshold calcium current (I_{T_s}) in acutely dissociated RE cells which lack most of their dendrites. Simulations based on a cell with truncated dendrites and Hodgkin-Huxley kinetics reproduced these recordings with a relatively low density of I_{T_s}. In intact RE cells, high densities of I_{T_s} in distal dendrites were required to generate the higher amplitudes of the current seen experimentally, as well as the typical properties of the burst of RE cells. More importantly, we found that, because of dendritic I_{T_s}, synaptic bombardment in the dendrites had a strong impact on the genesis of the burst, and could generate the typical burst responses of RE cells *in vivo*. These findings suggest that synaptic integration on the dendrites of RE cells is significantly influenced by dendritic calcium currents. In addition, we were able to simulate the same behavior in simpler models with as few as three compartments, provided there was a high density of I_{T_s} in the dendrites. These experiments provide firm evidence for resolving the difference observed in the intrinsic bursting properties of RE cells *in vivo* and *in vitro*, on the basis of dendritic calcium currents.

466.5

IONIC MECHANISMS UNDERLYING SYNCHRONIZED OSCILLATIONS AND TRAVELING WAVES IN A MODEL OF FERRET THALAMIC SLICES

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A network model of thalamocortical (TC) and thalamic reticular (RE) neurons was developed based on electrophysiological measurements in ferret thalamic slices. Single-compartment TC and RE cells included voltage- and calcium-sensitive currents described by Hodgkin-Huxley type of kinetics. Synaptic currents were modeled by kinetic models of AMPA, $GABA_A$ and $GABA_B$ receptors. The model reproduced successfully the characteristics of spindle and slow bicuculline-induced (SBI) oscillations. The most critical parameters were: (a) the mutual recruitment between TC and RE cells, due to their reciprocal connectivity. The time course of GABAergic IPSPs from RE to TC determined the frequency of oscillations (8-12 Hz spindle or 2-4 Hz SBI). (b) Cooperativity in the activation of $GABA_B$ responses, which are absent during spindles. If the intra-RE $GABA_A$ IPSPs were suppressed, the stronger discharges of RE cells enhanced $GABA_B$ IPSPs in TC cells, recruiting TC and RE neurons in the slower highly synchronized SBI oscillations. (c) A TC origin for the "waning" of these oscillations, through the upregulation of I_h by intracellular calcium, leading to a refractory period in these cells. (d) Local axonal arborization of the TC-RE and RE-TC projections, which allowed traveling phenomena to occur. The model predicts that intrinsic properties of TC and RE neurons and their reciprocal connectivity establish a highly excitable structure; stimulation at any location can induce a oscillatory travelling wave through the reciprocal recruitment of TC and RE cells. The presence of a refractory period in TC cells accounts for the waxing and waning properties of the traveling waves, collisions between waves and the slower propagation of highly synchronized SBI oscillations.

466.6

SYNCHRONIZATION OF THALAMIC SPINDLE OSCILLATIONS IS ENHANCED BY CORTICAL FEEDBACK INPUT. D. Contreras*, A. Destexhe†, T. Sejnowski† and M. Steriade, Lab. of Neurophysiol., School of Med., Laval University, Quebec, CANADA, G1K 7P4. †The Salk Institute, Computational Neurobiology Laboratory, La Jolla, CA 92186, USA.

Synchronization of spindle waves was studied in intact-cortex and decorticated cats under barbiturate anesthesia. Field potentials were recorded from the cortical suprasylvian gyrus (areas 5 and 7) with an array of 8 equidistant bipolar electrodes (surface-depth), with an interelectrode separation of 1 mm. Sequences of spindles at 7-12 Hz recurred every 5 to 10 seconds and they were simultaneous in all cortical leads. Interruption of corticocortical connections by a cut between recorded foci, in the middle suprasylvian gyrus, did not affect intracortical synchronization. In intact-cortex animals, recordings from the thalamus, with the same array of bipolar electrodes (1 mm apart), revealed that most spindle sequences occur simultaneously over a distance of about 7 mm. Massive unilateral decortication decreased intrathalamic synchrony; however, many spindles still occurred simultaneously among the 8 electrodes. In decorticated animals, thalamic recordings with an array of 8 equidistant tungsten microelectrodes, separated by smaller interelectrode distances (0.4 mm), revealed that, when spindling propagated sequentially, thalamic reticular neurons recorded from the rostral pole consistently discharged typical spike-bursts, with acceleration-deceleration patterns, before thalamocortical neurons recorded from more posterior dorsal thalamic nuclei. These results demonstrate the important role of the cortex in enhancing the intrathalamic synchrony within the frequency range of spindles and emphasize the role of rostral reticular neurons in initiating and synchronizing spindling in decorticated animals.

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466.7

CHANGES OF INPUT RESISTANCE AND SYNAPTIC EXCITABILITY OF CORTICAL AND THALAMIC NEURONS DURING VARIOUS COMPONENTS OF THE SLOW OSCILLATION IN CAT. L Timofeev*, D. Contreras and M. Steriade, Lab. of Neurophysiol., Univ. Laval, Sch. Med., Quebec, Canada G1K 7P4.

A slow oscillation characterizes the activity of cortical and thalamic network during anesthesia and natural slow-wave sleep. Typically, long-lasting (0.2 to 0.8 s) positive field potentials recorded from the cortical depth, recurring at a frequency below 1 Hz, are associated with prolonged hyperpolarization in cortical, thalamic reticular (RE) and thalamocortical (TC) cells. These hyperpolarizations are followed by a depolarizing phase associated with negative field potentials in the cortical depth. To shed light on the nature of cellular phenomena underlying the slow oscillation, intracellular recordings from cortical, RE and TC cells were performed in vivo in cats under ketamine & xylazine anesthesia. Hyperpolarizing current pulses showed that the hyperpolarizing phase of the spontaneous oscillation is accompanied by an increase in input resistance of 15-20% in all three types of cells. During the following depolarizing phase, input resistance decreased by 50 %. Testing synaptic excitability with stimuli applied to prethalamic paths revealed that during the hyperpolarizing phase of the oscillation, bisynaptic EPSPs in RE and cortical cells were virtually abolished. During the same phase, TC cells did not display a decrease in the prethalamic-evoked monosynaptic EPSP, but the hyperpolarization abolished the firing probability, so that the signal transmission through the dorsal thalamus was obliterated. During the depolarizing phases, the responses to prethalamic volleys were overwhelmed by the spontaneous synaptic activity. By contrast, the responsiveness to cortical stimuli was not altered during the slow oscillation. These results indicate that the messages from the periphery are blocked at the level of TC cells during slow oscillatory states, but also show that the responses to corticocortical and corticothalamic signals remain unaffected.

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466.8

COMPARISON OF INPUTS FROM THE ENTOPEDUNCULAR NUCLEUS AND THE CEREBELLUM TO VA-VL NEURONS IN THE CAT THALAMUS. T. Futami*, Y. Izawa[1], Y. Shinoda[1] and E.G. Jones[2]. Frontier Research Program, The Institute of Physical and Chemical Research (RIKEN), Wako, 351-01, Japan, [1] Dept. of Physiology, School of Med., Tokyo Medical and Dental Univ. Tokyo, 113, Japan and [2] Dept. of Anatomy and Neurobiology, Univ. of California Irvine, CA 92717

Synaptic inputs from the entopeduncular nucleus (ENT) to thalamocortical neurons (TCNs) in the ventroanterior-ventrolateral complex (VA-VL) of the thalamus were characterized in anesthetized cats. Effects of stimulation of the ENT and the brachium conjunctivum (BC) were examined by recording intracellular potentials from VA-VL neurons. TCNs were identified by their antidromic responses to stimulation of the pericruciate cortex.

Stimulation of the ENT evoked monosynaptic IPSPs in TCNs, while stimulation of the BC evoked monosynaptic EPSPs followed by disynaptic IPSPs. Only IPSPs were evoked in virtually all TCNs receiving ENT input. Mapping experiments revealed that TCNs in the rostrodorsal part of the VA-VL predominantly received input from the ENT, while TCNs in the more caudoventral part of the VA-VL received input from the BC. Less than 10% of the TCNs examined received convergent inputs from both the ENT and BC and all were located at the border between ENT- and BC-receiving areas of the VA-VL. TCNs receiving ENT input projected to area 6, while TCNs receiving BC input projected to areas 4 and 6.

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466.9

ORGANIZATION OF TERMINAL FIELDS OF SINGLE LABELED CEREBELLAR AXONS IN THE VENTROLATERAL NUCLEUS OF THE MONKEY THALAMUS. A. Mason, K. Kultas-Ilinsky and I.A. Ilinsky*. Dept. of Anatomy, Univ. of Iowa Sch. Med., Iowa City, IA 52242.

The projection zone topography and ultrastructural features of cerebellothalamic terminals in nonhuman primates have been extensively studied earlier. However, the extent and geometry of the terminal fields of individual cerebellothalamic axons and their relationships to different groups of neurons in the ventrolateral nucleus (VL) are not known. To study these aspects small pressure injections of biotinylated dextran amine were centered on the dentate nucleus of the cerebellum, and after 4-5 weeks of survival time the tissue was processed with DAB for visualization of the tracer. The labeled fibers were traced and their relationships with thalamic neurons were analyzed in continuous series of sections using either a drawing tube or Eutectics system. Some of the material was also analyzed under confocal microscope in transmitted light mode. The thick afferent axons upon entering the VL divided into, on average, four primary branches that after running a similar course for 200-300 µm divided each into 3-5 terminal branches. The latter either ended with irregular shape and size terminal enlargements spaced at irregular intervals or branched one more time before forming the terminals. Up to 10 enlargements could originate from the same terminal branch. The terminals of single afferent axons distributed within a relatively small space (apprx. 5.6×10^{-4} µm^3) clustering around somata and proximal dendrites of only some neurons present within the space and completely avoiding others. The results suggest that the input to the monkey VL originating in the dentate nucleus is very focused, targets small groups of cells and appears to be directed preferentially to proximal parts of projection neurons. Supported by RO1NS24188.

466.10

SEGREGATED VENTRAL PALLIDUM-THALAMIC PROJECTIONS FROM THE CORE AND SHELL REGIONS OF THE RAT NUCLEUS ACCUMBENS: TRANSYNAPTIC RETROGRADE LABELING USING PSEUDORABIES VIRUS. P. O'Donnell*, A. Lavin, A. A. Grace, and J.P. Card. *Depts. Neuroscience and Psychiatry, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.*

The primary output of the nucleus accumbens (NA) is known to exert regulatory control over the mediodorsal thalamic nucleus (MD) via its projections to the ventral pallidum (VP), with the *core* and *shell* regions of the NA projecting to the lateral and medial aspects of the VP, respectively. In this study, we assessed whether these projections remain segregated within the NA-VP-MD system. An attenuated strain of pseudorabies virus was injected into either the medial or lateral segments of the MD, or into the rostral or caudal aspects of the reticular thalamic nucleus (RTN). After appropriate survival times, infected neurons were labeled immunocytochemically using antiviral antibodies. These results show that the medial MD receive direct projections from the medial VP and other regions previously shown to project to this region of the thalamus. Transynaptic projections to the medial MD were observed to arise from the shell (but not the core) region of the NA. Injections into the lateral MD resulted in retrograde labeling of neurons in the globus pallidus (GP) but not the VP, and second order infections were absent from both regions of the NA. Viral injections into the rostral RTN resulted in labeling of cells in the lateral aspect of the rostral VP along with the amygdaloid, basal forebrain, and thalamic nuclei. In these cases, transynaptically infected cells were observed in the core, but not the shell, region of the NA. Injections in the caudal RTN resulted in direct retrograde labeling of cells within the GP (but not the VP), and transynaptic infections were found in cells located in the dorsolateral striatum (but not in the NA). These results suggest that the main output of VP cells receiving inputs from the shell of the accumbens is directed to the medial MD, whereas core neurons contact primarily VP cells that in turn project to the RTN, which is known to regulate thalamocortical activity.

466.11

STRIATAL PROJECTIONS FROM THE ETHMOID NUCLEUS IN THE RAT. S. Gagnon*, M. Levesque, J. Bourassa, M. Deschênes and A. Parent, Centre de recherche en neurobiologie, Hôpital de l'Enfant-Jésus, Univ. Laval, Québec, Canada, G1J 1Z4.

Posterior to the parafascicular (Pf) nucleus and anteroventral to the ventral part of the anterior pretectal area, lies a distinct group of large cells in a thalamic region perforated by vertically oriented fibers. This thalamic region is termed the Ethmoid nucleus (Eth). The axonal projection of Eth neurons was studied in rats after labeling small pools of cells with biocytin. The mapping of axonal arbors was made from serial horizontal sections that were also immunostained for calbindin D-28k , a specific marker of the striatal matrix compartment. Eth cells have a large perikarion from which emerge thick, long and poorly branched dendrites bearing spines and filamentous appendages. Their dendritic domain extends for up to 1.5 mm. Eth fibers project sparsely to the ventral wing of the thalamic reticular nucleus and to the globus pallidus, and arborize densely within the matrix compartment of the dorsal striatum. Most Eth cells also send an axonal branch towards the cortex but this projection appears light. The histological features of the dendrites and axonal terminals of Eth cells are reminescent of those of Pf neurons. It appears that Eth and Pf neurons are representative of a distinct category of thalamic relay cells, different from those found in the rest of the thalamus, that constitutes the main component of the thalamostriatal pathway.

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466.12

STRIATAL AND CORTICAL PROJECTIONS OF SINGLE CELLS FROM THE CENTRAL LATERAL THALAMIC NUCLEUS IN THE RAT. M. Deschênes*, J. Bourassa and A. Parent, Centre de recherche en neurobiologie, Hôpital de l'Enfant-Jésus, Univ. Laval, Québec, Canada G1J 1Z4.

The axonal projections of 15 cells from the caudal part of the central lateral thalamic nucleus (CL) were traced in the cerebral cortex and striatum of rats after labeling small polls of neurons with biocytin. CL neurons have a medium sized perikarion from which emerge numerous frequently branching dendrites bearing a few sessile spines. Their axons branch profusely in the thalamic reticular nucleus, and arborize sparsely in the striatum and massively in the cerebral cortex. At striatal level, CL fibers form a rather loosely organized network composed of axonal branches that appear to contact *en passant* several striatal neurons. In contrast, the cortical branch of CL axons forms multiple (4-5) patches of terminations in layers Va and III aligned along the rostrocaudal extent of the motor area. Projections to layers I and II are very sparse, consisting of occasional branches which do not show any ramifications. These results reveal that CL relay neurons, once thought to project diffusely to the superficial cortical layers, innervate mainly the mid cortical layers of a single cytoarchitectonic area. Therefore, it appears likely that the early surface negativity observed in the cortex after electrical stimulation of CL was due either to current diffusion or to activation of a dysynaptic pathway.

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466.13

STRIATAL AND CORTICAL PROJECTIONS OF SINGLE CELLS FROM THE PARAFASCICULAR NUCLEUS IN THE RAT. J. Bourassa*, A. Parent and M. Deschênes Centre de recherche en neurobiologie, Hôpital de l'Enfant-Jésus, Univ. Laval, Québec, Canada G1J 1Z4.

The thalamostriatal projection from the rat parafascicular nucleus (Pf) was studied by tracing anterogradely axons of small pools of thalamic neurons labeled with biocytin. A total of 21 Pf neurons were analyzed in the present study. Pf neurons have a large perikarion from which emerge thick, long and poorly branched dendrites bearing spines and filamentous appendages. The dendritic domain extends for up to 1.5 mm. After leaving the nucleus, Pf axons course through the thalamic reticular nucleus, where they emit two poorly branched collaterals, traverse the globus pallidus, where they also distribute collaterals, and arborize massively in the striatum and sparsely in the cerebral cortex. At striatal level 4-5 axon collaterals leave the main axon and terminate in clusters scattered over a large rostrocaudal sector of the striatum. These patches are located within the calbindin D-28k rich matrix compartment. The cortical branch forms small terminal puffs centered upon layer VI of the motor cortex. Before entering the striatum some Pf axons also give rise to descending collaterals that arborize in the entopeduncular nucleus, the subthalamic nucleus and the red nucleus. These findings provide the first direct evidence that virtually all Pf cells project to both striatum and cerebral cortex. They also reveal that, besides their massive projection to the striatum, Pf axons provide collaterals to other components of the basal ganglia.

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466.14

UNILATERAL 6-HYDROXYDOPAMINE (6-OHDA) LESIONS OF THE SUBSTANTIA NIGRA INDUCE BILATERAL INCREASES IN GLUTAMIC ACID DECARBOXYLASE (GAD) mRNA IN THE RETICULAR THALAMIC NUCLEUS J.M. Delfs*, J.J. Soghomonian#, V.M. Ciaramitaro and M.-F. Chesselet, Institute of Neurological Sciences and Dept. of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA and #Hopital De L'Enfant Jesus, Quebec, Canada.

The reticular thalamic nucleus (RTN) is a thin band of GABAergic neurons which surrounds the lateral border of the thalamus. Due to its extensive and reciprocal projections with the cortex and thalamus, the RTN is thought to be involved in attentional processes. The RTN also receives inputs from the basal ganglia, in particular the globus pallidus (GP, external segment) and the substantia nigra (SN) suggesting that it may be involved in relaying motor information to the thalamus and cortex. In order to explore the relationship between the basal ganglia and the RTN, rats received unilateral 6-OHDA lesions of the SN and were sacrificed 2 or 3 weeks after the lesion. Sections of the RTN at the level of entopeduncular nucleus were processed for in situ hybridization and emulsion autoradiography. A unilateral SN lesion resulted in a bilateral increase in GAD (Mr 67,000:GAD67) gene expression in neurons of the RTN. The level of mRNA encoding GAD (Mr 65,000:GAD65) was increased ipsilateral to the SN lesion. Neither short-term (7 day) or long-term (8 month) haloperidol treatment, which induce catalepsy or orotacial dyskinesia, respectively, altered GAD67 mRNA levels in the RTN, indicating that changes in GAD mRNA in the RTN are not related to motor symptoms. The results reveal novel bilateral effects of unilateral dopamine depletion in the RTN. In view of the role of the RTN is attentional processes, this effect could be related to the non-motor effects of dopamine depletion. This work was supported by PHS grants MH 44896 and MH 17168.

466.15

SINGLE INJECTION OF GABA INTO RAT MOTOR THALAMUS (VL/VM) INCREASES STRIATAL GLUTAMATE RELEASE AND THE DENSITY OF NERVE TERMINAL GLUTAMATE IMMUNO-REACTIVITY. C.K. Meshul*, C. Allen, J. P. Riggan, and D.J. Feller. V.A. Medical Center and Oregon Health Sciences University, Portland, OR. 97201.

Subchronic injections of GABA (10^{-5} M, 0.5 µl) into VL/VM for 21 days resulted in an increase in the percentage of striatal glutamatergic synapses with perforated postsynaptic densities (PSDs) and an increase in the density of glutamate immunoreactivity within nerve terminals of all asymmetric synapses (Meshul et al 1995). These findings are similar to those seen after subchronic haloperidol treatment. An acute injection of GABA (15 µl) into VL/VM results in an increase in striatal glutamate release as measured by in vivo microdialysis. This may be due to stimulation of presynaptic GABA receptors within the thalamus, leading to a decrease in GABA release and activation of the thalamo-cortico-striatal pathway There was no change in striatal glutamate synapses with perforated PSDs or in glutamate release if GABA was injected into the somatosensory thalamic nuclei (VPM/VPL). We determined if a single injection of GABA (10^{-5} M, 15 µl) into VL/VM would also result in changes in glutamate synapses 1 day later. We found that there was a significant increase in the percentage of striatal synapses with perforated PSDs and an increase in the density of nerve terminal glutamate immuno-reactivity. If these findings are due to activation of the thalamo-cortico-striatal pathway, then it is possible that the identical changes observed with haloperidol treatment may also be due to increased activity of corticostriatal synapses. Supported by Dept. of Veteran Affairs.

466.16

THALAMIC ARBORIZATIONS OF AXONS ARISING IN THE MESOPONTINE RETICULAR FORMATION OF SQUIRREL MONKEYS. C. Asanuma*. Laboratory of Neurophysiology, NIMH, NIH Animal Center, Poolesville, MD 20837.

The thalamic projections of the mesencephalic (MRF) and pontine (PRF) reticular formation were examined and compared using anterograde transport of wheat germ agglutinin–conjugated horseradish peroxidase (WGA-HRP) and tetramethylbenzidine (TMB) histochemistry in squirrel monkeys.

Injections of WGA-HRP directed into the rostral MRF result in diffusely scattered anterograde label (fine, dust-like particles of TMB reaction product) throughout the dorsal thalamus and thalamic reticular nucleus (TRN). Increased concentrations of diffuse anterograde label are evident bilaterally throughout the intralaminar nuclei, and patches of moderate label occur laterally in the ipsilateral mediodorsal nucleus (MD) and in parts of the anterior ventral lateral nucleus (VLo).

PRF injections of WGA-HRP, in contrast, result in a patchy distribution of anterograde label within ventral MD, at the level of the centromédian/parafascicular complex (CM/PF). Dense clusters of label occur ventrally within the central lateral nucleus (CL) as well at this level, whereas labeling elsewhere within CL and throughout the intralaminar nuclei is spread more evenly and occurs in only moderate density. Anterograde labeling elsewhere in the dorsal thalamus is light. As with the MRF cases, the anterograde labeling within the intralaminar nuclei resulting from PRF injections is bilateral. Prominent labeling in the ventral lateral geniculate nucleus extends dorsally along the medial margin of the TRN. However, only occasional axons are detected within the TRN itself.

When the CL labeling resulting from an MRF injection is examined at the EM level, the TMB reaction product is observed within both myelinated and unmyelinated axons and in terminal profiles that establish synaptic contact with unlabeled dendrites. The synaptic relations of terminals arising in different sectors of the mesopontine reticular formation as well as the relation of the ascending brainstem terminals with the $GABA^+$ and the $GABA^-$ synaptic profiles of the CL neuropil are currently being examined in more detail.

466.17

THE CHEMICAL ARCHITECTURE OF THE DORSOMEDIAL AND INTRALAMINAR THALAMIC NUCLEI AND ITS RELATIONSHIP WITH THE THALAMOSTRIATAL SYSTEM IN THE CAT. E. Mengual, S. de las Heras, J.L. Velayos and J.M. Giménez-Amaya*. Departamento de Morfología, Facultad de Medicina, U.A.M., 28029 Madrid, Spain.

A great discrepancy is found among the different authors concerning the delineation of the mediodorsal (MD) and intralaminar (IL) thalamic nuclei in the cat, following cytoarchitectonic and/or connectional criteria. In this study, the distribution of acetylcholinesterase (AChE), NADPH-diaphorase (NADPH-d) and cytochrome oxidase (CO) histochemical activities was studied, together with cytoarchitectonic and myeloarchitectonic techniques, in order to further characterize these thalamic nuclei and to establish a clearer delineation between them. Additionally, this chemical architecture was correlated with the distribution of retrogradely labelled thalamostriatal neurones, after stereotaxic injections of either HRP-WGA or fluorescent tracers into the caudate nucleus. For this purpose, twenty four adult cats were used for the histochemical studies and twelve animals for the hodological ones. The main findings of this study were: 1) The heterogeneous distribution of AChE activity within these nuclei allowed to delineate MD from the adjacent nuclei rostrally, medially and, at rostral levels, also laterally. 2) The AChE activity showed a network of AChE-rich patches located in the midline, in the ventral and lateral portions of MD and in the dorsal portions of the paracentral nucleus, that crossed the cytoarchitectonic borders. 3) NADPH-d activity in MD and IL displayed a distribution pattern that overlapped that of AChE, except in the centromedian-parafascicular (Cm-Pf) complex and in the central medial nucleus. CO activity, also heterogeneous, proved to be very useful in the identification of the central lateral nucleus (CL). 4) The distribution of the thalamostriatal neurones confirmed the chemical architecture of DM and IL, being located at histochemically defined areas, such as the area dorsal to MD, rhomboidal nucleus with its lateral wing, Cm-Pf complex and moderately stained areas within CL.

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466.18

DOPAMINE AGONIST-EVOKED LOW THRESHOLD SPIKES IN MEDIODORSAL THALAMIC NUCLEUS NEURONS IN VITRO. A. Lavin* and A.A. Grace *Depts. Neuroscience and Psychiatry, CNUP, Univ. Of Pittsburgh, Pittsburgh, PA, 15260.*

The mediodorsal thalamic nucleus (MD) of the rat is reported to be innervated by dopaminergic and non-dopaminergic cells of the ventral tegmental area (VTA), and DA receptors have been shown to be present in the thalamus. Using intracellular recordings in 400 µm sagittal slices, we studied the effect of the DA D2 agonist qunpirole (10 µm) on the physiology of MD thalamic neurons. The D2 agonist applied into the bath caused a hyperpolarization of the membrane of 14/22 cells by 5.8±4.4 mV, and depolarized 4/22 for 9.3 ± 12.1 mV. Following drug application there was an increase in the average input resistance of 9/16 neurons for 32% (all cells had exhibited a depolarizing response) and also a decreased in the input resistance by 17% in 7/16 cells (all cells had exhibited hyperpolarizing response) and also an increase in the time constant in 27% (7/10 cells, 6 of them hyperpolarized). In the majority of cells tested (17/22 cells), the D2 agonist caused an increase in the apparent spike threshold of the cells by 8.1 ± 3.8 mV. Plotting I/V regression lines before and after quinpirole administration showed a reversal potential for this response averaging approximately -92 mV, which is consistent with the reversal of a K^+ conductance. When haloperidol was applied, a depolarization of the membrane (9.2±1.4 mV, n=2) and a decrease in the input resistance (17%) were produced. In addition, a prominent (LTS)-like event was apparent in 11/18 cells following quinpirole administration. Thus, in MD thalamic cells D2 receptor stimulation was found to cause a hyperpolarization that may be due to an increase in K^+ conductance, which in turn may facilitate the expression of the LTS.

467.1

PROJECTIONS FROM DORSAL PARAFLOCCULUS TO CEREBELLAR NUCLEI OF MACAQUES. M. Glickstein, I. Kralj-Hans, B. Mercier, C. Swales, N. Gerrits, J. Voogd, R. J. Krauzlis, and F. A. Miles*, Department of Anatomy, University College London, London WC1E 6BT, England, Department of Anatomy, Erasmus University, Rotterdam, Netherlands, and Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

The dorsal paraflocculus is a major target for visual input to the cerebellar cortex. To study the projections from dorsal paraflocculus and adjacent areas of the cerebellar cortex, wheatgerm-agglutinin horseradish peroxidase injections of between 0.1 and 0.2 microliters were placed in the dorsal paraflocculus of three monkeys, and in other cerebellar cortical areas of six others. We report here on the nuclear targets revealed by these injections. Injection into the dorsal paraflocculus revealed a dense zone of terminals in a ventral, caudal region of the posterior interpositus nucleus and adjacent dentate nucleus. Cells in the dorsal paraflocculus (Noda and Mikami, 1986) and in the ventral caudal region of nucleus interpositus (Van Kan et al, 1993) are known to discharge in relation to eye movements. Injections into paramedian lobule, Crus II, the ventral paraflocculus, or vermian lobule VII revealed projections to other, non-overlapping nuclear targets.

Thus, in addition to other cerebellar circuits which involve the flocculus, ventral paraflocculus, or vermian lobule VII, there is another pathway by which visual inputs can influence eye movements: Visual cells in the pontine nuclei receive an input from dorsal extrastriate visual areas and relay to the dorsal paraflocculus, which in turn projects to the posterior interpositus nucleus.

467.2

POSTSYNAPTIC TARGETS OF PURKINJE CELLS IN THE CEREBELLAR AND VESTIBULAR NUCLEI. A. S. Berrebi* and C. I. DeZeeuw†, ENT Dept, West Virginia Univ School of Medicine, Morgantown, WV and †Dept of Anatomy , Erasmus Univ Rotterdam, Rotterdam, The Netherlands.

The cerebellar (CN) and vestibular nuclei (VN) consist of a heterogeneous group of inhibitory and excitatory neurons. A major proportion of the inhibitory neurons provide GABAergic feedback to the inferior olive, while the excitatory neurons exert more direct effects on motor control via non-olivary structures. It is not clear whether Purkinje cells (PC)s innervate all neuronal types in the CN and VN nor whether an individual PC axon can innervate different types of neurons.

We studied the postsynaptic targets of PC axons in the rat by combined preembedding immunolabeling with L7, a PC-specific marker, and postembedding GABA and glycine immunocytochemistry. In the CN and VN, and nucleus prepositus hypoglossi PC terminals were apposed to GABAergic and glycinergic (GLY) neurons as well as to larger nonGABAergic, nonGLY neurons. In the CN and VN individual PC terminals innervated both inhibitory and excitatory neurons. Both neuron types were not only contacted by GABAergic PC terminals, but also by GABA-containing terminals that were L7-negative and by nonGABAergic, nonGLY terminals that formed excitatory synapses. GLY-containing terminals were relatively scarce (<2% of the GABA-containing terminals) and frequently contacted larger nonGABAergic, nonGLY neurons.

In summary, PC axons modulate the activity of multiple cell types in the CN and VN complex. The observation that individual PCs can innervate both excitatory and inhibitory neurons suggests that the excitatory cerebellar output system and the inhibitory feedback to the inferior olive may be controlled simultaneously.

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467.3

SPINAL CONNECTIONS OF CAT POSTERIOR INTERPOSITUS. K. M. Horn*, C. M. Porter and A. R. Gibson. Barrow Neurological Institute, Phoenix, AZ 85013.

Injections of HRP into the spinal cord label neurons in medial regions of the cerebellar posterior interpositus (Matsushita & Hosoya, Br. Res. 142). Where does the interposito-spinal projection terminate, and does it target specific motor neuronal pools? To answer these questions, we injected WGA-HRP (.008 ul, 1%) into cat posterior interpositus.

Labeled fibers could be traced from the injection site through the decussation of the superior cerebellar peduncle. The fibers descended along the neuraxis as a widely spaced group passing above the inferior olive; at spinal levels, the fibers coursed in the ventrolateral funiculus. No motoneuronal pools were targeted by the projection, and labeling in the spinal gray was largely confined to Rexed's lamina VIII contralateral to the injection site. Although the labeled area decreased in size and moved medially at levels caudal to C3 (as does lamina VIII), some label was present as far caudally as T1. In addition to the anterograde label, large retrogradely labeled neurons were also present in lamina VIII.

Neurons in lamina VIII provide crossed spinal connections. Therefore, the interposito-spinal pathway may be involved with muscles requiring bilateral control, such as those of the neck or spine. Spinal neurons projecting back to interpositus could allow the cerebellum to respond to lamina VIII activity at very short latency.

467.4

INFLUENCE OF UNILATERAL LABYRINTHECTOMY ON EXPRESSION OF PROTEIN KINASE C IN A CEREBELLAR-VESTIBULAR PATHWAY IN RAT. N.H. Barmack,* J. Yoshimura, Z.-Y. Qian and H. Fushiki. R.S. Dow Neurological Sciences Inst., Portland, OR 97209.

Protein Kinase C-δ, a calcium-independent isoform of PKC, is found in sagittal banding patterns in the cerebellum of rat. In other systems, PKC has been linked to the regulation of ion channels related to signal transduction. Using immunohistochemical staining for PKC-δ, we have confirmed these banding patterns in the uvula-nodulus. Western blotting identifies a PKC-δ positive protein with a molecular weight of 75-80 KDa. In the present report we investigate the projection patterns of PKC-δ positive, uvula-nodular Purkinje cells.

In three rats, anesthetized with ketamine-xylazine-acepromazine, we made unilateral lesions of the uvula-nodulus and examined the distribution of PKC-δ positive terminals within the vestibular complex. Subtotal unilateral lesions deplete more than 50% of the terminals within the ipsilateral caudal medial vestibular nucleus (MVN) and nucleus prepositus hypoglossi (NPH). In fifteen anesthetized rats, we performed unilateral labyrinthectomies. The rats were sacrificed 6 hrs - 38 days after the labyrinthectomy. PKC-δ positive terminals within the MVN and NPH were counted. Unilateral labyrinthectomies caused a decreased count of PKC-δ positive terminals in the ipsilateral MVN and NPH. Paradoxically, unilateral labyrinthectomies caused no detectable decreases in labeling of Purkinje cell bodies in the ipsilateral nodulus. Nor, using hybridization histochemistry, could we detect a change in PKC-δ mRNA in nodular Purkinje cells. The decreased PKC-δ labeling in terminals within the ipsilateral vestibular complex may reflect a change in the regulation of a protein to which PKC-δ binds.

467.5

PROJECTIONS FROM THE RED NUCLEUS TO THE INTERPOSITUS NUCLEUS IN THE RAT DEMONSTRATED BY THE RETROGRADE TRANSPORT OF FLUOROGOLD. J.M. Lockard* and D.G. Lavond. Dept. of Neurobiology, Univ. of Southern California, Los Angeles, CA 90089

Injections of a 2% solution of Fluorogold (Fluorochrome, Inc.) in 0.9% saline were made through a glass micropipette (tip diameter 25 μm) in the cerebellar interpositus nucleus of Sprague-Dawley rats. Injections were made over a 10 minute period with a constant current unit, set to deliver a 7 second, +5 μA pulse, with 7-second interpulse durations. Survival times for the animals was 14 days. Animals were then deeply anesthetized with sodium pentobarbital (35-50 mg/kg) and then perfused transcardially with 400 ml of ice-cold 4.0% paraformaldehyde in 0.1 M sodium borate buffer at pH 9.5, following a brief saline rinse to remove most of the blood. The brains were then removed and postfixed for 24 hrs. at 4°C in the same fixative containing 10% sucrose. Serial 30 μm thick sections were collected, with one series being processed for immunohistochemistry with an anti-Fluorogold serum (Chemicon), at a concentration of 1:10,000. The primary antiserum is localized using a standard method for visualizing the avidin-biotin complex system. The second adjacent series is mounted and Nissl-stained for reference purposes. Retrograde cell labeling was seen throughout the rostral-caudal extent of the contralateral red nucleus. These findings support the earlier reports of Courville & Brodal (J. Comp. Neurol. 126:471-485, 1966) and more recently by Huisman et al. (Brain Res. 264:181-196, 1983) and further extends the key role played by the red nucleus in cerebellar functioning.

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ELECTROPHYSIOLOGICAL PROPERTIES OF NEURONS IN THE RAT PONTINE NUCLEI IN VITRO *C.Schwarz*, M.Möck and P.Thier. Sekt. für Vis. Sensomotorik, Neurologische Universitätsklinik, 72076 Tübingen, Germany.*

The pontine nuclei are the major relay of cortical information destined for the cerebellum. We established a slice preparation of rat pontine brainstem in order to study the membrane and synaptic properties of pontine neurons using standard intracellular electrophysiological techniques. We recorded from 69 pontine neurons which displayed a resting potential of -63±5.8 mV (mean±SD) and were not spontaneously active. The input resistance was 50±28 MΩ and the time constant was 7.3±2.9 ms (measured with injection of -1.0/-0.2 nA respectively). Action potentials (amplitude 70±7.7 mV) were elicited with current pulses depolarizing the membrane potential to -41±8.7 mV. Each spike was followed by a marked afterhyperpolarization. A salient property of pontine neurons was the adaptation of the firing rate when depolarized with long lasting current pulses. Ca^{++} dependency of this phenomenon could be demonstrated by the reduction of the spike adaptation under substitution of extracellular Ca^{++} by Mn^{++} or Co^{++} (2.4 mM). Ca^{++} influx most probably takes place via voltage dependent Ca^{++} channels as broad spikes could be elicited by depolarizing current pulses from rest during the block of K^+ and fast Na^+ channels by TEA (10 mM) and TTX (1 μM). These broad spikes were based on Ca^{++} current as was shown by their sensitivity to substitution of extracellular Ca^{++} by Mn^{++}. We next studied the postsynaptic influences of cortical afferents by electrical stimulation of the pedunculi cerebri. Graded EPSPs could be elicited depending on the amplitude of stimulation currents (minimal 20 μA). The EPSPs were sensitive to DNQX (25 μM) suggesting glutamate as transmitter acting on AMPA receptors. In most cases the time of decay of the EPSPs increased markedly with depolarized membrane potentials. This may be due to an additional involvement of NMDA receptors, an issue which we are investigating currently. *Supported by DFG SFB 307-A1*

467.7

CEREBELLAR AFFERENTS FROM THE SUPERIOR COLLICULUS AND A17 ARE KEPT SEGREGATED IN THE RAT PONTINE NUCLEI *Anja Horowski, Cornelius Schwarz and Peter Thier (SPON: European Neuroscience Association). Sekt. für Vis. Sensomotorik, Neurologische Universitätsklinik, 72076 Tübingen, Germany.*

We investigated whether inputs to the cerebellum arising from the superior colliculus (SC) and the visual cortex (A17) are integrated in the pontine nuclei (PN). To this end we first used double anterograde tracing (DiI, DiAsp) from different points within each structure in order to clarify if a topographical organization of pontine afferents exists. The tectopontine terminal fields were then compared with those originating in A17. In a second step we used a combination of anterograde tracing and intracellular fills of identified projection neurons in fixed slices in order to investigate if projection neurons extend their dendrites into the terminal fields of both structures.

Independent of the location of the injection sites in the SC and A17, DiI and DiAsp-labeled terminal fields were largely congruent. Tectopontine fibers converge essentially in one pontine compartment while those arising from A17 were distributed among 3 to 4 compartments. Comparing tectal and visual-cortical terminal fields within the PN revealed that while located close to each other they in any case remain segregated. Moreover, intracellular fills showed that the dendrites of pontocerebellar neurons respect the borders of the labeled axon terminal fields and do not tend to cross them. We therefore conclude that visual information from cortical and subcortical sources destined for the cerebellum is kept segregated from each other in the PN. *Supported by DFG SFB 307-A1*

467.8

EXPRESSION OF aFGF AND A NOVEL PROTEIN TYROSINE PHOSPHATASE mRNA FOLLOWS A ROSTROCAUDAL BOUNDARY IN THE MURINE CEREBELLAR CORTEX. [1]A. Rotter*, [2]P.E. McAndrew, [2]A.H.M. Burghes, [3]I.-M. Chiu, [1]J.E. Evans, [1]M. Sutter and [1]A. Frostholm. Depts. of [1]Pharmacology, [2]Neurology and [3]Internal Medicine, The Ohio State University, Columbus, OH 43210.

The cerebellum is a highly compartmentalized structure, being organized into various layers, parasagittal zones and anterior-posterior compartments. While the segregation of molecular markers into lamina is indicative of differences in neuronal populations of the various layers, parasagittal zonal differences are a reflection of afferent and efferent connectivity. Recently, genes which specify cerebellar rostral-caudal compartments during development have been identified and have been shown to define a distinct boundary between anterior and posterior regions. We have used in situ hybridization with cRNA probes to define the distribution of acidic FGF mRNA and a novel protein tyrosine phosphatase (PTPase) mRNA in the cerebellum. Within the cerebellar cortex, the expression of both genes was limited to the granule cell layer. Each signal was strong in the anterior region (lobules 1-6) and formed a distinct boundary with posterior lobules at the dorsal aspect of lobule 6. The expression of the two messages may be related to the homeobox gene, Otx-1, which has a similar developmental expression pattern. This developmental boundary was first noted in several mouse mutants, including meander tail, leaner, and swaying. The restricted pattern of acidic FGF and the novel PTPase implies that these molecules may have a specific signaling role in the anterior region of the cerebellum.

467.9

CALRETININ IMMUNOREACTIVE UNIPOLAR BRUSH CELLS FORM PARASAGITTAL BANDS IN THE RABBIT CEREBELLUM. M.R.Dino*, E. Mugnaini, Neuromorphology Lab, U-154, Univ. of Conn., Storrs, CT. 06269-4154

Golgi and immunocytochemical methods have been used to anatomically characterize the unipolar brush cells (UBCs) in the granular layer of the rodent cerebellum. Intermediate in size between the granule and Golgi cells, UBCs are distinguished by a brush-like formation, consisting of several short branchlets, emanating from the tip of a single, relatively thick dendrite. The brush usually establishes a giant synapse with a single mossy fiber rosette, is postsynaptic to small boutons of the Golgi cell axon, and, in addition, is presynaptic to granule cell dendrites. Utilizing the calcium binding protein calretinin as a neuronal marker, we have mapped the distribution of UBCs in Brazilian opossum, mouse, rat, guinea pig, rabbit, cat, Rhesus monkey, and human by immunocytochemistry. The features of the unipolar brush cells are remarkably similar in all mammals analyzed, including human. As a general trend, the UBCs are distributed in cerebellar lobules that receive the least pontine nuclei input, are particularly enriched in the vestibulocerebellum, and are more prominent and widely distributed in cat, macaque and human than in rodents. A remarkable interspecies variation, however, was observed in the rabbit, where UBCs, in addition to the patterns observed in other species, were distributed in distinct alternating on-off compartments, reminiscent of the parasagittal zones described by others. Experiments are in progress with other cell population markers to establish whether calretinin-negative UBCs are present in the off-bands. In addition to UBCs, calretinin positive mossy and climbing fibers were also prominent in the rabbit cerebellum. Supported by NIH grant NS09904.

467.10

Quantitative Anatomical Aspects of the Inhibitory Interneurons of the Rat Cerebellar Molecular Layer. A Light and Electron Microscopic Golgi Study. F. Sultan*[1], M.H. Ellisman[2] and J.M. Bower[1].
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The basket and stellate cells are important elements in the cerebellar cortex circuit. Together, these cells form the second most numerous type of neuron in the cerebellar cortex. The long term goal of this study was to provide accurate anatomical data to construct a realistic model of these neurons.

Using the Eutectic Neuron Tracing System we reconstructed at the light microscopic level seven stellate and two basket cells stained with the Golgi-Rapid method. The average length of the stellate cell axon was 1150 µm. The average axonal swelling or bouton density of the stellate cell axon was 0.13/µm. All stellate cells had local axonal collaterals. The majority (5/7) had a main axon that extended beyond the region occupied by the stellate cells dendritic tree (up to 550 µm from the soma).

In addition to the light microscopic level, the dendrite of a Rapid-Golgi stained basket cell was serially sectioned for electron microscopy after gold-toning. A total of 41.9 µm dendritic length was reconstructed and analyzed. The number of synapses per dendritic length calculated was 0.9 synpases/ µm. The serial reconstruction of the dendrite is employed in order to facilitate the identification of the excitatory presynaptic structure, i. e., parallel or ascending part of the granule cell axon.

The two major functions which have been proposed for the inhibitory cells of the molecular layer are threshold control and lateral inhibition. If the overlap between the dendrite and the axon were large, then the former hypothesis would be favored. If the overlap were small, then the latter is more likely. A method is proposed that allows a quantitative comparison of the overlap between the two structures.

Supported by Human Frontier Program

467.11

CEREBELLAR MODULAR ORGANIZATION IS MAINTAINED IN NUCLEO-BULBAR PROJECTIONS: AN ANATOMICAL TRACER STUDY IN THE RAT. T.M.Teune, J. van der Burg, T.J.H.Ruigrok and J.Voogd*, Dept. of Anatomy, Erasmus University Rotterdam, 3000 DR, The Netherlands.

Introduction. Cerebellar circuitry is characterized by longitudinally arranged cortico-nuclear and olivo-cerebellar projections. Less is known to what extent this modular organization is preserved in the cerebellar output to the brainstem. The potential di- and convergence of projections from the cerebellar nuclei (CN) to four functionally different target areas (the inferior olive: IO; the nucleus reticularis tegmenti pontis: NRTP; the red nucleus: RN; the prerubral area: preRN) was investigated. Retrograde tracers (WGA-BSA-gold + CTb) were applied to CN target areas. Anterograde tracers (BDA + Pha-L) were applied to the CN.

Retrograde labeling. Cases with injection combinations that involved the IO resulted in very sparse double labeling (less than 0.5% of all labeled cells). Combinations of NRTP, RN and preRN injections showed up to 45% double labeled CN cells. The posterior interposed nucleus (PIN) remained relatively devoid of labeling from the NRTP.

Anterograde labeling. All cases with separate injection sites in the CN resulted in distinct patches of terminal labeling within the IO, NRTP, RN and preRN, even when both tracers were centered on the same subnucleus. PIN injections did not result in labeling within the NRTP. Injections centered on the anterior interposed nucleus (AIN) did not result in labeling within the preRN.

Conclusions: A) the nucleo-olivary neurons represent a distinct entity within the CN; B) AIN and lateral cerebellar nuclear (LCN) neurons collateralize to the NRTP, RN and/or preRN; C) The CN projections to the brainstem maintain a non-overlapping topographic organization.

467.12

Perioral Regions of Somatosensory Cortex Project to Corresponding Cerebellar Regions Through Spatially Restricted Domains Within the Pontine Nuclei.

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We have been using electrophysiologically guided placement of punctate iontophoretic injections of tracers to reveal the details of how a somatotopic map in S1 of somatosensory cortex projects to a "fractured" somatotopic map in Crus IIa of the cerebellar hemisphere via the pontine nuclei. High density micromapping techniques in both cerebral cortex and the cerebellum have allowed precise localization of representations of specific regions of the body surface. Tracers were delivered with iontophoresis in the upper lip (UL) representation in S1, layer 5, and in an UL patch in the granule cell layer of Crus IIa. The distributions of anterogradely labeled corticopontine fibers and retrogradely labeled ponto-cerebellar cell bodies were computer reconstructed as point clouds inside a solid model representing the pontine nuclei. Using anatomical landmarks, the distribution patterns from several experiments were superimposed for comparison. Inspection of these and individual cases has revealed several important findings. The UL representation of corticopontine fibers occupies a much more restricted space than does the entire facial region. The ponto-cerebellar cell bodies which project to the UL patch are located in only a part of the domain for Crus IIa-projecting ponto-cerebellar cells. Fibers from the UL region of S1 and cells projecting to the UL patch of Crus IIa are not in completely overlapping regions, but appear to occupy adjacent subspaces. We find no evidence supporting the traditional concept of "columns" of cortico-pontine fibers.

467.13

NOS, NMDAR1 AND CYTOCHROME OXIDASE IN THE BRAINSTEM-CEREBELLAR CIRCUIT OF *MACACA MULATTA*. B.J. Anderson, W.S. Liebl, B. Myklebust* and M. Wong-Riley. Dept. of Cell. Biol. & Anat., Med. Coll. of Wisconsin, Milwaukee, WI 53226.

Nitric oxide (NO) is a gaseous intra- and inter-cellular messenger in the CNS. NO production can be triggered by the activation of NMDA or other glutamate receptors. The level of nitric oxide synthase (NOS) is high in the rodent cerebellum, but its localization is unknown in the monkey. The goal of the present study was to correlate the distribution of NOS with that of NMDAR1 (a major NMDA receptor subtype) and of cytochrome oxidase (C.O.; a metabolic marker) in the macaque cerebellum and precerebellar nuclei. Serial sections were reacted for NOS, NMDAR1, and C.O., respectively. All three labels were present in the cell bodies and dendrites of neurons in the inferior olive (IO), pontine nuclei (PN) and deep cerebellar nuclei (DCN). In the cerebellar cortex, Purkinje cell dendrites are labeled for NMDAR1, C.O. and lightly for NOS. Granule and basket/stellate cells and Purkinje cell bodies were labeled in a heterogeneous but spatially corresponding pattern for NMDAR1. NOS consistently labeled glomeruli in the granular cell layer and basket/stellate cells in their entirety in the molecular layer. The labeling patterns in the IO, PN and DCN are consistent with NMDA-triggered NO production in these regions. Unexpectedly, granule cells in the cerebellum were not labeled for NOS. The lack of a consistent co-localization of NOS and NMDAR1 is in agreement with earlier reports that non-NMDA receptors may also be involved in NO production. The high levels of C.O. in many NMDAR1+ structures suggests that excitatory input imposes a high energy demand on these neurons. (Supported by NIH NS18122)

467.14

CEREBELLAR PURKINJE CELLS DISPLAY VARIATIONS IN PROTEIN CONTENT AND LOCALIZATION PATTERN. C.J. Tandler and A. Pellegrino de Iraldi*. Inst. of Cell Biology & Neuroscience Prof. E. De Robertis, Buenos Aires School of Medicine, Paraguay 2155, 1121 Buenos Aires, Argentina.

Differences in Purkinje cells nuclei and perikarya in adult rats and chicken have been found as a result of cytochemical and conventional procedures. At ultrastructural level there were electron dense and light cells. Purkinje cells in which the nucleus was filled with abundant electron-dense material showed granular endoplasmic reticulum cisternae in lamellar arrays. Those with a clear appearance in their nuclei displayed clusters of ribosomes in polysomal configurations and a less developed granular endoplasmic reticulum. The contribution of proteins and nucleic acids to the electron opacity was indicated by selective blockage and extractive techniques. At light microscopy the mercury-silver method (*Biotech. Histochem. 69*: 329, 1994) also revealed dark and light Purkinje neurons in both nuclei and perikarya, according to their relative protein concentration. A direct relationship with extranucleolar transcriptional rates is suggested. This work was supported by grants from CONICET and UBA.

467.15

PHYSIOLOGICAL EFFECTS OF CALCITONIN GENE-RELATED PEPTIDE IN THE MOUSE INFERIOR OLIVE. K.V. Gregg* and G.A. Bishop. Neuroscience Program, The Ohio State University, Columbus, OH 43210.

We have previously shown that calcitonin gene-related peptide (CGRP) immunoreactive varicosities have a dense distribution throughout all subnuclei of the inferior olivary complex (IOC) of the mouse (Gregg et al., Soc. Neurosci. 24:714.2, 1994). The aim of the present study was to determine the physiological effects of CGRP on olivary neurons and to analyze interactions between the peptide and excitatory amino acids (EAAs) through the use of extracellular recordings in an in vitro slice preparation. In the slice preparation, olivary neurons fire spontaneously at irregular intervals at a frequency of 0.2-1 Hz which is in general agreement with data obtained previously in other species (Armstrong, Physiological Reviews 53:358, 1974). Application of EAAs such as quisqualate or glutamate increased the firing rate to 2Hz and induced neurons to fire more regularly. Application of CGRP to spontaneously active olivary neurons suppressed the firing rate. In some instances, after prolonged application of the peptide, neurons were able to recover. CGRP also suppressed EAA induced activity. Two different paradigms were used. In one, the EAA was applied first at levels that increased olivary activity. Application of CGRP during the EAA pulse reduced neuronal activity. If CGRP was applied first, the excitatory effects of quisqualate and glutamate were suppressed or delayed in their onset. As with spontaneous activity, in a few cases neuronal firing rate recovered during later stages of the CGRP pulse. These effects of CGRP on spontaneous and EAA induced activity suggest a neuromodulatory role for CGRP in the IOC. In the dorsal horn of the spinal cord, CGRP has been shown to modify Ca^{2+} currents (Ryu et al., Brain Res. 441:357, 1988). Ca^{2+} is responsible for generating specific portions of the olivary action potential (Llinas and Yarom, J. Physiol. 315:549, 1981a; Llinas and Yarom, J. Physiol. 315:569, 1981b) including an afterhyperpolarization. Future intracellular studies will allow us to determine if CGRP alters olivary activity by modulating Ca^{2+} currents in olivary neurons. (supported by NS-18028).

467.16

THE EXPRESSION OF CORTICOTROPIN RELEASING FACTOR (CRF) mRNA AND CRF RECEPTOR mRNA IN THE MOUSE BRAINSTEM AND CEREBELLUM T.L. Overbeck* and J.S. King Department of Cell Biology, Neurobiology and Anatomy and Neuroscience Program, The Ohio State University, Columbus, Ohio 43210 U.S.A.

Corticotropin releasing factor (CRF) has been immunocytochemically localized to climbing fibers and mossy fibers in the cerebellar cortex, and in varicosities in the cerebellar nuclei. In the present study we used *in situ* hybridization to detect CRF mRNA in brainstem nuclei that are the source of these afferents. A positive signal was present in all subnuclei of the inferior olivary complex, the source of climbing fibers. In addition, CRF mRNA hybridization signal is present in neurons located in brainstem nuclei previously shown to give rise to CRF mossy fibers (Errico and Barmack, 1993, JCN: 336:307) including the nucleus prepositus hypoglossi, nucleus raphe magnus, nucleus reticularis gigantocellularis and the vestibular complex. Receptor binding studies show positive ligand binding over the molecular, Purkinje and granule cell layers in all lobules of the adult cerebellar cortex (Overbeck et al. 1994, FASEB Journal Abst.: 8:109). We used *in situ* hybridization to identify the neuronal elements in the cerebellum that express the CRF receptor mRNA. An antisense riboprobe corresponding to the full length sequence of the rat CRF receptor was used (Perrin et al. 1993, Endocrinology: 133:3058). Positive hybridization is evident in Purkinje cells, granule cells and cerebellar nuclear neurons. No signal was detected in the molecular layer. In vitro physiological studies demonstrate that CRF acts as a neuromodulator by enhancing both the spontaneous and excitatory amino acid-induced activity of Purkinje cells (Nelson and Bishop, unpublished observations). Tissue culture experiments (Fox and Groul, 1993, Neurosci. Lett.: 149:103) suggest that the effects of CRF is mediated through a cAMP second messenger system which acts to close calcium-dependent K+ channels, thereby depolarizing the cell. Taken together, these data suggest that CRF may modulate the activity of Purkinje cells, granule cells and nuclear neurons in the adult mouse cerebellum. Currently, we are investigating the temporal expression of CRF mRNA in the inferior olive and the expression of CRF receptor mRNA in the cerebellum of the developing mouse. (Supported by NS-08798)

467.17

THE PHYSIOLOGICAL EFFECTS OF DOPAMINE (DA) ON PURKINJE CELLS IN MOUSE CEREBELLAR SLICES. T.E.Nelson* and G.A.Bishop. Neuroscience Program, The Ohio State University, Columbus, OH 43210.

Recent studies have indicated that dopaminergic fibers and binding sites are present within the rodent cerebellar cortex (Panagopoulos et al., 1991, Neurosci. Lett. *130*:208; Panagopoulos and Matsokis, 1994, Gen. Pharmac. *25*:131) and that DA is released within the rat cerebellum (Chrapusta et al., 1994, Brain Res. *655*:271). Furthermore, D3 receptor mRNA is present in Purkinje cells (Bouthenet et al., 1991, Brain Res. *564*:203) and D3 receptor binding is found within the molecular layer (Levant et al., 1993, J. Pharmacol. Exp. Therap. *264*:991) of lobules IX and X of the rat cerebellum. In this study, we investigated the physiological effects of this catecholamine on mouse cerebellar Purkinje cells using an *in vitro* slice preparation. Multibarrel glass electrodes were used to record the single-unit activity of Purkinje cells in lobules IX and X of the cerebellar vermis and to iontophoretically apply combinations of DA, glutamate, quisqualate and GABA, as well as the DA agonists, PPHT and 7-OH-DPAT, to the neurons. The effects of DA and its agonists on spontaneous activity, excitatory amino acid-mediated excitation, and GABA-mediated inhibition of Purkinje cells were tested.

Purkinje cell activity was decreased in response to DA although it never completely suppressed Purkinje cell firing. DA reduced the spontaneous firing rate of 57% of the Purkinje cells (n=54) while 37% were unaffected by DA. Only 6% of the cells responded to DA with an increase in spontaneous firing rate. A majority (94%) of Purkinje cells (n=36) showed a marked suppression of glutamate- or quisqualate-induced excitation in response to DA; the remaining 6% showed an enhancement of excitatory activity. The suppressive effect of DA on excitatory amino acid-induced activity was typically greater than its suppression of spontaneous activity. Nearly half (47%) of the Purkinje cells tested (n=17) exhibited no interaction between DA and GABA. In 29% of the cells the inhibition due to GABA was augmented by DA while in 24% GABA-mediated inhibition was reduced. Moreover, all of the suppressive effects of DA were mimicked by both PPHT (a nonselective D2-like receptor agonist) and 7-OH-DPAT (a D3-selective agonist) suggesting a D3 receptor mediation of these responses. This is in agreement with the reports of Bouthenet et al. (1991) and Levant et al. (1993) describing a population of D3 receptors on Purkinje cells in lobules IX and X of the rat cerebellum. In summary, DA acts as a neuromodulator to suppress the spontaneous activity of Purkinje cells and decrease their responsiveness to excitatory amino acids. This suppression of Purkinje cell activity would likely result in a higher firing rate of target neurons within the cerebellar nuclei, and thus a subsequent increase in cerebellar output. (Supported by NS18028 and NS08798.)

467.18

INCREASE IN VELOCITY-SENSITIVITY OF FLOCCULAR PURKINJE CELLS BY ACETYLCHOLINE. J van der Steen and H.S. Tan *Dept of Physiology, Faculty of Medicine, Erasmus University Rotterdam, Rotterdam, The Netherlands.* (Spon: European Neuroscience Organisation)

Behavioural evidence is emerging suggesting a positive modulatory action of acetylcholine (ACh) in the cerebellum. The present study describes such action of ACh on floccular Purkinje cells (P-cell) in rabbit. Using a sinusoidal optokinetic stimulus (0.2 Hz; Ampl 5°; max vel 6.3°/s) we tested simple-spike responses of P-cells sensitive to optokinetic stimulation about an earth-vertical axis (VA-cells) or a horizontal axis oriented at 135° azimuth (HA-cells). The firing rate of VA-cells (n=22) showed a drum-velocity sensitivity of 26 spikes/deg/s with stimulation in the excitatory direction. This excitatory direction corresponds with the temporal-to-nasal direction of optokinetic stimulation of the eye ipsilateral to recorded flocculus. In the inhibitory direction sensitivity was only 12 spikes/deg/s. Iontophoretic application of ACh enhanced sensitivity to 80 and 40 spikes/deg/s for the excitatory and inhibitory directions respectively. With drum velocities within the range of ± 3°/s the simple-spike rate of the HA-cells tested (n=17) showed a sensitivity of 7 spikes/deg/s in both directions. Higher velocities in both directions were cut off. ACh application increased sensitivity to 11 spikes/deg/s without affecting the cut-off level. Conclusion: The described pattern of velocity-sensitivity of floccular P-cells correlates with behavioural data on the optokinetic reflex. ACh enhances optokinetic responses of P-cells through increasing the P-cells' velocity-sensitivity, without extending their sensitivity range.

467.19

EXTRINSICALLY DERIVED GABAERGIC INHIBITION MODULATES THE INFORMATION TRANSFER THROUGH RAT PONTINE NUCLEI.

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In opposition to the traditional view as a pure relay station in the corticocerebellar pathway, the pontine nuclei (PN) contain a considerable number of GABAergic synapses. Immunohistochemistry suggests that the number of GABAergic neurons residing in the PN is substantially lower in the rat as compared to the monkey. We have asked whether this finding reflects a genuine species difference in the cellular composition of the PN. We have therefore reexamined the number of GABAergic neurons using the more sensitive technique of *in situ* hybridization which monitors the amount of mRNA for glutamic acid decarboxylase (GAD). Our finding of an almost complete absence of GAD mRNA expressing neurons in the PN confirmed the earlier results based on immunohistochemistry, suggesting that at least in rat the dense pontine network of GABA-positive fibers originates exclusively from extrinsic sources.

In order to study the synaptic characteristics of GABAergic inhibition of pontine neurons, we performed intracellular recordings from coronal and parasagittal slice preparations. IPSPs, elicited by electric stimulation (20-240 μA) of distinct sites within the pontine tegmentum, could be demonstrated in the majority of neurons sufficiently tested. The precise location of these stimulation sites and their relationship to afferents of the PN are currently under investigation. On their way to the PN the inhibitory axons probably cross the pedunculi cerebri as stimulation there under blockade of glutamatergic transmission (DNQX 25 μM) revealed IPSPs similar to those observed after tegmental stimulations. The IPSPs had amplitudes of up to 4.4 mV, latencies between 0.8 and 4.3 ms, a maximal duration of 40 ms, and a reversal potential of -75 to -79 mV. IPSPs were completely blocked by bicuculline (50 μM) suggesting an involvement of the $GABA_A$ receptor. In conclusion, we have demonstrated that inhibition affecting the pontine information transfer originates from outside the PN. *Supported by DFG SFB 307 A1.*

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ABSENCE OF GABAERGIC FEEDBACK PROJECTION FROM THE RAT LATERAL CEREBELLAR NUCLEI ON PRECEREBELLAR NUCLEI.

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The pontine nuclei (PN) and the nucleus reticularis tegmenti pontis (NRTP) are important targets of the lateral cerebellar nucleus (LCN). On the other hand these brainstem nuclei are sources of a direct excitatory projection to the cerebellar nuclei. It has been suggested that these backprojections from the LCN are partially GABAergic. In order to test this hypothesis we applied a combination of anterograde tracing (biotinylated dextran amine injection into the LCN) and postembedding GABA and glutamate immunogold histochemistry to the PN and NRTP. The pattern of labeling was compared to that of cerebellonuclear terminals in two target structures which in turn project to the spinal cord and neocortex: the nucleus ruber (NR) and the thalamus (VM/VL). The projection to the inferior olive (IO) known to be predominantly GABAergic served as a control. Quite unexpectedly we did not find any GABA immunoreactive (ir) cerebellonuclear terminals neither in the PN, NRTP nor in the NR and VM/VL. In contrast 73% of the terminals in the IO were GABA-ir. On the other hand, when applying glutamate immunohistochemistry more than 90% of the cerebellonuclear terminals in PN, NRTP, NR and VM/VL were labeled contrasting with only 5% in the IO. The cerebellonuclear terminals in the feedback projections to the PN and NRTP were smaller (1,1 μm^2), than those found in VL/VM and NR (1,75 μm^2). The smallest terminals were found in the IO (0,6 μm^2). Cerebellonuclear terminals in the PN, NRTP; VL/VM, and NR reached predominantly proximal dendrites. In summary our data do neither support a role for GABAergic inhibition in the feedback systems from the LCN to the PN and NRTP nor within the projections to the NR and VM/VL. Hence, the rat LCN and PN/NRTP may be connected reciprocally through excitatory synapses only. *Supported by DFG SFB 307-A1*

OCULOMOTOR SYSTEM: SACCADES—SUPERIOR COLLICULUS

468.1

INTRACOLLICULAR CONNECTIONS OF THE OPTIC LAYER IN THE TREE SHREW.

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Intracellular injections of biocytin in living brain slices were used to study the connections of neurons in the optic layer of the superior colliculus. The results indicate that the neurons in this layer differ from those of the superficial gray layer in at least two ways. First, in contrast to the narrow and wide field vertical cells in the adjacent part of the superficial gray layer, which have apical dendrites that ascend toward collicular surface, the optic layer neurons have radiate dendrites. These dendrites often span the optic layer and sometimes extend into the adjacent superficial and intermediate gray layers. Second, whereas the intracollicular axons of the narrow and wide field vertical cells terminate primarily within the subjacent layer, the optic layer neurons project most profusely near their somas. Prominent but less dense projections terminate within the adjacent gray layers. Other optic layer neurons have axons that extend for considerable distances without terminating, and may project to structures outside of the superior colliculus. These results support the hypotheses that optic layer neurons are distinct from those in the adjacent layers and that many modulate the flow of information from the superficial to the deeper gray layers. Supported by NIH grant EY08233.

468.2

INTERRUPTING SACCADES BY ELECTRICAL STIMULATION OF THE SUPERIOR COLLICULUS DETERMINES AN EXTENDED FIXATION ZONE.

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The spatial shift theory for control of saccades suggests that a organized wave of activity moves from caudal superior colliculus (SC) to the rostral pole. The wave starts in the buildup neurons (BUNs) and travels to the fixation neurons (FNs) whose activation serves to end the saccade. Results from rostral pole electrical microstimulation at saccade onset have supported this hypothesis. The effect of such stimulation is to briefly interrupt the ongoing movement, which resumes shortly after the termination of the stimulation and ends near the original target. This study attempts to determine the extent of the *fixation zone*, the SC region where stimulation produces interrupted saccades. We obtained interruption of saccades by stimulating the deep intermediate layers of the SC at locations caudal of the rostral pole. Prior to stimulation BUNs, not FNs, were recorded at these sites. One interpretation of these results is that fixation zone neurons (FNs and rostral BUNs) are actually one class of functional cells involved in saccade termination. Our reasoning is supported by an anatomic study which determined that direct projections to the omnipause neurons (OPNs) in the brainstem from the SC originate from the rostral SC but not exclusively the rostral pole.

Interrupted saccades are also produced by stimulation of the OPNs. While interrupted saccades produced by SC and OPN stimulations are similar, analysis showed some consistent differences. OPN stimulation induced interrupted saccades often overshot the target position (behavior consistent with decay of a resettable integrator for eye position), but those due to SC stimulation were hypometric (behavior consistent with spatial averaging at the level of the SC). However, the pulse train durations needed to produce an interruptive effect on saccades were considerably shorter in the SC. Further increases in stimulus train duration in OPN truncated saccades, while increasing stimulus duration at some sites in SC produced a redirected saccade.

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468.3

SACCADIC REACTION TIME IN MONKEY. I. SPATIAL SELECTIVITY AND EFFECTS OF GAZE ANGLE.

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The introduction of a temporal *gap* between the disappearance of a fixation point and the appearance of a peripheral target causes a reduction of saccadic reaction times (SRT). In addition to this reduction of SRT, presumably due to a gap-related disengagement of fixation, the occurence of *express* saccades having very short SRT is facilitated in this *gap* paradigm. The percentage of express saccades has been shown to increase with training, whose effect is spatially selective. The extent of this spatial selectivity, however, remains to be determined. Also relevant, the *gap* effect may depend on the eye position from which saccades are initiated. Two monkeys were trained to perform saccades to a single visual target (10° of eccentricity) presented either simultaneously with the disappearance of the fixation point (step task), or after a gap period (gap task). In both monkeys, SRT distribution in the gap task displayed bimodality, i.e., an express saccade peak at ~100ms and a regular saccade peak at ~150ms. After a period of training at this one target location, target location was randomly varied in either amplitude or direction. The monkeys made no express saccades to the novel target locations, unless they were nearby the previously trained target location: within about ±50° or ±20° of direction and ±5° of amplitude. The gap-related reduction in SRT was nevertheless observed for all targets. The position of eye fixation was also randomly varied when the animals were required to make saccades to two targets presented randomly on either side of the fixation point. Varying the initial gaze angle changed the relative frequency of the different modes of SRT. The percentage of express saccades increased as the movements started more contralateral. To explain our results, we suggest that training-dependent express saccades are caused, at least in part, by local changes – coding for the trained movements – restricted to a specific locus in a neural map of saccades, e.g., superior colliculus. Furthermore, these local changes are modulated by a gaze angle signal such that movements toward central eye position are facilitated.

468.4

SACCADIC REACTION TIMES IN MONKEY. II. NEURAL CORRELATES IN THE SUPERIOR COLLICULUS.

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The reduction in saccadic reaction times associated with the introduction of a temporal gap of no stimuli between the disappearance of an initially fixated visual target and the appearance of a peripheral saccadic target is called the *gap effect*, and occurs irrespective of target location. Extremely short latency *express saccades* (reaction times ~ 100 ms) may be elicited using this gap paradigm after training to a specific target location. We recorded from single neurons in the intermediate layers of the superior colliculus (SC) of monkeys trained to generate saccades in the gap paradigm. Fixation neurons (FNs), located in the rostral SC, were tonically active during fixation and paused for saccades. During the gap, FN discharge rate decreased by about 50% to a minimum level 250 ms into the gap, and then slowly increased for longer gap durations. The level of FN discharge at target onset was highly correlated to the ensuing saccadic reaction times. Saccade-related burst neurons (BNs) and buildup neurons (BUNs), discharged visual and saccadic motor bursts following target appearance at a specific point in the visual field. During the gap, BNs remained silent, whereas BUNs, located at an SC site coding the amplitude and direction of a potential saccadic target, became tonically active. Prior to the generation of express saccades, the anticipatory BUN discharge rate during the gap was higher. We conclude that the reduction in FN discharge that occurs during the gap period partially disinhibits the entire SC saccade generating map leading to the 'global' reduction in saccadic reaction times known as the gap effect. The occurrence of express saccades, however, requires an additional gap-related increase in discharge of local populations of BUNs coding for trained target locations.

468.5

AUDIO-VISUAL INTEGRATION IN THE SUPERIOR COLLICULUS OF THE AWAKE MONKEY M.A. Frens[1,2] and A.J. van Opstal[1]*.
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We have recorded single-unit activity in the deep layers of the Superior Colliculus (SC) of two rhesus monkeys, trained to make saccadic eye movements towards visual targets, while ignoring a simultaneously presented auditory noise burst. The position of the sound stimulus relative to the visual target was systematically changed. The properties of these bimodal saccades were similar to saccades elecited by unimodal visual stimuli, except for a clear change in movement latency. Furthermore, the saccade reaction time was significantly modulated by the spatial distance between the visual and the auditory stimuli in 36% of the recordings. The timing of the saccade-related burst in SC units remained tightly locked to the saccade under unimodal and bimodal conditions. In most units, the saccade-related activity for otherwise identical saccades was significantly affected by the auditory distractor (mean enhancement of 95% in 26/50 units; mean suppression of 31% in 19/50). In 18/50 units, spatial separation of the stimuli induced a systematic change in the firing rate, which co-occurred strongly with the effect on saccade latency.

We conjecture that the audio-visual interaction effects on the activity in the SC result from (1) changes in the excitability of the cells, necessary to initiate the saccade-related burst, and (2) a change in the balance of various afferent signals. The observed large variations in SC activity were not related to either saccade kinematics or accuracy, and are thus incompatible with a dynamic motor error code.

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468.6

SUPERIOR COLLICULUS ACTIVITY DURING ORBITALLY DEPENDENT REMEMBERED SACCADES. E.J.Barton, R.P. Kalesnykas, T.R. Stanford*, D.L.Sparks. Univ of Pennsylvania, Philadelphia, PA. 19104

The direction and amplitude of visually-guided saccades in the light are relatively independent of the initial eye position, within the range +/- 20 deg. However, in monkeys, saccades to remembered target displacements result in horizontal and vertical biases, which are markedly influenced by the initial eye position (Barton and Sparks, *Neurosci. Abst.*, 295.18, 92). The influence of the starting orbital position is large, systematic, and consistent across monkeys. The behavioral and neural data strongly suggest that the effect of orbital position during remembered saccades is a direct function of neural coding and not a function of the mechanical properties of the plant (Barton and Sparks, *Neurosci Abstr.*, 321.8, 93).

We recorded from saccade related burst neurons (SRBNs) within the superior colliculus (SC) of a rhesus monkey to examine whether the SC codes the orbitally-dependent biases. If the SC activity includes the biases, the response of any SRBN should correspond to the amplitude and direction of the movement, whether or not the movement is an accurate visually-guided saccade or a deviated remembered saccade. Whereas, if the SC does not include the biases, the discharge will better correspond to the target location than to the amplitude and direction of the inaccurate remembered saccade. In the former case, changes in the starting position of remembered saccades will affect the discharge according to the magnitude of the bias introduced, but in the latter case, the activity will be independent of bias magnitude.

The activity of the SRBNs studied reflected the target location rather than the amplitude and direction of the movements. The cells fired similarly for the grossly inaccurate remembered saccades as for the accurate visually-guided saccades to the same targets. Furthermore, when the targets for the visually-guided and remembered trials were different, but the movements were of comparable amplitude and direction, the cells fired dissimilarly.

This suggests that the orbitally-dependent biases are introduced to the saccadic system after the level of the SC and represents yet another example of a dissociation between the SC command and the executed movement.

468.7

COMPONENT STRETCHING DURING OBLIQUE SACCADES: COMMON SOURCE VS. CROSS-COUPLING MODELS. M. James Nichols and D.L. Sparks.* Institute of Neurological Sciences, and the Department of Psychology, University of Pennsylvania, Philadelphia, PA 19104.

Electrical stimulation at sites in the primate superior colliculus (SC) can evoke saccades with latencies down to 20 ms or shorter. For a given site and fixed stimulation parameters, evoked saccades have very stereotyped direction and amplitude. The activity at particular SC sites, then, provides a vectorial saccadic request that must subsequently be decomposed for downstream horizontal and vertical pulse generators. It has long been known, however, that the horizontal and vertical pulse generators do not operate independently during oblique saccades. Rather, the minor component is typically reduced in velocity and stretched in duration to better match the duration of the major component.

It has been proposed previously that component stretching might be accomplished by appropriate decomposition of a vectorial velocity signal ("common source model"; van Gisbergen et al., 1985). Furthermore, a growing body of evidence demonstrates that the level of SC activity systematically influences vectorial saccadic velocity. It therefore seems quite plausible that the SC could provide the common source vectorial velocity signal. An active site in the motor map would send a velocity command to each component burst generator that was weighted appropriately for the saccade direction normally specified by that site. Alternative models have proposed that independent horizontal and vertical pulse generators could be cross-coupled downstream from the SC to produce component stretching (e.g., Grossman and Robinson, 1988).

An experimental paradigm originally proposed by Nichols and Sparks (1994, 1995) to test other aspects of saccadic control can also distinguish between these two schemes for component stretching. Experimental results will be presented.

468.8

MULTIMODAL ADAPTIVE CONTROL OF SACCADIC EYE MOVEMENTS BY SUPERIOR COLLICULUS. ((S. Grossberg [1,2,3], K. Roberts[1,2], and D. Bullock[1]). Department of Cognitive and Neural Systems, Boston University, Boston, MA 02215.

How does the saccadic eye movement system select a target when visual, auditory, and planned movement commands differ? Recent data on superior colliculus (SC) reveal a traveling wave of activation whose peak codes the current gaze error (Munoz et al., 1992). In contrast, Waitzman et al. (1991) found that a stationary locus of peak activity in SC decays as a function of residual gaze error. We develop a neural network model that answers the question posed while providing a functional rationale for both signals. Calibration between visual inputs and eye movement commands is learned early in development within a visually reactive saccade system. The accuracy of planned saccades derives from using the gains learned by the reactive system. Visually reactive locations on the SC motor map encode gaze error in *retinotopic* coordinates. Planned targets are coded in *head-centered* coordinates. These distinct types of signals must be adaptively aligned to ensure selection of a single target. For this, a transformation between a planned and a retinotopic target representation needs to be learned. The targets can then compete for attention to generate a movement command in motor error coordinates. In addition, converging, spatially-coincident, visual and auditory signals produce response enhancement in collicular cells that is greater than the sum of the responses to each signal, as well as response depression when paired auditory and visual stimuli are not spatially coincident. The model simulates this effect as well as cell profiles measured in visually guided, memory guided, overlap, and gap paradigms. [1]Supported in part by ONR (N00014-92-J-1309). [2]Supported in part by ARPA (ONR N00014-92-J-4015). [3]Supported in part by AFOSR (AFOSR F49620-92-J-0499).

468.9

AFFERENT CONNECTION TO FIXATION ZONE OF MONKEY SUPERIOR COLLICULUS FROM FRONTAL CORTEX AND BASAL GANGLIA H. Aizawa*, C. R. Gerfen, and R. H. Wurtz. Lab. of Sensorimotor Research, NEI, NIH, and Lab. of Systems Neuroscience, NIMH, NIH, Bethesda, MD 20892.

Recent experiments in the monkey identified fixation cells in the rostral pole of the superior colliculus (SC) with properties distinct from saccade related cells in the caudal SC (Munoz and Wurtz, 1993). The aim of this experiment is to determine whether the fixation cells of the SC receive projections distinct from those of the saccade related cells.

The rostral fixation cell area was distinguished from the caudal saccade area by recording single cells and by stimulating electrically. We identified the fixation area by recording cells that paused during saccades and by electrically stimulating to delay saccades. Using a 30 gauge syringe needle with a protruding micro wire to verify its tip location by recording and stimulation, we then pressure injected fastblue (FB: 2%, 0.2 - 0.4µl) and diamidinoyellow (DY: 5%, 0.2 - 0.4µl) into the fixation cell area and the saccade cell area.

Retrogradely labeled cells were identified in ipsilateral frontal eye field (FEF) and substantia nigra pars reticulata (SNr) of rhesus monkeys. In FEF, cells labeled by rostral SC injection were found in the more lateral part of anterior bank of the arcuate sulcus and cells labeled by caudal SC injection were clustered more medially on the anterior bank in the arcuate sulcus. In SNr, cells labeled by rostral SC injection were found in the more caudal dorsolateral part, and cells labeled by caudal SC injection were found more ventromedially. We also found cells labeled by rostral SC injection in contralateral rostral SC. In contrast, none, if any, labeled cells were found in contralateral SC following caudal SC injection.

These findings show 1) that the projections to the fixation and saccade regions of SC receive input from different areas of FEF and SNr, and 2) that the cross connection between the two rostral SC could underlie the coordination of fixation activity on the two sides.

469.1

THE FRONTAL EYE FIELDS OF THE AWAKE, BEHAVING CAT. T.G. Weyand* and A.C. Gafka. Dept. Anatomy, LSU Medical Center, New Orleans, LA 70112

We have recorded the activity of > 300 isolated neurons in a region of cortex that corresponds to the frontal eye fields in the awake, behaving cat. In these experiments, head position is fixed and eye position (gaze) is determined using the scleral search coil technique (resolution < .2°). The cats were trained to fixate on a small red spot that could be positioned anywhere on a rear projection screen. Our assumption was that this cortical region was involved in generating saccadic eye movements. Towards that end, we were particularly concerned with evaluating activity of neurons prior to saccadic eye movements and during active fixation. Only a small fraction (< 3%) of the neurons exhibited clear pre-saccadic activity. Most neurons were modulated by fixation, with suppression of activity during fixation being the most common response. At least one third of the neurons were visually responsive. Most of these had huge (hemi-field) receptive fields with a preferred direction of motion either towards or away from the hemisphere we were recording from. Visual responsiveness tended to be profoundly suppressed when fixation was demanded in the behavioral tasks. We were unable to determine any modulation of activity in one third of the neurons encountered. This estimate is conservative, as we were attracted to study those neurons that appeared influenced by the tasks.

Although this region of cortex shares some common cell types with the primate frontal eye fields, its influence on oculomotor behavior appears less direct. Rather than dictate via pre-saccadic elements where gaze should be directed next, an important role of this area in gaze control is elimination of potential targets for saccadic eye movements. (Supported by NIH EY06818 and MRC Canada)

469.2

FRONTAL EYE FIELD ACTIVITY IN CONJUNCTION WITH SACCADES MADE IN RESPONSE TO STEP-RAMP MOTION. D. Shi*, H. R. Friedman, and C. J. Bruce. Section of Neurobiology, Yale Univ. Sch. Med., New Haven, CT 06520-8001.

Saccades to moving targets are accurately targeted despite the fact that saccades are executed ballistically and take 100–200 ms to program, being unaffected by target displacements occurring less than 100 ms prior to initiation of the saccade. Therefore, we studied saccade-related activity in the macaque monkey's FEF during saccades from stationary fixation to moving targets to determine if target velocity is predictively compensated for at the level of FEF. After the optimal vector for each cell's saccade-related response was estimated by testing with static targets, a parametric step-ramp experiment was conducted wherein targets moved towards the cell's response field along paths perpendicular to the cell's optimal vector (e.g., leftward and rightward ramps for an upward response field). The monkeys' saccadic behavior generally compensated fully for target velocity, and in many cases the saccades even ended slightly ahead of the targets. We then analyzed the neuronal data by modifying our previous Gaussian model for directional tuning of response fields by adding a single parameter (K) to quantify compensation for target velocity: $R_i = B + R \cdot EXP(-0.5((\emptyset - S\emptyset_i + K \cdot d T\emptyset_i)/\partial)^2)$ where R_i is the cell's spike rate for each trial, $S\emptyset_i$ is the saccade's polar direction, $d T\emptyset_i$ is the target's angular velocity, and the remaining parameters are all to be fit, with $\emptyset$ estimating the cell's optimal polar direction, ∂ its directional tuning, and K estimating (in ms) the velocity compensation being added downstream of the FEF cell in question. Some FEF cells appeared to be upstream of all velocity compensation (e.g., K of 230±30 ms), but other cells had considerably smaller K, e.g., 90±20 ms, indicating that they were at an intermediate stage of velocity compensation. This data may help explain why projections from motion areas in posterior cortex (MT, MST, STP, etc.) are not confined to FEF's smooth pursuit representation in the depths of the arcuate sulcus, but terminate extensively in saccadic FEF as well. We hypothesize that these projections enable saccade-related FEF activity to begin the process of predictive compensation for target velocity. Supported by PHS grants EY04740 & MH44866.

469.3

REVERSIBLE ACTIVATION AND INACTIVATION OF MONKEY FRONTAL EYE FIELD WITH GABAERGIC DRUGS. E. C. Dias*, M. Kiesau and M. A. Segraves. Dept. of Neurobiol. and Physiol., Northwestern Univ., Evanston, IL 60208.

Although compelling anatomical and physiological evidence link the primate frontal eye field (FEF) to the generation of eye movements, chronic lesions produce minimal long-term oculomotor deficits. To test the effects of acute activation or inactivation of the FEF, we made microinjections of GABA-related substances in 2 rhesus monkeys and recorded the animal's spontaneous as well as task-related oculomotor performance.

Injections of muscimol, a GABA agonist with inhibitory effect, produced an oculomotor scotoma. When the monkey was performing a visually-guided saccade task it was unable to saccade to targets in the injection site's movement field, while easily making saccades to targets at other locations. In a memory-guided saccade task these effects were enhanced revealing at least two deficits in oculomotor performance. When the target appeared in the movement field of the injection site, the monkey failed to saccade towards it. When the target appeared in the opposite direction, the monkey made irrepressible saccades towards it, i.e. the monkey looked at the peripheral target as soon as it came on, not waiting for the go-signal, which in this task was the extinction of the central fixation target. The monkey also showed difficulty in maintaining fixation after the injection. In contrast, when bicuculline, a GABA antagonist that produces disinhibition of cortical cells, was injected at the same site, on a different day, the monkey's response to a target at any location in a visually-guided saccade task was to first make a saccade toward the movement field of the injection site followed by a saccade to the target. Difficulty in maintaining fixation prevented the monkeys from performing a memory-guided saccade task. When idle the monkey spontaneously produced series of short-latency saccades of approximately the same direction and amplitude as the saccade elicited electrically from the injection site, similar to the staircases of saccades obtained by electrically stimulating the FEF with long trains of electrical pulses. Control injections of saline did not produce observable changes in the monkey's performance.

These findings suggest monkeys with surgical FEF ablations show substantial recovery from the acute effects of FEF lesions and that FEF activity in the intact primate is important for the generation of all voluntary saccades and for fixation. *Supported by NIH Grant EY08212.*

469.4

METRICS OF SACCADES EVOKED BY ELECTRICAL STIMULATION IN THE FRONTAL EYE FIELDS ARE NOT AFFECTED BY SHORT-TERM SACCADIC ADAPTATION. J. A. Edelman* and M.E. Goldberg. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

Monkeys and humans can rapidly adjust saccadic amplitude to compensate partially for intrasaccadic target perturbations. Previous work in this laboratory has shown both that the movement fields of saccade-related burst neurons in the primate superior colliculus (SC) change after such short-term saccadic adaptation (STSA), and that the metrics of saccades evoked by electrical stimulation of the SC are not affected by STSA. This paradox can be resolved if stimulation of the SC fails to entrain a corrective mechanism located in the deep cerebellar nuclei, lesions of which abolish STSA. To test whether stimulation of the frontal eye fields (FEF) entrains this corrective mechanism we stimulated in the FEF before, during and after STSA in two monkeys. A target was initially presented at the same location as the end-point of saccades evoked by low-current (<50 μA) electrical stimulation. First, the monkey performed ~150 pre-adaptation trials. Next, at saccade onset the target was stepped back a fixed percentage (50%) of the initial target displacement. Adaptation was usually complete within ~400 trials, after which another ~150 target-displacement trials were performed. On ~10% of both kinds of trials no target was presented and stimulation was applied ~400ms after the central fixation point was extinguished. Eye movements were recorded during a block of trials before, during, and after saccadic adaptation at each of 7 stimulation sites. Across all sites, non-stimulated saccade amplitude decreased by an average of 27% while stimulated saccade amplitude increased by 1%. At all sites the time course of any change in stimulated saccade amplitude was not related to that of STSA. These data suggest that like SC stimulation FEF stimulation does not activate a cerebellar pathway that adjusts saccadic amplitude.

469.5

THALAMOCOCORTICAL INPUT TO THE SLOW EYE MOVEMENT AND SACCADIC EYE MOVEMENT SUBREGIONS OF THE FRONTAL EYE FIELD IN CEBUS MONKEY J.-R. Tian*[1], Hao Liu[1] and J.C. Lynch[1,2,3]. Depts. of Anatomy[1] Ophthalmology[2], and Neurology[3], University of Mississippi Medical Center, Jackson, MS 39216

Thalamocortical projections to the slow eye movement subregion (SEM) and the saccadic eye movement subregion (SAC) of the frontal eye field (FEF) were studied in 4 *cebus apella* monkeys. The SEM and SAC were localized using standard low-threshold (<50 μA) electrical microstimulation procedures and Telazol anesthesia. The supplementary eye field (SEF) and the hand/arm region of dorsal premotor cortex (PMd) were also localized in some monkeys. Retrogradely-transported fluorescent dyes were then injected into these areas. Eye movements were recorded and later analyzed using a video recording system. The major sources of thalamic input to SEM were VA, VLc, and MD. In each of these, labeled neurons are clustered in the most dorsal part of the nucleus. In contrast, neurons labeled by SAC injections were concentrated in paralaminar MD and in VA; neurons labeled from SEF were concentrated in VA and X; and neurons labeled from PMd were concentrated in ventral VLc, VLo, and VPLo. Although the SEM was adjacent to $PMd_{(hand)}$, the connections of the two regions were clearly distinct. Previous studies have established that VA, rostral VLc, and MD receive input from the globus pallidus or substantia nigra, whereas caudal VLc, X, VPLo, and MD receive input from the cerebellum. Thus each oculomotor subregion, as well as PMd, receives a major neural input from both a basal ganglia target and a cerebellar target in the thalamus.

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469.6

SUPPLEMENTARY EYE FIELD NEURONAL ACTIVITY DURING MONKEY PERFORMANCE OF ANTISACCADE TASKS. N. Amador, M.Schlag-Rey*, J.Schlag & H.Sanchez. Brain Research Institute, UCLA, Los Angeles, Ca. 90095-1761

Human antisaccades made to look at a location opposite to that of a visual target are known to be selectively impaired by frontal lobe and basal ganglia lesions while visually guided prosaccades are not.

We studied neuronal activity in the monkey Supplementary Eye Field (SEF) during the performance of immediate and delayed anti- and prosaccades in directions chosen to coincide with each cell preferred or null direction (interleaved trials). The cue for making anti- or prosaccades was a small dot or square appearing as the initial fixation point. The saccade target (always a dot) was flashed during or after fixation of the instruction cue which, when turned off, provided the go signal for the eye movement. The cells studied were confined to the SEF region defined by low threshold electrically evoked saccades (some of them, goal-directed).

Unit responses to instruction cues did not differentiate pro- and antisaccades trials. Fixation cells, characteristic of the SEF area, were activated (or silenced) during the initial fixation period. Visuomovement cells, sustained (VM) or twice activated (V&M) revealed a dissociation of their visual and movement components as a function of the task. Some of these cells increased their firing more before antisaccades than before prosaccades having identical vectors. Such cells were not observed in the Frontal Eye Field of the same monkey performing the same task.

The results support the hypothesis of a distinct role for the SEF in initiating intentional saccades to goals internally rather than externally defined, i.e. in gaze behavior competing with visual reflexes. *(Supported by USPHS grants EY02305 and EY05879).*

469.7

RESPONSE FIELDS OF NEURONS IN AREA LIP DURING PERFORMANCE OF DELAYED SACCADE AND SELECTION TASKS M.L. Platt* and P.W. Glimcher. Center for Neural Science, New York University, New York, NY, 10003.

We examined the response fields of single LIP neurons in head-fixed rhesus macaques performing delayed saccade and selection tasks. Both tasks began with the fixation ($\pm 2^0$) of a central yellow LED. In the delayed saccade task, a second yellow LED was then briefly illuminated (200-1000 msec). After 200-1000 msec, the fixation LED was then extinguished, cueing the monkey to align gaze with the no longer visible target ($\pm 3^0$). In the selection task, fixation of the central LED was followed by the illumination, for 200-800 msec, of two yellow eccentric LEDs, one above and one below the horizontal meridian. The fixation LED then changed color to either red or green. Red indicated that the monkey would be rewarded for aligning gaze with the upper LED ($\pm 3^0$) upon offset of the fixation LED, green specified the lower LED as the saccade goal. Eccentric targets were located at 4^0 intervals spanning 40^0 of horizontal and vertical visual angle. Neurons were also studied with a delayed version of the selection task in which potential targets were extinguished before the fixation LED changed color.

Cells studied typically had low baseline activity that increased to a maximum (<100 Hz) during saccades of particular directions and amplitudes. For each delayed saccade trial, we measured activity during four canonical epochs: 1) 200 msec after the illumination of the eccentric target; 2) 200 msec after the offset of the eccentric target; 3) 100 msec before the beginning of the saccade; and 4) 200 msec after the beginning of the saccade. On selection trials, activity was also measured 200 msec after the color change cue. Detailed response fields were constructed for LIP units during each epoch. Some units showed a maintained increase in activity across measured epochs for particular target locations/movement metrics. Other cells displayed discrete bursts of activity after the illumination of particular target locations and immediately preceding saccades of particular amplitudes and directions. On selection and delayed selection trials, sensory non-movement response fields (Glimcher and Sparks, *Nature*, vol. 355, 1992) of unit activity during the epoch following the color change cue were not co-extensive with movement fields for the same epoch. Comparison of these target-movement and distractor non-movement response fields suggests that LIP neurons may be differentially sensitive to targets and distractors.

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469.8

NEURONS OF THE LATERAL GENICULATE (LGN) CARRY AN EYE POSITION-RELATED SIGNAL POST-SACCADICALLY. L. Chukoskie, A. Handel & P.W. Glimcher*. Center for Neural Science, New York University, New York, NY, 10003.

Single units of the lateral geniculate were studied in two head-restrained awake-behaving rhesus monkeys while they performed delayed and memory saccade tasks in dim illumination and in total darkness. Units were found which burst vigorously after saccades. The magnitude of this burst, even when it occurred in total darkness, was correlated with the post-saccadic orbital position of the monkey's eye.

In our total-darkness memory-saccade task, an animal fixated a central LED ($\pm 3^0$). A second eccentric LED was then illuminated for 600-900 msec and extinguished. After a further 300-500 msec, the fixation LED was extinguished, placing the animal in total darkness and cueing him to make a saccade (typically with a 200 msec reaction time) which aligned gaze with the remembered location of the eccentric target ($\pm 4^0$).

We studied the first 300-400 msec of this post-saccadic burst by plotting the mean frequency of post-saccadic firing (as measured in the 300 msec interval that began 100 msec after saccade onset) as a function of final eye position with regard to the animal's fixed head. These LGN units were typically found to have a preferred final eye position for which they responded most vigorously. As final eye positions deviated from this preferred position, the magnitude of the post-saccadic response declined. By systematically varying the location of the initial fixation target, we were able to determine that the spatial tuning of these units *did not* correlate with the amplitude and direction of the just completed movement but *did* correlate with the final position of the eye.

To confirm that these LGN signals were correlated with eye position we performed an additional experiment in which units were studied both before and after an animal was mechanically rotated in the visually fixed environment of the experimental chamber. We found that this manipulation, which did not alter the animal's visual environment, did shift the position tuning of the unit in the direction predicted by the rotation of the monkey. Histological analysis of several marking lesions in one animal revealed that responses of this type were encountered within the magnocellular layers of the LGN_d.

469.9

THE CONTROL OF SACCADES, SMOOTH PURSUIT EYE MOVEMENTS AND FIXATION: A PET STUDY. E.P. O'Sullivan, R.C. Miall, P. Haggard, J.F. Stein, C. Kennard, D.J. Brooks SPON: Brain Research Association. MRC Cyclotron Unit, Hammersmith Hospital, London W12 0HS.

Visual fixation of a stationary object has been thought of as a smooth pursuit eye movement (SPEM) of zero velocity. However, there is electrophysiological and behavioural evidence suggesting that fixation affects the performance of both the saccadic system and the SPEM system, implying that it is not merely a specialised form of SPEM, but that it is derived from a separate neural pathway.

To investigate the neural substrates controlling these oculomotor systems, we studied 6 male volunteers using $H_2{}^{15}0$ positron emission tomography (PET). Reflexive saccades to unpredictable targets, smooth pursuit at a range of velocities and fixation were tested.

Comparison of fixation with saccades, and of fixation with SPEM showed significant increases ($p < 0.001$) in regional cerebral blood flow (rCBF) in the dorsolateral prefrontal cortex and the cingulate cortex.

Both the saccadic and SPEM tasks resulted in significant rCBF increases in the striate, extrastriate and temporal lobes relative to fixation. In addition, the saccadic task also showed increased rCBF in the posterior parietal lobe and the frontal eye fields. This pattern of activation was not seen for SPEM. The comparison of saccades and SPEM showed no overall significant change in rCBF.

This study suggests that the control of visual fixation includes a neural pathway which is separate from those controlling saccades and SPEM. It also demonstrates that there is much overlap between the pathways that control saccades and SPEM.

469.10

VISUALLY-GUIDED SACCADIC EYE MOVEMENTS ELICIT ACTIVATION IN FRONTAL OCULOMOTOR AREAS IN HUMAN BRAIN USING FUNCTIONAL MAGNETIC RESONANCE IMAGING (fMRI). K.-M. Lee[1,2], J. Hirsch*[1,2], K. Kim[1,2], R. L. DeLaPaz[1,3], N. Relkin[1]. Dept. of Neurology and Neuroscience, Cornell University Medical College[1]; Dept. of Neurology[2] and Radiology[3], MSKCC, New York, NY 10021

We studied human cortical oculomotor systems with fMRI by the following parameters: 1.5 T magnetic resonance scanner (GE) retrofitted for echo-planar imaging (Advanced NMR Instascan), T2*-weighted images by gradient echo sequence (TE = 60 ms, TR = 3 secs, flip angle = 30 deg) with in-plane resolution of 1.56 x 1.56 mm (128 x 256 matrix in 20 x 40 cm FOV) and 16 contiguous 4.7 mm thick slices parallel to the AC-PC line. Independent t-test with requirement of replication was employed to compare the mean intensity of each voxel between baseline and activation periods. During activation periods, four right-handed healthy volunteers were asked to make saccadic eye movements to a small dot that was back-projected onto a screen. The direction and amplitude of saccades were controlled so as to obtain cortical activation as specific as possible for invariant-vector saccades vs. saccades to and from an invariant eye position. Activation was observed at punctate regions in the frontal cortex. There was an asymmetry of those regions in that the activated area was more dorsomedially located in the left hemisphere of all subjects. Furthermore, separate but adjacent areas were identified where the saccade-related activation depended upon eye position as well as the direction of eye movement. In summary, with fMRI, we have localized areas in the frontal cortex that show increased activity with visually-guided saccades and observed that some areas code for the direction of saccades without eye-position dependency, while others show activation that is dependent upon eye position as well as saccadic direction. (Supported by MSKCC (JH, RD), C.V. Starr Foundation (NR), Morris Foundation (KK))

469.11

DIFFERENTIAL EFFECTS OF REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION OVER CORTICAL VISUOMOTOR AREAS IN PREFRONTAL AND PARIETAL CORTEX IN MAN. S.A. Brandt, C.J. Ploner, B.-U. Meyer P. Stoerig* and A. Villringer. Laboratory of Neurophysiology, Department of Neurology, Charité, 10098 Berlin, Germany.

In an earlier study we showed that repetitive, rapid-rate transcranial magnetic stimulation (rTMS) over the dorsolateral prefrontal cortex (DLPFC) impairs performance of memory-guided saccades in humans. Now we are using rTMS to compare the differential effects of stimulation over different cortical visuomotor areas during different phases of the premotor planning of saccades. Precision, latency and velocity of saccades to memorized locations were measured after rTMS (10 Hz) over the prefrontal and parietal cortex of either hemisphere. We compared rTMS shortly before or after presentation of a visual cue with rTMS performed at different intervals during the delay period before saccades had to be executed. Results show that prefrontal rTMS impairs saccade latency and precision maximally when performed during the delay period, whereas parietal rTMS had a maximal effect when performed shortly before or after cue presentation. Our findings are compatible with the hypothesis that the parietal cortex is a critical structure during the early phase of visuospatial delay tasks, and that the prefrontal cortex (area 46) is involved in the transient representation of visuospatial information in absence of the visual cue. RTMS is a powerful tool to study functions of visuomotor system by interferring with information processing in selected cortical areas non-invasively in humans.

469.12

TRANSCRANIAL MAGNETIC STIMULATION (TMS) ALTERS HUMAN SACCADE INITIATION IN A COUNTERMANDING PARADIGM. J. D. Olson†, S. Anand† and J. Hotson‡†*. Dept Neurology & Neurological Science, Stanford Univ Sch Med† and Calif Inst Med Res‡, Stanford and San Jose, CA.

Purpose: Performance in a saccade countermanding paradigm can be modeled as a competition between initiation vs inhibition of saccades. Since TMS over frontal cortex delays saccade initiation, we asked whether TMS could selectively shift the balance between initiating and withholding saccades in this paradigm. **Methods:** In >67% of the trials, two subjects rapidly initiated a saccade to a go-signal target presented 4.5° to the right or left of a central fixation target that was simultaneously extinguished. Stop trials were interwoven in which the central fixation target reappeared as a signal to withhold the saccade. Stop signal delays (SSD) ranged from 0-250 ms after the onset of the go signal. Single pulse TMS was delivered at 80-100% maximum intensity with a butterfly coil 10, 25 and 50 ms after the onset of the stop signal. **Results:** In control trials without TMS, results were similar to those reported in the macaque monkey. Saccades were rarely initiated at SSD of 0-50 ms and were rarely stopped at SSD of 175-250 ms. Saccade gain was near unity for go trials. TMS over right or left dorsolateral frontal cortex (DLFC) or a midline parietal area reduced the frequency of normometric saccades at SSD of 125-200 ms when compared to control results. This effect was greatest for TMS 10 ms after the stop signal. At the shortest SSD, however, TMS delivered 10 ms after the stop signal tended to increase the frequency of saccade initiation. Saccade gain was less than 0.5 for TMS trials at the shortest SSD and increased at longer SSD. **Conclusion:** TMS can decrease saccade initiation at long SSD, but may also increase the initiation of hypometric saccades at short SSD. These responses are not unique to TMS delivered over DLFC.

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469.13

TRANSCRANIAL MAGNETIC STIMULATION OF THE POSTERIOR PARIETAL CORTEX AND PREFRONTAL CORTEX DURING MEMORY-GUIDED SACCADES. R.M. Muri*, A.I. Vermersch, S. Rivaud, B. Gaymard, C. Pierrot-Deseilligny. INSERM 289, Hôp. de la Salpêtrière, 47 Bld de l'Hôpital, F-75651 Paris, France, and Dep. of Neurology, University of Bern, Inselspital, CH-3010 Bern, Switzerland

The posterior parietal cortex (PPC) and the dorsolateral prefrontal cortex (PFC) play an important role in the control of memory-guided saccades (1,2). In both regions, single cell recordings in monkeys have shown sustained activity during the different phases of the paradigm. In humans, however, only little is known about the functional and temporal organization of memory-guided saccades. Therefore, transcranial magnetic stimulation (TMS), which can disturb saccadic programmation (3), was used to determine such organization. Single TMS was applied in 8 normal subjects over the right PPC and in 7 subjects over the right PFC. Temporal mapping of both regions was performed by stimulating 160ms, 260ms, 360ms after the flashed target, during the mid-memorization period (800 - 1400ms), and 100 ms after the go-signal, i.e. the extinguishing of the central fixation point. Horizontal component of saccades were recorded by electrooculography. Compared with the percentage of error in amplitude (PEA) without stimulation (mean: 8%), a significant increase of PEA was found for contralateral saccades if TMS was applied over the PPC 260ms after the flashed target (mean: 18%, Mann-Whitney, $p < 0.0008$), and, secondly, if TMS was applied over the PFC during the mid-memorization period (mean: 27%, $p < 0.0002$). Control experiments with stimulation over the occipital cortex during the same time intervals had no significant influence. These results suggest that the observed effect of TMS seems to be specific to the stimulated region. Furthermore, for the functional organization of memory-guided saccades, the PPC might play a major role during the early phase of the paradigm, whereas the PFC seems to be more important during the memorization phase.

1. Gnadt J.W. et al. Exp. Brain Res. 70: 216-20 (1988).
2. Funahashi S. et al. J. Neurophysiol. 61: 331-49 (1989).
3. Müri R.M. et al. Exp. Brain Res. 101: 521-4 (1994).

OCULOMOTOR SYSTEM: HEAD MOVEMENTS AND LISTING'S LAW

470.1

CATS USE MULTIPLE COORDINATION PATTERNS TO ACHIEVE THE SAME MOTOR TASK. K. D. Statler, E. A. Keshner, S. L. Delp, and B. W. Peterson*. Sensory Motor Performance Program, Rehab. Inst. of Chicago, Dept. of Biomedical Engineering and Dept. of Physiology, Northwestern University, Chicago, IL 60611.

Simultaneous video-fluoroscopic and neck muscle EMG data were recorded from one cat performing ±15° sinusoidal (0.25 Hz) head tracking movements in the sagittal plane. The cat performed the movement in two different body postures and with two different starting neck orientations. Radio-opaque markers were inserted into the anterior/posterior and lateral aspects of the occipital ridge and C_1-C_7 to measure vertebral displacement. Kinematic data of the head and neck were analyzed using a computer model. The model was used to characterize the limits of movement in the cervical spine and to estimate the moment arms of the neck muscles at different orientations of head-neck movement. Experimental results showed that the cat utilized different vertebral alignments, relative joint movements, and muscle activation patterns to achieve the same movement outcome. The model predicted that muscle moment arms would vary little for the different vertebral alignments chosen by the cat to complete the task varied as the movement was performed from different initial conditions. When the cat was standing, the movement was accomplished with joint movements about Skull-C1, joints on either side of C3, and joints on either side of C6. When the cat was prone, the movement was accomplished with joint movements about Skull-C1 and joints on either side of C3. Rectus capitis, complexus, and splenius EMG activity were similar in all positions of the neck and body. Biventer cervicus and occipitoscapluaris EMG activity had variable responses for different body positions. Although the model predicted that the tracking task could be accomplished in the same manner regardless of the initial conditions, the results suggest that the central nervous system engages in multiple strategies of musculoskeletal coordination to achieve the same gross movement outcome. *Supported by grants BNS9109705 and NS22490.*

470.2

METRICS OF INCORRECT GAZE SHIFTS AND DISSOCIATED EYE-HEAD MOVEMENTS IN A DISTRACTOR PARADIGM IN HUMANS. B.D. Corneil*, D.P. Munoz. MRC Group in Sensory-Motor Physiology, Queen's University, Kingston, Ontario, CANADA K7L 3N6.

We studied the coupling of eye-head movements using a multimodal task in which human subjects were instructed to make a gaze shift to a target located 40° to the left or the right. In the distractor condition a distracting target was presented on the opposite side of the saccade target; in the enhancer condition the two targets were spatially coincident. Both auditory and visual stimuli were used as targets. Correct gaze shifts consisted of a coordinated eye and head movement directed to the saccade target. Incorrect gaze shifts were occasionally observed in the distractor condition, when the subject's gaze was initially directed to the distracting target. The frequency of these incorrect movements could be altered by presenting the distracting target earlier or later relative to saccade target onset. Incorrect gaze shifts initiated earlier relative to saccade target onset typically had larger amplitudes in the incorrect direction and had longer intervals before the correct gaze shift was initiated. Some correct gaze shifts in the distractor condition consisted of a dissociated early slow head movement directed to the distracting target, while the eyes made a compensatory movement to keep gaze aligned with the initial fixation position. Following a variable time, subjects then generated a correct gaze shift directed to the saccade target. The results provide support for a gaze control system wherein reflexive orienting is suppressed until a decision to make a gaze shift has been made. Incorrect gaze shifts occur when this suppression is released before the saccade target has been identified, and subjects orient to the first perceived stimulus. For correct gaze shifts with an early incorrect head movement, we hypothesize that the premotor area controlling head movements is receiving a separate localizing signal from the distracting target before the signal to initiate the gaze shift, and this signal is not under the same suppressive control as the gaze signal.

470.3

REDUCED PROPRIOCEPTIVE INPUT FROM CERVICAL AFFERENTS IN HUMANS IMPAIRS GAZE ACCURACY DURING LARGE COMBINED EYE-HEAD SACCADES. JL Johnston*, GTD Thomson, GD Miller, D Ireland Dept of Medicine & Otolaryngology, University of Manitoba, Winnipeg, Canada R3E 0Z3

We measured head and gaze movements using magnetic search coil oculography in 14 patients with idiopathic fibromyalgia and compared them to normal subjects. Head-free saccades were made to a laser dot target that moved in random directions, timings and amplitudes of 30-60 deg under 2 conditions: 1) with no direction to the subject as to how to move their heads; and 2) with instructions to move their heads as fast as possible. Under condition 1, all fibromyalgia patients could be classified as non head-movers, compared to 50% of our control group. All normal control subjects and 13 of 14 patients increased the amplitude of head movements significantly under condition 2. Of these 13 fibromyalgia patients, 7 (Group A) had improved gaze accuracy, as measured by the gain (gaze/target amplitude) of the first head-free saccade, for condition 2 (mean gain 0.92) compared to condition 1 (mean gaze gain 0.81, $p=0.04$). The remaining 6 patients (Group B), and all normal subjects did not show any change in gaze gain between conditions. Group A was more likely to employ synchronous eye and head movement of low amplitude during condition 1 (mean head latency 74ms) rather than suppress the head movement, or produce head motion at prolonged latency (188ms), as in Group B. During condition 2, Group A made larger amplitude head movements at shorter latency (about 7 ms). We conclude that musculoskeletal injury to the neck may impair gaze accuracy during large amplitude combined eye-head tracking by reducing proprioceptive inputs from neck afferents during a time when the VOR is suppressed. By voluntarily increasing head amplitude, a more head dominant strategy is employed, and the incorporation of neck afferent information during the initial gaze saccade improves accuracy.

470.4

Head-free primate gaze shifts in response to double target steps. J.O. Phillips*, L. Ling, and A.F. Fuchs. Department of Physiology and Biophysics, Univ. of Washington, Seattle, WA 98195

During shifts of gaze, the eye and head may either be driven by a common command producing an early eye saccade and a later head movement or be driven independently toward a common goal. To evaluate these two alternatives, we observed primate eye and head movements during gaze shifts in response to forward-forward or forward-backward double target steps, looking for reliable relationships between eye and head movement direction, amplitude and timing.

Short inter-step durations (≤100 ms) reliably produced two movements of the head, the first of which varied considerably in size; the second brought it onto the final target. However, these double head movements were often accompanied by only a single saccade to the second target. When two saccades did occur, the second typically began after the start of the second head movement, even when the onset of the initial head movement trailed the onset of the first saccade. The minimum inter-saccadic interval was relatively constant, while the interval between head movements was quite variable. Longer inter-step durations (≥150 ms) typically produced two saccades, which were often accompanied by a single head movement. Overall, there was a poor correspondence between eye and head movement amplitude for each step.

These data suggest that eye and head commands are separate in the primate. Further, they suggest that the minimum inter-saccadic interval is a feature of the eye movement system alone, and is not a function of sensory-motor processing common to both eye and head movement.

This work was supported by NIH grants EY00745 and RR00166.

470.5

TWO COMPONENTS OF HEAD MOVEMENTS DURING READING: PERCEPTUAL AND COGNITIVE ORIGINS. Choongkil Lee*. Dept. of Psychology, Seoul National University, Seoul Korea, 151-742.

The procedures adopted in most studies measuring eye movements during text reading require immobilizing the head, and so the pattern of head movements and the consequent ocular compensations have been undetermined. If head movements are suppressed for fixation stability while the eyes remain within the oculomotor range, programming of a head movement is likely to be coupled with the programming of eye movements, because the head movement is based on information concerning the position of the eyes in the orbit. Alternatively, since the direction and expected excursion of impending gaze shifts are known, the head may be programmed independently of the eyes to move continuously with a predetermined velocity influenced by several factors such as text familiarity.

Simultaneous recording of eye and head movements while reading revealed that during forward gaze saccades, head movements consisted of two components: a constant velocity component, and a velocity-modulatory component which was coupled to gaze saccades as revealed by cross correlation analysis. The constant velocity, but not the amplitude of the velocity-modulatory component, increased as subjects repeatedly read the same text. These results suggest that these two components of head movements are controlled by separate processes: a perceptual process coordinating eye and head movements, and a cognitive process incorporating text familiarity, operating on a more global scale, and that the former is not influenced by the latter for head movements.

470.6

EYE AND HEAD COORDINATION DURING HEAD-FREE GAZE PURSUIT IN THE *Rhesus* MONKEY: PREDICTABLE vs. UNPREDICTABLE STIMULI. W.A.D. Smith, B. Segal*, and K.E. Cullen, Aerospace Medical Research Unit, McGill University, Montreal, Canada.

The relationship between the head and eye movements generated during head-free gaze pursuit of a moving visual target was investigated. *Rhesus fascicularis* monkeys were trained to track a target moving in either a "predictable" (a single sine wave: 0.25 - 2.5Hz, 20 or 40°/s peak velocity) or an "unpredictable" (pseudorandom stimulus: the sum of five non-harmonic sinusoids; 10 or 20°/s component peak velocity) trajectory in the horizontal plane. Two specific series of studies were carried out. In the first, a monkey's ability to track target motion and pursuit strategy were compared across frequencies and between conditions. Monkeys were completely head-free and pursued either sinusoidal or pseudorandom target motion. Although the target remained well within its oculomotor range (± 50°), the monkey made significant use of both head and eye movements to maintain accurate pursuit, and these movements were well-coordinated. Overall tracking accuracy decreased with increasing stimulus frequency; gaze velocity gain decreased and phase lag increased relative to target velocity. Head velocity consistently lagged (> 15°) eye velocity in each paradigm. A prediction strategy, defined by a significant phase lead in gaze velocity relative to target velocity, was only observed systematically during sinusoidal pursuit at the lowest frequency tested (0.25 Hz). In the second series of studies, the functional state of the vestibulo-ocular reflex (VOR) was evaluated during head-free pursuit. VOR gain (G_{VOR}) was assessed by temporarily braking head motion and evaluating the resultant short-latency (20-60 ms) eye movement responses. During both sinusoidal (> 0.25 Hz) and pseudorandom pursuit, G_{VOR} was significantly attenuated at a very short latency (< 20 ms; G_{VOR} = .40 - .69), compared to control values (G_{VOR} = .84 - .98). The reduction in G_{VOR} observed during gaze pursuit indicates that the strong degree of synergy observed between head and eye movements is beneficial to tracking, since head movements do, in fact, contribute to the displacement of the visual axis in space.

470.7

HEAD POSITION SPECIFIC ADAPTATION OF VERTICAL EYE ALIGNMENT. J.S Maxwell* and C.S. Schor. University of California at Berkeley, Berkeley, CA 94720.

Previous experiments have shown that open loop vertical eye alignment (measured as vertical phoria) can be trained to vary with eye position. Such adaptation would be appropriate for compensating for muscle palsies wherein the magnitude of the misalignment varies with orbital eye position. Vertical deviation might also result from deficits in specific sensorimotor pathways. The ocular tilt reaction, for example, requires the coordination of head roll, ocular counterroll and vertical eye deviation. The present experiment demonstrates that vertical eye alignment can be trained to vary with head position. Subjects trained binocularly with different vertical disparities present at two different head positions. The disparities were created with base up and base down prisms. If a right over left disparity was presented with the head pitched up, for example, a left over right disparity was presented with the head pitched down. The head was stationary during training and testing. Changes in head position were in either pitch, roll or yaw. Roll rotations were about an earth-horizontal axis (EHA) and yaw rotations were about and earth-vertical axis (EVA). Pitch rotations were about either an EVA or and EHA. Head rotations were either by head-on-neck (HON) or whole body (WB) movements. All subjects adapted in all paradigms where rotation was about an EHA. Most also adapted to HO pitch rotation about an EVA. Head position specific adaptation would be expected to normally compensate for bilateral imbalances in otolith-ocular and, perhaps, cervical-ocular pathways.

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470.8

NEW CLUES TO THE COORDINATES OF THE OCULOMOTOR SYSTEM. D. Tweed*. Depts. of Physiology and Ophthalmology, Univ. of Western Ontario, London, N6A 5C1, Canada.

This work deals with 3 interconnected puzzles involving oculomotor coordinates:

1. When humans are rotated in roll while supine, their eyes counterroll but then quickly drift back to zero torsion when the vestibular stimulation is over; this drift suggests that the oculomotor integrator is leaky in torsion (Seidman et al. 1995). I present a model to explain how the integrator can leak torsionally but hold vertically, even though torsional and vertical signals are mixed together in the integrator neurons of the interstitial nucleus of Cajal (Crawford et al. 1991).

2. Humans adopt nonzero torsional eye positions when they converge their eyes (Allen 1954). *How can we hold these positions if our integrators are leaky in torsion?* I suggest that this torsion is maintained by a separate vergence integrator. This does not imply that vergence is a 2D system, because its "horizontal" and "torsional" effects can both be explained by a 1D command. The resulting model is greatly simplified if vergence, saccades and pursuit all operate in a novel coordinate system which combines some of the advantages of quaternions, rotation vectors and Helmholtz angles.

3. During eye-head saccades, each eye rotates with 3 degrees of freedom, breaking Donders' law (Tweed et al. 1995). *But saccadic commands from the superior colliculus are just 2D* (van Opstal et al. 1991). I show that a 2D colliculus can drive the observed 3D eye-in-head motion if the variable coded in the collicular map is *conjugate gaze shift in space*. Together, these 3 puzzles suggest a model for binocular eye-head control whose predictions will be compared to those of other theories of saccades, pursuit, vergence and VOR.

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470.9

ON LISTING'S LAW DURING SLEEP. Y Suzuki*, K Hepp[1], V Henn. Neurology Dept, University Hospital, CH-8091 Zürich, Switzerland

With the head erect and not moving, Listing's law confines conjugate eye positions to two degrees of freedom. Its validity has been shown for fixation, saccades, and pursuit. It has not been solved to what degree Listing's law is the result of orbital mechanics or is determined neuronally. During light sleep, when the eyes drift, the coordinated activation of motoneurons deteriorates, and is finally lost during deeper stages of sleep, when eye movements cease. We measured the oculomotor range, the orientation and thickness of Listing's plane during alertness and compared it to periods of light sleep.

Rhesus monkeys were trained for a visual fixation task, and were chronically prepared with a head holder and a dual magnetic search coil to monitor eye position in three dimensions.

In light sleep, eye positions during slow drifting movements covered a range of about +/- 20 deg in the horizontal and vertical directions with torsional components in a range of about +/- 10 deg. The distribution of data points can often still be approximated by a plane which is similarly oriented to Listing's plane during alertness. However, torsional positions then show a variability of up to 5 deg standard deviation from the mean value as compared to less than 1 deg during alertness. Similar observations could also be obtained after bilateral riMLF lesions, which in the awake condition is characterized by a loss of vertical and torsional rapid eye movements.

We agree with Nakayama (1975) that the main factor responsible for the high precision of Listing's plane during alertness is neuronal, and not mechanical.

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470.10

THE GEOMETRY OF SACCADES AND LISTING'S LAW. A. A. Handzel, T. Flash*. Dept. of Applied Mathematics and Computer Science, Weizmann Institute of Science, Rehovot, 76100, Israel.

We have analysed the geometry of conjugate saccadic eye movements using basic Lie group theory and differential geometry. Normal saccades involve rotations about a single axis (Tweed and Vilis, 1990), hence they are described mathematically as geodesics in the manifold of the rotation group (Hepp, 1990). We show that trajectories of saccades follow curved arcs in the space of Rodriguez rotation vectors (coordinates in the 3D projective plane). In addition, we point to the relations between different parameterizations of rotations (Hepp and Henn, 1987), namely, rotation vectors (Rodriguez), gimbal systems (Fick and Helmholtz) and canonical coordinates in the Lie algebra of the rotations group, so(3); transformations between the coordinates can be computed using the Campbell-Baker-Hausdorff formula.

Further, Listing's law can be described by means of the Lie algebra so(3), which is decomposed into Listing's plane and the orthogonal axis of torsion rotations. The axes of rotation in Listing's plane generate the geometrical structure of a 2D sphere S^2, which corresponds to the space of gaze directions, giving a direct connection to Donders' law. Although Donders' law admits many 2D submanifolds of the rotation group, only the sphere is an integral surface of the Lie algebra elements (i.e. translation invariant vector fields), which lie in Listing's plane. Finally, to rotate between two (non-primary) eye positions contained in Listing's plane, the rotation axis itself lies outside the plane; this reflects the structure of the rotation group, i.e. the rotations which correspond to Listing's plane do not form a subgroup of SO(3).

470.11

DYNAMIC CONTROL OF PRIMARY EYE POSITION AND LISTING'S COORDINATES AS A FUNCTION OF HEAD POSITION IN SPACE IN RHESUS MONKEYS. B.J.M. Hess* and D.E. Angelaki. Dept. of Neurology, University Hospital Zürich, CH-8091, Switzerland and Dept. of Surgery (Otolaryngology), University of Mississippi Medical Center, Jackson MS.

Primary eye position is defined on the basis of Listing's law under static conditions with the head upright (reference position) and undergoes systematic transformation as a sinusoidal function of head position relative to gravity. Thus, as the head assumes different static roll or pitch positions, the torsional and vertical coordinates of primary position transform relative to reference position according to the sine of the roll and pitch angle, respectively (Haslwanter et al 1992). We investigated whether this transformation was maintained constant when head orientation changed dynamically relative to gravity at different speeds and whether it would be compatible with a generalized dynamic implementation of Listing's law. We studied 3d eye position and angular velocity of slow and fast phases of nystagmus as a function of head position during constant velocity and mid and high frequency oscillations about off-vertical axes in rhesus monkeys. It was found that modulation of torsional and vertical mean eye position was (a) independent of frequency up to at least 0.5 Hz suggesting a frequency independent control of the torsional and vertical coordinates of primary eye position (b) generated by slow eye movements only at low frequencies and mainly by *saccadic*, i.e. forward directed fast eye movements at higher frequencies. Furthermore, angular velocities of fast or saccadic eye movements followed on average the velocity criterion of Listing's law. The results show that Listing's coordinates are dynamically controlled by otolith input suggesting a tight link between oculomotor coordinates and vestibular signals related to absolute orientation in space.

470.12

WHY IS LISTING'S PLANE THICK? **J.F.X. DeSouza, S. Mikael, D.A. Nicolle and T. Vilis*. Neuroscience Program, Departments of Physiology and Ophthalmology, University of Western Ontario, London, Ontario, Canada N6A 5C1.**

This study examined the precision with which the angular position of the eye is confined torsionally to Listing's Plane (LP) during and after saccades. Five normal subjects were asked to saccade to targets within a 30° square grid in different directions: horizontal (as in reading), vertical, radial, random, clockwise (CW) and counterclockwise (CCW). The thickness of LP was measured by fitting the 3D eye position data to a second order surface and then computing the variability in the torsional direction about this fitted surface. The thickness during the random task averaged 1° and was used as a reference. LP was 40% thinner in the Hor & Vert tasks and 25% thinner during the CW & CCW tasks. This decrease in thickness occurred for data sampled during a saccade, immediately after a saccade, and before the next saccade, suggesting that torsional "blips" (Tweed et al., 1994) are not a major factor. We also examined the coefficients of the second order surface fit for indications of task dependent changes in shape. LP was more twisted, towards a surface produced by a Helmholtz gimbal, during the Vert task than during the Hor task. As observed by Ferman et al. (1987), we found that the surface shifted forward in the CCW task as compared to the CW task. When subjects were asked to orient their head towards the same targets, a similar reduction in the thickness of the plotted surfaces was observed for the head's Donders surface (DS) and this surface also exhibit task dependent changes in shape. In summary, both the eye's LP and the head's DS exhibits task dependent changes in shape. We propose that the surface in the random task is "thicker" because it is the combination of surfaces with different shapes.

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SPINAL CORD AND BRAINSTEM: FUNCTIONAL NEUROPHYSIOLOGY

471.1

DORSAL SPINOCEREBELLAR TRACT NEURONS CAN BE INFLUENCED BY THE NEURAL CIRCUITRY PRODUCING FICTIVE LOCOMOTION IN THE CAT. B. Fedirchuk*, H. Hultborn, D.J. Bennett, and M. Gorassini. Dept. Med. Physiol., Univ. of Copenhagen, DK-2200, Denmark.

Dorsal spinocerebellar tract (DSCT) neurons have been reported *not* to be rhythmically active in a de-afferented locomoting cat (Arshavsky et al. 1984; In Studies of Brain Function Vol 13: Cerebellum and Rhythmical Movements). In contrast, the ventral spinocerebellar tract (VSCT) has been described to report activity in spinal motor circuitry (see Baldissera et al. 1981; In Handbook of Physiology - The Nervous System II).

Extra- and intracellular recordings were made from antidromically identified DSCT neurons within the L1 to L4 spinal segments in 9 precollicular-postmammillary decerebrated cats. After paralysis, fictive locomotion was elicited by electrical stimulation of the mesencephalic locomotor region. 42 of 60 DSCT neurons exhibited rhythmic activity; either firing exclusively, or at higher frequencies, in one phase of the fictive step cycle. There was no relationship between the type of afferent input exciting the neuron and whether or not the cell was rhythmically modulated. 21 DSCT cells were more active during flexion, 21 were more active during extension. 14 of 17 intracellularly recorded DSCT cells exhibited locomotor drive potentials (LDPs) ranging from 0.4 to 6.0 mV in amplitude. 7 VSCT cells were also recorded and all 7 exhibited rhythmic activity during the fictive step cycle. VSCT neurons generally had higher firing frequencies and larger LDPs than DSCT neurons.

These results indicate that the motor circuitry producing locomotion can directly affect both DSCT and VSCT neurons and suggest that previous conceptions of the dorsal spinocerebellar pathway be re-assessed.

471.2

HOMOSYNAPTIC DEPRESSION OF ROSTRAL TRIGEMINAL SENSORY NEURONS IN THE CHRONIC CAT: A ROLE FOR PRESYNAPTIC INHIBITION? B.E. Cairns*, M. Fragoso, and P.J. Soja. *Fac. of Pharm. Sci., UBC, Vancouver, BC, Canada V6T 1Z3.*

In acute anesthetized animal preparations, homosynaptic conditioning (HC) of V afferents exerts a suppressive influence on synaptic transmission through the trigeminal sensory nuclear complex (TSNC). Homosynaptic suppression of TSNC neuronal activity has been casually linked to a presynaptic inhibitory mechanism involving a process of primary afferent depolarization (PAD, *J. Neurophysiol.* 28: 695-709, 1965; *Brain Res.* 143: 369-372, 1978). In the present study, the association between neuronal suppression and PAD induced by such stimuli was examined in the chronic unanesthetized cat. Two adult cats were prepared for chronic recording as reported previously (*J. Neurophysiol.*, 1995, *in press*). Overall, the maxillary tooth pulp-evoked activity of 11 TSNC neurons was suppressed by 20% when HC stimuli (0.2ms, 50-100μA) were applied to the mandibular tooth pulp (CT interval: 30 ms). These HC stimuli did not alter the latency to spike onset (control: 4.6ms ± 0.3; conditioned: 4.4ms ± 0.3). Paired experiments were also performed to evaluate excitability changes resulting from HC in 7 TSNC neurons and their adjacent tooth pulp afferent terminals. Tooth pulp evoked activity was decreased (by 19-58%) in 4 neurons, increased (by 47%) in 1, and unchanged in 2 cells. The excitability of individual afferent terminals was examined using Wall's technique (*J. Physiol.*, 288: 437-447, 1979) at sites where these individual sensory neurons were previously recorded. While variable effects on TSNC neurons were observed, the same homosynaptic stimuli depolarized *all* the adjacent tooth pulp afferent terminals by a mean of 334%. Although V HC stimuli uniformly induce PAD in individual tooth pulp afferents, postsynaptic mechanisms, i.e., facilitation and/or inhibition, must also contribute to the V afferent modulation of TSNC neuronal activity observed in the chronic cat preparation. Supported by BCHRF, MRC, and NIH (NS32306).

471.3

BARBITURATE-INDUCED SUPPRESSION OF DORSAL SPINOCEREBELLAR TRACT (DSCT) NEURONS IN THE CHRONIC CAT. M. Fragoso*, G. Nixon, B. E. Cairns, and P.J. Soja. *Faculty of Pharm. Sci., UBC, Vancouver, BC, Canada V6T 1Z3.*

Barbiturates are often used to maintain the state of general anesthesia during electrophysiological studies of DSCT neurons (see *Brain Behav. Evol., 7: 34-83, 1973; Prog. Neurobiol., 36: 391-423, 1991*). The present study was performed to access the extent to which barbiturate anesthesia alters the excitability of individual DSCT neurons in the intact behaving cat. Three adult cats were prepared for chronic extracellular recording of DSCT neurons (*J. Neuroscience Meths., 1995, in press*). Spontaneous and sciatic nerve-evoked spike activities from antidromically identified DSCT neurons were recorded during wakefulness (W, *ibid*), the induction into and recovery from the state of anesthesia induced by the short acting barbiturate thiopental. During control periods of W, ongoing DSCT neuron spike rate measured 13.2Hz ± 3 (SEM, n=8). Thiopental (15mg/kg, i.v.), infused over a 3 min period, rapidly induced unconsciousness that was accompanied by a sustained "slow wave" EEG wave pattern, miosis, areflexia, and generalized atonia. Five minutes following injection, spontaneous and sciatic nerve-evoked (0.05ms,150-200μA) spike activities were maximally suppressed by 76.5%, and 33%, respectively ($p<0.05$, ANOVA). Complete recovery from thiopental's effects on DSCT cellular activities occurred within 30 min. Antidromically propagated spikes remained secure in all but one DSCT neuron. These findings confirm that thiopental attenuates ongoing synaptic transmission through the DSCT accounting in part for the loss of proprio- and exteroceptive sensation during barbiturate anesthesia. Marked differences are also likely to occur in the electrical and integrative properties of DSCT neurons recorded in acute, anesthetized vs. chronic, unanesthetized cat preparations. Supported by MRC.

471.4

MEDULLARY RETICULAR CONTROL OF DORSAL SPINOCEREBELLAR TRACT (DSCT) NEURONS IN THE UNANESTHETIZED CAT. P.J.Soja*, G. Nixon, and M.C. Fragoso. *Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada V6T 1Z3.*

The nucleus reticularis gigantocellularis (NRGc) is known to exert an inhibitory influence on ascending lumbar sensory tract neurons in anesthetized animal preparations. The present study was performed to characterize the effect of NRGc conditioning stimuli on the spontaneous activity of DSCT neurons in the chronic unanesthetized cat. Two adult cats were prepared for chronic extracellular recording of DSCT neurons (*J. Neuroscience Meths., 1995, in press*). DSCT neurons were antidromically identified and a baseline level of spontaneous spike activity established (*ibid*). Short trains of stimuli (4 pulses, 0.8 msec, 400 Hz, 20-160 μA) were applied to the NRGc (HC: P 7.5-9, L 1.5-2.5, H -6.0 to -7) during states of wakefulness and quiet sleep. Computerized analyses indicated that DSCT baseline spike activity did not differ between wakefulness and quiet sleep (*Sleep Res. 24: 1995, in press*). NRGc stimulation suppressed (~50-90%) DSCT neuron spike activity in every cell examined (n=20). The latency-to-onset and duration of the suppression ranged from 5-15 msec and 20-60 msec, respectively. The degree of suppression was graded with the intensity of NRGc stimuli used. There was no difference in the onset or duration of the NRGc suppression during the state of wakefulness vs. quiet sleep. Strychnine (0.08-0.12mg/kg, i.v.), antagonized the NRGc-induced suppression of spontaneous spike activity in each of 3 DSCT neurons tested without altering baseline activity. The present findings indicate that synchronous activation of the NRGc exerts a potent, phasic suppression of ongoing transmission of sensory information through the DSCT in the chronic unanesthetized cat. A process of glycine-mediated postsynaptic inhibition may partly underlie DSCT neuronal suppression exerted by the NRGc during wakefulness and quiet sleep.
Supported by BCHRF and MRC.

471.5

PAD AND PRESYNAPTIC INHIBITION ELICITED IN GROUP Ia MUSCLE AFFERENTS PERSIST 1-3 MONTHS AFTER CRUSHING THEIR AXONS IN THE PERIPHERAL NERVE. E. Manjarrez, M. Enriquez & P. Rudomin* Dep. Physiol. CINVESTAV-IPN. México, D.F 07300.

Horch & Lisney (J.Physiol. 313: 287, 1981) showed that 1-3 months after crushing their peripheral axon, cutaneous fibers fail to show PAD following stimulation of other cutaneous nerves. In contrast, stimulation of the posterior biceps and semitendinosus nerve (PBSt) produces PAD in group Ia fibers of the medial gastrocnemius (MG) whose axons were crushed in the periphery 1-3 months before (Abst. Soc.Neurosc. 18: 217.17, 1992). We now report observations aimed to investigate if this PAD is associated with presynaptic inhibition. The experiments were performed in anesthetized, paralyzed and artificially ventilated cats, in which the left MG nerve was crushed 1-2 months previously, while the lateral gastrocnemius plus soleus (LGS) nerve was left intact. The left L6-S1 ventral roots were sectioned and a NaCl filled glass micropipette (2-5 MΩ) was placed within the GS motor nucleus, either to record the extracellular field potentials (EFP's) produced by MG or LGS stimulation, or to test the excitability of the intraspinal arborizations of the MG and LGS afferent fibers. In two experiments, conditioning stimulation of the PBSt nerve (3 pulses at 300 Hz applied 20 ms before the test pulse) increased the excitability of both the MG and LGS fibers (mean 167% and 170% of control, respectively). In 3 experiments, conditioning stimulation of the PBSt nerve applied 30-45 ms before, depressed both pre- and postsynaptic components of the EFP's evoked by stimulation of the MG nerve (to 75±27% and 60±15%, of control respectively). The pre- and postsynaptic EFP's produced by stimulation of the intact LGS nerve were depressed to about the same extent (to 58±14% and 62±5% of control, respectively). These results suggest that the PAD that is produced in muscle spindle afferents whose peripheral axons have been crushed 1-3 months before is associated with presynaptic inhibition. *Partly supported by NIH NS09196, and CONACyT 031N9107.*

471.6

Large inhibitory effects of single interneurons on the motoneurons in neonatal rat spinal cord.

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The effect of individual spinal interneurons on the motoneurons was investigated.

Compound synaptic potentials were recorded with good signal to noise ratio from the ventral roots L4 or L5 of isolated spinal cords from 0 to 5 day old rats. The cords were split mid-sagitally. Intracellular recordings from interneurons were obtained using the tight seal technique. Spikes were elicited either with steady current injection or 1 ms current pulses.

Individual action potentials in some interneurons medial to the motoneuron pool generated a large hyperpolarization in the ventral root recording. In order to quantify this hyperpolarization, its amplitude was compared to the amplitude of a monosynaptic excitation that was large enough to elicit spikes in the motoneurons. The absolute value of the amplitude of the inhibitory potential was up to 40% of the depolarization needed to discharge motoneurons. The delay between the spike in the interneurons and the inhibitory potential ranged from 25 to 40 ms in different cells, but varied by less then 7 ms in individual cells. The inhibitory potential was blocked by kynurenic acid and low Ca^{++}. These results together suggest a polysynaptic pathway for the inhibitory effect.

The interneurons were characterized by large afterhyperpolarizations and a stable firing over a wide range of frequencies. Several cells were spontaneously active. The firing frequency affected the shape of the inhibitory potential, and it was abruptly abolished at frequencies above 5-7 Hz.

These experiments suggest that individual neurons can have a powerful role in the complex network controlling the motoneurons.

This work was supported by The Human Frontier Program.

471.7

RUBROSPINAL PROJECTIONS TO FORELIMB MOTONEURONS AND PHRENIC MOTONEURONS IN CATS. Y. Fujito* and M. Aoki
Dept. Physiol., Sch. Med., Sapporo Med. Univ., Sapporo 060, Japan

Rubrospinal projections to forelimb and phrenic motoneurons (Mns) in the cat were examined and compared with those of corticospinal projections electrophysiologically. Cats were anesthetized with Nembutal. Single pulse stimulation of the red nucleus (RN) produced EPSPs with a mean amplitude of about 0.35 mV in the majority of forelimb Mns (88/122). Half of forelimb Mns (23/46) of C8-T1 segments in which RN-EPSPs were detected by single shocks exhibited segmental latencies of RN-EPSPs shorter than 1 ms, while only 3 of 43 forelimb Mns in C6-7 segments showed such latencies. These results suggest that rubro-motoneuronal connections are, at least in part, monosynaptic for forelimb Mns that innervate in particular the wrist and digit muscles. When paired pulse stimuli (interpulse interval of 3 ms) were applied to RN, the second EPSPs were potentiated only moderately (mean facilitation ~24%) for EPSPs with short segmental latencies (<1.0 ms). Probable monosynaptic RN-EPSPs were still induced in distal forelimb Mns after acute transection of the ipsilateral ventral quadrant of the C4 spinal segment. Stimulation of the interstitiospinal tract did not produce any monosynaptic EPSPs in Mns. There was no evidence suggesting monosynaptic connections between RN and phrenic Mns, and between corticospinal fibers and forelimb Mns. Single socks to the cerebral peduncle (CP) produced EPSPs in 18/57 phrenic Mns and their segmental latencies shorter than 1 ms were observed in 2 cells in which paired pulse facilitation of CP-EPSPs were not evident. The present results suggest the presence of preferential direct rubrospinal projection to Mns innervating distal forelimb muscles in the cat.

471.8

BRAINSTEM CONTROL OF SENSORY INPUTS TO LUMBAR MOTONEURONS IN THE NEONATAL RAT. L. Vinay* and F. Clarac. Lab. Neurobiologie et Mouvements, CNRS, 31 chemin Joseph Aiguier, 13402 Marseille cedex 20, France.

The pathways descending from the brainstem to the spinal cord and the connections between dorsal root afferents and motoneurons are both present very early during development. In the present study the interactions between the brainstem and the peripheral inputs were investigated using intracellular recordings from lumbar motoneurons in the *in vitro* brainstem / spinal cord preparation of the neonatal (P0-5) rat.

The brainstem inputs to motoneurons were characterized by microstimulations delivered to the different funiculi of the spinal cord at the level of the brainstem spinal cord junction, in C1. Synaptic responses consisted of excitation and inhibition that were mediated mainly by NMDA, non-NMDA and glycine receptors. Stimulation of the ipsilateral dorsal root at long interstimulus intervals (30 s to avoid synaptic depression) evoked short-latency EPSPs assessed to be monosynaptic since they persisted under mephenesin. In normal Ringer, stimulation of the ventromedial and ventrolateral funiculi of the spinal cord 40-130 ms prior to the peripheral stimulation resulted in a depression (up to 40%) of the dorsal root evoked EPSP. In some cases, stimulation of the rostral spinal cord at a low intensity reduced the amplitude of the dorsal root evoked potentials without any post-synaptic effect on the motoneuron suggesting that the modulation of the dorsal root evoked EPSP may occur at a presynaptic level. The inhibition of the dorsal root evoked EPSP was reduced markedly by bath application of $GABA_A$ (bicuculline) and $GABA_B$ (saclofen, phaclofen) antagonists.

These data suggest that pathways descending from the brainstem already exert a control on segmental afferents at birth.

471.9

INITIATION OF VOCALIZATION INDUCED BY DISINHIBITION OF THE RESPIRATORY NEURONS IN THE NUCLEUS OF THE SOLITARY TRACT IN CATS. T. Sakamoto*, A. Katada, T. Sugimoto and K. Kikuchi, Dept. of Physiol., Asahikawa Med. Col., 4-5 Nishikagura, Asahikawa, Japan 078

Repetitive electrical stimulation (20-100 μA, 0.2 ms, 100 Hz, lasting for 2 to 5 sec) delivered to the periaqueductal gray (PAG) in decerebrate cats resets the normal respiratory rhythm, and induces initial inspiration followed by alternate vocalization and inspiration. The changes in the neuronal activities of the medullary respiratory neurons induced by PAG stimulation were extracellularly recorded by tungsten microelectrodes to understand the initiation mechanisms for vocalization.

Under halothane nitrous oxide gas anesthesia, cats were decerebrated at precollicular postmammillary level. The temperature of rectum was kept at 36-37°C by a radiant heat lamp.

When the PAG stimulation was delivered during the expiratory period, the augmenting expiratory neurons recorded from the retrofacial nucleus (E-aug neurons) ceased to discharge within 20 to 30 ms after the onset of the PAG stimulation. While the inspiratory neurons in the ventrolateral nucleus of the solitary tract (I-neurons) started to increase their discharge rate about 30 to 50 ms after the initiation of the PAG stimulation. However, none of these I-neuron was orthodromically activated by the PAG stimulation.

It has been known that the E-aug neurons exert the inhibitory effects upon the I-neurons, and most of those neurons are premotor neurons of the inspiratory muscles. The present results suggest that the preceding inspiration before vocalization is induced by disinhibitory process acting on the I-neurons from the E-aug neurons.

471.10

DESCENDING PROJECTIONS OF THE NUCLEUS RAPHE OBSCURUS (nRO); POSSIBLE ANATOMICAL BASIS OF ROLE IN LUMBOSACRAL REFLEXES G.E.Hermann*, G.M.Holmes, J.C. Bresnahan, M.S.Beattie, and R.C.Rogers, Depts.Cell. Biol., Neurobiol, and Anat., and Physiol., Ohio State University, Columbus, OH 43210

Our previous electrophysiological studies (Holmes, NS 1994) suggested that the nRO is an important source of descending control for lumbosacral reflexes. Therefore, anterograde labeling of nRO projections to the spinal cord and retrograde labeling of motoneurons (MN) innervating the external anal sphincter (EAS), bulbospongiosus (BS) and the ischiocavernosus (IC) muscles was used to examine the neuroanatomical basis of these observations. Fluorescent labeling of the nRO ("fluororuby", Mole. Probes) demonstrated terminations in the intermediolateral cell column of the thoracic cord, and in the sacral parasympathetic nucleus, lamina X, dorsal commissural nucleus and ventral horn in the lumbosacral cord. We observed presumptive terminal contacts between fibers originating in the nRO and cell bodies and/or dendrites of motoneurons of the EAS (approx 25%) and BS (approx 11%) but not the IC. [Motoneurons were retrogradely labeled with either fluorogold or fluorescein.] These apparent direct connections to lumbar MN and to regions participating in sacral reflexes, suggest the nRO provides a pathway for descending control and/or integration of autonomic and somatic motor activity. The results substantiate physiological studies which indicated that the nRO provides potent input to caudal spinal cord regions controlling eliminative and sexual functions. (Support: NS-31193; PVA SCRF 1254)

471.11

PHYSIOLOGICAL AND BEHAVIORAL EFFECTS OF SEROTONIN OR THYROTROPIN RELEASING HORMONE ON RAT DORSOMEDIAL MOTONEURONS. G.M. Holmes*, J.C. Bresnahan, R.C. Rogers, G.E. Hermann, M.S. Beattie. Depts. of Cell Biology, Neurobiology, and Anatomy; and Physiology. The Ohio State University, Columbus, OH 43210.

In the rat, motoneurons (MNs) innervating the external anal sphincter (EAS) and bulbospongiosus (BS) muscles lie within the dorsomedial (DM) motoneuron pool. Differences in spontaneous activity and response to nucleus raphe obscurus (NRO) stimulation of BS and EAS MNs suggest that BS and EAS MN's are differentially controlled by the NRO. Putative transmitters of NRO cells include serotonin (5-HT) and thyrotropin releasing hormone (TRH) both of which augment firing rates of classical α-motoneurons. We have shown that intrathecal (IT) infusions of TRH augment both smooth muscle anorectal activity and EMG activity of the EAS but not the BS muscles. Experiment 1 sought to clarify the locus of TRH activation. Nanoliter volumes of TRH were intraspinally pressure injected directly into the DM nucleus of anesthetized rats. Intraspinal TRH profoundly increased EAS, but not BS, EMG activity. In Experiment 2, awake, chronically catheterized animals, were infused with IT TRH (10-500 μM). All doses inhibited both corpus cavernosum and corpus spongiosum erections. Defecation rates were unaffected.

Unlike TRH, serotonin pressure injected into the DM eliminated firing of spontaneously active (presumably EAS) cells. Doses of IT 5-HT that inhibit urethro-genital reflexes in anesthetized acute preparations did not significantly affect penile reflexes (e.g. erection latency and erection total) in awake, chronically catheterized animals, despite the presence of "5-HT syndrome" hyperreactivity which would be expected to interfere with (i.e. inhibit) penile reflexes. These data suggest differential circuitry regulates adjacent DM motoneurons involved in sexual and eliminative reflexes. (Support: NIH, NS-31193; PVA, SCRF 1254).

471.12

CHANGES IN THE EXPRESSION OF RAT MICROGLIAL IMMUNOMOLECULES AFTER UNILATERAL PUDENDAL NERVE SECTION OR CASTRATION T. J. Brown*, J.C. Bresnahan and M. S. Beattie., Dept. of Cell Biol., Neurobiol., and Anatomy; and Neuroscience Program, The Ohio State University, Columbus OH 43210.

The dorsolateral (DL) and dorsomedial (DM) motor nuclei innervate the perineal muscles of the rat via the pudendal nerve (PN), are androgen sensitive, and exhibit synaptic plasticity in response to lesions or castration. Previous studies using the plant lectin GSA I-B_4 showed that while pudendal nerve section resulted in microglial activation in all animals, only a percentage of the castrated animals showed this phenomenon and only at 3 days post-op. We decided to try more sensitive markers to determine whether or not castration results in microglial activation. The markers used were the monoclonal antibodies OX-42 (anti- CR3 receptor), OX-18 (anti-MHC I antigens), and OX-6 (anti-MHC II antigens). Animals were allowed to survive for 2, 3, 5, 6, 7 or 10 days following castration or PN section. Animals with nerve sections labeled with OX-42 showed activation in the dorsal horn (DH), DM and DL at all time points. Adjacent sections labeled with OX-18 showed activated microglia in the DH at 3 days post-op, DL at 5 days, and DM at 7 days post-op. Microglia stained with OX-6 did not appear until 10 days post-op. In the castrated animals no activated DH microglia were observed using any of the OX antibodies. At six days post-op activated microglia were observed in the DL and DM in sections labeled with OX-42 and OX-6 in one animal out of 15. Using microglial activation as an indicator, we conclude that the effects on motoneurons from removing androgen (castration), are not equivalent to the removal of target derived trophic factors (axotomy). (Support:NS31193 & NS10165).

471.13

ONUF'S NUCLEUS (ON) MOTONEURONS (MNs) HAVE MORE GABA-ERGIC SYNAPTIC COVERAGE THAN OTHER SOMATIC MNs: A QUANTITATIVE IMMUNOCYTOCHEMICAL STUDY IN THE CAT. Q. Li., M.S. Beattie, J.C. Bresnahan* Dept. of CBNA and Neuroscience Program, The Ohio State University, Columbus, OH 43210.

The pudendal motoneurons (PMNs) in ON are involved in visceral reflexes although they innervate somatic muscles. They have different properties than limb and other "classical" MNs (CMNs), e.g. they are more resistant to ALS. We compared the pattern of GABAergic input to these two populations of MNs in the cat (n=4) using EM immunocytochemistry. Quantitative analysis of was carried out in randomly selected plastic sections through ON, the dorso-lateral nucleus (DLN) and the ventromedial nucleus (VMN). At the light microscopic (LM) level, PMNs in ON (n= 169) had a mean (+/-SEM) synaptic coverage by GABA terminals of 43.3+/-0.56% vs. CMNs of 18.5+/-0.43% (n=129; $p<.01$). DLN did not differ from VMN. EM montages of the somatic surface of randomly selected MNs (20 cells/group) showed more GABA terminals apposed to PMNs vs. CMNs (mean no./cell= 4.45 vs. 0.7 respectively), and that a greater percent of the PMNs' cell perimeter (7.5+/-1.13%) was apposed to GABA terminals than the CMNs' (0.9+/-0.3%). The mean terminal apposition length and area of labeled terminals apposed to PMNs vs. CMNs did not differ (1.5+/-0.06um vs. 1.9+/-0.28um; area 0.9+/-0.07um2 vs 1.3+/-0.23um2; $p>0.05$), nor did the active zone length (0.37+/-0.03um vs. 0.33+/-0.08um). The shape of the synaptic vesicles in the GABA terminals apposed to MNs was spherical (56.3%) or pleomorphic (41.8%); some DCVs were present. (Supported by NIH NS-10165 and NS-31193)

471.14

EMERGENCE OF DISTENSION-EVOKED BLADDER CONTRACTIONS IN SPINAL CATS REQUIRES PERINEAL STIMULATION. J.W. Downie*[1,2], M.J. Espey[1], and J.B. Gajewski[2]. Depts. Pharmacol.[1] and Urol.[2], Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada.

Distension-evoked bladder contractions usually appear 2-8 weeks after spinal cord injury in cats. For a testing paradigm in our laboratory, cats had had chronically-implanted bladder cannulas. After spinal transection at T11/12, 3 cats failed to develop reflex bladder contractions in response to bladder distension up to 16 weeks post-transection when twice daily bladder drainage was accomplished through the implanted cannula. In two of these cats tested after decerebration, the bladder contracted in response to electrical stimulation of the S2 dorsal or ventral roots, indicating that appropriate spinal circuitry and a peripheral motor pathway were present. Four similarly-prepared cats managed by twice daily catheterization showed reflex bladder contractions within 2-4 weeks of spinalization. Five cats were then prepared with indwelling cannulas and were spinally transected. In these, tactile perineal stimulation was provided during bladder drainage through the implanted cannula. All 5 showed contractile activity in response to bladder distension within 1-3 weeks. In further experiments, 2 cats were prepared with sensory pudendal and superficial perineal neurectomies, 3 others were sham-operated controls. All were managed by headcap drainage plus tactile perineal stimulation. Controls showed rhythmic bladder contractions in response to bladder distension (up to 63-72 days post spinal transection). Cats with peripheral neurectomies showed no rhythmic contractions ≥15 cm H2O by 54 and 56 days post-spinalization. It is concluded that the emergence of distension-evoked bladder contractions in spinal cats is not an inevitable consequence of spinal cord transection but may depend on peripheral afferent activity from the perineum. (Supported by the Rick Hansen Man in Motion Legacy Fund.)

471.15

EFFECTIVE ELECTROMICTURITION AND HISTOPATHOLOGIC CHANGES AFTER CHRONIC IMPLANTATION OF MICROELECTODES IN THE SACRAL CORD OF THE CAT. B.J. Woodford*, R.R. Carter, D. M. McCreery, L.A.Bullara, W.F. Agnew. Huntington Medical Research Institutes, Pasadena, CA 91105

With the goal of designing a neural prosthesis for bladder control, we have implanted iridium microelectrodes into the sacral spinal cord of male cats, targeting parasympathetic preganglionic neurons. Electrodes were 50 μm in diameter and 2.0 to 2.8 mm long. Through a support substrate on the dorsal surface of the cord, four electrodes were positioned one behind the other and directed to alternating sides. Stimulation-induced bladder pressure changes and electrode impedances were monitored on a weekly basis. At the termination of the experiment, the cats were perfused with fixative, and the electrodes were left *in situ* until autopsy. The final position of each electrode was determined by following patent tracks in 1 μm serial transverse sections of epoxy-embedded sacral segments.

One out of five cats had histologic signs of chronic inflammation near the electrodes. The most consistent aspect of chronic implantation was a macrophagic and fibrous ensheathment of the support substrate, which sometimes involved dorsal roots and encroached into the dorsal cord along the path of the electrode entry. Other findings within the cord were varying thicknesses of glial ensheathment around the electrodes, axonal degeneration of varying severity in the dorsal columns, and neuronal loss in gray matter around the electrodes.

Electrophysiologic monitoring showed that the electrode array was electrically stable and that bladder pressure was controllable for at least 60 days. The effectiveness of stimulation decreased over time, however, and our results suggest that there may have been electrode migration away from the parasympathetic target, and/or trauma to spinal cord tissue that caused the neurons to be refractory to stimulation.

472.1

SYSTEMATIC CHANGES IN POSTURAL EQUILIBRIUM (HEAD STABILITY), GAZE NYSTAGMUS AND COGNITIVE PERFORMANCE WITH GRADED DOSAGES OF ALCOHOL. R.S. Kennedy*, J.M. Drexler, Essex Corporation, Orlando, FL 32803; W.P. Dunlap, Tulane University, New Orleans, LA 70118; and J.M. Ordy, Memory Research Associates, Pittsford, NY 14534.

Postural equilibrium is used by clinical and experimental neuroscientists as a global index of central nervous system integrity. However, posturography devices are generally cumbersome, expensive, and follow a bottom-up (feet, knees, hips, etc.) measurement approach. In this study we measured and analyzed head kinematics (HK) (position, velocity and acceleration) using simple and low-cost equipment (video recording, PC-based framegrabber, head mounted reticle and specially written software) in a top-down approach. The effects of graded dosages of alcohol on the objectively scored video of postural stability was compared to performance on a standardized cognitive test (CT) battery and ratings by trained enforcement officers on the standardized roadside field sobriety tests (FST).

Eleven volunteers (male and female) were dosed with grain alcohol using the Widmark equation which expresses the relationship between distribution of alcohol, in the body as a whole, compared to the blood. After subjects reached a breath alcohol concentration (BAC) of .08% they were tested (HK, CT, FST) at each .02% BAC interval thereafter until .02% BAC was reached at which time subjects were again dosed to .08% BAC and the procedure was repeated. The results showed that all tests correlated with BAC levels. The best measure of alcohol intoxication was gaze nystagmus from the FST and the next best was y Velocity from HK. However, the gaze nystagmus test correlated minimally with y Velocity, the cognitive tests and the other FST posture measures. This means that aspects of y Velocity can add additional information not available from gaze nystagmus and performance alone and also provides support for our hypothesis that a video record of head position has promise as an index of alcohol dosage and perhaps other agents and treatments.

472.2

THE INFLUENCE OF COGNITION ON BALANCE CONTROL IN PATIENTS WITH ALHEIMER'S DISEASE. D. J. Beckley*, V. P. Panzer, K.R. Thompson, B.R. Bloem, and M.P. Remler, Depts. of Neurology, UC Davis, CA 95616 and Leiden University Hospital, The Netherlands.

Falls are a significant problem in the elderly population and Alzheimer's disease (AD) is said to be a further risk factor. While exact mechanisms of postural instability are poorly understood in AD, one possible factor may be failure to use relevant environmental information. To assess the role of cognition on balance control in patients with dementia of the Alzheimer's type, eight patients with AD (5 female, mean age 68.8 ± 11.6 (SD); Folstein MMSE 16.4 ± 6.9) and eleven elderly normal subjects (4 female, mean age 68.9 ± 6.5; MMSE 29.6 ± 0.5) were tested with two sets of force plate perturbations (random mixture of small (5^0) toe-up, toe-down, and large (15^0) toe-up rotations). In one set, prior to each trial, subjects received perturbation pre-information. No pre-information was provided in the other set. The dependent variable was the frequency of two biomechanical markers of falls which occurred only during the 15^0 perturbations. There was no significant difference in the frequency of the biomechanical markers of falls between the AD and control group when no pre-information was provided (chi-square = 2.4). In contrast, a significant effect was noted when subjects received perturbation pre-information (chi-square = 22.3, $p < .005$). The number of falls decreased in the control group while there was no improvement in the AD group. Our results suggest that the inability to use relevant environmental pre-information may be a potential mechanism leading to the higher incidence of falls in patients with AD. (Study supported by NIA-NIH)

472.3

INTERACTION BETWEEN ATTENTION DEMANDING FOCAL TASKS AND POSTURAL SWAY. D.L. Weeks*, R. Forget, L. Mochnino, D. Gravel and D. Bourbonnais. Dept. of Physical Therapy, Regis Univ., Denver, CO, 80021, & Rehabilitation Inst., 6300 Darlington Ave., Montreal, Quebec, Canada, H3S 2J4.

The ability to simultaneously allocate attentional resources to both a postural and focal task is not well understood. Therefore, this study investigated the interaction between attention demanding cognitive and motor focal tasks, and postural sway during quiet stance with borders of the feet together in healthy young and healthy elderly subjects. The cognitive task involved silently solving an orally presented multi-step mathematics problem over 30 sec, while the motor task was a 30 sec bilateral static finger-thumb pinch task at 10% MVC. Each focal task was performed separately, and in a condition in which both tasks were performed simultaneously. Sway was recorded in a baseline (no focal task) condition, and during the performance of the focal tasks with full vision and with vision occluded on the premise that focal task performance would be degraded when attention devoted to postural control increased in the absence of vision. Analyses of cognitive task answers and RMS from the motor task target force revealed no Age Group, Gender, or Vision main effects. Thus, subjects adhered equally well to all focal tasks regardless of visual condition. Young subjects exhibited significantly less M-L sway, mean radial sway normalized for the height of the subject, and absolute sway velocity. Occlusion of vision significantly increased M-L and A-P sway, mean radial sway, and absolute sway velocity in the young and elderly. The single significant effect of the various focal tasks was on M-L sway in which the cognitive task resulted in significantly less sway than the motor task condition for the elderly subjects. Postural sway in the young subjects was not affected by the focal tasks. This suggests that for the elderly, M-L sway was influenced centrally during the cognitive task. In contrast, the increase in sway in the elderly during motor focal task performance suggests a reduced ability to suppress sway when the motor system is concurrently occupied with a voluntary task.

472.4

ARE THERE VARYING ATTENTIONAL DEMANDS FOR DIFFERENT POSTURAL STRATEGIES? M.H. Woollacott*, L.A. Brown and A. Shumway-Cook. Inst. of Neuroscience, Dept. of EMS, University of Oregon, Eugene OR.

Postural control is considered automatic, implying that sensorimotor processing requires minimal cognitive involvement. However, studies suggest that decreased neural function due to aging may increase postural attentional requirements. Consequently, postural control may be jeopardized if performing an attentionally demanding task. Attentional demands may also increase when the postural task becomes more difficult. This study examined the effects of attentional distraction on postural control as well as the attentional requirements associated with increasing postural demands. Six young adults participated in this study. Postural control and attentional performance were tested using a platform perturbation paradigm and an attentionally demanding counting task. The range of centre of mass displacement increased with increasing perturbation velocity, but did not significantly change when the secondary task was added. Attentional distraction therefore, did not disrupt the ability to control balance following unexpected perturbations. However, the findings confirmed that postural recovery is an attentionally demanding task: all subjects showed a decrease in attentional performance for the perturbation vs the control trials. This implies that, postural control takes precedence over a secondary task when the two are performed simultaneously. In addition, there did not appear to be varying attentional demands for different types of nonstep strategies; differences in attentional performance however, were noted when subjects executed a stepping response. For one subject in particular, there were no differences in attentional performance for the execution of an ankle-dominated or a hip-dominated postural response (mean subtraction duration was 9.3± 6.3 sec for the control trial and, 12.7±5.8 vs 12.6±5.6 sec for the ankle and hip responses respectively). For this same subject however, the shift from a nonstep to a stepping strategy demanded additional attentional resources, and further reduced performance on the secondary task (mean subtraction duration was 16.1±3.9 sec for the stepping response). Thus, postural control is not automatic, but requires varying attentional resources depending on the postural response elicited.

472.5

ARE ACCOMMODATIONS TO POSTURAL PERTURBATIONS AFFECTED BY FEAR OF FALLING? L.A. Brown* and J.S. Frank. Neural Control Laboratory, Dept. of Kinesiology, University of Waterloo, Waterloo, Ontario Canada.

There is increasing evidence that many older adults are apprehensive about falling. Fear of falling is based on a perceived risk of instability leading to potential injury. Perceptions and fears are often reduced by familiarity with a situation. One possibility however, is that individuals apprehensive about falling do not adapt to familiar situations. This study addressed whether accommodations to postural perturbations are affected by fear of falling. In this study, fear of falling was simulated by manipulating the consequences of imbalance.

Eight healthy male subjects (23 ± 5.7 yrs) were exposed to 5 repeated external perturbations (force applied to the upper back) under conditions which manipulated the consequences of a loss of balance: (a) standing at ground level and (b) standing at the edge of a .83m high elevated platform. Whole body centre of mass (COM) kinematics and post-perturbation EMG activity of selected trunk and leg muscles were examined. The range of linear COM displacement was significantly lower during the elevated condition ($p<.05$). In addition, COM displacement increased across trials in both testing conditions (statistical significance was noted between trial 1 and trials 4 and 5). Comparison of post-perturbation EMG activity revealed that the onset latency for the soleus muscle occurred significantly earlier for the elevated condition than for the ground-level condition. In addition, a progressive reduction in soleus EMG magnitude with repeated perturbations was revealed for the elevated condition only ($p<.01$). Thus, despite the noted accommodation of postural responses in both conditions, the neural strategy underlying the resultant accommodation depended on the consequences of instability. These findings have implications for understanding how postural control is managed by individuals afflicted with a fear of falling.

Supported by NSERC Canada.

472.6

POSTURAL ADAPTATIONS WHEN STANDING ON AN ELEVATED PLATFORM. J. S. Frank*, D. Cockell and L. A. Brown. Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1.

The selection of a postural strategy to control upright stance can be influenced the cognitive set of an individual. A possible factor influencing cognitive set is the perceived threat to one's personal safety. We explored this factor by examining the control of stationary stance when standing at ground level and on a platform elevated to 0.81 metres above ground level. Eight adult males (17-34 years) of approximately the same height (1.8-1.9 metres) participated in the experiment. Subjects stood with their feet positioned side-by-side: when standing on the elevated platform, the toes were positioned at the edge of the platform. Postural control was examined during three consecutive 30-second intervals defined by three vision conditions: eyes open, eyes closed and return to eyes open. Measures of the root mean square of centre of pressure (CoP) and centre of mass (CoM) for the whole body showed little difference when standing with the eyes open at ground level versus on the elevated platform. However, removal of vision differentially influenced postural control for the two height conditions. Upon closing the eyes, CoP and CoM increased (58%) when standing at ground level and decreased (-40%) when standing on the elevated platform. The decrease in CoP and CoM for this latter condition was accompanied by a small increase in the mean level on soleus and tibialis anterior electromyographic activity. It appears that postural control is modified by perceived threat to safety and involves an increase in joint stiffness. Increase in joint stiffness would serve to limit displacement of the body to an impending perturbation.

Supported by a grant from NSERC.

472.7

REDUCED OR INAPPROPRIATE SENSORY INFORMATION INCREASES CENTER OF PRESSURE EXCURSION IN CHILDREN. H. Sveistrup*, M. Trudel and R. Thibeault. Program in Occupational Therapy, University of Ottawa, ON Canada K1H 8M5

The ability to regulate posture is a function of the detection and integration of inputs from the somatosensory, visual and vestibular systems. The challenge of maintaining balance increases when sensory information is removed or if information from one system is either distorted or in conflict with information from other systems. We recorded center of pressure excursion of children aged 4-6 (YC) and 7-10 (OC) under six conditions of altered sensory input obtained by modifying the support surface (normal, foam) and visual cues (normal, eyes closed, sway referenced). Center of pressure excursion increased from baseline in YC and OC when visual information alone was removed (8% and 54%) with slightly greater increases when somatosensory information alone was altered (23% and 69%). Altering inputs from both systems resulted in increased excursion from 43% and 110% (eyes closed, foam) and 63% and 165% (sway referenced, foam). The greatest increases in both YC and OC were recorded when both visual and somatosensory inputs conflicted suggesting that subjects had greater difficulty minimizing sway when inappropriate information was provided than when sensory information was removed.

Supported by N.S.E.R.C. to H.S.

472.8

POSTURAL CONTROL DURING QUIET STANDING IN STROKE SUBJECTS. M. C. Verrier*, D. Fogal Smith, A. Yuen, S. Dick, C.D. MacKinnon. Departments of Physical Therapy and Physiology, University of Toronto. Toronto, Ontario, M5T 1W5, Canada.

Centre of pressure (CP) is the point of application of the resultant ground reaction force vector and can indirectly represent the continuous neuromuscular control of the location of the body's centre of mass. To study the location and velocity of the CP, ground reaction forces and moments were recorded using a single AMTI force platform during three 20 second trials (eyes open) in stroke subjects (> than 6 months post-stroke) and normal subjects. In contrast to previous studies which forced subjects into a standardized foot position, stroke subjects stood on the force platform with their feet in the position that best reflected their perception of maximal stability. CP location and velocity were calculated post-hoc. Location of the CP was standardized to mid-ankle joint centre for each individual subject for inter-subject and group comparison. The mean CPx (anterior/posterior) and CPz (medial/lateral) locations were used to estimate weight-bearing relative to a mid-point between the two limbs (paretic and non-paretic). The mean CPx location was similar for both groups, but the mean CPz location was displaced laterally towards the non-paretic limb ($\bar{x}$=1.3cm). The mean variance in the velocity of the excursion of CPx and CPz was calculated to provide an indicator of the 'rate of control of posture'. When CP velocity was compared in a sub-group of age- and anthropometrically-matched normal subjects who adopted the preferred standing position of their matched stroke subjects (n=9), there was an increase in the mean variance of both the CPx (26%; p=0.059) and CPz (65%; p=0.006) velocity of stroke subjects. The marked increase in the variance of the CPz velocity indicates that preferred foot positioning does not adequately compensate for the medial/lateral instability seen in stroke. (Supported by NHRDP Grant # R6606-4547-59).

472.9

RELIABILITY AND VALIDITY OF THE CTSIB AND BALANCE SYSTEM IN THE EVALUATION OF INDIVIDUALS WITH HEMIPARESIS D.S.Nichols*, K.J. Hutchinson, L.A. Colby. Physical Therapy Division, The Ohio State University, Columbus, OH 43210.

Test-retest reliability and discriminant validity were evaluated for the Clinical Test for Sensory Interaction in Balance (CTSIB) and the sway index of the Balance System. 14 subjects with hemiplegia (x = 62.1, range 48-85) and 14 age and gender matched controls (x = 60.6, range = 48-83) participated. Each subject was tested using both the CTSIB and Balance System under 6 testing conditions: 2 support surfaces (CTSIB - firm, compliant; Balance System - firm,angular rotation) and 3 visual conditions (eyes open, eyes closed, visual conflict). Selected subjects were retested within 1 wk. ICC's (1,1) for the test-retest reliability ranged form .12-.82 for the Balance System and .20 to .97 for the CTSIB. Student's t-test comparisons identified significant differences between the 2 groups on both measures. A discriminant analysis using both measures correctly identified 12 of the 14 hemiplegic subjects. Pearson's correlation coefficients between the 2 tests were low to moderate.

472.10

POSTURAL REFLEXES IN SUBJECTS WITH SOMATOSENSORY IMPAIRMENT. M.Zampolini*, A. Reali, M. Franceschini. Department of Rehabilitation, Trevi, 06039, Perugia, Italy.

Balance reflexes elicited by a body sway pruduced by brisk movement of a platform are regulated by different afferent systems (somatosensory, visual and vestibular). In this study we investigated the role of the somatosensory system on the regulation of postural reflexes.

We studied four patients with specific somatosensory system lesions. Three were patients affected by spinal cord lesion limited mainly in the dorsal column pathway at dorsal or cervical level. The fourth patient was affected by a thalamic haemorrhage with proprioceptive impairment.

The subjects stood on a platform ("Dynamic Platform Apache BTH 1000") moved briskly backwards or forwards. The platform was moved by an engine stall holding force 60 Kgf and the displacement was regulated with a resolution of 1 mm using a computer with a RS232 interface. The activity of anterior tibialis (Tib) and medial gastrocnemius (Gast) was recorded by bipolar surface electrodes.The data were acquired using an A/D coverter (Codas - 20 Khz). The body sway was backward and was induced with a series of 4 random acquisitions. Three different patterns of acceleration were used. All the series were studies with both open and closed eyes. The data were compared with the reference value of the normal subjects.

The data show a significant increase of the latency of Tib responses (18-35 ms) in all the patients. In the patient with thalamic lesion the latency on pathologic side was increased compared with the normal side. Measuring the postural responses during the follow-up (3 months later) the latency showed improvement.

The data indicate that the somatosensory system plays a pivotal role in regulating the postural reflexes through a long loop response.

This analysis could be used for monitoring the improvement of the postural adjustment during recovery after a central nervous system lesion.

472.11

SHORT-, MEDIUM-, AND LONG-LATENCY RESPONSES TO POSTURAL PERTURBATIONS IN PATIENTS WITH DIABETES MELLITUS. R. W. Simmons*, C. Richardson and R. Pozos. Motor Control Lab, San Diego State Univ., San Diego CA 92182.

Insulin-dependent diabetes mellitus patients (IDDM: n = 23), non-insulin dependent diabetes mellitus individuals (NIDDM: n = 10), and diabetics with clinically apparent distal, symmetrical neuropathy (DN: n = 23) were tested using dynamic posturography techniques. Diabetic subjects were matched to controls for age, weight and gender factors. For all subjects pre-amplified electromyographic (EMG) surface electrodes were placed over the anterior tibialis (AT) and medial gastrocnemius (GS) muscles of both legs. Subjects stood on dual forceplates that rotated upwards about the ankle joint 8 degrees in 400 ms. Twenty trials were completed separated by a randomly determined inter-trial delay of 1-5 s. Raw EMG signals were full-wave rectified and averaged for each subject. An interactive computer cursor device was used to determine the onset of the short-loop (SL), medium-loop (ML) and long-loop (LL) latencies manifested by the AT and GS muscles. For all pair-wise comparisons t-tests revealed no significant differences between diabetic and control groups for SL, ML, and LL latencies ($p < .05$). Deficits in postural control associated with diabetic polyneuropathy are not attributable to delayed latencies in the lower limbs.

472.12

DECREASING SWAY MAGNITUDE TO ENHANCE POSTURAL CONTROL IN OLDER, NON-FALLER ADULTS. S.L. Wolf*, R.L. Segal, P.A. Catlin, C.A. Byrne, T.E. Palm, D.E. Sedgwick, A.H. Snyder. Department of Rehabilitation Medicine, Division of Physical Therapy, Emory University School of Medicine, Atlanta, GA 30322

The consequence of falls in the elderly is recognized as a severe public health issue. In part, falls may be due to disequilibrium. This study determined if sway magnitude in older adults following a toes-up perturbation could be conditioned using visual feedback. Ten healthy, non-faller adults (mean age: 73.1 years) received 30 toes-up perturbations during each of six baseline and six training sessions. Visual feedback of sway magnitude changes in center of pressure was provided during training. EMG data were gathered for training sessions to monitor subjects' responses to perturbation. Somatosensory and motor control tests were administered before baseline, between baseline and training sessions and following training. Sway magnitude was divided into response and maintenance intervals. The response interval was further divided into three 75 msec bins. During feedback training, sway magnitude increased within the first 75 msec, but decreased between 150-225 msec. Difference scores in somatosensory and motor control tests did not change significantly over time. Although adaptation may occur at a single joint, responses of the entire kinetic chain do not adapt. Visual feedback may have helped subjects decrease sway magnitude, but an extended baseline is needed to confirm this observation. Postural retraining of sway magnitude should be pursued more rigorously before ascertaining if this approach can improve postural dyscontrol in older individuals.

This study was supported by VA Merit Award #E723-RA awarded to Drs. Wolf and Segal.

472.13

OVERALL VS SELECTIVE DECLINE OF POSTURAL SUBSYSTEMS IN BALANCE-IMPAIRED OLDER ADULTS. S. Moore*, M.H. Woollacott, M.S. Hu. Department of Exercise and Movement Science, University of Oregon, Eugene, OR 97403.

This study addressed the issue of whether balance impairment, unrelated to any known pathology in community dwelling older adults, involved overall vs selective decline of postural subsystems. Sensory and motor subsystems were evaluated. Two groups of 10 older adults (Balance Impaired, 80±8 yrs; Nonimpaired, 72±6 yrs) were selected from previous studies in our lab because they differed markedly in their ability to maintain balance when sensory information was manipulated. The Balance Impaired group experienced loss of balance 7±1 times on the Sensory Organization Test of Nashner (1982) whereas the Nonimpaired group did not experience any loss of balance. Both older groups, plus a Young adult control group (n=10, 31±5 yrs), were subjected to a test of motor coordination (sudden, unexpected support surface displacements) for which long loop muscle response latencies and organization were evaluated. The older groups also were screened neurologically.

The Nonimpaired and Young groups had significantly shorter distal agonist latencies than the Balance Impaired group (Young 88 ms, Nonimpaired 94 ms, Impaired 109 ms). Age was moderately correlated with distal agonist latency (Gastroc r=.51, Anterior Tib r=.66). There were no group differences for orders of muscle onset, or for the symmetry of left and right agonist responses. Neurological screening revealed that the Impaired group had more impairment of vibration and position senses, and of tandem gait than the Nonimpaired group.

Evidence from this study suggests selective rather than overall decline of postural subsystems, and that this is partially explained by aging.

472.14

POSTURAL RESPONSES TO A SLIP DURING WALKING IN HEALTHY OLDER AND YOUNG ADULTS. P-F. Tang*, M. H. Woollacott, R. K-Y. Chong. Institute of Neuroscience, and Dept. of Exercise and Movement Science, University of Oregon. Eugene, OR 97403.

Slips are one of the major causes of falls in older adults. The present study compared postural responses to simulated slips during walking between healthy older and young adults. Subjects were perturbed during free-speed walking with an unexpected forward or backward translation of a movable platform incorporated into the center of a 5 m-long walkway. The onset of the platform movement (10 cm at 40 cm/s) was precisely timed to heel strike, midstance, or the late stance phase of the gait cycle, to induce various slip conditions. A total of 48 trials were tested, with the first 12 trials as a no-perturbation blocked condition, followed by 12 anterior perturbations (4 trials/phase), 12 posterior perturbations (4 trials/phase), and 12 additional no-perturbation trials in random order. Long-loop muscle response sequences from bilateral legs and the trunk, and the onset latency of distal leg muscles were investigated. Results from 4 healthy older (mean age = 77±6 yrs) and 4 young (mean age = 24±2 yrs) adults showed that the anterior platform translation at heel strike induced the most muscle responses, suggesting that this was the most challenging perturbation. In response to this anterior slip condition, both age groups revealed an increased activity of the tibialis anterior (TA), followed by increased activity of the quadriceps (Q), of the perturbed leg. These increased muscle activities were accompanied by decreased gastrocnemius (G) activity of the perturbed leg following heel strike, but a significant increase of G activity in late stance. Increased TA and Q activity was also found in the contralateral leg, but with a smaller magnitude. Unpaired t-test showed that the onset latency of the TA muscle in the perturbed leg was significantly longer ($p = .01$) in the older adults (122±5 ms) than in the young adults (106±7 ms). These results demonstrate that healthy older and young adults have similar muscle sequencing in response to a slip during walking. The response from the distal muscle (TA), however, is delayed in older adults. (Supported by NIH AG05317 to Dr. Woollacott)

472.15

OPEN-LOOP AND CLOSED-LOOP POSTURAL CONTROL MECHANISMS IN PARKINSON'S DISEASE: INCREASED MEDIOLATERAL ACTIVITY DURING QUIET STANDING S.L. Mitchell, J.J. Collins, C.J. De Luca*, A. Burrows, and L.A. Lipsitz Hebrew Rehab Center for Aged, Harvard University. NeuroMuscular Research Center, Boston University, Boston, MA 02215

The postural dyscontrol associated with Parkinson's Disease (PD) is a poorly understood phenomenon. In this study we used stabilogram-diffusion analysis (SDA) to gain insights into the postural impairments in PD. SDA is a technique based on a random-walk approach to quiet-standing center-of-pressure (COP) trajectories. SDA yields COP parameters which relate directly to the steady-state behavior of the neuromuscular mechanisms involved in maintaining erect stance. Posturographic data were obtained using a Kistler balance platform from 22 subjects with PD (72 ± 10 yrs) and 24 healthy elderly subjects (75 ± 2 yrs). Each subject performed ten 30s trials of quiet standing with eyes open. Effective diffusion coefficients, which reflect the level of effective stochastic activity of the postural control system, were calculated by plotting mean square COP displacements as a function of increasing time interval. Effective diffusion coefficients over short-term time intervals (D_S) and long-term time intervals (D_I) are proposed to represent an open-loop and closed-loop control system, respectively. The D_S ($p<0.0001$) and D_I ($p=0.002$) were significantly greater in the mediolateral (ML) direction for the PD subjects compared to the healthy elderly. These parameters did not distinguish the two groups in the anteroposterior (AP) direction. ML posturographic measures were also associated with a history of falls ($p=0.01$), difficulty arising from a chair ($p<0.001$), poor performance on a clinical scale of balance ($p<0.001$), and slower gait speed ($p<0.0001$). Thus, the postural control mechanisms in PD subjects, compared to healthy elderly, were characterized by an increase in effective stochastic activity in the ML direction. This may reflect an attempt to maintain potentially stabilizing movements during quiet stance in the face of impaired movement in the AP direction. This study suggests that ML activity is an important posturographic marker of functional balance impairment in PD during quiet standing.

LIMBIC SYSTEM AND HYPOTHALAMUS: FUNCTION I

473.1

SINGLE UNIT ACTIVITY IN THE MEDIAL SEPTUM FOLLOWING SELECTIVE LESION OF THE SEPTOHIPPOCAMPAL CHOLINERGIC SYSTEM. E. Apartis, M.H. Bassant, F. Jazat-Poindessous and Y. Lamour*. INSERM U 161, 2 Rue d'Alésia, 75014, Paris, France

The medial septum contains cholinergic and GABAergic neurons which project to the hippocampal formation. A significant proportion of the septohippocampal neurons exhibit a rhythmically bursting (RB) activity which is involved in the generation of the hippocampal theta rhythm. The neurochemical nature of septal RB neurons is not firmly established. To address this question, the cholinergic septal neurons were lesioned by an icv injection of the toxin 192 IgG-saporin (5µg), which destroys selectively the cholinergic neurons bearing the low-affinity NGF receptor (NGFr). The extracellular activity of the medial septal neurons was then studied in urethane anesthetized and unanesthetized rats 14 to 21 days after lesion. The loss of cholinergic neurons (assessed by NGFr immunohistochemistry) ranged from 80 to 100%. In urethane-anesthetized lesioned rats, the percentage of RB neurons decreased dramatically (8% (19/235) vs 20% (57/286) in controls, $p<0.001$). Even in rats with a complete cholinergic lesion, a few RB neurons were still recorded. The frequency of the RB neurons was higher in lesioned rats as compared to controls (4.5 vs 3.7 Hz, $p<0.0001$). In unanesthetized lesioned rats, no RB neurons were found out of the 51 neurons recorded during different behavioral states including REM sleep, whereas a high proportion of neurons recorded during REM sleep in control rats displayed RB activity. These data support the conclusion that RB septal neurons form two populations of unequal size: a cholinergic one, as shown by the decrease of RB activity in lesioned rats; a non-cholinergic one, as shown by the few RB neurons remaining in case of complete cholinergic lesion. (Supported by a grant from Bayer-Pharma, France).

473.2

Non-cholinergic neurones of the medial septum display cluster firing at the theta frequency as well as 40 Hz (20-50 Hz) subthreshold oscillations. M. Serafin*, S. Williams, B.E Jones° and M. Mühlethaler. *Dept. of Physiology, CMU, 1211 Geneva 4, Switzerland and °Montreal Neurological Inst., McGill University, Canada H3A 2B4.*

We have recently demonstrated that nucleus basalis (NB) non-cholinergic neurones display high frequency (20-70 Hz) subthreshold oscillations and have the ability to discharge in rhythmic clusters (2-10 Hz) of action potentials (20-70 Hz during a cluster). In view of the possible rôle of the medial septum (MS) in conveying theta rhythmicity to the hippocampus as well as the recent evidence of "40 Hz" activity within that structure, we have reinvestigated the properties of non-cholinergic MS neurones, originally described by Griffith and Matthews (1986) in the guinea-pig and Markham and Segal (1990) in the rat. Using guinea-pig basal forebrain slices we found in the MS essentially two cell types. Out of 24 cells, 18 had properties characteristic of cholinergic neurones, that is a tonic discharge most probably subserved by a low-threshold calcium spike followed by a prominent slow afterhyperpolarizaton (AHP). Their firing rate could not exceed 10-15 Hz when depolarized. The remaining 6 cells were typically non-cholinergic with a fast AHP and fast spiking. We found that these cells, like the non-cholinergic NB cells could fire in rhythmic clusters at theta frequency when depolarized. Their firing frequency during the clusters was voltage-dependent and varied between 20 to 50 Hz. In addition subthreshold oscillations at the same frequency were observed between clusters. These results indicate that non-cholinergic (presumably GABAergic) MS neurones could convey to the hippocampus not only theta but also "40 Hz" rhythmicity. *(Swiss NSF and Canadian MRC).*

473.3

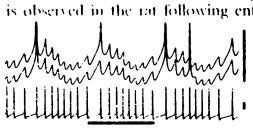
"SYNCHRONIZATION OF GAMMA OSCILLATIONS BY SYNAPTIC INHIBITION AMONG INTERNEURONS: A MODEL STUDY".
X-J Wang* (1), A. Sik (2) and G. Buzsaki (2), (1) Department of Mathematics, University of Pittsburgh, Pittsburgh, Pa. 15260. (2) Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, N.J. 07102

Rhythmic neural activity (gamma, 40-100 Hz) has been observed in the neocortex and hippocampus during behavioral arousal. We investigated the synchrony induced by inhibitory synaptic interactions among interneurons during gamma oscillations, using computer simulations. We show that the degree of synchrony is critically dependent on intrinsic cellular properties (in particular the amplitude of afterhyperpolarization (AHP)), as well as the network properties (especially the sparseness of connectivity). These two conditions appear to be fullfiled by fast spiking (basket or alveus/oriens) interneurons in the hippocampus. They display AHPs of less than 15 mV from the spike threshold. Futhermore, in vivo biocytin and parvalbumin double staining has allowed a direct estimate on the probability of contact between a pair of parvalbumin positive interneurons. This estimated probability is shown in the model to be sufficient but minimal for ensuring a high degree of synchronous firing in a local population of interneurons, which in turn are presumably capable of synchronizing the firing of the pyramidal cell populations.

473.4

MODELS OF GAMMA (~40 Hz) AND THETA RHYTHM IN THE RAT HIPPOCAMPUS IN VITRO AND IN VIVO R.D. Traub[1], J.G.R. Jefferys, M.A. Whittington, G. Buzsáki, M. Penttonen & S.B. Colling IBM Watson Res. Ctr., Yorktown Heights, NY 10598, St. Mary's Hospital Med. Sch., Imperial College, London W2 1PG, U.K. and Center for Neuroscience, Rutgers Univ., Newark, NJ 07102

Synchronized oscillations can be elicited in synaptically coupled networks of interneurons, by stimulation of metabotropic glutamate receptors and without a need for ionotropic glutamate receptors (Whittington, Traub & Jefferys, Nature, 1995). The network frequency is about 40 Hz. We studied further the oscillation mechanisms in a computer-simulated network (with 1,024 pyramidal cells and 256 inhibitory cells), in hippocampal slices and in anesthetized and behaving rats. 1) In the model and in slices, network frequency depends on 3 parameters - $GABA_A$ conductance and time constant in interneuron-interneuron synapses, and the driving current to the interneurons; 2) The model predicts a zero phase lag between pyramidal cell and interneuron firing during 40 Hz, in contrast to models dependent on recurrent excitation. *In vivo* data are consistent with our prediction; 3) The model can generate a 40 Hz rhythm that is frequency-modulated at theta frequency, using theta-modulated inputs to networks of chandelier, basket and dendritic interneurons. Firing patterns then resemble those recorded *in vivo*; 4) The model predicts that synchronized pyramidal cell bursts can be followed by a transient gamma-frequency tail. This phenomenon is observed in the rat following entorhinal cortex lesions. Simulated θ + γ:
2 pyramidal cells above,
basket cell below
Calibrations:
200 ms, 25 mV, 25 mV



473.5

ACTION POTENTIALS AND RELATIONS TO THETA RHYTHM OF SEPTOHIPPOCAMPAL NEURONS IN VIVO. S. E. Fox* and E. Brazhnik. Dept. of Physiol., SUNY Health Sci. Ctr., Brooklyn, 11203.

Our previous intracellular studies distinguished three types of neurons in the medial septal nucleus / nucleus of the diagonal band (MS/DB) based upon duration of action potential (AP) and afterhyperpolarization (AHP). Two of the types are numerous: cholinergic septohippocampal neurons (long-AP, long-AHP) and GABAergic septohippocampal neurons (short-AP, short-AHP). Extracellularly recorded neurons are also divisible into two types based on AP duration. Both short-AP and long-AP cells can fire in rhythmic bursts phase-locked to the hippocampal theta rhythm. To better understand the neuronal organization of MS/DB we measured the phase relations to the theta rhythm, recorded at the hippocampal fissure, for intracellular recordings taken from urethane-anesthetized rats (n=30) and for extracellular recordings taken from freely moving rats (n=96).

Phase histograms of both intracellular and extracellular recordings from short-AP (putative GABAergic) cells showed that they fired mainly on the negative phase of the theta rhythm, whereas long-AP (putative cholinergic) cells fired on the positive phase. Rhythmic bursting of long-AP cells (n=5) was affected by local injection of GABA-A antagonist (picrotoxin, 1-1.5 μg), while the rhythmicity of short-AP cells was relatively unaffected (n=3). The results suggest that, for the theta rhythm that accompanies walking, the "GABAergic" septohippocampal neurons contribute to the phasic modulation of the "cholinergic" septohippocampal neurons.
(Supported by NIH NS 17095.)

473.6

SIMILAR EFFECTS ON THE HIPPOCAMPAL EEG FOLLOWING INJECTIONS OF GABA AGONISTS AND ANTAGONISTS INTO THE MEDIAN RAPHE NUCLEUS. Jeffrey S. Thinschmidt[1*], Bernat Kocsis[2], and Gene G. Kinney[3]. (1) University of Florida, College of Medicine, Dept. of Neuroscience, Gainesville, FL (2) National Institute of Neurosurgery, Budapest, Hungary (3) Fisons Pharmaceuticals, Dept. of Biology, Rochester, NY.

It has been shown that stimulation of the median raphe nucleus (MR) produces a desynchronized hippocampal EEG. We previously demonstrated that injections of 5-HT_{1a} agonists (which inhibit 5-HT cell firing in the MR) and excitatory amino acid antagonists into the MR produce long trains of hippocampal theta rhythm (Vertes et al., *Neurosci.*, 60, 441, 1994; Kinney et al., *Brain Res.*, 654, 96, 1994). More recently, we found that injections of the $GABA_a$ agonist, muscimol, produced similar effects (Kinney et al., *Psychopharm.*, In Press). We now report that injections of the GABA antagonist, bicuculline, also produce long trains of hippocampal theta rhythm.

The hippocampal EEG of urethane anesthetized rats was recorded during baseline conditions (desynchronization), in response to sensory stimulation, and following a 0.1-1µl injection of bicuculline methiodide or saline into the MR. Bicuculline (0.1-1.0µg/0.1µl of saline) produced theta with latencies of 0.5-5.6min and durations of 23.5-76.34min, while saline had no effect on the hippocampal EEG. By comparison, muscimol (0.5-3.0µg/0.1µl of saline) injections into the MR produce theta with latencies of 0-12.45min and durations of 9.62-48.83min (Kinney et al., *Psychopharm.*, In Press).

The unexpected finding that a GABA agonist and antagonist produce similar effects may indicate a complex GABAergic modulation of the hippocampal EEG at the level of the MR. This modulation may be mediated through the following mechanisms: (1) GABAergic receptors on 5-HT cells in the MR; (2) a population(s) of GABAergic interneurons in the MR; (3) an extrinsic GABAergic modulation of the MR.
(Supported by NIAAA grant AA09115 to B.E. Hunter)

473.7

MEDIAN RAPHE INFLUENCES ON HIPPOCAMPAL FIELD POTENTIALS AND NEURON SUBPOPULATIONS. K.Kao.* M.J. Sanders and E.J. Green, Dept. of Psychology, University of Miami, Coral Gables, FL 33124

Prestimulation of the median raphe has been demonstrated to markedly augment the flow of information through the hippocampus. In the present experiments we investigated the mechanism underlying this phenomenon by assessing the influence of MR stimulation on the activity of different neuronal subpopulations.

Urethane anesthetized rats were prepared for stimulation of the perforant path (PP) and median raphe (MR), and recording of units and field potentials in the hippocampus. Prestimulation of the MR consistently produced substantial (> 50%) facilitation of the perforant path-evoked population spike without affecting the evoked field EPSP. Spontaneously active units were classified on the basis of location, spike duration, spontaneous firing rate, and response to paired stimulation of the perforant path. During the period of maximal spike facilitation (30-50 msec following the MR stimulus), MR stimulation produced suppression of many (50%) putative interneurons recorded in the stratum granulosum/hilus; the remainder of those cells were not consistently affected during this period. The majority (72%) of hilar complex spike cells were unaffected by MR stimulation, but 28% showed decreases in spontaneous activity. Similar effects were observed in CA1, where MR stimulation suppressed spontaneous firing in the majority (67%) of CA1 theta cells, and a smaller proportion (38%) of complex spike cells. Excitatory responses to MR stimulation during this interval were not observed.

The present results suggest that facilitation of hippocampal throughput by MR prestimulation likely results from inhibition of inhibitory interneurons. These data, taken with similar observations regarding the actions of other hippocampal afferents (e.g. medial septum) provides support for the idea that disinhibition is a common mechanism by which information flow through the hippocampus is facilitated.

473.8

THE INFLUENCE OF THE THALAMIC NUCLEUS REUNIENS ON HIPPOCAMPAL THETA AND SHARP-WAVE FIELD ACTIVITY IN URETHANE-ANESTHETIZED RATS. L.M. Materi, I.J. Kirk, S.D. Oddie, R.S. Sainsbury and B.H. Bland, Behavioural Neuroscience Research Group, Dept. of Psychology, University of Calgary, Calgary, AB, Canada T2N 1N4.

There is considerable evidence that hippocampal theta (θ) activity is generated independently in CA1 and dentate gyrus cell populations. These two generators give rise to θ fields approximately 180° out of phase. The nucleus reuniens of the thalamus (Re) projects densely to the CA1 field of the hippocampus, but not to the dentate (Wouterlood et al., J. Comp. Neurol. 296:179, 1990). This raises the possibility of differential effects of Re inputs on θ activity generated in the CA1 and dentate. Previous electrophysiological work has established that electrical or pharmacological stimulation of the Re can elicit epileptiform activity in hippocampus (Vachon and Miliaressis, Beh. Neurosc. 106:981, 1992; Hirayasu and Wada, Brain Res. 577:36, 1992), although to date, no role in theta generation has been examined. In this study, hippocampal θ was recorded from close to the CA1 pyramidal layer and from the stratum moleculare of the dentate gyrus. Procaine (0.5 µl; 20%) infused into Re significantly reduced the amplitude of CA1 θ, but had less effect on dentate θ. Electrical stimulation of the Re elicited hippocampal θ in both generators at low intensity stimulation (100-300 µA). Systematically increasing the level of stimulation (up to 1000 µA) initially resulted in the appearance of epileptiform sharp-waves in CA1 (θ still present in dentate). Further increases in stimulation intensity resulted in sharp-wave activity in both CA1 and dentate. These results provide further support for the independence of CA1 and dentate θ generation, and suggest that the nucleus Re preferentially affects activity in the CA1 hippocampal region. (supported by NSERC and AHFMR).

473.9

SPONTANEOUSLY OCCURRING, RETICULARLY-ELICITED AND SEPTALLY-DRIVEN HIPPOCAMPAL THETA RHYTHM: A COMPARISON OF DEPTH AND CURRENT-SOURCE DENSITY PROFILES. I.J. Kirk[1]*, S.D. Oddie[1], N. McNaughton[2] and B.H. Bland[1], [1]Behavioural Neuroscience Research Group, Dept. of Psychology, University of Calgary, Calgary, AB, Canada T2N 1N4. [2]Dept. Psychology and Neuroscience Research Group, University of Otago, PO Box 56, Dunedin, New Zealand.

Hippocampal theta (θ) can occur spontaneously in urethane anesthetized rats or can be elicited by high frequency (eg. 100 Hz) stimulation of the pontine reticular formation. High frequency stimulation of the medial septum desynchronizes hippocampal activity. However, θ-like activity can also be driven by θ-patterned stimulation of the medial septum. It has been suggested that the latter method may result in synchronous activation of septo-hippocampal fibres and fibres of passage and that the "driven θ" is the sum of a variety of rhythmically evoked hippocampal field potentials that resemble θ, rather than being analagous to actual θ (Stewart and Fox, TINS 13:163,1990). In the present study, we analyzed the depth profiles (phase and amplitude) and the current-source density (CSD; Nicholson and Freeman, J. Neurophysiol. 38:356, 1975) profiles generated from them in spontaneously occurring, reticularly-elicited, and septally-driven hippocampal θ (4-8 Hz). Consistent with previous research spontaneous and reticularly-elicited θ reversed about an amplitude null zone in the stratum radiatum. Septally-driven θ was found to reverse in phase at the same level. Also consistent with previous research, CSD analysis revealed predominant rhythmic sink-source pairs in the stratum oriens, stratum radiatum, and stratum lacunosum moleculare of CA1, and at, or just below the fissure in the stratum moleculare of the dentate gyrus. Again, CSD profiles derived from septally-driven θ displayed essentially the same pattern. These results suggest that, at least in the urethane anesthetized rat, septally-driven hippocampal θ is principally the result of activation septal projections normally active during spontaneous or reticularly-elicited θ.

473.10

INTRASEPTAL MICROINFUSION OF MUSCIMOL: EFFECTS ON HIPPOCAMPAL FORMATION FIELD ACTIVITY AND PHASIC THETA-ON CELL DISCHARGES. B.H. Bland*, C.Trepel, S.D. Oddie, & I.J. Kirk. Behavioural Neuroscience Research Group., Dept. of Psych., University of Calgary, Calgary, AB, Canada T2N 1N4.

The effect of intraseptal microinfusions of the GABA-A agonist muscimol on hippocampal (HPC) field activity, and simultaneously-occurring discharges of phasic theta-ON cells, was investigated in urethane-anesthetized rats. The microinfusion of 5.0 - 12.5 nmoles of muscimol into the medial septum (MS) resulted in a progressive reduction (beginning 5 min post-infusion) in the power (amplitude), and finally the total loss of theta field activity. In contrast, the frequency of the theta field activity remained unaffected until it was abolished. In the period immediately following the first 1 min intraseptal microinfusion of 5 nmoles muscimol, a brief period of phasic theta-ON cell excitability was manifested as an increase in the number of discharges per rhythmic burst. Subsequently, associated with the progressive reduction of the amplitude of theta field activity, phasic theta-ON cell discharge rates were progressively reduced for a period beginning 5 min post-infusion of 5 nmoles muscimol. Despite the progressive reduction in the number of discharges, phasic theta-ON cells maintained their preferred phase preferences while theta activity was present. Just prior to the complete abolishment of theta activity, phasic theta-ON cells ceased discharging. During the period when theta activity was replaced by low amplitude asynchronous activity, phasic theta-ON cells discharged in bursts correlated with every occurence of sharp wave field activity. The results support previous suggestions that GABA-ergic neurons in the MS regulate the activity of cholinergic septohippocampal neurons, that the MS modulates theta amplitude primarily and theta frequency secondarily, and that HPC theta and sharp wave field activity represent separate neural inputs to phasic theta-ON cells. (Supported by NSERC & AHFMR).

473.11

PHASIC THETA-ON CELLS IN THE MEDIAL MAMMILLARY NUCLEUS ARE SCULPTURED BY INHIBITORY INPUT DURING HIPPOCAMPAL THETA FIELD ACTIVITY IN URETHANE-ANESTHETIZED RATS. S.D. Oddie*, I.J. Kirk, J. Konopacki, and B.H. Bland. Behavioural Neuroscience Research Group, Dept. of Psychology, University of Calgary, Calgary, AB, Canada T2N 1N4.

Hippocampal theta (HPC-θ) results from the activation of synchronizing inputs which originate in the pons region (PO) and ascend via the posterior hypothalamic and supramammillary (SuM) nuclei to the septo-hippocampal system. Cells in these nuclei, and in the medial mammillary nucleus (MM), show distinct relationships to HPC-θ (Bland et al., 1995). Specifically, MM cells discharge rhythmically and in phase with HPC-θ (phasic θ-ON). The linear relationships which θ-related cells have to HPC-θ are used to characterize frequency coding in the hippocampal synchronizing system. The current study examined the linearity of MM cells during varying frequencies of HPC-θ produced by electrical stimulation in the PO. MM cell discharges were recorded during spontaneously-occurring HPC-θ and during HPC-θ elicited by 0.1 - 0.9 mA PO stimulation. For any recorded signal, the average discharge rate per θ-wave and the frequency of the simultaneously-occurring HPC-θ were determined. For each MM neuron, cell discharge values were regressed onto the frequency values and the significance and type of linearity (i.e. negative, positive, or non-linear) were assessed. Of the 17 MM cells recorded, 12 revealed significant negative-linear relationships to HPC-θ. That is, as the frequency of HPC-θ increased, the rhythmic discharge rate of the MM neurons decreased linearly. Of the remaining 5 cells, two were positive-linear, and three were non-linear. All but one negative-linear MM neuron were found in the anterior portion of the MM, immediately ventral the SuM, whereas the remaining cells were located in the posterior MM. These findings indicate that most rhythmic MM cell discharge, in the 2-8 Hz bandwidth, results from a frequency-coded inhibitory input originating in the septo-hippocampal system.

473.12

RECOGNITION OF SPATIALLY SEPARATED FIELD POTENTIALS AND UNIT ACTIVITY BY "SPATIAL CLUSTERING" METHOD. Z. Nádasdy*, A. Bragin and G. Buzsáki. CMBN, Rutgers University, Newark, NJ 07102.

Sixteen-channel recordings of spontaneous local field potentials, recorded from the CA1 and dentate regions of the hippocampus, were analyzed by a "spatial clustering" method using multiple site silicon probes (100 μm intervals). The rationale of the method is that the amplitude ratios of the simultaneous field components are expected to change systematically with the distance of the electrodes from the actual current source of synaptic currents. Here we describe the utility of the method for known hippocampal field events, sharp waves (SPW) and two types of dentate spikes (DS1, DS2) which are known to be generated by spatially segregated synapses. The "spatial clustering" method could reliably cluster these events in the space-time domain.

The same rationale was applied to identify unit activity. Spatial separation was applied to four-channel multiunit recordings (25 μm intervals) from the CA1 region of the hippocampus. A combination of different spatial descriptors of the spikes led to a convergence of spike localization. The correspondence of the spatial spike-clusters with single neuronal activity was attested by auto-correlation analyses. After single spike separation, we examined somadendritic spike propagation in individual neurons. During slow firing the amplitude ratio of extracellularly recorded spikes in the dendrites and soma were similar. However, during epileptic activity a gradual spike attenuation and failure was observed in the dendrites. The findings show that multiple site recording in the awake animals can be used to monitor spike propagation and network interactions.

473.13

TEMPORAL STRUCTURE IN THE DISCHARGE OF SUPERFICIAL LAYER (II-III) ENTORHINAL CORTICAL NEURONS. J.J. Chrobak* & G. Buzsáki, Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102

The superficial layers (II-III) of the entorhinal cortex (EC), parasubiculum and presubiculum provide the neocortical input to the hippocampus (HPC). Information about the activity of this cell population is crucial for understanding memory formation and the etiology of Alzheimer's dementia. The present study examined the discharge characteristics of these neurons in the freely-behaving rat.

During awake immobility and slow wave sleep, layer II-III neurons discharged single spikes that were phase-related to local high frequency field oscillations (~100Hz) during EC sharp potentials. Entorhinal sharp potentials were prominent throughout the deep and superficial layers of the EC and occur with greater frequency than hippocampal sharp waves. During exploratory activity and REM sleep, superficial neurons fired phase-related to EC gamma (~40-100Hz) oscillations that were coupled to EC theta waves. Most neurons fired in association with gamma for an irregular series of theta waves and then remained silent for indefinite periods. A single cell was observed that fired on each and every theta-nested gamma oscillation. Rhythmic and intermittent high-frequency oscillations associated with EC theta and EC sharp potentials, thus provide temporal structure to the discharge of these neurons. These patterns are analogous to neuronal activity observed at their targets within the dentate/hilar region of the HPC. This structured discharge suggests that functional coupling between these regions is mediated by network oscillations, when the discharge of neurons in each region is brought together in time within the millisecond range.

473.14

ACTIVITY OF NUCLEUS TRIANGULARIS SEPTI (TS) NEURONS TO THETA AND SHARP WAVES IN THE BEHAVING RAT. D Carpi, A Sik and G Buzsáki*, CMBN, Rutgers University, Newark, NJ 07102

The TS is a homogeneous group of neurons located in the fimbria-fornix. TS cells are immunoreactive for calcium binding proteins, calretinin and calbindin, and contain numerous peptides, including LHRH, neurophysin and GnRH. TS neurons may receive afferents from the hippocampal formation and through their efferent projections may convey endocrine information. The aim of this study was to investigate the relationship between firing patterns of TS neurons and hippocampal activity in the behaving rat, specifically during theta waves associated with exploration and sharp waves (SPW) associated with consummatory behaviors and slow wave sleep. Population firing of TS cells were phase-locked to the ascending part of CA1 theta waves. Isolated individual cells rarely fired rhythmically at theta frequency. All TS cells recorded were observed to powerfully increase their firing rates during SPW, indicated by large peaks in the cross-correlograms. The correlation between hippocampal population patterns and TS activity provides physiological support for an excitatory input from the hippocampal formation to this nucleus. The strongest population bursts in association with SPW may explain why several endocrine functions are most affected during sleep and we hypothesize that hippocampal SPW are instrumental in such operations.

473.15

GAMMA FREQUENCY (30-100 Hz) PATTERNS IN THE HIPPOCAMPUS: PARTIAL COHERENCE ANALYSIS. B. Kocsis*, A. Bragin and G. Buzsaki. Natl. Inst. Neurosurgery, Budapest; CMBN, Rutgers University, Newark, NJ

We examined the cellular generation and spatial distribution of gamma frequency activity in the hippocampus of the awake rat. Multiple site recording techniques with silicon probes (16-site arrays) combined with wire electrodes were used. The power at gamma frequency was greater during theta than during immobility or slow wave sleep. Phase measurements indicated 180° reversal of gamma waves at two different locations: across the granule cell layer and across CA1 pyramidal layer. Partial coherence analysis revealed separate dipoles associated with the dentate gyrus (DG) (50-90 Hz), the stratum lacunosum-moleculare (lac-mol) and pyramidal layer (30-60 Hz).Mathematical elimination of the components coherent with electrical activity in the hilus from signals recorded at different depths in CA1 did not affect the coherence between gamma in lac-mol and str. oriens indicating that these oscillators were independent from the DG oscillator. Independence of the lac-mol and pyramidal oscillators was suggested by marked separation of coherences between CA1 electrodes placed above or below the pyramidal layer. Similarly, the coherence between oscillators in the hilus and the hippocampal fissure remained significant when allowance was made for gamma oscillations in CA1.The granule cell layer also separated coherent oscillations in the hilus and the molecular layer. Following surgical removal of the entorhinal cortex (EC), active generators of gamma activity were found in str. radiatum and pyramidale. The coherence between these oscillators could be eliminated by partialization on the signal recorded from CA3c region suggesting that the latter is transmitted from the CA3 region. We conclude that in the intact brain layers II and III of EC are coupled by gamma oscillators of DG and CA1 regions, respectively.

473.16

SEPTAL STIMULATION SUPPRESSES HIPPOCAMPAL SYNAPTIC INHIBITION IN A COMBINED SLICE PREPARATION. K. Tóth, T.F. Freund and R. Miles*. Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary and Neurobiologie Cellulaire, Institut Pasteur, Paris, France.

The septohippocampal pathway includes, in addition to cholinergic afferents, GABAergic fibers which terminate specifically on hippocampal interneurons (Freund & Antal, 1988). We investigated the functional effects of this pathway using septohippocampal slices of mouse and rat. Immunocytochemical staining for parvalbumin and ChAT revealed the slices contained intact septohippocampal axons. The activity patterns of intracellularly recorded septal and hippocampal neurons were as described previously.

We examined the effect of septal stimulation on spontaneous IPSPs recorded with KCl-filled electrodes from CA3 hippocampal pyramidal cells in the presence of CNQX (20μM), APV (100μM) and atropine (1μM). IPSP frequency was reduced by 73 ± 8 % during trains (20-50 Hz, duration 0.2-1 s) of septal stimuli (n=16 slices). Averages of responses to single septal shocks (n=4 cells) revealed a hyperpolarisation at membrane potentials of -65 to -80 mV, while IPSPs were depolarising at these potentials. In contrast, at potentials positive to the IPSP reversal in records with CsCl filled electrodes, averages showed a depolarisation (n=3 cells). These findings suggest that one functional effect of septal activity is hippocampal disinhibition.

473.17

DENTATE MOSSY CELL INVOLVEMENT IN TRANSIENT FOREBRAIN ISCHEMIA: AN IMMUNOCYTOCHEMICAL STUDY.
M. Hsu*†, C. Leranth§, A. Sik† and G. Buzsáki†. †Ctr for Molec & Behav Neurosci, Rutgers Univ, Newark, NJ. §Dept Obstetrics & Gynecology, Yale Univ, New Haven, CT.

Previous studies have implicated the degeneration of dentate mossy cells after ischemia and epilepsy. Because mossy cells do not express the common neuropeptides and calcium-binding proteins known to date, they have been impossible to visualize other than using Golgi methods. Recently, we have shown,using the Gallyas silver stain for "dark" neurons and immunocytochemistry against heat-shock proteins (HSP72), that mossy cells are affected by ischemia. However, it is still controversial whether neurons expressing HSP72 actually degenerate, or whether the damage is reversible. In the companion abstract, we report that in the dentate hilus, only mossy cells stain for the GluR2/3 subunit of the AMPA receptor (Leranth et al, 1995). This allowed us to directly visualize mossy cells in the postischemic hilus. Rats were made ischemic using the 4-vessel-occlusion method and allowed to survive for 4-12 mo. Their brains were then processed for immunocytochemistry against GluR2/3 and the calcium-binding proteins calbindin, calretinin, and parvalbumin. Non-overlapping hilar cell populations were immunostained using these antibodies. Electron microscopic examination revealed the presence of thorny excrescences on the thick proximal dendrites of the GluR2/3-positive cells, confirming that they were indeed mossy cells. Although a few mossy cells were still present in the postischemic hilus, their numbers were drastically reduced compared with controls. Yet, mossy cells could be seen even in ischemic brains where CA1 had completely degenerated. These results indicate that the extent of hilar and CA1 ischemic damage may not parallel each other, and that ischemic cell damage in the hilus and CA1 may occur by means of independent mechanisms.

473.18

REGULATION OF EXCITATORY SYNAPTIC INPUT TO BASKET CELLS IN NEONATAL RAT DENTATE GYRUS DURING HYPOXIA.
J. Doherty*, T. Kneisler and R. Dingledine. Dept. of Pharmacol., Emory Univ., Atlanta, GA.

Alterations in excitatory input to basket cells in the dentate gyrus of neonatal rat hippocampal slices resulting from brief hypoxic episodes (1-5 min. 95% N_2/5% CO_2) were examined under whole cell clamp. Basket cells were morphologically identified by a biocytin staining protocol following electrophysiological study. A brief hypoxic episode evoked two slow currents, a low amplitude (10-20pA) hypoxic outward current, followed by a larger (40-60pA) post-hypoxic outward current. Hypoxic episodes also increased the frequency of spontaneous EPSCs.

Minimal electrical stimulation of dentate granule cells, CA3 pyramidal cells, and the perforant path generated AMPA and NMDA receptor mediated excitatory postsynaptic currents (EPSCs) in basket cells. Brief hypoxic episodes rapidly and reversibly suppressed basket cell EPSCs evoked in response to all three inputs. Hypoxic episodes also suppressed CA3 evoked polysynaptic IPSCs in dentate granule cells, but did not suppress monosynaptic IPSCs evoked by stimulation in the granule cell layer. Adenosine A1 receptor antagonists (DPCPX, N-0840 1 μM) delayed, but did not block, hypoxia-induced suppression of basket cell excitatory input.

Adenosine (50 μM) rapidly and reversibly suppressed both evoked and spontaneous basket cell EPSCs, but did not generate slow postsynaptic currents. The metabotropic glutamate receptor agonist 1S,3R-ACPD (30-100μM) also rapidly and reversibly suppressed evoked EPSCs, and sharply increased the frequency of spontaneous EPSCs. Application of 1S,3R-ACPD generated slow inward currents in 71% of basket cells. Loss of excitatory input to dentate basket cells may contribute significantly to hippocampal hyperexcitability following hypoxia. The extreme sensitivity of basket cell excitatory inputs to suppression under hypoxic conditions suggests that these inputs may be crucial in regulating hippocampal excitability.

473.19

MAPPING HUMAN LIMBIC RESPONSES TO SUBANESTHETIC NITROUS OXIDE BY PET.
F. Gyulai, L. Firestone, M. Mintun*, P. Winter. Dept. of Anesthesiology/CCM, and PET Facility, University of Pittsburgh, PA 15261

Recent human behavioral studies have shown that even subanesthetic concentrations of nitrous oxide (N_2O) impair psychomotor, cognitive performance and semantic learning and memory processes. To elucidate the cerebral areas mediating these effects, following IRB approval (#931063), 8 volunteers were studied, by positron emission tomography (PET). ^{15}O-water was used to detect changes in regional cerebral blood flow (rCBF), that in turn reflected central neuronal activity, during room air or 20% N_2O inhalation. In 4 subjects, N_2O-related regional cerebral metabolic activity (rCMR) was also measured with ^{18}F-deoxyglucose, to control for possible effects of N_2O on rCBF/rCMR coupling. Two rCBF and 1 rCMR scan was obtained in each of these conditions. The room air condition always preceded N_2O administration. N_2O-associated regional activity changes were identified using the statistical parametric mapping method at a significance level of 0.01. The magnitude of changes detected by the rCBF and rCMR scans were statistically analyzed (t-test) for differences ($p<0.01$).

Twenty percent N_2O did not produce significant spatial or magnitude differences in rCBF/rCMR coupling. N_2O inhalation was associated with significant *activation* in the anterior cingulate cortex and *deactivation* in the poster cingulate and hippocampal areas. The anterior cingulate cortex is associated with psychomotor and cognitive function, while the posterior cingulate and hippocampus mediate learning and memory. Thus these neuroanatomic findings strongly correlate with the behavioral effects of N_2O. Supported by a grant from the IARS (LF), UACCMF, UPMC PET Facility.

473.20

SELECTIVE ACTIVATION OF A LIMBIC NETWORK IN HUMANS
D. Servan-Schreiber*, W. Perlstein, J.D. Cohen, & M. Mintun. Dpt. of Psychiatry and PET Facility, Univ. of Pittsburgh, Pittsburgh, PA, 15213.

Previous studies have suggested that Procaine HCl administered IV can reliably induce activation of limbic structures. In this PET O15 study, 10 normal human volunteers received two injections of placebo and two injections of procaine HCl 1.84 mg/Kg in a single scanning session (ABBA sequence). One minute scans were obtained two minutes after each injection. Subjects were instructed to pay attention to somatic, perceptual, cognitive and emotional effects and interviewed for 10 mins after each injection. Procaine induced powerful and varied subjective effects including anxiety, depression and euphoria, as well as somatic and perceptual changes. It also reliably increased plasma cortisol levels. Blood-flow increased very selectively in a bilateral anterior limbic network encompassing the insular cortex (IC), the anterior cingulate cortex (AC) and the amygdala/parahippocampal region (AMG/PHC). No neo-cortical region was comparably activated. Individual subject analyses showed that activation of this anterior limbic network occurred in all subjects. A principal component analysis with varimax rotation on selected neocortical and limbic voxels suggested three independent patterns of activation that together accounted for 77% of the variance in blood-flow among the selected voxels over all four scans. The first pattern reflected primarily anterior limbic activation (IC, AMG/PHC, and rostral AC). Exploratory correlations revealed a strong selective association with ratings of mood, somatic and perceptual symptoms, and with changes in plasma cortisol. The second pattern captured changes in blood-flow in the inferior frontal cortex (IF), with right and left IF loading in opposite directions. It was not associated with the subjective or physiological variables measured in this study but was related to time spent in the scanner. The third pattern captured primarily blood-flow changes in the caudal AC and was associated selectively with changes in heart rate.

474.1

New CBF-based Functional MRI Technique, Seong-Gi Kim*, Center for Magnetic Resonance Research, Dept. of Radiology, Univ. of Minn., Minneapolis, MN 55455, In order to measure relative cerebral blood flow (relCBF) changes quantitatively, we have developed a **F**low-sensitive **A**lternating **I**nversion **R**ecovery (**FAIR**) MRI technique (Kim, Magn. Reson. Med., accepted), in which perfusion-based functional images can be generated by subtracting two inversion recovery (IR) images, one with a non-slice-selective IR pulse and another with a slice-selective IR pulse. Consecutive FAIR echo planar images were collected, first under a resting state, then during a "task" period, and ending with a recovery state. Tasks are repetitive thumb-digit opposition movements. Imaging parameters are spatial resolution = 3.8x3.x5 mm, inversion time = 1.4 s and inter-image time = 2.8 s. In all 4 subjects, the contralateral motor cortex was activated during finger movements, which is consistent with previous studies. The relCBF change of contralateral motor cortical area in four subjects detected by the FAIR technique was 49 +/- 16% SD. Although the FAIR technique provides the same relCBF information compared to PET, the MR method provides advantages due to availability of higher spatial and temporal resolution, ease of use and absence of any invasive procedures. Also, the FAIR technique is capable of generating microvascular-based functional maps and relatively independent of magnetic field strength. (Supported by NIH grants NS32919 and RR08079).

474.2

SUBTRACTION ANALYSIS OF FDG PET DATA: DESCRIPTION OF METHOD AND COMPARISON OF TWO AGE GROUPS. J.R. Absher* (1,2), F.H. Fahey (3), C. Eades (3), F.B. Wood (1). Departments of Neurology (1), Psychiatry and Biobehavioral Sciences (2), and Radiology (3), Bowman Gray School of Medicine, Winston-Salem, N.C. 27157-1078, U.S.A..

Worsley and colleagues described a technique for statistical analysis of PET activation experiments. We describe a modification of the technique for between-group comparisons, and report an analysis of 11 "old" and 20 "young" subjects. Normal volunteers were recruited from the community, and carefully screened for psychiatric, neurological, and neuropsychological problems. All subjects underwent ^{18}FDG PET scanning on a Siemens CTI 951/31 PET Scanner, following a standardized period of tracer uptake. All subjects performed a letter identification task during glucose uptake to reduce variability in glucose metabolism. The PET scans were registered to T-1 weighted MRI scans. MRI scans were automatically transformed to stereotaxic space using a cross correlation minimization procedure. All the PET scans were placed in stereotaxic space by combining transformation parameters for each step. Each PET surface was marked with a 3D contour so the degree of overlap among scans ("n image") could be computed. Between-group differences in glucose metabolism were computed for each voxel, along with the pooled variance for each group. The n image and the pooled variances were used to scale the difference image to the appropriate t value. Significant differences were identified as those exceeding the critical threshold. Significant age-related reductions ($p < 0.01$) in glucose metabolism were noted bilaterally in the parietal, frontal, and prestriate cortices, and caudate nuclei. The right putamen, right insula, left anterior cingulate, and left Heschl's gyrus also demonstrated age-related declines in glucose metabolism. We conclude that unpaired analysis of average ^{18}FDG PFT scans is a viable analytic strategy.

474.3

FLUCTUATION IN SOMATOSENSORY OPTICAL SIGNAL DURING SUSTAINED STIMULATION. M. Heneqar, J. Dowling, D. Liu, †C. Rovainen, T. Woolsey, R. Grubb*. Departments of Neurosurgery and of †Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110

Measurable changes in optical characteristics of the cerebral cortex at various wavelengths accompany increased neural activity. Activity dependent changes in absorbance at 520-560 nm, are attributable to changes in blood volume and thus reveal fluctuation in blood flow or perfusion. Optical changes were monitored over the rat whisker-barrel cortex during whisker stimulation with a Macintosh computer, CCD camera with image intensifier, research microscope and public domain software (NIH Image 1.49). Somatosensory cortex was imaged through a closed cranial window (11mm, i.d.) in 300 g Wistar rats anesthetized with 1 g/kg urethane, ip. Single and C-row whiskers were stroked at 4-5 Hz for up to 30 minutes. Pixel by pixel comparisons of images obtained prior to, during and after stimulation revealed blood flow related changes in the optical characteristics of barrel cortex and of cerebral vessels. Arterial, parenchymal and venous responses were selected and analyzed at intervals as short as 133 ms directly from the CCD camera or *post hoc* from video tape recordings. These studies demonstrate the temporal and spatial fluctuations in optical signal which occur in response to prolonged stimulation.

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474.4

APPRAISAL OF UNIVARIATE AND MULTIVARIATE ANALYSIS TECHNIQUES FOR PET BRAIN IMAGE QUANTIFICATION

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Analyses of PET brain image data currently quantify statistical effects using pixel-wise univariate comparisons within and/or between datasets. However, functional intercorrelation and multiple comparisons of rCBF measurements may complicate the interpretation of results obtained via this approach. Multivariate methods avoid these problems by measuring effects along latent dimensions present in the data. To date, no direct comparisons of univariate and multivariate methods of PET data analysis have been conducted. We assessed the relative performance of univariate and multivariate techniques in the analysis of $H_2{}^{15}O$ PET data from age-, sex-, and performance-matched medicated (N=10, haloperidol 0.4mg/kg/d) and unmedicated (N=10) schizophrenic groups and normal controls (N=10) during the Wisconsin Card Sort test. In particular we contrasted; 1) multivariate discriminant analysis vs. pixel-wise t-tests, for measuring differences between subject groups; and 2) canonical correlation vs. pixel-wise correlations, for assessing the within-group relationships between task and control conditions. Discriminant analysis offered an index of the overall difference between the groups ($F=4.13$, $p=0.006$), a measure not offered by the pixel wise omnibus map, and indicated two separate dimensions presumably due to diagnostic and possibly medication related effects. Pixel-wise comparisons indicated similar results, but required multiple contrasts of the groups to draw similar conclusions. Canonical correlation analysis between task and control conditions suggested strong overall associations with variations across groups (with correlations highest in the medicated patient group) which were not as evident via pixel-wise correlations. Overall, multivariate methods were more robust and succinct than univariate methods in describing statistical effects present in the data, which in these clinical groups suggested effects of diagnosis and drug treatment on rCBF.

474.5

A METRIC FOR VALIDATING CROSS-MODALITY IMAGE REGISTRATION TECHNIQUES. K.J. Black*, T.O. Videen, J.S. Perlmutter. Departments of Radiology, Psychiatry and Neurology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

We placed thin tubing in the shape of 3 N's in a rigid headholder used for imaging studies in primates. The headholder bolts firmly to a surgically implanted "cap" affixed to the skull. The tubing can be filled with a solution containing either ^{18}F or $CuSO_4$, for visualization with PET or MRI, respectively. Using manual segmentation of each limb of each N, followed by least-squares regression, we can locate the two "corner," or intersection, points on each fiducial. This gives 6 well-localized fixed points. Test-retest accuracy in a Siemens 953b PET scanner varied from 0.2mm (largest fiducial) to 1.1mm (smallest fiducial). Comparing the location of these fixed points in serial PET scans during a given study confirmed that there was no detectable movement over a period of several hours (mean error 0.4mm, or within the limits of test-retest accuracy). Accuracy was also assessed by computing distances between independently located fixed points. The method preserved distances across MR studies (<1% error), across PET studies (<1% error), and between modalities (<2% error). Given this validation of our ability to find these fixed points in either PET or MRI, we can test the accuracy of cross-modality image registration techniques which *do not* use these fiducials. We have applied this test to the AIR method of Woods et al. (**J Comput Assist Tomogr** 17:536-546, 1993) in our ongoing PET and MRI studies of baboons. Preliminary results show that summed blood flow images ($H_2{}^{15}O$-PET) can be aligned to MR anatomic images with an accuracy of 1.5mm-5.22mm (mean, 3.3mm), *measured at the external fiducials*. Maximum error *in the brain* is roughly 2mm. Single, unfiltered $H_2{}^{15}O$ emission images are aligned with only slightly worse accuracy. In sum, we report validation of a stringent test which can be applied to any post-hoc method for intra- or cross-modality image registration.

474.6

THE TEMPORAL/SPATIAL EVOLUTION OF OPTICAL SIGNALS IN HUMANS. A.F. Cannestra*, A.W. Toga, K.L. Black. Lab. of Neuro Imaging, Division of Brain Mapping, Department of Neurology, UCLA School of Medicine, Los Angeles, CA. 90095.

Human somatosensory and motor cortex was mapped in response to peripheral stimulation using optical reflectance imaging. Informed consent was obtained from patients whose tumor resection neurosurgeries required parietal cortex exposure. Upon reflection of the dura evoked potentials (EP) and optical images were collected in response to median and ulnar nerve stimulation. Non-contact image data acquisition was demonstrated by monitoring and synchronizing to electrocardiographic and respiratory waveforms. The spatial extent of the optical response correlated topographically with the electrophysiologic response to peripheral transcutaneous nerve stimulations. Activated regions were observed in both precentral (motor) and postcentral (sensory) contralateral cortex. The evolution of the signal demonstrated a genesis at 1-2 s, a peak at 3 s, and a return to baseline by 9 s. The optical response differed spatially when median and ulnar nerves were stimulated separately. Ulnar stimulation produced a more posterior response in the postcentral gyrus in relation to median nerve stimulation. When both median and ulnar nerve simultaneous stimulation was performed a larger and more focal response appeared than either nerve alone. The simultaneous median and ulnar nerve stimulation produced an optical response greater than the arithmetically additive response of separate stimulations. EP mapping generated a similar pattern. These optical reflectance maps, overlaid by the EP mapping of human somatosensory and motor cortex correlates neuronal firing, vascular and metabolic function.

474.7

A STUDY OF HUMAN SENSORIMOTOR AREAS USING WHOLE BRAIN ISOTROPIC FUNCTIONAL MRI WITH 0.05 ML RESOLUTION. R.H. Sexton[1*], V.S. Mattay[1], A.K.S. Santha[1], K.A. Tallent[1], A.C. McLaughlin[1], J. Pekar[2], J. Ostuni[2], P. Jezzard[3], E.C. Wong[4], J.A. Frank[2], and D.R. Weinberger[1]. [1]CBDB/NIMH, [2]LDRR/OIR, and [3]LCE/NHLBI, National Institutes of Health, Bethesda, MD 20892; [4]Depts. of Radiology and Psychiatry, Univ. of California at San Diego.

In an attempt to map in 3-D space the response of human brain sensorimotor areas to a simple motor task, we performed whole-brain blood oxygenation level dependent functional Magnetic Resonance Imaging at 1.5 T in five normal volunteers. Sagittal gradient-echo echo planar images of the whole brain with 3.75 mm isotropic voxels were acquired continuously while the subjects performed sequential tapping of the thumb and fingers of one hand followed by a resting period. Each active/rest period lasted for 33 seconds. A complete study consisted of eight such pairs of active/rest segments.

The threshold for determination of "activation" was set at p value of 0.05 (two-tailed), Bonferroni-corrected for the ~20,000 pixels in the brain, yielding a Z score of ~4.7. Using this criterion, all five subjects showed activation in ~1% of brain pixels located predominantly in the contralateral sensorimotor cortex and the supplementary motor areas. The average signal change in these activated pixels was ~4%. These results demonstrate the potential of whole brain fMRI with EPI at 1.5 T for robust investigational brain mapping.

474.8

FMRI DEMONSTRATES SUSTAINED BLOOD OXYGENATION AND FLOW ENHANCEMENT DURING EXTENDED DURATION VISUAL AND MOTOR CORTEX ACTIVATION. P. A. Bandettini*, K. K. Kwong, T. L. Davis, A. Jiang, J. R. Baker, J. W. Belliveau, R. M. Weisskoff, and B. R. Rosen. Massachusetts General Hospital - NMR Center, Charlestown MA

Sustained blood flow and oxygenation elevation during extended cortical stimulation was demonstrated using T1-weighted (blood flow sensitive) and T2*-weighted (blood oxygenation sensitive) echo-planar MRI. Seven subjects were studied. Flow sensitive (inversion recovery EPI: TI = 1000ms, TR = 3000ms, TE=20ms: spin-echo) and blood oxygenation sensitive (gradient-echo EPI: TR = 3000ms, TE = 40ms) time course series were obtained using a 1.5 T GE Signa scanner retrofitted with an ANMR resonant gradient system. Five subjects underwent 10 Hz full field black and white alternating checkerboard visual stimulation. Timing was: 1 min.off, 1 min.on, 1 min.off, 20 min.on, 1 min.off, 1 min.on, 1 min.off. Two subjects performed repetitive finger tapping for up to 20 minutes during T2*-weighted image collection. Motion correction was also performed.

All subjects demonstrated sustained elevation of flow and oxygenation during the 20 minute stimulation period. A post-stimulation undershoot was observed with the T2*-weighted sequence but not with the T1-weighted sequence. These studies demonstrate that flow and oxygenation elevation are *not* transient effects for these types of stimuli. Minimal neuronal habituation occurs and oxygen extraction does not sufficiently increase to cause a decrease, over time, in blood oxygenation sensitive MR signal. Ongoing studies of extended duraton stimulation of visual cortex tissue known to have high cytochrome oxidase concentrations, "blobs", and therefore higher oxidative metabolism, are being performed to determine the sensitivity of the blood oxygenation-sensitive MR signal to differences in oxidative metabolic rate.

474.9

INTERNAL CAROTID AND VERTEBRAL ARTERIAL BLOOD FLOW SIMULTANEOUSLY AND CONTINUOUSLY MEASURED BY ULTRASONIC DOPPLER FLOWMETRY IN ANESTHETIZED AND CONSCIOUS RABBITS Ying-Hui Yu*, B-S Zhu and W.W. Blessing. Centre for Neuroscience, Flinders University, Bedford Park, SA 5042, Australia.

Ultrasonic Doppler flowmeters, implanted around the internal carotid and vertebral arteries were used to continuously measure cerebral blood flow (CBF) in the rabbit, both in anesthetized and in conscious animals. Flow in these arteries was compared with sagittal sinus flow, simultaneously measured. All three flows increased in the expected manner with increases in the concentration of CO2 (up to 10%) in the inspired gas. There were linear relationships with high correlation (0.83 to 0.96 in six animals) between mean internal carotid flow and mean sagittal sinus flow. The sagittal sinus blood flow increased to 205±27% of baseline (n=6, P<0.01). At the same time internal carotid and vertebral arterial flows also increased (to 200±31% of baseline for internal carotid flow, n=6, P<0.01 and to 139±7% of baseline for vertebral flow, n=6, P<0.01). When animals were breathing normal air (conscious animals) or oxygen and halothane (anesthetized animals) arterial pCO2 was 39±2 mmHg (n=6). When inspired CO2 was increased to 9%, arterial pCO2 was 54±2 mmHg (n=6). The relationship between internal carotid flow and inspired CO2 was different in conscious and anaesthetised rabbits. Vertebral and internal carotid angiography demonstrated the recording probes and the cerebral distribution of the vessels around which they were implanted. Measurement of cerebral arterial inflow by chronically implanted ultrasonic Doppler flowmeters is quite feasible, accurate, relatively non-invasive, and suited to repeated measurements in different experimental conditions, particularly in the conscious rabbit. The similarity in cerebrovascular arrangements between rabbits and humans means that the rabbit may become an important model for studies of the control of CBF relevant to humans.

474.10

LAMINAR ANALYSIS OF CEREBELLAR BLOOD FLOW BY A LASER-DOPPLER COMBINATION PROBE. M. Fabricius*, N. Akgören and M. Lauritzen. Dep. of Med. Physiology, Univ. of Copenhagen, DK-2200 Copenh., Denmark.

Laser-Doppler flowmetry (LDF) estimates relative changes of cerebral blood flow (CBF). The measurement depth depends on the wavelength of the laser light and on the separation between the transmitting and recording optic fiber of the LDF probe. We used a LDF combination probe (Perimed, Sweden) holding four combinations of wavelength and fiber separation to measure CBF in four different depths in the cerebellar cortex of halothane anesthetized rats. The cortex was exposed to local electrical stimulation, hypercapnia, and adenosine applied topically or injected 1 mm into the tissue through a glass microelectrode.

Local tetanic stimulation, mainly of the parallel fibers, increased CBF by 78 ± 8% in the upper layer corresponding to the superficial part of the molecular layer in which these fibers and their synaptic connections are located. The CBF increase declined at increasing depth of measurement to 59 ± 8%, 43 ± 7%, 23 ± 3% (P = 0.001, ANOVA test). Hypercapnia increased CBF by 102 ± 14% and 94 ± 11% in the middle depths, but only by 44 ± 10% in the upper layer and by 77 ± 8% in the largest depth (P = 0.002). Topical adenosine (1mM) increased CBF by 118 ± 20% in the upper layer, the values decreasing steadily to 52 ± 5 in the largest depth (P = 0.004). Injection of 100 nl of adenosine (20mM) increased CBF by 38 ± 7% in the largest depth, the values decreasing steadily to 20 ± 6% in the upper layer (P < 0.001).

We conclude that the LDF combination probe is valid for laminar analysis of CBF with a resolution in depth of 2-300 µm. Important differences of laminar CBF reactivity is seen during hypercapnia.

474.11

DIFFUSION OF [3]H_2O AND [14]C-PEG IN ISCHEMIC BRAIN SLICES. **G.Newman*, F.E.Hospod, C.S.Patlak. Depts. of Neurology and Surgery, SUNY at Stony Brook, NY, and Northport VAMC, NY.**

Spectacular advances in imaging of molecular diffusion in humans by diffusion-weighted MRI are finding immediate application in stroke management. However, interpretation of the apparent diffusion coefficient (ADC) is complicated because it reflects diffusion in the extracellular space (ECS) and intracellularly, capillary blood flow and CSF flow. Brain slices provide an opportunity to study diffusion without blood or CSF. Our model of ischemia employs 1050µm hippocampal brain slices which share features of *in vivo* ischemia including histology, increased water, lactate and glucose utilization and reduced adenylates.

Both influx and efflux of [14]C-PEG(4000) and [3]H_2O were studied in 450µm or 1050µm hippocampal brain slices in Krebs-Ringer with 3% dextran for periods of 30 sec to 3 hours with about 60 slices per data set. Slice radioactivity and times were analyzed with kinetic models which explicitly include diffusion in ECS and one serial compartment, using non-linear least squares methods. [14]C-PEG experiments were used to determine the size of the ECS for the [3]H_2O experiments. Diffusion coefficients (D_C) and transport rate constants, with calculated intracellular space, were determined by the best least squares fit.

Thick slices show a large reduction in ECS to 0.16, from 0.25 in thin slices, along with marked intracellular swelling (cytotoxic edema). Surprisingly, the D_C of both tracers increase in the thick slices, contrary to the observed decrease in ADC of water by MRI in stroke patients. We will discuss this seeming paradox in light of ongoing experiments but these results emphasize the complexity of events that underly the MRI ADC measurements. Support: VA Merit Review and NIH #NS28429.

474.12

GENERALIZED THEORY FOR INTERPRETING MICRODIALYSIS

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Previous mathematical modeling of microdialysis has been directed primarily at interpretation of measurements obtained with low-molecular-weight, hydrophilic solutes. For such solutes, movement through tissue is assumed to occur predominantly by diffusion through the interstitial fluid. The steady-state and transient models have now been extended to treat lipophilic solutes by incorporating transcellular solute movement. When applied to microdialysis measurements in muscle and brain for the lipophilic solute, ethanol, the steady-state model indicates a significant transcellular contribution.

The revised models suggest that a useful measure for characterizing microdialysis is the ratio, $H = P_t \cdot r_o / D_t$ in which P_t and D_t are the permeability and diffusivity for the solute in the tissue of interest. The ratio is made nondimensional by including the outer radius of the microdialysis probe, r_o. The magnitude of this ratio is an increasing function of the nondimensional clearance rate, $\theta = r_o \sqrt{k_t / D_t}$, where k_t is the rate constant characterizing clearance of the solute from the tissue interstitium. The radial distance, r_t, over which microdialysis disturbs tissue concentrations exhibits a strong inverse relationship to θ. The dominance of the nondimensional clearance rate, θ, signifies the important role played by local clearance processes in microdialysis behavior. The influence of a third parameter ratio, L_m / r_o, involving the probe membrane length, L_m, has not yet been incorporated. Its effects are generally neglected, since L_m is typically much larger than r_o. This assumption may be questionable, at least for slowly cleared solutes.

The applicability of the generalized theory will be illustrated for a wide range of hydrophilic and lipophilic solutes and interstitial clearance mechanisms: very rapid active cellular uptake of dopamine, rapid utilization of glucose, active efflux-to-blood of dopamine metabolites, passive efflux of ethanol and tritiated water and slowly effluxing extracellular markers, such as sucrose.

475.1

A FUNCTIONAL MRI (fMRI) STUDY OF CEREBELLUM DURING MOTOR AND WORKING MEMORY TASKS. J.E. Desmond*, J.D.E. Gabrieli, B.L. Ginier, J.B. Demb, A.D. Wagner, D.R. Enzmann, and G.H. Glover. Depts. of Radiology and Psychology, Stanford University, Stanford, CA 94305.

Recent studies have suggested cerebellar contributions to cognition as well as to motor performance. We investigated cerebellar activation in human volunteers using fMRI during two tasks: (1) A motor task consisting of finger-to-thumb movements of the right hand, and (2) A working memory task (Sternberg, Science, 153:652, 1966) in which difficult and easy conditions were alternated. For the difficult condition, an array of 6 different letters was presented on a screen for 0.5 sec, followed by a 5.5 sec no-stimulus delay, followed by a single letter probe. Subjects responded using a squeeze ball if the probe matched one of the letters in the 6-letter array. For the easy condition, the 6 letters were all the same. Subjects received 4 cycles of easy/difficult for the memory task and movement/rest for the motor task over a 6 min scan. Brain images of the cerebellum were collected continuously from 2-4 slices oriented parallel to the brain stem using a T2*-weighted gradient echo spiral sequence (1.5T, 5-10mm thick slices, TR=75 ms, TE=40 ms, flip angle=23). Preliminary results from 4 subjects revealed for the motor task mostly ipsilateral increases in activation in lobules HIV and HV. Ipsilateral or bilateral dentate activation also appeared to be present. To a lesser degree, mostly bilateral activation was observed in HVIII. For the working memory task, hemispheric increases in activation for the difficult condition were observed in two locations: (1) mostly on the right side of lobule HVI, extending in two cases to superior portions of HVIIA; (2) bilaterally in inferior HVIIA and HVIIB. These results suggest that fMRI can be used to study cerebellar contributions to cognition. Supported by NIH (F32NS09628), Stanford Office of Technology and Licensing, and the Lucas Foundation.

475.2

WORKING MEMORY AND DECISION-MAKING ARE MEDIATED BY TWO SEPARATE SECTORS OF THE PREFRONTAL CORTEX. A. Bechara,* D. Tranel, H. Damasio, S.W. Anderson and A.R. Damasio, Div. of Cognitive Neuroscience, Dept. of Neuro., Univ. of Iowa, Iowa City, IA 52242.

The prefrontal cortex has been implicated in the mediation of both working memory and decision-making. How related or distinct are the respective operations? Do they depend on separate anatomical substrates? To approach these questions, we compared the performances of normal controls and frontal lobe patients on two sets of behavioral tasks. One included modified delay tasks (delayed response; delayed non-matching to sample), which test experimentally the role of prefrontal cortex in working memory as studied in nonhuman primates. The other included "gambling" tasks which we recently designed to study the real-life decision-making deficits expressed by frontal lobe patients.

Five patients with bilateral damage to the ventromedial prefrontal cortex, but without damage to the basal forebrain, were severely impaired on the decision-making tasks, but not on the working memory tasks. (When the damage included the basal forebrain, patients (n=4) were impaired on both the decision-making and memory tasks). By contrast, four patients with damage to the right dorsolateral sector of the prefrontal cortex were severely impaired on the working memory tasks, but less so on the decision-making tasks. Four patients with dorsolateral lesions on the left were only mildly impaired on the working memory tasks. These results suggest: (1) A relative segregation of the neural structures supporting working memory (right dorsolateral sector) and decision-making (ventromedial sector). (2) A relative asymmetry in the cognitive deficits observed after damage to each sector. Decision-making defects can occur in the absence of working memory defects; however, working memory defects are usually accompanied by some compromise in decision-making.

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475.3

PERFORMANCE OF CONTINGENT OPERATIONS AND SHORT TERM MEMORY FOR A STIMULUS ACTIVATE DIFFERENT PARTS OF THE PRE-FRONTAL CORTEX
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The prefrontal cortex is known to be important for the performance of consecutive operations according to a plan. We investigated if the number of contingencies in an instruction effects the cortical activity. Ten human subjects performed three short-term memory tasks with delayed matching of coloured patterns. Two of the tasks contained instructions with additional contingencies of how to alternate between the matching of colours and patterns. Regional cerebral blood flow was measured with PET, during the three tests and one control situation which contained the same type of visual stimulation and motor responses as the tests.

All three short-term memory tasks had two cortical activations in common: one in the left intraparietal sulcus, and one in the left inferior frontal sulcus. Keeping a stimulus representation in short-term memory was apparently carried out through the co-activation of these two areas. In the two tasks with additional contingencies, the right middle frontal gyrus was also activated. This activity was interpreted as underlying the ability to perform contingent, consecutive operations.

475.4

A PET STUDY OF IMMEDIATE AND MEMORIZED VISUOMOTOR TRANSFORMATIONS. F. Lacquaniti*, F. Guigon, D. Perani, V. Bettinardi, F. Fazio Istituto Scientifico S. Lucia, 00179 Rome, Italy; INSERM CREARE, UPMC, 75005 Paris, France; INB-CNR, 20131 Milan, Italy; Cattedra di Medicina Nucleare, Università di Milano, 20132 Milan, Italy.

Positron emission tomography was used to reveal the neural bases of visuomotor transformations in the human brain. Subjects ($n = 8$) were instructed to fixate a small spot displayed on a screen in front of them, to detect the appearance of a peripheral target (every 5 s) and to perform a movement using a stylus on a horizontally oriented table. In a first condition (*direct*), the movement was performed toward the current target. In the second conditions (*memory*), the movement was toward the previous target. The results show that different networks are involved in *direct* and *memory* conditions. Common processing pathways involved the contralateral sensorimotor cortex, the contralateral inferior frontal gyrus, the vermis and the cerebellar hemispheres bilaterally, the right fusiform gyrus. The *direct* condition involved specifically a focus in the left precentral gyrus, in the left middle occipital gyrus. The *memory* condition activated specifically the contralateral supplementary motor area, the anterior thalamus, the cingulate cortex, the lingual gyrus, the calcarine sulcus and the hippocampus, bilaterally. The *memory* condition is a spatial short-term memory task and activates a network (hippocampus, anterior thalamus, cingulate) generally associated with long-term memory. In the *memory* condition, the subject must simultaneously execute the appropriate movement toward the position of the previous, memorized target, and memorize the current target as the goal of the next movement. The present results indicate that the neural substrat of long-term memory might be involved when sensorimotor encoding and decoding processes are in competition.

475.5

INVESTIGATING WORKING MEMORY IN HUMANS AS INDEXED BY COGNITIVE EVOKED POTENTIALS. G. S. Robinson*. Dept. of Psychology, Skidmore College, Saratoga Springs, NY 12866

The utility of cognitive evoked potentials to objective -ly study memory processes in humans is on the rise. Cognitive evoked potentials were recorded from 20 college students, while they performed three different conditions designed to activate working memory. Condition one was the the letter "X" as the target letter, and the rest of the letters as non-targets. Condition two was a two letter word task, in which the target letter was the last letter of any two letter word. Condition three was a three letter word task, in which the target letter was the last letter of any three letter word. The letters were presented one at a time in the center of the screen. Thus, for the word conditions, the subjects had to hold the last one or two letters in working memory in order to identify a two or three letter word.

Activity recorded from Cz, Fz and Pz, indicated an increase in P300 latency, $F(2, 28) = 58.21$, $p < .01$, with increasing working memory demands. The P300 amplitude generally decreased with increasing working memory demands. However; there was a significant interaction for amplitude and scalp location. Results suggest that cognitive evoked potentials are sensitive to changes in working memory processes. Further, amplitude scalp locations, suggest different neural arrays for different levels and demands of working memory processing.

475.6

NEUROPHYSIOLOGY OF SEMANTIC CONCEPTS. S.L. Provencal, J.D. Lewine, E.C. Benzel*, P.C. Amrhein, C. Edgar, and K. Paulson. New Mexico Institute of Neuroimaging and the Department of Psychology, University of New Mexico, Albuquerque, NM

The neural mechanisms that support the formation of, and access to, equivalent semantic representations (e.g., picture of a dog and printed word "dog") are poorly understood, although the issue has been studied with vigor within the behavioral context, especially with regards to the visual and cognitive processing of pictures and written words. This study augments the traditional behavioral approach by analysis of simultaneously collected electrophysiological (EEG) data. Cross-modal associations between visual pictures and auditory words were examined using a simple match-to-sample task. Behavioral and EEG (32 channel scalp electrodes) data were recorded as subjects responded to the sameness-of-meaning for two sequentially presented stimuli. Probes (second stimulus) could either be the same or different as the target (first stimulus). Within mode and cross-mode interactions could thus be examined in regards to the extent in which the pattern of brain activity evoked by stimulus two was a function of stimulus one. Behavioral data suggests no significant within mode advantage, other than that believed to be due to sensory priming effects. This is consistent with the notion of a common processing pathway for within and cross conditions. EEG data demonstrates that modality specific processing cascades exist, with activation determined mostly by probe rather than target stimulus. The EEG also demonstrates early differences in evoked responses that are a function of the target stimulus. This implies modality specific memory representations, rather than an amodal representation. Although an architectural model is available to explain these findings, higher resolution spatial data may aid in a better understanding of certain components of the model, as may magnetoencephalographic recordings.

475.7

SEMANTIC RETRIEVAL: A STUDY OF REGIONAL CEREBRAL BLOOD FLOW IN NORMALS. H.P. Ham, M.F. D'Souza, W.W. Beatty*, and J.R. Prince. University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104.

Regional cerebral blood flow (rCBF) for semantic retrieval was examined in 10 healthy, right handed normals using a split-dose ^{99m}Tc-HMPAO Single Photon Emission Computed Tomography (SPECT) protocol. Subjects underwent back-to-back baseline (resting) and activation (verbal fluency task) scans on a triple-headed rotating gamma camera system. Co-registration using individual MRI images ensured anatomical accuracy for isolating functional areas of interest. As hypothesized, the most significant hyperperfusion was noted in left pre-frontal cortex, however, high flow was also found in left temporal and inferior parietal cortices (respectively, Broca's area [Brodmann's 44, 45, and 46], Wernicke's area [Brodmann's 21, 22, 37], and inferior parietal lobule [Brodmann's 39, 40]). Interestingly, significant hyperperfusion was also noted in inferior visual association cortices (Brodmann's 18, 19) even though subjects had their eyes closed throughout the entire imaging procedure. rCBF was negatively correlated with verbal fluency in the left pre-frontal area ($r = -.37$, $p = .2$) and the left temporal area ($r = -.65$, $p < .05$). Thus, high fluency subjects exhibited no or very low activation during the semantic task, whereas low fluency subjects revealed higher activation. These results are consistent with PET findings that have found inverse relationships for glucose metabolism with semantic retrieval and intelligence. One hypothesis for this somewhat counter intuitive phenomenon is that the "more efficient" brain requires less effort for certain tasks and thus lower blood flow (or glucose) to the appropriate cortex. Another closely related hypothesis suggests that the pre-frontal cortex may initially be directly involved in learning semantic retrieval tasks, but as these tasks become over-learned and more stimulus driven, alternate pathways may perform the task and thus reduce the effort (i.e., activation) of the pre-frontal cortex. Supported by OCAST Grant No. HR3-005 and the William K. Warren Foundation, Tulsa, OK.

475.8

GAMMA-BAND ACTIVITY (30-60 Hz) INDUCED BY A VISUAL RECOGNITION TASK IN HUMAN. C. Tallon-Baudry, O. Bertrand, M.-H. Giard * and J. Pernier. Brain signals and processes laboratory INSERM U280, 151 cours A. Thomas, 69003 Lyon, France.

Various 40 Hz or near 40 Hz synchronized activities have been described in cat, monkey and human. According to the different authors, they are thought to be implied in perception (feature binding, figure-ground separation), attention, and/or consciousness. Our study aimed at testing in human the hypothesis of a role of gamma-band activity in feature binding.

Our paradigm used the famous "dalmatian dog" picture. In a first recording session, the subjects were not aware of the presence of the dog. Before the second recording session, they were trained at perceiving it. Our hypothesis was that feature binding occurred only in the second recording session, when the subject had to bind together the blobs to perceive the dog, thus inducing a specific 40 Hz activity.

We recorded continuous EEG from 8 subjects. During the first recording session, subjects were shown a neutral stimulus (randomly displayed blobs), the dalmatian dog with its head turned rightward (unperceived as a dog), and a target stimulus (twirled blobs) they had to count. During the second recording session, they were shown the same neutral condition, the same dog (perceived as a dog after training) and had to count a target which was a dalmatian dog with its head turned leftward.

A time-frequency analysis of single trial responses shows a significant increase in the second recording session of a non phase-locked activity peaking at 250 ms, centered at 35 Hz. It must be underlined that this activity differs significantly from the first recording session for both neutral and dog stimuli (Quade signed rank test, $p < 0.03$, occipital and parietal electrodes). No such effect could be found on the low frequency (0-25 Hz) averaged response.

These results cannot be solely interpreted in terms of visual integration, as the neutral stimulus also induced a gamma-band activity in the second part of the experiment. They could also be interpreted in terms of both increased attention and activation of a mental representation of the dog.

475.9

TEMPORAL IMAGING OF EEG COHERENCE SHOWS DISTINCT REGIONAL CHANGES FOR STORAGE AND RECALL DURING A VERBAL WORKING MEMORY TASK. D. Lacroix*, T. M. Dung Vo, R. Lamer and Y. Chaput. Département de Psychiatrie, Hôpital Notre-Dame, Université de Montréal, 1560 Sherbrooke E., Montréal, CANADA H2L 4M1.

Working memory is used when short-term storage of information is needed to execute a cognitive task. EEG coherence is a measure of the synchrony of amplitude variations between two electrical signals. It is computed between each electrode paired to all electrodes and detects localized or inter-regional brain changes during a cognitive activation task. EEGs were obtained from 10 right-handed healthy males at rest -eyes opened- and during the following twelve-second task: 1- to memorize four words that were read on a computer screen and 2- to recall if a fifth presented word was part of the previous four words. Subjects used a mouse to answer. The task was repeated 20 times. 19 electrodes were used according to the 10/20 system. Signals were referenced to averaged signals from both ear lobes (TC 0.3s, Filter 35 Hz). Coherence values were computed for each component of the task and compared to those of the resting EEG. Probability mapping showed that: 1- when memorizing words, changes of inter-regional coherence were centralized on left medial (T3) and posterior (T5) temporal electrodes paired with left prefrontal (F7, F3, Fp1) and right centro-parietal electrodes (P4, C4). 2- when recognizing a word, changes of inter-regional coherence were mostly centralized on the left medial temporal (T3) and the left lateral prefrontal (F7) electrodes paired between themselves and with other prefrontal sites, mostly on the left. These results show that, during a verbal working memory task, short-term storage of information involves distinctively the left posterior temporal region in contrast to the left prefrontal lateral region during recall.

475.10

ERPS DISSOCIATE ITEM AND SOURCE MEMORY. E.L. Wilding, and M.D. Rugg. (SPON: European Brain and Behaviour Society). Wellcome Brain Research Group, Sch. of Psychology, Univ. of St Andrews, UK.

In two recognition memory tests, each of which employed 16 subjects, event-related potentials (ERPs) were recorded at midline and lateral scalp sites. At study, subjects heard a series of single words, half spoken in a male and half spoken in a female voice. At test, subjects saw a series of single words, half of which had been heard at study. Subjects made initial old/new judgments (item judgment) and, for words judged old, indicated whether the word had previously been spoken by the male or the female voice (source judgment).

The ERPs to words correctly judged old were separated according to the accuracy of the source judgment. Comparison of these ERPs with those evoked by words correctly judged new revealed that both were more positive between 500 and 800 msec post-stimulus, whilst post-800 msec this was true only for the ERPs to correct source judgments. The scalp distributions of the ERPs to correct and incorrect source judgments did not differ over the 500-800 msec latency region. However, the scalp topography of the differences between the ERPs to correct source judgments and the ERPs to words correctly judged new differed over time. Between 500 and 800 msec the largest differences between these ERPs were at left parietal sites, whilst post-900 msec the largest differences were at right frontal sites. The findings are consistent with the idea that multiple neural circuits contribute to recognition memory.

475.11

THE ROLE OF THE RIGHT HIPPOCAMPAL REGION IN THE ENCODING AND RECALL OF OBJECT-LOCATION: A PET STUDY A. M. Owen*, B. Milner, M. Petrides and A. C. Evans Montreal Neurological Institute, McGill University, Montreal, H3A 2B4, Canada.

A plethora of studies, across many species, have demonstrated that the hippocampal region plays a critical role in memory for spatial location. In the present study, the functional anatomy of object-location memory was investigated further using PET. Regional cerebral blood flow (rCBF) was measured while 12 normal volunteers encoded, and then recalled, the locations of eight familiar objects presented on a computer screen. In two analogous conditions, the subjects were required to encode, and then to recall, eight distinct locations represented by identical white boxes on the screen. An increase in rCBF was observed in the caudal part of the right medial temporal region when the encoding-location condition was subtracted from the encoding-object-location condition. An increase in rCBF was observed more anteriorly in the region of the right parahippocampal gyrus corresponding to entorhinal cortex when the recalling-location condition was subtracted from the recalling-object-location condition. In addition, the two encoding conditions activated left-hemisphere regions preferentially, whereas the two recall conditions activated right-hemisphere regions.

Together, these findings suggest that the human right hippocampal region is critically involved in recalling information that links object to place. The secondary finding, that encoding and retrieval appear to be lateralized to the left and right hemispheres respectively, is discussed with reference to current models of episodic memory (cf. Tulving et al, 1994), and alternative hypotheses are considered.

475.12

AN ELECTROPHYSIOLOGCAL INVESTIGATION OF STRATEGIC INFLUENCES ON CONCRETENESS EFFECTS. W.C. West and P.J. Holcomb* Dept. of Psychology, Tufts Univ., Medford, MA 02155

A variety of studies have demonstrated a distinct processing advantage for words representing concrete concepts than words that represent abstract concepts. One theory used to explain these effects is Paivio's dual-coding model, which proposes that both word types are represented in a verbal system, but that concrete words also benefit from referential connections to an image based system. Evidence supporting the dual-code model comes from studies of acquired dyslexics who have relatively greater problems with abstract words. This has lead to the speculation that there may be distinct cognitive and therefore neural systems for processing the two word types. The aim of the present study was to determine the influences of different processing strategies on concreteness effects manifested in both a behavioral measurement (reaction time) and in a physiological measurement [event-related potentials (ERPs)]. Three groups of 12 subjects performed a sentence verification task in which the final word of each sentence was either concrete or abstract. For each group the truthfulness judgement involved either 1) imagery, 2) a semantic decision which is not aided by imagery, or 3) surface level processing (letter search). Response times were found to increse in the order: surface, semantic, imagery. ERPs to concrete and abstract final words did not differ for the surface group. Waveforms were more negative to concrete than to abstract words in the region of the N400 component for both the semantic and imagery groups. This effect was evident at locations across the entire scalp for the semantic group but were restricted to anterior sites for the imagery group. These results suggest a role for imagery in language processing and support the dual-coding model of semantic representation. The anterior spatial distribution of the N400 in these tasks suggests the existence of multiple neural generators which may contribute to this component.

475.13

IMPLICIT WORD PRIMING: A NEW METHODOLOGY TO CIRCUMVENT EXPLICIT MEMORY CONTAMINATION.

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An extensive literature suggests that priming effects and explicit memory are mediated by separate mechanisms. Some authors, however, have suggested that priming effects may be seriously contaminated by covert explicit strategies, limiting our ability to analyze potentially separable memory mechanisms in aging and brain disease states. We have devised a new priming methodology to circumvent such contamination. Subjects were tested for implicit priming on a speeded category membership decision task carried out in two blocks. In each block, the subject first studied 10 category members, and was then tested on a set of 40 word stimuli. Of these, 10 were the previously studied category members, 10 were new category members (20 "yes" responses), and 20 were category non-members. List study conditions were manipulated in two experiments. In experiment 1, 14 normal undergraduate subjects were presented in each block with the 10 study words, each shown twice for a one second duration. In experiment 2 , carried out with 20 other normal undergrad subjects, the identical stimuli were presented. This time, however, word presentation consisted of a brief duration (17 ms) pattern masked exposure of the word. Each word was presented 10 times in succession at this exposure duration. No subject could reliably report the words seen. In both experiments, and for both semantic categories, the reaction time for "yes" decisions to the primed words was significantly faster ($p < .001$) than to the unprimed words (priming effect of 52 ms in exp. 1 and 62 ms. in exp. 2). Furthermore, there was no significant difference in the magnitude of the priming effect between the two experiments. These experiments establish that word priming effects can occur even in the absence of explicit recognition or recall of presented material in normal individuals. Furthermore, no evidence for additional priming from "covert explicit strategies" was found in experiment 1. This methodology may allow better evaluation of intact and impaired memory mechanisms in individuals with brain damage.

475.14

INFERIOR TEMPORAL-OCCIPITAL AND HIPPOCAMPAL CONTRIBUTIONS TO WORD PRIMING AND RECOGNITION MEMORY. D. Swick* & R.T. Knight. Dept. of Neurology & Center for Neuroscience, UC Davis, VAMC, Martinez, CA 94553.

Posterior cerebral artery stroke patients with unilateral damage to posterior hippocampus and adjacent inferior temporal and occipital structures can exhibit anterograde memory disturbances (Von Cramon et al., 1988). A case study has suggested that right occipital cortex is critical for word repetition priming (Gabrieli et al., 1995). Similarly, a PET study of young controls during word stem completion priming found decreased blood flow in right occipital cortex, centered in the region of the lingual gyrus (Squire et al., 1992). In the present experiment, electrophysiological (ERP) and behavioral data were recorded from age-matched controls and 3 patients with damage in right parahippocampal and lingual gyri, with variable extent into posterior hippocampus (n=2) and striate/extrastriate cortex (n=2). Words and pronounceable nonwords repeated after one of 3 delays: immediate (lag 0), 1-3 intervening items (lag 1-3), and lag 9-19. In separate sessions, subjects performed lexical decision (LD) or recognition memory (RM) tasks. Analysis of reaction time (RT) data from LD revealed a lag X group interaction ($p<.01$). The patients failed to show significant RT priming at lag 1-3 and lag 9-19, but immediate priming was intact. Likewise, the late positive ERP component related to stimulus repetition was diminished at all scalp sites, implying the loss of a neural generator associated with priming. In RM, behavioral accuracy did not differ between groups ($p>.2$), with only one of 3 patients showing a verbal memory deficit. The ERP repetition effect appeared reduced at posterior electrodes in this task as well, but to a lesser extent than in LD. These results demonstrate the importance of right inferior temporal-occipital cortex to behavioral and ERP measures of word priming.

475.15

EVENT-RELATED POTENTIAL MEASURES OF PRIMING AND RECOGNITION AS A FUNCTION OF LAG. J.V. Patterson[1], C. Sandman[3], C. Cotman[1,2], and W.E. Bunney, Jr.[3]* Departments of [1]Neurology, [2]Psychobiology, and [3]Psychiatry and Human Behavior, University of California, Irvine, CA 92717

Event-related potential (ERP) measures of priming and recognition were compared in 10 young controls. Three lists of words matched for word frequency and length were prepared for 3 conditions (priming, recognition, target detection). Each list was presented in the auditory modality (digitized speech of the experimenter) and contained the following: (1) 40 words repeated immediately (0 lag); (2) 40 words repeated after 5 intervening words (5 lag); (3) 16 words not repeated (fillers); and (4) 34 infrequent target words (numbers) not repeated. In the recognition condition, subjects pressed one of 2 reaction time (RT) buttons to indicate whether each word was a first ("new") or second ("old") presentation of a word in the list. In the priming condition, subjects made syllable judgements of each word and were not required to recognize the words as new or old. In a target detection condition, subjects pressed one RT button to infrequent numbers interspersed among the list of words. RTs were longer to new than to old words in both the priming and recognition conditions (ANOVA, $p<.001$). RT differences between new and old words were larger at 0 lag than at 5 lag for both the priming and recognition conditions. RTs in the priming and recognition conditions were not significantly different. ERP differences between new and old words were observed for each of the 3 conditions in the region of N400 and the late positive component (300-600 msec) (t-test, $p\leq.05$). N400 was attenuated to old compared to new words and the late positive component was earlier and larger. ERP differences between new and old words were larger for 0 lag than 5 lag, and for recognition compared to priming. The late postive component to repeated words was delayed for 5 compared to 0 lag. These results show that the ERP can reflect memory processes and may differentiate between implicit (e.g., priming) and explicit (e.g., recognition) memory tasks.

COGNITION VIII

476.1

HUMAN PREFRONTAL CORTEX GATES DISTRACTING SENSORY INPUT

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Jacobsen's (1935) seminal observation that monkeys with bilateral frontal lesions involving the sulcus principalis were impaired at delayed discrimination tasks was initially interpreted as a memory deficit. However, subsequent work by Malmo (1949) indicated that prefrontal monkeys were unable to gate irrelevant sensory information, which decremeted their ability to maintain information in working memory over delay intervals. This finding supported an attentional deficit in the frontal monkeys. Experimental research has shown that humans with prefrontal lesions perform poorly on neuropsychological tests that require response inhibition; however, few studies have examined whether this inhibitory deficit extends to control of irrelevant sensory information. The present study examined the role of human dorsolateral prefrontal cortex, hippocampal region, and temporal-parietal junction in the gating of irrelevant auditory stimuli during an auditory working memory task.

Stroke patients with unilateral lesions located in either the dorsolateral prefrontal cortex, the temporal-parietal junction, or the posterior hippocampus and control subjects were tested on a task requiring short-term retention of environmental sounds. Subjects had to indicate whether initial presentation and subsequent test sounds were identical in two conditions. The initial and test sounds were separated by either a silent period varying from 4 to 12.6 seconds (no-distractor condition) or by a series of irrelevant tones (distractor condition). Prefrontal patients were significantly impaired by distractors at all delays, hippocampal patients were impaired only at longer delays, while temporal-parietal patients performed comparably to controls. The findings suggest that dorsolateral prefrontal cortex is crucial for gating of distracting information. Loss of this capacity may contribute to deficits in working memory observed after prefrontal damage. Supported by NIMH fellowship 1 F31 MH10958-01 and NINDS grant NS21135.

476.2

PERFORMANCE OF PATIENTS WITH DORSOLATERAL FRONTAL LESIONS ON AN OLFACTORY IDENTIFICATION TASK. J. Baldo* and A. Shimamura, Psych. Dept., Univ. of California, Berkeley, CA 94720.

Studies of neurological patients have shown that lesions of the orbitofrontal and anterior temporal regions disrupt performance on olfactory discrimination tasks. Although such olfaction studies have not implicated more dorsal regions of prefrontal cortex, patients with lesions in this area are impaired on certain types of memory paradigms, such as free recall. The present study was undertaken to determine whether patients with unilateral dorsolateral prefrontal lesions would show deficits on a recall manipulation of a standardized olfactory, "scratch and sniff" test, the University of Pennsylvania Smell Identification Test (UPSIT). For each item, patients with dorsolateral prefrontal lesions and controls first attempted to name the odorant from memory (recall). This was followed by the four-alternative forced choice procedure (recognition). Subjects smelled 20 odors monorhinally and 20 odors with both nostrils. In the monorhinal condition, patients sniffed using the nostril associated with their lesioned hemisphere. It has been suggested that patients with dorsolateral frontal lesions are impaired at verbal recall and retrieval from remote memory due to decreased use of memory strategies and organization; they are generally less impaired on recognition tasks. Thus, we hypothesized that the patients would show deficits only on the recall portion of the experiment. Instead, we found that patients were impaired on both recall and recognition when they sniffed monorhinally, but not when they used both nostrils. These findings indicate olfactory memory impairment in patients with dorsolateral frontal lesions.

476.3

THE PREFRONTAL INFERIOR CONVEXITY (IFC): INVOLVEMENT IN CATEGORICAL VS. SENSORY PROCESSING

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In rhesus monkeys, gaze duration is greater for faces and objects than for color fields (Wilson & Goldman-Rakic, Behav Br Res 60). In order to examine the role of the frontal lobe in these behaviors, we compared the responses of 155 IFC stimulus-selective neurons to presentations of faces, objects and monochromatic color fields (CF). Each neuron was tested with sets of: 5 objects, 1 face and 1 CF (40 sets, 280 stimuli). Each stimulus was placed in 1 of 3 categories: faces, CF's, and objects.

IFC neurons responded selectively. For each neuron, the stimuli eliciting the maximal and minimal response were determined. Assuming that neuronal selectivity was based on arbitrary stimulus attributes, one would expect the face and CF categories to recruit approximately 22 neurons (155/7) for maximal and minimal responses. This assumption was clearly wrong: faces elicited maximal responses in 40 IFC neurons, almost double the incidence of other categories. Moreover, in 16 of 40 neurons with maximal responses to faces, the minimal response was to colour fields; this incidence was twice as great as found for any other stimulus category. One possible implication of these data is that the firing rate of IFC neurons reflects the degree to which a stimulus can be categorised. In contrast, the 8 neurons with maximal responses to color fields had minimal responses to faces, an incidence twice as large as for any other stimulus category. This second finding is consistent with separate mechanisms for color and face processing in prefrontal cortex.

476.4

HUMAN REASONING AND THE FRONTAL CORTEX. R. Adolphs*, A.R. Damasio, Div. of Cognitive Neuroscience, Dept. of Neurology, Univ. of Iowa Coll. of Medicine, Iowa City, IA52242.

We have previously reported evidence that the human prefrontal cortex, especially its ventromedial sector (VMFC) appears necessary to re-create somatic states and associated feelings in response to stimuli that have had personal significance in prior experience. Those findings support a theoretical framework in which human reasoning, via the VMFC, can draw upon the outcomes of previous experience, by enacting "somatic states" that were engaged on those prior occasions (A.R. Damasio, Descartes' Error, New York: Putnam, 1994). The present study aims to test these ideas further by examining the performance of brain-damaged subjects on a conditional reasoning task, the Wason selection task. This task requires subjects to test the truth of a conditional ("If A then B") on the basis of the four possible conditions (A, not-A, B, not-B). Studies with normal subjects have revealed that (1) they often incorrectly choose A&B as the relevant conditions on abstract tasks, and (2) they often correctly choose A&not-B on certain concrete tasks. We found that patients with brain lesions outside of the VMFC (n=22) performed similarly to normal subjects. However, subjects with lesions to the VMFC (n=6) made significantly more errors on concrete, familiar conditionals ($p<0.01$, Fisher's exact test), while performing similarly to controls on abstract and unfamiliar conditionals. While correct choices on the task are facilitated for control subjects when the subject matter is familiar, no such facilitation is seen for subjects with VMFC lesions (their reasoning on unfamiliar and familiar subject matter are equivalent). These results show that the VMFC is important in relating the choices that can be made in reasoning to their real-life contingencies. We suggest that successful reasoning in real life requires the feelings (hunches, intuitions, gut-feelings) evoked by past experiences.

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476.5

THE INTERFERENCE EFFECT OF TEMPORAL PROXIMITY CONTRIBUTES TO THE FRONTAL RESPONSE SEQUENCE DEFICIT

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Patients with frontal lesions have long been known to fail in the execution of response sequences while individual responses pose little problem. Among the many factors that could contribute to the sequencing deficit is the well-documented interference caused by the temporal proximity of responses, which may be increased in frontals. One test of the magnitude of this interference is the acceleration of sequential responses by stimulus preview which measures how much processes can be squeezed together or ovelapped by making stimuli available in advance. We compared 9 patients with a frontal excision to 9 patients with a temporal excision and 9 controls in a task requiring rapid keypress responses to each of five letters in a sequence. The letters (A,B, or C) were randomly presented at adjacent positions on a computer screen and were mapped to three adjacent keys. In the no-preview condition, the five letters were presented one at a time, immediately following the previous response. In the full-preview condition, the five letters were presented simultaneously. Subjects were tested in 10 practice trials and 60 experimental trials in each condition. Frontals had slower response times than the other groups. In normals and temporals, stimulus preview produced the expected slowing of the first response and the acceleration of the next four responses in the series. In frontals, however, preview did not increase response speed and the slowing of the first response was exacerbated. The absence of facilitation by preview indicates that frontals show increased interference of adjacent responses which suggests a longer decision interval.

476.6

FRONTAL LESIONS INCREASE THE PSYCHOLOGICAL REFRACTORY PERIOD IN HUMANS. M.J.Chouinard*,S.Bédard & F. Richer, Hôpital Notre-Dame, Université du Québec, Montréal.

Simple decisions interfere with each other when executed in close sequence. This interference appears to be mainly due to a basic temporal limitation in systems involved in response selection known as the psychological refractory period (PRP). From evidence of response selection difficulties in patients with frontal lesions (Décary & Richer,1993), we hypothesized that frontal lesions would be associated with longer response selection times which would produce an additive increase in the PRP. We compared 8 patients with a unilateral frontal excision to 8 patients with a temporal excision and 8 controls in a double decision task using visual stimuli. In each trial, a letter (A or B) was followed by a number (1 or 2) separated by either 55, 300 or 600 ms. After the number, the S made two rapid, grouped keypress responses (A or 1 = left, B or 2 = right). The PRP effect can be observed as increase in the response time of the second decision (RT2) with shorter delays. Compared to temporals and normals, frontals showed an increase (RT2) that was constant for all inter-stimulus delays. The data indicate that the interference between adjacent decisions is more durable in frontals, which agrees with an increased duration of response selection processes. The basic problem could contribute to a number of behavioral deficits of frontals such as those observed in sequential responses, categorization, and general behavioral planning.

476.7

SPATIAL REASONING AND PROBLEM-SOLVING IN THE RHESUS MONKEY. E Procyk and J-P Joseph*. INSERM U94, 16 av. Doyen Lepine, 69500 BRON, FRANCE.

A large body of experimental data indicates that Rhesus monkeys (macaca mulatta) can perform complex spatial tasks. They are able, for instance, to memorize the order of illumination of 3 fixed spatial targets and, after a delay, to press them in the same order. This task entails complex neural processes such as the capacity to integrate the spatio-temporal information given by the environment (the order of the targets) into a spatial plan.

The present report analyses the behavioural data obtained in two rhesus monkeys in a problem-solving situation derived from the spatial task described above. The task consisted in finding, by trial and error, the order (that the animal ignores) of touching 2 or 3 targets in a set of 3 or 4 fixed spatial targets. Whenever an order has been discovered and practised several times, a signal was sent to the animal indicating that a new order had to be discovered in the same set of targets.

The data show that monkeys are capable of conducting a methodic research of the hidden order and of finding the solution in a **minimal** number of trials. When the first target is found, it is kept in ongoing trials until the second target is found and so on, until the whole sequence is discovered. This optimal research seems to be guided by an integration of the location and rank of the successful and erroneous target-touches, i.e. by manipulations of internal representations of the environment.

It is concluded that the monkey brain is able, in a spatial problem solving task of this type, to construct complex cognitive structures which mimic a spatial reasoning. This reasoning is logic in as much as it reflects the logic of action ("get the reward as fast as possible"). It has a direct relationship with practical (success) or pre-symbolic intelligence.

476.8

NEURONAL ACTIVITIES IN THE ORBITOFRONTAL CORTEX OF MONKEYS INVOLVED IN PERFORMING A VISUAL COGNITIVE BEHAVIOR. K. Matsumoto, K. Nakamura, A. Mikami* and K. Kubota. Dept. of behavioral and Brain Sciences, Primate Research Inst., Kyoto Univ., Inuyama, Aichi 484, Japan.

To determine the role of the monkey orbitofrontal cortex (OFC) in visual cognitive behavior, the activity of single neurons were recorded from the OFC while the monkeys (N=3) were performing a visual recognition memory task, with or without eye fixation, as employed in previous studies of the temporal cortices done in our laboratory (Nakamura *et al.*, 1994; Mikami *et al.*, 1994). In the task, a photograph (a human face, a monkey, food and non-food objects) was presented repeatedly 1 to 4 times as a sample stimulus (S) on a TV monitor with a 2 s interstimulus delay period, then another photograph was presented as a response stimulus (R). The monkeys memorized the S and discriminated it from the R by a non-matching rule. When the monkeys responded correctly to the R by releasing a holding lever, a drop of water was delivered to the mouth as a reward. In total, 161 neurons responded to the S. The stimulus selectivity of half of the neurons was lower than those in the temporal cortices, and the selectivity of the remainder were as high as those in the temporal cortices. The activity of 13% of the neurons (21/161) responding to S also changed during the delay period. In 38% of these, (8/21), the activity during the delay period gradually increased toward the end of the period. The activity of the remainder was similar to that of the neurons in the temporal cortices. Seventy-one percent of the neurons (114/161) responding to S also responded to R. When the same stimulus as S was presented as R, 18% (3/17) responded strongly and the peak of the responses was near the time of the lever release. Thirteen percent of the neurons (21/161) responding to S also responded to the reward. These neurons responded to a delivery of water while the monkeys were not performing the overtrained task. We also tested the responsiveness of the neurons responding to S, to the same stimulus as S in a task in which the monkeys were required to detect change of orientation of a fixation point. Both the responsiveness and the selectivity of the neurons to the stimulus were dramatically lowered. These behavior-related OFC neurons were different from the temporal neurons. These results suggest that the monkey OFC is involved in employing visual information to prepare for the upcoming behavior and in receiving information from the result of the behavior.

476.9

CONSISTENCY AND SPECIFICITY OF ACQUIRED SOCIAL AND EMOTIONAL DEFECTS FOLLOWING VENTROMEDIAL FRONTAL LOBE DAMAGE. S.W. Anderson,* D. Tranel, H. Damasio, & A.R. Damasio, Dept. of Neurology, Univ. of Iowa, Iowa City, IA 52242.

Several case studies have documented striking impairments in social behavior and emotion following damage centered in orbital and mesial sectors of the human frontal lobes (ventromedial frontal lobe or VMFL), but no study to date has examined the consistency of this behavioral profile in a *group* of subjects with VMFL damage. Here, the intersubject reliability and consistency of a circumscribed defect in the realm of social and emotional functions were examined in a group of 13 subjects with focal VMFL lesions, and in 2 control groups, one with dorsolateral frontal lesions (DLFL; N=15) and one with posterior, nonfrontal lesions (NF; N=15). All subjects had normal developmental histories and focal lesions acquired in adulthood due to stroke or resection of a benign tumor. They were studied with standardized neuropsychological and neuroimaging protocols at least 2 years following lesion onset. We examined social behavior and emotion, intellect, attention and working memory, anterograde memory, visual perception and visual construction, and executive functions. We found that: (1) all 13 VMFL subjects had major impairments of social behavior, accompanied by emotional alterations (primarily blunting) and defects in decision-making and behavioral organization, in the context of otherwise well preserved cognitive abilities; (2) subjects in the control groups had generally normal social and emotional profiles despite significant and varied cognitive impairments. These findings indicate that the behavioral profile associated with human VMFL damage is highly consistent across subjects, and support further the hypothesis that this region is a critical component of the neural system which supports social and emotional behavior. (Supported by NINDS P01 NS19632).

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS III

477.1

LAMINAR ORGANIZATION OF THE RAT SUBICULUM. N. Ishizuka*. Dept. Cell Biol., Tokyo Metropol. Inst. for Neurosci., Fuchu, Tokyo 183, Japan.

The distribution of subcortically projecting subicular neurons was investigated with a retrograde labeling technique in the rat. Under chloral hydrate anesthesia, WGA-HRP was injected into the anterior thalamic nuclei (ATH), the nucleus accumbens (ACB), and the medial mammillary body (MB). To facilitate a cytoarchitectonic analysis of the hippocampal region, frozen sections were cut serially at perpendicular to the long axis of the extended hippocampus. This transverse plane revealed that subiculum coexisted with Ammon's horn (CA) and the dentate gyrus (DG) along the whole extent of long axis.

At all septotemporal levels, neurons projecting to the ATH (ATH cells), MB (MB cells), and ACB (ACB cells) were labeled in the pyramidal cell layer of the subiculum. However, few neurons of CA and DG were labeled in any cases of the present study. Arrangement of ATH, MB and Acb cells changed along the long axis. At the level of a septal quarter, each group of labeled neurons was organized in lamina parallel to the pial surface, i.e., ACB cells were located in the most superficial portion, MB cells were distributed in the upper two-thirds of the layer, and ATH cells were situated in the deepest portion. This laminar arrangement of the subicular neurons is quite similar to that of subcortically descending neurons in laminae V-VI of other cortices. At the more temporal levels, where gyrification of the hippocampal formation well developed, the vertical arrangement of the laminae gradually changed to the oblique arrangement: ACB cells were confined to the transitional part with CA1 sector and some of them penetrated beneath the CA1 pyramidal cell layer, while ATH cells were gradually accumulated in the distal portion of the subiculum and the adjacent lamina principalis interna of the presubiculum. MB cells were still located in between ACB and ATH cells in both vertical and proximodistal axis of the subiculum. It was suggested that the inrolling of hippocampus caused a slide of laminae in the subiculum, resulting a modification in cellular arrangement from vertical to oblique. Taken together, it was also suggested that the subiculum corresponds to the infragranular layer, while CA and DG are rather comparable to the supragranular layer of the cortex.

477.2

HIPPOCAMPAL LESIONS AND ACQUISITION OF A 5-CHOICE SELECTIVE ATTENTION TASK. Bratt, A.M.*, Stacey, K., Chase, R.M., Mittleman, G. Dept. Psychology, The University of Memphis, Memphis, TN 38152.

Neuropathological structural changes have been found in the brains of schizophrenics (*Shapiro, 1993; Schizophrenia Res. 10: 187-239*), with the hippocampus being particularly affected. It is thought that such developmental brain abnormalities may play an aetiological role in the progression of this complex disease. One of the behavioural deficits typically shown by schizophrenics is an impairment in selective attention (*Mesulam & Geshwind, 1978; J. Psychiat. Res. 14: 249-259*). Therefore, this experiment investigated the effects of lesions of subfields of the hippocampus on the acquisition of a task of selective attention (5-choice serial reaction time task, (*Carli et al., 1983; Behav. Brain Res. 9:361-380*) in the rat. Male Long-Evans rats received either total hippocampal ablations using multi-site bilateral intra-cerebral micro-injections of ibotenic acid, (*Jarrard, 1993; Behev. Brain Res. 60:9-26*), ibotenate lesions of the subiculum or dentate gyrus, electrolytic lesions of the fimbria-fornix or sham lesions. Rats were trained to discriminate a light stimulus randomly presented in 1 of 5 locations and to nose poke into the light to receive a food reward. Daily 30 min training sessions comprised a total of 100 trials in 4 x 25 trial blocks. Rats passed progressively through 5 individual test stages, upon attaining 75% correct responses at each stage, and the attentional demands of the task were incrementally increased. The task difficulty was elevated by altering the parameters from standard (stimulus duration [SD] 30s; inter-trial interval [ITI] 60s) to a criterion level of >75% correct; (SD 1s, ITI 10s). Preliminary data indicate that rats having total hippocampal and subiculum lesions required significantly more trials to reach criterion at the final test stage than sham controls, suggesting the presence of attentional deficits. These data may provide evidence for the role of the hippocampus in processes of selective attention in the rat.

477.3

CHOLINERGIC MODULATION MAY ALLOW COMBINATION OF SELF-ORGANIZING FEEDFORWARD CONNECTIONS AND ASSOCIATIVE FEEDBACK CONNECTIONS IN PIRIFORM CORTEX AND NEOCORTEX.

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Different models of piriform cortex focus on either the competitive self-organization of lateral olfactory tract (LOT) synapses (Ambros-Ingerson et al., Science 247: 1344-1348. 1990) or on the associative memory function of excitatory intrinsic synapses (Hasselmo et al., J. Neurophysiol. 67: 1230-1246, 1992). Here we show that these functions can be combined, as long as cholinergic suppression of intrinsic synaptic transmission prevents associative recall during learning. A model with mean firing rate representations of pyramidal cells and inhibitory interneurons could store multiple different patterns as fixed point attractors without modification of afferent input. However, capacity was limited by interference between different overlapping patterns. With modifiable LOT synapses, self-organization decreased the overlap between stored input patterns, increasing capacity. This effect was also studied in a biophysical simulation of the piriform cortex developed using the GENESIS program (Barkai and Hasselmo, J. Neurophysiol. 72: 644-658, 1994).

In the neocortex, cholinergic modulation could allow self-organization of afferent and feedforward synapses terminating in layer IV to be combined with associative memory function of feedback synapses terminating in layer I. This would require selective suppression of synaptic transmission in layer I but not layer IV. In brain slice preparations of rat somatosensory cortex, we tested the amount of suppression of synaptic potentials recorded extracellularly in layer I or layer IV. Layer I synaptic potentials showed suppression during perfusion of 100 µM carbachol (37.8% suppression, n=8), while layer IV synaptic potentials showed slight enhancement with 100µM carbachol (7.8% enhancement, n=5). This supports the theory that cholinergic modulation allows combination of self-organization and associative memory function in the neocortex. *Supported by ONR N00014-93-1-595 and NIMH R29 MH52732-01.*

477.4

POLYSYNAPTIC POTENTIATION IN THE DENTATE GYRUS INDUCED BY LEARNING AND MEMORY OF AN OLFACTORY ASSOCIATIVE TASK. F.A. Chaillan, E. Marchetti-Gauthier*, F.S. Roman and B. Soumireu-Mourat. Lab. de Neurobiologie des Comportements, URA-CNRS 372, IBHOP, 13388 Marseille cedex 13, FRANCE.

Previous work showed that electrical high frequency stimulations applied to the lateral olfactory tract can be used as an artificial olfactory cue (olfacto-mimetic stimulations, OMS) in an olfactory discriminative task. Likewise a progressive long-term potentiation phenomenom was also induced in piriform (olfactory) cortex while rats were learning the meaning of these OMS (Roman et al., 1993). Electrophysiological recordings were used to study the dynamic of dentate gyrus (DG) involvment in learning and memory in an olfactory associative task. A first group of animals (OMS-group) was trained in discriminating a natural odor versus an OMS. A second group of pseudoconditionned animals was used to check that OMSs have no effect without a learning context. During the learning sessions, behavioral performances were gradually improved. The electrophysiological recordings, before and after each learning session, revealed the development and the potentiation of a long latency polysynaptic evoked potential in the DG. The changes of the electrical activity recorded in the DG were correlated to the performances only in the first learning sessions. The polysynaptic potentials evoked by a control electrode (which served only to deliver single test pulses) remained unchanged. No significant variation of the electrophysiological recordings was obtained in pseudoconditionned animals. The long onset latency of the potentiate polysynaptic response obtained in the DG suggests that its reactivation is mediated by a hippocampal loop *via* the entorhinal cortex. The fast development of the polysynaptic evoked potential observed in the sessions is consistent with an early involvment of the hippocampus during the learning. This early activation could induce long lasting changes in a specific neuronal network of the piriform cortex, which themselves could be one of the neurobiological substracts of the memorized information.

477.5

PROACTIVE INTERFERENCE AND SHORT-TERM MEMORY DURING PERFORMANCE OF A DNMS TASK IN NORMAL RATS AND RATS WITH HIPPOCAMPUS REMOVED

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Neural ensemble activity recorded from the hippocampus of rats performing a spatial Delayed-Nonmatch-to-Sample (DNMS) task has been shown to encode information specific to cognitive features of the task (Hampson *et al.* Soc.Neurosci.Abstr. 20:430, 1994). Recent studies with neurotoxic lesions confirm that this task is hippocampal dependent. Population analyses of hippocampal ensemble firing have identified two sources of errors in the DNMS task: 1) "Miscoding" of the sample response (lever position) by the ensemble, and 2) "Weakly encoded" sample information that decays at longer delays. Unlike the second source of errors, miscoding was not delay-dependent, and occurred consistently across all delays. The source of miscoding was likely proactive interference from the previous trial. Analyses were performed in normal rats (n=30) and rats with confirmed ibotenate removal of the entire hippocampus (n=12), to assess hippocampal involvement in trial-to-trial responding.

Trials were sorted on the basis of the same or different preceding trials and preceding trial delay. There was no proactive interference if the delay on the preceding trial was <10 sec. however there was a 5-20% decrement in correct responding if the preceding trial delay was >10 sec. When trials were the same, preceding delays of >10sec were slightly facilitatory. Similar analyses showed proactive interference in rats with hippocampal lesions, but it was not delay related. In these instances it was likely that other response strategies (i.e. maximizing on one lever) at longer delays, was the most prominent behavior exhibited by lesioned rats.

The DNMS task thus demonstrates serial memory (Hampson and Deadwyler 1990). It is likely that delay dependent hippocampal deficits result from increased interference of the previous trial, on the normal encoding by the hippocampus during sample phase of the current trial. When this fails to occur, as indicated by ensemble recording, normal animals respond like animals without a hippocampus.

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477.6

HIPPOCAMPAL LESIONS DISRUPT THE RETENTION, BUT NOT ACQUISITION, OF OBJECT DISCRIMINATIONS.

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A defining feature of the anterograde amnesia seen in patient populations is an impaired ability to retain information over long delay intervals despite the normal functioning of short-term memory. Animal models of human amnesic syndromes, however, have largely ignored the effect of neural trauma on long-term retention. We recently found that entorhinal-hippocampal disconnection from angular bundle transections does not affect rats' capacity for acquiring a series of object discrimination problems but does impair the retention of this information (*J. Neurosci.*, 15: 3193, 1994). The present study was designed to determine if direct damage to the hippocampus also leads to a selective impairment in the retention of object discriminations.

Rats with bilateral dorsal hippocampal lesions, rats with control lesions of overlying neocortex, and normal controls were trained on three successive object discrimination problems and then retrained on these problems after a 3 week delay interval. All three groups showed similar levels of performance during the training phase of testing. During retraining, normal and control animals showed robust -- and equivalent -- levels of retention. In contrast, animals with hippocampal lesions demonstrated impaired retention compared to normals and controls. This acquisition/retention dissociation, moreover, is consistent with that seen in rats with entorhinal-hippocampal disconnection. Together, these findings suggest that the hippocampus and anatomically related structures are not involved in establishing memories of complex visual discriminations, but are critical for maintaining this information for later use. Thus, the role of the hippocampus in nonspatial memory may be clarified by paradigms that employ measures of retention. Supported by Alzheimer's Association Grant PRG-94-172.

477.7

EFFECTS OF SELECTIVE LESIONS ON CA3 AND DENTATE GYRUS UPON WORKING MEMORY OF RATS.

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Although the hippocampus has been associated for some time with working memory process in rodents, less is known about the role of the hippocampal sub-areas. In the present study, we investigated the actions of colchicine-induced lesion on the dentate gyrus (DG), and kainic acid lesions on CA3 sub-area of the hippocampus on retention of Wistar rats pretrained to perform a win-shift task on an elevated T-maze. The task consited of a series of two-run trials, the first one forced (information trial - I), and the other with free choice (C). The animals that reacquired criterion after the surgery were also tested after several delay intervals interposed between the I and C trials of the task. DG lesion initially disrupted performance in comparison to controls, but not the number of sessions required to reacquire criterion; an impairment was also seen in the delay tests with all intervals. CA3 lesion also disrupted performance, but in a more permanent way, since most of the CA3-lesioned rats did not reacquire criterion. It is suggested that CA3 and DG have different functions in mediating working-memory. Finantial support: AFIP, CNPq, CAPES.

477.8

TIME OF DAY AS A CONDITIONAL CUE IS NOT DEPENDENT UPON THE HIPPOCAMPUS.

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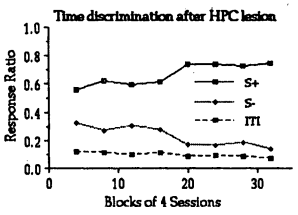
Rat were trained in a conditional discrimination based upon time of day in operant chambers. Rats were trained that the light cue, not the tone cue, is correct at Time 1, and that the tone cue, not the light cue, is correct at Time 2. This experiment showed that rats are capable of using time of day as a conditional cue after extensive training in an operant task using lever pressing. Hippocampal formation was damaged by multiple intrahippocampal NMDA microinjections and fornix lesions. Preliminary results indicate that the hippocampus is not necessary for this type of associative learning. The same rats were impaired at the fixed location hidden platform location of the Morris water task.



477.9

MEMORY IMPAIRMENT INDUCED BY LESIONS OF DOPAMINERGIC MESO-HIPPOCAMPAL PATHWAYS IN THE RAT.

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The hippocampal formation (HF) has long been thought to play a role in learning and memory. Previous studies from our laboratory examined the organization of mesencephalic projections towards the HF in the rat. In order to evaluate the effects on learning and memory of retrograde selective lesions of mesencephalic dopaminergic neurons, following bilateral injection of 6-hydroxydopamine (6-OHDA) in dorsal and ventral subiculum and adjacent CA1 field of HF, young adult Sprague-Dawley rats were trained in: a) the classical inhibitory (passive) avoidance (IA), b) IA using a multiple-trial (training to criterion) and c) standard Morris water maze task (MWM), cued and spatial version. Concerning IA, retention was examined 1, 3, and 10 days after training. Concerning MWM task, 6-OHDA lesioned and sham-operated rats received 4 training trials on each of 4 days. After training sessions, the rats were tested during a 60s probe trial (free-swim trial) in which the platform is removed from the maze. During the free-swim we recorded: a) the time to cross the original platform position (latency); b) the time spent in the target quadrant (quadrant time), c) the number of crossings over the original platform position (platform crossings). After behavioral tests, the loss of mesencephalic dopaminergic neurons in the 6-OHDA lesioned rats, compared to sham-operated rats, was verified by tyrosine hydroxylase immunohistochemistry. Though the 6-OHDA lesioned rats were indistinguishable from sham-operated rats in performing the IA and cued version of MWM task, in the spatial version of MWM, lesioned rats, compared to controls, exhibited significantly differences in the latency ($P<0.05$), quadrant time ($P<0.01$) and platform crossings ($P<0.05$). These results suggest that the rat's ability to acquire spatial learning and memory for place navigation in the MWM is likely dependent also on the integrity of meso-hippocampal dopaminergic connections.

477.10

THE EFFECTS OF HIPPOCAMPAL AND PARIETAL CORTEX LESIONS ON MEMORY FOR AN OBJECT/SPATIAL LOCATION PAIRED ASSOCIATE TASK IN RATS.

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Previous research in rats suggest that lesions of the hippocampus do not significantly impair object/object paired associate (PA) learning and result in facilitation of odor/odor PA learning. However, more "natural" PA learning is impaired by hippocampal damage (Eichenbaum and Bunsy, Curr. Dir. 1995).

In the present study Long-Evans rats were trained in a "natural" object/spatial location PA task. This was a successive discrimination, go/no-go task in which the rats had to remember which object/spatial location pairs had been associated with reward . Four objects and four spatial locations served as stimuli. This resulted in four PA's (reward condition) and twelve mispairs (non-reward condition). Five PA's and five mispair trials were given daily until rats reached criterion performance (a minimum of a 5 sec. difference between reward and non-reward trials based on 100 trials). Rats then received either hippocampal lesions, parietal cortex lesions, or control operations under Nembutal anesthesia After a one-week recovery period they were given 300 additional test trials in order to assess retention of the PA task.

Preliminary data indicate that compared to controls, rats with hippocampal lesions have only a mild impairment , whereas rats with parietal cortex lesions are significantly impaired in their retention of the previously learned object/spatial location PA's. These results suggest that the parietal cortex, but perhaps not the hippocampus, is involved in memory for the more "natural" object/ spatial location PA's. The involvement of the parietal cortex may be because either object, spatial location, or object plus spatial location needs to be represented in memory. Other data support the third possibility.

477.11

PARIETAL NEURAL ACTIVITY OF AWAKE RATS DURING DIRECTIONAL DELAYED NONMATCHING TO SAMPLE TASK IN FAMILIAR AND UNFAMILIAR CONDITIONS. K. Nakamura*, T. Ono#, T. Isaji and M. Yoshida. Dept. Electronics and Informatics, Toyama Prefectural Univ., Toyama 939-03, and #Dept. Physiol., Fac. Med., Toyama Med. & Pharmaceu. Univ., Toyama 930-01, Japan.

To elucidate the involvement of parietal cortex (PG) in a spatial working memory function, rats were trained to perform a directional delayed nonmatching-to-sample (dDNMS) task. Six speakers surrounded the experimental field. In dDNMS, rat needed to remember the stimulus direction during 2 s delay. Neural activity in PG was in the first tested in familiar condition, then retested either by using unfamiliar tones, or after rotating the rat to the unfamiliar orientation. In total, 118 neurons were recorded during dDNMS in familiar condition and some (24 neurons) in both familiar and unfamiliar conditions. Forty two neurons responded to the directional first tones (39, differential; 3, non-differential). Of 39 differential PG neurons, 29 neurons had also significant activity during the delay period (memory related). Of 24 differential neurons (18 of these, memory related) which were also tested in unfamiliar conditions, 21 neurons had significant activity during delay period in unfamiliar conditions. Seven neurons had enhanced activity in unfamiliar conditions either to the first tone or both to the first tone and during delay. The response enhancement ratio were ranged from 1.4 to 4.3. The response tuning index (peak/mean) were usually reduced in unfamiliar conditions. These results suggest that PG neurons are involved in spatial working memory. The spatial memory in PG may be persistent, and spatial attention or learning mechanisms may be enhanced in unfamiliar conditions.

477.12

HIPPOCAMPAL INSULTS PRODUCE MEMORY IMPAIRMENTS WHEREAS CHRONIC STRESS CAUSES FASTER EXTINCTION IN Y-MAZE BEHAVIOR. Y. Kuroda*, C.D. Conrad, L.A.-M. Galea, B.S. McEwen. The Rockefeller University, New York, NY 10021

A recently modified version of the Y-maze has been shown to assess memory in rats (F. Dellu, et. al., Brain Res. (1992) 588: 132-139). Advantages of this Y-maze is that memory can be assessed quickly (within hrs) and the task requires no food deprivation or pain.

The Y-maze consists of three identical arms. An arm was randomly blocked (novel) and a rat was allowed to investigate the other two arms for 15 minutes. After a delay of 2, 4, or 6 hrs, the rat was re-introduced in the maze with all arms available for investigation. The hippocampus was lesioned with kainic acid (KA) or colchicine (COL), i.c.v., in male rats. After 3 wk recovery, the rats were tested in the Y-maze. KA and COL groups showed no preference for the novel arm in the first choice (2 & 6 hr delay) whereas controls entered the novel arm first (93% = 2hr, 86% = 6 hr). Further, controls spent more time in the novel arm compared to the other arms relative to KA and COL rats (2 hr delay). None of the groups showed an arm preference at the 6 hr delay. These data indicate that the Y-maze is sensitive to hippocampal damage.

Because chronic restraint stress (STR, 6 hrs daily/21 days) can cause CA3 dendritic atrophy, we investigated whether such damage results in memory impairment. STR and controls entered the novel arm on first choice (80% preference for both, 4 hr delay). However, controls spent more time in the novel arm in the first two mins whereas the STR rats preferred the novel arm in the first min only. These results show that STR rats can perform a memory task but may have faster extinction rates than controls. Supp. by MH10804 to CC and MH41256 to BM.

477.13

KAINIC ACID-INDUCED NEUROPATHOLOGY AND COGNITIVE DYSFUNCTION AS AN ANIMAL MODEL OF DEMENTIA. D.J. Fontana*, M. Haraguchi, M. Rowe, R. Lewis and G.R. Stewart. Dept. Neurosciences, Syntex Research, Palo Alto, CA 94303.

Systemic injection of kainic acid (KA) causes an acute syndrome of seizures and seizure related brain damage. The present study characterized the long term effects of kainic acid-induced brain damage on cognitive and sensorimotor behaviors. Adult male Wistar-Furth rats were treated with KA (10 to 14mg/kg, ip). The majority of animals (63%) entered status epilepticus (STAT) and remained in a state of continuous seizure activity for six hours. Animals were then treated with MK-801 (0.5mg/kg, ip) and diazepam (2.5mg/kg, ip). Overt seizure activity abruptly ceased within 20 minutes and animals remained sedated for several hours. KA treated animals not entering status epilepticus (nonSTAT) were administered MK-801/diazepam and used as controls. Untreated, age-matched animals (AMC) were included as a second control group.

Following a one month recovery, all animals were subjected to a battery of tests to assess neurologic and cognitive status. STAT animals were significantly impaired on three distinct tests of learning and memory (passive avoidance, Morris Water Maze and habituation to a novel environment) and on a test of sensorimotor gating (pre-pulse inhibition to acoustic startle). General hyperactivity and increased responsiveness to acoustic stimuli were observed, but STAT animals were otherwise intact neurologically. NonSTAT animals performed similar to AMC on all tests. Histologically, STAT animals had bilaterally symmetrical damage to the hippocampus and piriform/entorhinal cortex. In some animals, there was damage to the thalamus including the presence of calcium deposits.

These results demonstrate that KA-induced seizure-disseminated brain damage yields animals in a state of global cognitive dysfunction and with underlying limbic pathology similar in many respects to that found in advanced Alzheimer's Disease.

477.14

HIPPOCAMPAL GRAFTS OF CELLS ENGINEERED TO PRODUCE ACh REVERSE SPATIAL MEMORY DEFICITS IN RATS WITH UNILATERAL FIMBRIA FORNIX LESIONS. H.A. Dickinson-Anson*, F.H. Gage and L.J. Fisher, The Salk Institute, La Jolla, CA 92037-1099

Lesions of the fimbria fornix (FF) produce spatial memory deficits that can be attenuated by grafts of cholinergic tissue into dennervated target regions or by systemic administration of cholinergic drugs. However, it has not been clarified if the presence of acetylcholine (ACh) in the hippocampus is a critical element for achieving improvements in spatial processing post-damage. We previously developed a genetically modified fibroblast population that expresses high levels of choline acetyltransferase, and produces and releases ACh both *in vitro* and *in vivo*. In the present study, we used these ACh-producing fibroblasts to directly test whether cholinergic function in the hippocampus is involved in spatial learning and memory.

Male Fischer 344 rats received unilateral grafts of either ACh-producing fibroblasts or ß-galactosidase-expressing fibroblasts into the hippocampus. Immediately following the transplantation, the ipsilateral FF of the grafted rats was dissected by aspiration. Control groups consisted of unoperated rats or non-grafted rats with FF lesions. One week post-grafting, all rats were trained in a 2-day spatial water maze task. Rats with lesions of the FF showed a significant impairment in water maze performance compared to unoperated rats. Rats implanted with the ACh-producing cells, however, showed a performance in the water maze on the second day of training that was equal to that of unoperated controls. Since rats implanted with ßgal fibroblasts did not improve post-grafting, these results indicate that ACh was a critical component in the recovery of spatial learning and memory. Further support for the role of hippocampal cholinergic function in spatial memory processing was provided in a second experiment in which ACh-producing fibroblasts were implanted into the hippocampus of normal, unlesioned rats. The performance of these animals in both the water maze and in a simple habituation task was enhanced compared to nongrafted controls. Taken together, these data suggest that hippocampal ACh is involved in spatial learning and memory, and that ACh replacement within the damaged hippocampus is sufficient to ameliorate some cognitive deficits.

477.15

HIPPOCAMPAL EVOKED POTENTIALS TO PITCH-DEVIANT AND TIME-DEVIANT TONES IN THE TRAIN OF AUDITORY STIMULI IN THE CAT: MISMATCH-LIKE NEGATIVITY (MMN) AND ITS CONNECTIONS TO THE NEURAL BASIS OF LEARNING AND MEMORY. K.Kivirikko*, T.Korhonen, T.Ruusuvirta, J.Arikoski and P.Astikainen. Dept. of Psychol., Univ. of Jyvaskyla, P.O.Box 35, 40351 Jyvaskyla, Finland.

Hippocampal auditory evoked potentials elicited by pitch-deviant or time-deviant tones were recorded during a passive oddball situation in the cat. Experiment 1 showed that the hippocampal N130d recorded in the cat can be evoked by the presentation of a pitch-deviant tone (2500 Hz) in the sequence of repeated standard tones (2000 Hz) despite the lack of behavioral orientation to the stimuli. The recordings in CA1, CA3 and dentate fascia of the hippocampal formation showed that the main deflection in response to the deviant tone appeared in the latency range of 100-170 ms (N130d). The only similarity to the hippocampal MMN-like potentials previously reported in the cat, was N40d. No N130d was observed to the standard tones preceding the deviant tones. The stimulus parameters eliciting the N130 and its latency range support an assumption according to which the N130 may reflect the hippocampal neural orienting response. Consistent with the results obtained in the human cortical MMN studies concerning delayed noise bursts we could detect no N40 or N130 to the time-deviant tones following the pre-standard tones. The deviant event was a doubled interstimulus interval (ISI=1000 ms, p=.05) which occurred randomly in a sequence where the regular ISI was 500 ms. High selectivity of the hippocampal N130, to be elicited only in response to the deviant tone, is consistent with an assumption that the hippocampal novelty detectors are responsible for the response to the deviant tone in the sequence of standard tones.

In experiment 2 our purpose is to examine whether a shortened (250 ms) or a doubled (1000 ms) ISI used as a deviance in a sequence of tones where the regular ISI is 500 ms produces hippocampal or cerebellar MMN in the rabbit.

477.16

BEHAVIORAL AND HIPPOCAMPAL EVOKED RESPONSES IN AN AUDITORY ODDBALL SITUATION WHEN AN UNCONDITIONED STIMULUS IS PAIRED WITH DEVIANT TONES IN CATS. T. Ruusuvirta*, T. Korhonen, M. Penttonen, J. Arikoski, and K. Kivirikko. Dept. of Psychol., Univ. of Jyväskylä, Finland.

An acceleration of head movements and hippocampal event-related potentials (ERP) were measured in an oddball situation in freely moving cats. An electrical stimulation of the lateral hypothalamus (US) was paired with pitch deviant tones. In addition to the developing conditioned orienting head turns (orienting response, OR) towards the deviant stimuli, an amplitude increase of the parallelly elicited hippocampal ERPs was found. In fact, the changes in the ERP amplitude preceded the changes in the behavioral level. Both the behavioral and neural responses appeared not until the 50 ms latency range. Furthermore, time-amplitude characteristics of the ERPs corresponded to time-acceleration characteristics of the conditioned behavioral ORs indicating a close functional connection between the ERPs and the behavioral OR. The observed ERPs that were elicited at the latency range following the typical latency range of a hippocampal mismatch-like negativity in cats (30-50 ms) may represent a cat analogy of ERPs following a mismatch negativity (MMN) in humans (N2b, P3).

477.17

SYNAPTIC LOSS FOLLOWING REMOVAL OF SEROTONERGIC AND/OR CHOLINERGIC FIBERS IN THE RAT HIPPOCAMPUS. M. Ogawa, M. Matsukawa, and N. Okado*. Dept. Anat., Inst. Basic Med. Sci., Univ. Tsukuba, Tsukuba, Ibaraki 305, Japan.

Serotonergic neurons innervate broad areas of the vertebrate central nervous system implicating an unique role in the brain. Indeed, serotonin (5-HT) has been shown to have an important role for synaptic formation and maintenance in developing and adult animals (J. Neurobiol. 24: 687-698, Neurosci. Res. 19: 111-115). In addition to 5-HT, acetylcholine (ACh) has shown involved in memory formation in the hippocampus. The present study was undertaken to correlate between behavioural changes and synaptic structures following destruction of 5-HT and/or ACh systems in the hippocampus.

Following injections of AF64A (1 nmol/each lateral ventricle at once), ACh neurotoxin, or p-chrolophenylalanine metyl ester (pCPA, 100 mg/kg for every other day for two weeks), synthetase inhibitor for 5-HT, water maze learning was not significantly impaired, whereas simultaneous injections of AF64A and pCPA produced an apparent memory deficit. A slight reduction in the density of synapses (less than 10%) was found in the dorsal part of the stratum radiatum and stratum radiatum + stratum moleculare following injections of AF64A and pCPA, respectively. In contrast, synaptic density decreased in the dorsal part of the stratum radiatum by 20% following simultaneous injections of AF64A and pCPA.

This study has demonstrated that 5-HT and ACh have cooperative effects for synaptic structures and memory formation of the placement learning.

477.18

PAVLOVIAN AVERSIVE CONTEXT CONDITIONING USING CARBON DIOXIDE (CO2) AS AN UNCONDITIONAL STIMULUS (US): EXTINCTION OF CONDITIONED RESPONDING

DL Mongeluzi*, BJ Caldarone, HS Stock, RA Rosellini. Dept. of Psychology, The University At Albany: SUNY, Albany, NY 12222.

Three experiments were conducted to examine the utility of CO2 as an aversive US in a Pavlovian aversive context conditioning paradigm. The results of Experiment 1 indicated that animals which were exposed to CO2 in a distinctive context showed elevated levels of freezing behavior in the conditioning context than control animals. Experiment 2 replicated this basic effect with a modified conditioning procedure and additionally demonstrated conditioned analgesia as indexed by increased tail flick latencies following removal from the conditioning context. Based on the outcomes of Experiments 1 and 2, it was important to begin to determine whether conditioning phenomena which have been demonstrated with shock can also be observed in the present CO2 conditioning paradigm. Thus, the purpose of Experiment 3 was to investigate extinction and an extinction related phenomenon, renewal. The results of Experiment 3 indicated that repeated exposure to the context in the absence of CO2 resulted in a decrease in the ability of the context to induce freezing behavior. In addition, animals which received CO2 exposure in one context and extinguished in a different context showed an increase (renewal) in freezing behavior upon return to the original context.

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS IV

478.1

DYNAMICS OF MESOLIMBIC AND NIGROSTRIATAL DOPAMINE ACTIVITY, DURING APPETITIVE PAVLOVIAN CONDITIONING, AS DETERMINED BY *IN VIVO* ELECTROCHEMICAL DETECTION. T.J. Brozoski*, D.T. Brozoski, and M. Kim. Dept. of Psychology, Grinnell College, Grinnell, IA 50112.

There is considerable evidence that the mesolimbic (ML) and nigrostriatal (NS) dopamine (DA) systems of the mammalian brain are deeply involved in motivation and adaptive behavior. The ML is thought to be directly involved in mediating the effects of positive reinforcement, and the NS involved in the selection and maintenance of adaptive behavior. Pavlovian (Pav) conditioning is one of the fundamental processes through which many species acquire adaptive behaviors. The temporal dynamics of Pav conditioning are reasonably well known. The present experiment examined the temporal dynamics of DA activity in the ML and NS systems, in rats, during a simple appetitive Pav task. The objective was to discern the relationship between DA activity in these systems and behaviorally significant events in conditioning. DA activity, as indicated by *in vivo* electrochemical detection of DA metabolites, was rapidly and repeatedly measured throughout a course of Pav appetitive conditioning. The measures were obtained using chronic electrodes in freely behaving animals. Daily conditioning sessions consisted of 300 to 400 CS-US pairings, and extended over 10 to 20 consecutive days. A delay procedure was used, with an auditory cue as the CS and a food pellet as the US. Once conditioning developed, it was found that the ML system displayed a dramatic drop in DA activity during the CS and a prominent increase in DA activity immediately following the US. This was most clearly seen in the nucleus accumbens. The response of the NS system qualitatively resembled that of the ML system, but was more subtle and complex. The most notable difference between the two systems was that, in the NS system, DA activity increased only slightly, if at all, following the US. This corresponds to results previously reported by our laboratory, and suggests complementary and somewhat different roles for the ML and NS systems in the acquisition of adaptive behavior.

478.2

ACQUISITION OF OCCASION SETTING IN PREFRONTAL AND HIPPOCAMPAL LESIONED RATS. D.M. Skinner*, A. Bechara and D. van der Kooy. Dept of Anatomy and Cell Biology, University of Toronto, Toronto, Ontario, M5S 1A8. Dept. of Neurology, University of Iowa Hosp. & Clinics, Iowa City, IA 52242

In occasion setting one discriminative stimulus predicts whether or not a second, target stimulus will be followed by a motivational event (the US). We have previously suggested that most of the discriminative control is not due to simple, Pavlovian associations between the discriminative cue and the US or between the discriminative cue and the target stimulus, but to a more complex, higher-order type of association among all three elements. The hippocampus appears not to be essential for this type of complex associative learning. In a previous experiment, animals that had undergone aspiration lesions of the hippocampus were compared to sham lesioned controls on an occasion setting task employing contextual cues. While hippocampal lesioned rats were slower to acquire the occasion setting problem than sham controls, they eventually reached control levels and behavioral tests revealed that the two groups were solving the task using a similar strategy. We now report that lesions aimed at the prefrontal cortex do not impair performance on the same occasion setting task. In one distinct context, rats are given a novel flavored solution followed by an injection of LiCl. In a second distinct context, the same flavored solution is not followed by LiCl. Over time, both prefrontal and sham lesioned rats learn to suppress fluid consumption in the first context but consume large amounts in the second context. Prefrontal rats, unlike hippocampal rats, acquired the task at the same rate as controls rats. Behavioral tests, including a transfer test with a novel target solution and a choice test between the two training contexts, revealed that the prefrontal and control groups were solving the task in a similar manner. Surprisingly, the prefrontal cortex (or other cortical tissue, given previous findings of acquisition after neonatal decortication) does not appear essential for acquiring occasion setting, although the delayed acquisition after hippocampal lesions may point to a role for the hippocampus in decreasing interference among cues.

478.3

THE EFFECTS OF HIPPOCAMPAL LESIONS ON PAVLOVIAN CONDITIONED ACTIVITY DEPEND ON AMOUNT OF REINFORCEMENT OF CONTEXT. T. L. Davidson, S. C. Benoit, and L. E. Jarrard*. Purdue Univ., West Lafayette, IN 47907; Washington & Lee Univ., Lexington, VA 2445 0.

Rats with selective ibotenate lesions of the hippocampus and controls underwent Pavlovian conditioning in which a 10 sec tone conditioned stimulus (CS) terminated with the delivery of a food unconditioned stimulus (US). Behavioral activity during the CS and during the 10 sec period preceding the CS (Pre CS) was recorded by a computerized system. All rats received one CS-US trial during each of fifteen, 50 min sessions. The four groups groups differed with respect to the number of unsignaled USs received during training: Group 1-0 received no unsignaled USs, Group 3-1 received one unsignaled US every three sessions, Group 1-1 received one unsignalled US each session, and Group 1-3 received three unsignaled USs each session. Activity was greater during the CS than Pre CS periods for both hippocampal and control rats when the number of unsignaled USs was small (Groups 1-0 and 3-1). However, hippocampal rats were impaired when the number of unsignaled USs was relatively large (Groups 1-1 and 3-1). This difference was specific to the training context since both hippocampal and control rats showed greater activity during CS than during Pre CS periods when tested in a novel context. Furthermore, giving more unsignaled USs to Groups 1-0 and 3-1 increased Pre CS activity for hippocampal rats but not for controls. The results suggest that rats without a hippocampus may have difficulty suppressing activity in a highly reinforced context.

478.4

IBOTENATE LESIONS OF THE HIPPOCAMPUS DO NOT ATTENUATE THE EFFECTS OF US DEVALUATION FOLLOWING CS-US PAIRINGS IN RATS. J.R. Morell and S.E. Swithers*. Dept. of Experimental Psychology, Duke University, Durham, NC 27706.

The role of the hippocampus in simple associative learning was examined using US devaluation. Of the 37 rats in the experiment 20 were given ibotenate lesions of the hippocampus, 10 served as sham controls and 7 were only anesthetized and allowed to recover. All rats were then allowed to recover for at least two weeks post surgery. The lesioned and control rats were then distributed between Paired and Unpaired groups. For both groups, initial tone conditioning was accomplished in standard skinner boxes by pairing a tone with a food US for 8 days. All animals then received 10 minute access to the food US in their home cages followed by intraperitoneal injections (5ml/kg) of .3M lithium chloride (LiCl). The paired rats received the injections immediately following access to the food US while the unpaired rats were injected 6 hours after access to the food US. The following day the rats were placed back in the skinner boxes and tested for responsiveness to the tone CS. Both control and lesioned animals in the paired group showed an attenuated CR to the tone in the last test while both control and lesioned animals in the unpaired group did not. These results suggest that the hippocampus is not necessary for tracking devaluations of the US. This task may been seen as a conditional logic task where the animals are taught that A=B and B=C and then asked if they know that A=C. While many have suggested that the hippocampus is necessary for the solution of such tasks, these data suggest that the hippocampus may not be necessary for solving such tasks in the rat.

478.5

LATENT INHIBITION OF THE RABBIT CONDITIONED EYEBLINK IS NOT REFLECTED IN HIPPOCAMPAL ACTIVITY. D. B. Katz*, D. M. Shock, and J. E. Steinmetz. Dept. of Psychology/Neural Sciences, Indiana Univ., Bloomington, IN 47405.

Latent inhibition (LI) has been described as a robust effect whereby pre-exposure to a conditioned stimulus (CS) inhibits later learning of an association between the CS and an unconditioned stimulus (US). Several current neurobiological theories have suggested that the hippocampus mediates this phenomenon. In an examination of this possibility, male New Zealand White rabbits implanted with multiple-unit CA1 or dentate electrodes were pre-exposed to 1 kHz tones (120 per session) for eight sessions, after which they received standard delay eyeblink conditioning (pairings of the tone with a 3 psi air puff to the cornea). Pilot experiments with a 250 msec interstimulus interval (ISI) suggested that CS pre-exposure did not slow the learning rate of these animals compared to controls that had been exposed to the testing context for a similar amount of time. With the ISI set at 500 msec, however, CS pre-exposure caused a 120-trial delay in the acquisition of learning criterion. The hippocampus modeled the conditioned eyeblink in very few cases, regardless of whether the animal was pre-exposed to the CS or to the testing context only; when modeling did occur, it developed several sessions after the animal had reached criterion. These data stand in contrast to earlier work suggesting that as many as 75% of CA1-dentate pyramidal cells model the eyeblink in concert with learning. Our results suggest that the hippocampus may encode overall context, of which the CS is only a part. This last conclusion is in accord with recent lesion experiments suggesting that hippocampectomy may enhance or 'de-contextualize' LI.

478.6

TRACE AND DELAY EYEBLINK CONDITIONING INDUCE ALTERATIONS IN THE IMMUNOREACTIVITY FOR PKCγ IN THE RABBIT HIPPOCAMPUS. E.A. Van der Zee*, I.F. Palm, M.A. Kronforst, E.T. Maizels, M. Shanmugam, M. Hunzicker-Dunn, and J.F. Disterhoft. CM Biology, Northwestern University Med. Sch., 303 E. Chicago Ave., Chicago, IL 60611 USA.

Protein Kinase C (PKC) modulates neuronal activity in the hippocampus in relation to learning and memory. Previously, learning-induced changes for PKCγ immunoreactivity (ir) were found in the hippocampus after spatial learning (Van der Zee et al., J. Neurosci. 12:4808-4815; 1992). The aim of the present study was to determine whether both hippocampally-dependent 500 ms trace and 250 ms delay eyeblink conditioning induce changes in the ir for calcium-dependent PKC isoforms. Young adult (2-3 months; n=37) female NZW rabbits were used. Trained animals were killed 24 h after reaching an 80% CRs/session criterion. Brain sections were stained for the PKC isoforms α, βI, βII and γ. The hypothalamus served as a control region.

	hippocampus staining	hippocampus translocation	cerebellum staining	hypothalamus staining
pseudoconditioned (n=7)	+	-	nd	-
trace conditioned (n=7)	+++	-	nd	-
pseudoconditioned(n=8)	+	nd	-	-
delay conditioned(n=8)	++	nd	-	-

Comparison for PKCγ with naive young adults (n=7). nd = not determined; - = no change

PKCγ ir changed after conditioning. The smaller change after delay than trace conditioning might reflect differential hippocampal involvement. No gross changes in ir were observed for PKCα, βI, or βII. Western blot results showed that the total amount of hippocampal PKCγ did not change after conditioning, and no translocation occurred. These results suggest that PKCγ is the crucial PKC isoform mediating hippocampal memory changes during eyeblink conditioning. The absence of PKCγ-ir changes in the cerebellum may suggest that this isoform plays a role in declarative memory rather than procedural memory. (Supported by NIH RO1 MH47340, RO1 AG08796, and Dr. J.L. Dobberke Foundation for Comparative Psychology to I.F.P.)

478.7

POST-CONDITIONING ISOLATION DISRUPTS CONTEXTUAL BUT NOT AUDITORY FEAR CONDITIONING J. W. Rudy * Department of Psychology University of Colorado, Boulder, Co. 80309

Rats conditioned to an auditory cue paired with shock display a conditioned freezing response to both the cue and the context in which the shock occurred. Unlike cue conditioning, however, contextual conditioning appears to require a lengthy retention period before it is fully manifested. For example, 23-to-30-day-old rats display much less contextual conditioning when tested 10 min to 3 hrs after conditioning than when tested 24 hrs after conditioning, whereas freezing to the auditory cue is the same when animals are tested 10 min or 24 hrs after training (Rudy & Morledge, 1994, Behav. Neurosci. 108, 227-234). Rudy and Morledge proposed that this phenomenon reflects a lengthy consolidation period during which a representation of the context is constructed that can be used to evoke fear. To investigate this hypothesis, I compared 25-day-old rats that were either returned to their home-cage and littermates after conditioning with rats that were isolated in a novel room with food and water available. Consistent with the consolidation hypothesis, I found that (a) an isolation treatment lasting 1, 3, or 24 hrs disrupted contextual but not auditory fear conditioning, (b) a 15 min isolation treatment had no effect, (c) contextual conditioning was disrupted when the conditioning-isolation interval was 2 hrs but not when it was 24 hrs, and (d) preexposure to the conditioning context 24 hrs prior to the conditioning session prevented isolation from disrupting conditioning. These results support the consolidation hypothesis and provide additional evidence that contextual and auditory-cue fear conditioning depend on different processes.

478.8

CONTEXTUAL FEAR CONDITIONING AND HIPPOCAMPUS Robert J. McDonald*, Amy Koerner and Robert J. Sutherland, Dept. of Psychology, University of New Mexico, Albuquerque, New Mexico, U.S.A.

Investigations directed at understanding the neural substrates underlying fear conditioning to context remain controversial. Many of these studies use different response measures to assess conditioning and provide no evidence for context discrimination. In this study we address these issues by assessing 4 responses (freezing, locomotion, defecation, preference) in a context discrimination. Rats with damage to the hippocampus and shams were trained on a 4 phase procedure: (1) Pre-exposure. Each rat exposed to the 2 chambers and a connecting alley. (2) Conditioning. Each rat placed into either chamber for 2 minutes and received 3 shocks (1.5 mA for 2 s) separated by 60 s. (3) Retention. All rats were placed for 5 minutes into the three chambers (black, white, novel). (4) Preference. Each rat was allowed to show a preference for one of the chambers. Rats with damage to hippocampus do/do not exhibit acquired fear to context depending on the response measure. These results suggest that multiple respresentations of context exist and are differentially connected to actions.

478.9

HIPPOCAMPAL LESIONS CAUSE LEARNING DEFICITS IN MORRIS WATER MAZE AND CONTEXTUAL CONDITIONED FEAR TASKS IN INBRED MICE. S. F. Logue*, R. Paylor and J. M. Wehner, Institute for Behavioral Genetics and School of Pharmacy, University of Colorado, Boulder, CO 80309.

C57BL/6 (C57) mice perform better than DBA/2 (DBA) mice on the hippocampal-dependent components of the Morris water maze task and a conditioned fear task while performance of the two inbred mice strains is not different on the hippocampal-independent components of these tasks (Paylor et al., Behav. Neurosci., 108:1-8, 1994; Upchurch & Wehner, Behav. Neurosci., 103:1251-1258, 1989). Although the differential performance between the strains on the hippocampal-dependent components of the tasks is thought to be due to functional differences in the hippocampi of these strains, it has not yet been demonstrated that the mice are actually using the hippocampus (HPC) to learn these tasks.

The current experiments examined the effect of kainic acid/colchicine lesions of the HPC on acquisition of the Morris task and/or conditioned fear responses. Groups of C57 and DBA mice were given HPC lesions or sham surgery and after recovery trained on the tasks. In the contextual fear test, lesions decreased freezing in both strains of mice. In the conditioned stimulus test this lesion effect was not seen. In the Morris task, based on probe trial data, the lesioned C57 mice failed to learn relative to the sham controls while both the lesion and sham DBA mice failed to learn the task. These data demonstrate that C57 mice do use the HPC to solve the Morris and conditioned fear tasks and that the DBA mice use the HPC in the conditioned fear task. (Supported by S.F.L.-5T32HD-07289; J.M.W.-MH-48663 & AA-00141)

478.10

EFFECTS OF HIPPOCAMPAL LESIONS ON A CONTEXTUAL DISCRIMINATION TASK. P.D. Sparks* and J.E. LeDoux, Department of Psychology and Center for Neural Science, NYU, NY, NY 10003.

Dorsal hippocampal lesions interfere with the conditioning of fear responses to contextual stimuli in rats. In the present study we examined the effects of such lesions on a contextual discrimination task, in which an auditory conditioned stimulus (CS: 10kHz, 72dB, 20 sec) was paired with footshock in one chamber and without footshock (0.5 ma, 500ms) in another. Two weeks after surgery, blocks of two trials consisting of tone-shock pairings were given twice a day (7 hrs apart), for 8-10 days. Time spent freezing to context and tone were measured in each chamber in order to evaluate the animals' ability to discriminate between the chambers. After 7 days on average, controls spent less time freezing in the no shock chamber than in chamber the shock chamber. Hippocampal lesions reduced freezing to the context in the no shock chamber but not in the shock chamber. This result was unexpected, as it suggests that hippocampal lesions facilitated the learning of a contextual discrimination. The effects of septal lesions on contextual discrimination are currently under investigation. Supported by NSF9209646 and MH38774.

478.11

UNILATERAL AMYGDALA LESIONS ATTENUATE CONDITIONED FEAR IN RATS K.S. LaBar* and J.E. LeDoux. Center for Neural Science, New York University, NY, NY

We recently demonstrated impaired fear conditioning in human patients with unilateral medial temporal lobe resection, including the amygdala, to control medically refractory epilepsy (LaBar et al., 1994). This human study differed from typical animal models in several ways, including that a loud white noise burst served as a US, and that the extent of amygdala damage was primarily unilateral. The present study was thus designed to test the efficacy of a white noise US for conditioning to both an explicit CS and to the context, and to compare the effects of unilateral vs. bilateral amygdala lesions on conditioned fear in rats.

Male Sprague-Dawley rats were randomly assigned to experimental (left/right unilateral or bilateral electrolytic amygdala lesion) or control (sham operated or unoperated) groups. One week following surgery, the rats received 2 days of CS-US acquisition training (2 trials/day: CS=10 kHz tone, 75 dB, 20 sec; US=white noise, 110 dB, 1 sec) and 5 days of CS-alone extinction training (2 trials/day). Rats with bilateral amygdala lesions showed a nearly complete lack of freezing to both the CS and the context. Rats with unilateral amygdala lesions froze significantly less than controls to the CS and the context; the context effect, however, was less pronounced due to low overall levels of contextual conditioning in the control groups. We conclude that white noise can serve as an effective US, especially for conditioning to an explicit CS, and that unilateral amygdala lesions attenuate but do not eliminate conditioned fear in rats.

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478.12

STIMULATION OF HIPPOCAMPAL KAPPA OPIOID RECEPTORS BLOCKS PAVLOVIAN FEAR CONDITIONING TO CONTEXTUAL STIMULI F.J. Helmstetter*, P.S.F. Bellgowan & E.A. Kaspersen, University of Wisconsin, Milwaukee, WI 53201

Endogenous opioid peptides that bind to κ receptors may act as retrograde inhibitory neurotransmitters which regulate excitatory synaptic transmission in the hippocampus. *In vitro* studies have shown that endogenously released and exogenously applied κ agonists will selectively block the induction of at least one form of LTP (e.g., Wagner, Terman & Chavkin, Nature 363:451). In the present study rats were prepared with chronic cannullae in the dorsal hippocampus and tested in a Pavlovian fear conditioning paradigm. Prior to training during which animals received a series of paired presentations of an auditory stimulus and foot shock in a distinctive observation chamber groups were pretreated with the κ agonist U50,488H (U50), the κ antagonist norbinaltorphimine (nBNI) or saline. Twenty four and 48 hours later fear conditioning to the shock-associated observation chamber (contextual CS) and the auditory signal (discrete CS) was assessed independently. Prior work has shown that hippocampal lesions or local infusion of NMDA antagonists block learning about the contextual but not the discrete CS. Rats treated with U50 showed a significant disruption in fear to the shock -associated context. Initial response to the auditory CS was intact in these animals although large decrements due to pretraining U50 were seen during extinction. nBNI failed to facilitate the acquisition of either response. The results generally support a role for κ opioids as regulators of hippocampal plasticity during learning in intact animals.

478.13

EFFECTS OF MUSCIMOL APPLIED TO THE DORSAL HIPPOCAMPUS ON THE ACQUISITION AND EXPRESSION OF CUED VERSUS CONTEXTUAL FEAR CONDITIONING

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Previous research has shown that rats with lesions of the dorsal hippocampus show dramatic deficits in Pavlovian fear conditioning when complex contextual stimuli are used as the CS but show normal learning when a discrete unimodal cue such as a tone is used to signal shock (Kim & Fanselow, 1992). In order to investigate whether the hippocampus was selectively involved in either the acquisition or the expression of contextual fear conditioning we prepared rats with chronic cannulae and injected the GABA agonist muscimol (MUS, 0.25 μg) into the hippocampus prior to a training session in which white noise (10 sec / 72 dB) and shock (2 mA / 1 sec) were paired within a distinctive observation chamber, or prior to testing sessions during which fear responses to a single 5 min presentation of white noise and the shock-associated training context were measured independently during extinction. When hippocampal activity was disrupted with MUS prior to the training session, freezing behavior subsequently measured in the training context was drastically reduced. Furthermore, fear responses to the contextual CS were also affected when MUS was injected prior to CR expression in animals trained with saline. Neither of these effects were due to "state-dependence." These results suggest that the hippocampus is important for both the acquisition and the expression of contextual fear conditioning. Pretraining MUS produced minimal disruption of fear to the auditory CS measured 24 or 48 hr after acquisition. However, when MUS was injected prior to testing we observed a substantial attenuation of performance to the discrete CS during the second testing session suggesting that hippocampal manipulations may affect performance of discrete CRs during extinction.

478.14

IBOTENIC ACID HIPPOCAMPAL LESIONS REVERSE CONTEXT BUT NOT LATENT INHIBITION EFFECTS DURING APPETITIVE AND AVERSIVE SIGNALLED BARPRESS TRAINING. D. P. Miller[1]* and J. E. Steinmetz[2], Dept. of Psych., Carthage College[1], Kenosha, WI 53140; Prog. in Neural Sci. and Dept. of Psych., Indiana University[2], Bloomington, IN 47405.

A number of studies have demonstrated that conventional hippocampal lesions abolished latent inhibition in aversive (e.g., Solomon & Moore, *J. Comp. Physiol. Behav.*, 89:1192, 1975) and appetitive (Kaye & Pearce, *Quarterly J. Exper. Psychol.* 39B:107, 1987) tasks. Recently it was reported that ibotenic acid hippocampal lesions did not abolish latent inhibition in an appetitive Pavlovian conditioning task (Honey & Good, *Behav. Neurosci.* 107:23, 1993). In the present experiments we assessed the effects of ibotenic hippocampal lesions on appetitive and aversive signalled barpress conditioning following latent inhibition training. During appetitive training, rats that received pre-exposure to the tone signal exhibited a prolonged increase in latency to perform the signalled barpress response within trials. Hippocampal lesions did not disrupt this inhibitory effect. Latent inhibition training did not retard acquisition of the barpress response across trials. During aversive training, a separate group of rats with hippocampal lesions acquired the signalled avoidance response faster than rats with an intact hippocampus regardless of whether they had received pre-exposure to the tone signal or the conditioning chamber without the tone signal. Latent inhibition training retarded acquisition of the signalled escape response and hippocampal lesions did not reverse this retardation. Thus specific hippocampal lesions did not reverse inhibition related to processing the tone signal in either appetitive or aversive conditioning but did appear to reverse inhibition related to the processing of more general contextual cues in the aversive task. Supported by NIMH grant MH44052.

478.15

SELECTIVE NEUROTOXIC LESIONS OF THE HIPPOCAMPUS BLOCK RETENTION OF A SOCIAL MEMORY. H. Eichenbaum* and M. Bunsey. Center for Behavioral Neuroscience, SUNY at Stony Brook, NY 11794-2575.

Previous studies have indicated that hippocampal lesions impair the memory for the social transmission of food preferences (Winocur, Behav. Brain. Res. 38:145, 1989; Bunsey & Eichenbaum, Soc. Neurosci. Abstr. 19:358, 1994). The present study assessed which subcomponents of the hippocampus are critical to support this form of memory.

Male Long Evans rats underwent control surgery or ibotenic acid-induced lesions of the hippocampus proper plus dentate gyrus (H+D), the subiculum (S), or all three hippocampal subfields (H+S+D). Following a three week recovery, subjects were briefly housed with a conspecific that had just consumed a distinctively scented diet. Either immediately or 24 hr after this social interaction, subjects were given a choice between a diet identical to that eaten by the conspecific and a different scented diet. Consistent with previous results, normal rats preferred the conspecific's diet following the interaction, and showed no forgetting over a 24 hr retention interval. Neither selective H+D nor selective S lesions affected performance at either delay. By contrast, the complete hippocampus lesion (H+D+S) completely blocked memory at the 24 hr test. This deficit was delay-dependent -- there was no effect at the immediate retention test -- thus indicating a specific memory impairment. These findings demonstrate a critical role for the hippocampus in a naturally-occurring, and distinctly nonspatial form of memory and provide further evidence that the functional unit underlying hippocampal-dependent memory includes all of its subfields. (Supported by NIMH, ONR, NIA)

478.16

SELECTIVE HIPPOCAMPAL LESIONS BLOCK ASSOCIATIVE TRANSITIVITY. M. Bunsey* and H. Eichenbaum. Center for Behavioral Neuroscience, SUNY at Stony Brook, NY 11794-2575.

The hippocampus is thought to support declarative memory processing. Defining characteristics that distinguish declarative memory is the representaion of relations among items in memory and a capacity for representational flexibility, the ability to access memories in novel situations, independent of the circumstances of origianl learning. This study assessed the effects of selective hippocampal lesions on one such form of representational flexibility, i.e., associative transitivity.

Male Long Evans rats underwent ibotenic acid-induced lesions of the hippocampal subfields or control surgery. Subjects were then trained on two consecutive sets of odor paired associates with shared elements: Set 1 involved learning pairs A-B and X-Y, and set 2 involved pairs B-C and Y-Z. Associative transitivity between sets is demonstrated to the extent that subjects infer the associative relationships that exist across pairs, e.g., by judging an association to exist between A and C based on the shared element B. Controls quickly learned the paired associate problems and showed substantial transitivity on unrewarded probe trials in which inferential judgement was required. Rats with selective hippocampal lesions learned the paired associates as quickly as controls, but showed no evidence of transitive inference. Thus, despite intact acquisition of the paired associates, hippocampal rats developed an abnormal and inflexible representation of the odor pairings. These data add to growing evidence that the hippocampus itself is not critical to some forms of paired associate or conditional learning, but that it is crucial to the development of a "declarative" form of representation that supports relational and flexible memory expression. (Supported by NIMH, ONR, NIA)

478.17

SPATIAL & TEMPORAL ORGANIZATION IN TRANSITIVE INFERENCE BY RATS. J. Dusek *, M. Bunsey, & H. Eichenbaum. Center for Behavioral Neuroscience, SUNY-Stony Brook, NY 11794.

The term "transitive inference" signifies the ability to infer a relationship between items that have not been previously experienced together, based on previous learning of the relations between overlapping subsets of the items. The present experiment is intended to extend the Roberts and Phelps (Psychol. Sci. 5:368, 1994) study in which transitive inference was exhibited in rats trained with odor stimuli presented in a linear spatial arrangement, but not when the same stimuli were randomly arranged. These findings were interpreted as consistent with the spatial hypothesis of transitive inference, that is, that the spatial ordering of stimuli provided the organization necessary for inferential judgments. The present study was aimed to determine if other, nonspatial orderings provided during training would also enable rats to encode an odor sequence and demonstrate transitive inference.

Three groups of male Long Evans rats (spatial, temporal, control) were trained with five adjacent pairs of odors (A+ vs B-, B+ vs C-, C+ vs D-, etc.) that formed a sequential hierarchy (A>B>C>D>E>F). After reaching criterion on the set of pairwise discriminations, rats were subsequently tested in a novel environment with the non-adjacent pair, BD, to assess capacity for transitive inference. Preliminary results suggest that the groups acquired the sequence in different ways and that the patterns of responses are consistent with the organization of the odors for spatial or temporal ordering. Supported by ONR.

478.18

EXCITOTOXIC LESIONS IN THE BASOLATERAL AMYGDALA INDUCE A TEMPORALLY-STABLE RETROGRADE AMNESIA OF FEAR IN RATS. S. Maren *, G. Aharonov and M. S. Fanselow. Department of Psychology, University of California, Los Angeles, CA 90024-1563.

It is now generally agreed that the amygdala is required for the acquisition of conditional fear in a variety of learning tasks, but there is some debate concerning the role of the amygdala in the expression of learned fear following conditioning. Some investigators have suggested that the role of the amygdala is temporally-graded, because post-training amygdala inactivation disrupts retention only when performed shortly after training. In contrast, other investigators have found that electrolytic lesions of the amygdala produce deficits in the expression of conditional fear when given either 1 or 30 days following training, suggesting a temporally-stable role for the amygdala in the expression of conditional fear. Because the lesions used in the latter experiments included damage to the central nucleus, which is required for the performance of both conditioned and unconditioned fear, we have re-examined the retrograde gradient of Pavlovian fear conditioning using more selective *N*-methyl-D-aspartate (NMDA) lesions of the basolateral amygdala. On the conditioning day, rats were placed in observation chambers and three minutes later given three tone (90 dB, 2 kHz, 10 s)-footshock (1 mA, 2 s) pairings with a 74 s ISI. One, fourteen, or twenty-eight days following conditioning bilateral excitotoxic lesions were made in the basolateral amygdala using NMDA (20 μg/μL, 0.3 μL/side); control rats received sham surgery with no drug infusion. One week following surgery fear to the context of the conditioning chamber was assessed by returning the rats to the chambers and scoring freezing; fear to the tone was assessed in a different chamber the following day. Results showed that basolateral amygdala lesions produced an impairment of both context and tone fear that did not vary as a function of the training to lesion interval. This pattern of results reveals that the role of the amygdala in Pavlovian fear conditioning is not temporally-graded and suggests that neurons in the basolateral amygdala are the storage site for fear memories. These results will be contrasted with those obtained from excitotoxic lesions of the hippocampus. Supported by NIMH grant (MH39786) to MSF.

478.19

CATECHOLAMINE DEPLETION DOES NOT ATTENUATE CONSOLIDATION IN A SIMPLE PAVLOVIAN FEAR CONDITIONING PARADIGM

S.G. Anagnostaras* and Michael S. Fanselow. Department of Psychology, University of California, Los Angeles, CA 90024-1563.

Rats given signaled shocks in a particular context will later express conditional fear to that context or to the signal. Previous experiments in our laboratory have shown that contextual fear appears to be consolidated over a 4-wk period, such that hippocampal lesions given immediately after training produce a profound retrograde amnesia specific to contextual fear, but lesions given four weeks after training do not affect contextual fear memory. Although initial acquisition is dependent on NMDA receptor activation, the neurochemical events occuring during the 4-wk consolidation period are unknown. As a first step toward discovering the nature of consolidation, we are attempting to deplete particular neurotransmitter systems to determine which are necessary for consolidation. Studies by McGaugh and others have shown that catecholamines neurotransmitters are important in the modulation of consolidation. In order to test whether or not catecholamines are *necessary* for consolidation, rats were treated with *dl*-α-methyl-*p*-tyrosine methyl ester HCl (AMPT), a tyrosine hyroxylase inhibitor. On the conditioning day, the animals were given five tone-shock pairings in a particular context. *Anterograde* animals were given an injection of AMPT (200 mg/kg, i.p.) 1-hr prior to training. *Retrograde* animals received an injection of AMPT (200 mg/kg) immediately after training and twice daily for two additional days. *Saline Control* animals were treated similarly but received saline injections. Seven to eight days later, all animals were tested in the absence of the drug for conditional freezing to the training context and to the tone in a novel context. Although these treatments produce severe reductions in catecholamine levels, there was no effect of either AMPT treatment paradigm on the expression of conditional fear on either test. Thus, normals levels of catecholamines do not appear necessary for consolidation of simple Pavlovian fear conditioning. Studies are in progress examining other neurotransmitter systems on consolidation in this behavioral paradigm. Supported by NIMH grant (MH39786) to MSF.

478.20

EFFECTS OF LESIONS AND GLUTAMATE ANTAGONISTS IN THE VENTRAL AND DORSAL PAG ON THE LEARNING AND PERFORMANCE OF CONDITIONED FEAR. B. M. De Oca* and M. S. Fanselow. Dept. of Psychology, University of California, Los Angeles, CA 900241563.

The midbrain periaqueductal grey is involved in fear and defense-related behaviors. Lesion studies suggest that the caudal region dorsal and lateral to the aqueduct of the periaqueductal grey (D-PAG) is implicated in circa-strike defensive responding in rats while the caudal region ventral to the aqueduct (V-PAG) is implicated in defensive freezing. We have suggested that the D-PAG tonically inhibits the V-PAG. The purpose of this study was to: 1) Dissociate learning and performance effects of both the D-PAG and V-PAG on conditioned fear-induced freezing. 2) Ascertain that effects of lesions of the area are due to damage to neurons intrinsic to the PAG and not to the many fibers of passage which traverse the PAG. Pre-training lesions enhance defensive freezing to Pavlovian-conditioned fear cues, possibly by eliminating tonic inhibition of the V-PAG, while post-training lesions of the D-PAG have no effect. In other experiments, rats received the AMPA receptor antagonist, DNQX or vehicle prior to training and either DNQX or vehicle prior to testing of Pavlovian-conditioned fear. The results suggest that neurons within the PAG are not simply motor-ouptut relays, but rather are critical for acuisition of conditioned fear. Supported by NIMH grant (MH39786) to MSF.

478.21

THE IMMEDIATE SHOCK FEAR DEFICIT: A TEST OF TIMING VS. ASSOCIATIVE ACCOUNTS. M. S. Fanselow* & D. Stote. Department of Psychology, University of California, Los Angeles, CA 90024-1563.

Rats that receive aversive footshock soon after placement in an observation chamber show conditional fear-induced responses to this shock paired context when they are reexposed to the observation chamber. This contextual fear increases with increases in the interval between placement in the chamber and shock. If shock is given immediately upon placement in the chamber no fear is observed, an effect called the immediate shock deficit (ISD). We have suggested that the ISD arises because there is insufficient time to process contextual cues prior to shock and therefore, the animal fails to associate the context with shock. However, if one makes the assumption that rats accurately learn the placement to shock interval, alternative accounts can be entertained. For example, rats that expect shock once every 5 sec may abandon this assumption during the shock-free test more readily than rats expecting shock once every minute (disconfirmation hypothesis). We tested the timing assumption by determining the latency to freeze and the time at which peak freezing occurred in rats that received different intervals between placement and shock (5, 20, 60, & 180 sec). The timing assumption predicts a positive relation between this interval and these two measures, while the associative account predicts an inverse relationship. Both measures supported the associative account. We also tested the disconfirmation hypothesis' prediction that the ISD function should be eliminated if preshock, postshock and testing times are held constant. There was a substantial ISD under these conditions, supporting the associative account. This pattern of results generalized to the freezing deficit obtained with multiple massed shocks. By administering naltrexone prior to shock, we also determined if the ISD results from a brief opioid analgesia that could accompany placement in the chamber, but the ISD was not affected by the opioid antagonist. Overall, the data support the hypothesis that the ISD results from an associative deficit that arises because of a failure to appreciate the context prior to shock, a process that is likely to depend on the hippocampus. Supported by NIMH grant (MH39786) to MSF.

479.1

DOES REWARDING ELECTRICAL STIMULATION OF LATERAL HYPOTHALAMUS FACILITATE LEARNING OF CONDITIONED NICTITATING MEMBRANE RESPONSE IN RABBITS? J. Arikoski*, T. Korhonen, M. Penttonen, T. Ruusuvirta and J. Wikgren. Dept. of Psychol., Univ. of Jyväskylä, P.O. Box 35, SF-40351, Jyväskylä, Finland.

There are only few observations of rewarding effects of an electrical brain stimulation (ESB) on classical conditioning of nictitating membrane (NM) response in rabbits. There are also a few if not at all such studies in which both the rewarding pre- and post-ESB is present. In this study control rabbits (CC group) were classically conditioned with a tone conditioned stimulus (CS) and an airpuff unconditioned stimulus (US). In test groups, the ESB either preceded (CCpre group) or followed (CCpost group) the CS-US presentation.

Results indicated that during the CS periods of the CS test trials (an unpaired control tone) all the groups showed significant increase in the CR amplitude over days ($F(1.50, 40) = 22.48$, $p < .001$). After five days of conditioning it could be identified that the CCpost group showed faster CR acquisition than the CC group, whereas only a slight CR could be identified from the CCpre group. In this phase a significant Treatment x Day interaction ($F(2.99, 40) = 3.56$, $p < .05$) was observed.

Due to these facts we conclude that the ESB of the lateral hypothalamus given as post stimulation is sufficient to facilitate classical conditioning of the NM response. In this study also evoked potentials and multiple-unit recordings were obtained from hippocampus (CA1, CA3 and dentate gyrus). We are working on this data.

479.2

CEREBELLAR LESIONS PREVENT THE LEARNING OF CLASSICALLY CONDITIONED EYEBLINK RESPONSES IN MICE. S. Bao*, L. Chen, J. J. Kim, R. F. Thompson. Neuroscience Program, Univ. of Southern Calif., Los Angeles, CA 90089-2520

Converging lines of evidence from rabbit, rat and human studies indicate that the cerebellum is essential for the delay eyeblink conditioning. In the present study, we examined whether eyeblink conditioning is feasible and whether the cerebellum is crucial for this type of learning in mice.

Adult male mice (C57BL/6J strain) were implanted with four wires (Teflon coated) subcutaneously to the left upper eyelid. Two of the wires were used to record differential electromyograph (EMG) from obicularis oculi and the other two wires were used to deliver periobital shock. A four-pin strip connector to which the wires were soldered to was cemented to skull with dental acrylic. Animals were trained on a standard delay paradigm where conditioned stimulus (CS) was a 350-ms tone (1kHz, 83dB) and unconditioned stimulus (US) was a 100-ms periorbital shock coterminating with the CS (interstimulus interval = 250 ms). Eyelid EMG was recorded to score conditioned responses (CRs) to CS.

Eyeblink conditioning was examined in the following three groups of animals: paired, pseudo-ramdom, and cerebellar lesioned. Animal that received paired CS-US gradually acquired CRs to the CS and reached asymptote within five days. When switched to CS-alone trials, they exhibited gradual extinction of the CRs. In contrast to paired group, animals that received pseudo-random presentations of CS and US did not acquire CRs. When switched to paired training, they gradually acquired CRs. Animals with cerebellar lesions failed to show CRs even with extensive training (10 days). Together, our results shows that eyeblink conditioning is feasible in mice and that the cerebellum plays a critical role in eyeblink conditioning. Supported by grants from NSF (IBN9215069), NIA (AF05142), and Sankyo to RFT.

479.3

IMPAIRED CLASSICAL EYEBLINK CONDITIONING IN PURKINJE CELL DEGENERATION (*pcd*) MUTANT MICE. L. Chen*, S. Bao, J. J. Kim and R. F. Thompson. Neuroscience Program, Univ. of Southern Calif., Los Angeles, CA 90089-2520.

An accumulating body of evidence from rabbit, rat and human studies strongly suggest the crucial involvement of cerebellum in classical eyeblink conditioning. Within the cerebellum, however, it has been difficult to dissociate the relative role of cerebellar cortex versus deep nuclei in eyeblink learning; it is practically impossible to lesion the entire cerebellar cortex while sparing the deep nuclei. In the present study, we employed mutant mice deficient of Purkinje cells, the exclusive output neurons of the cerebellar cortex, to further investigate eyeblink conditioning.

The subjects, adult male *pcd* mutant (n=11) and normal littermate (n=10) mice (C57BL/6J background), underwent a standard delay eyeblink conditioning paradigm (350-ms tone conditioned stimulus, 100-ms periorbital shock unconditioned stimulus, interstimulus interval = 250-ms). The eyelid muscle electromyograph (EMG) was recorded to score CRs. Our results indicate that *pcd* mice exhibited a profound impairment in the acquisition of delay eyeblink conditioning in comparison to their normal littermates. The *pcd* animals, nevertheless, did evidence partial learned eyeblink responses with extensive training. There were no differences between the groups in sensitivity to the unconditioned stimulus nor unconditioned responses to the periorbital shock. These results suggest that cerebellar cortex plays a critical role in eyeblink conditioning.

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479.4

SELECTIVE INCREASE IN HIPPOCAMPAL AMPA RECEPTOR LEVELS WITH TRACE EYE-BLINK CONDITIONING. W. Sun*, L. Chen, S. Bao, S. Standley, G. Tocco, J. J. Kim, M. Baudry, and R. F. Thompson. Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520.

It has been shown previously that rabbits trained on the trace eye-blink conditioning paradigm showed an increase in ^{3}H-AMPA binding in their hippocampal sections. The increase in ^{3}H-AMPA binding may be due to an increase in the number of AMPA receptors or to an increase in the affinity of the receptor for its ligand. To distinguish the two different possibilities, we examined the hippocampal proteins for changes in AMPA receptor levels with conditioning by western blot analysis. Three groups of animals are analyzed: 1) 500 ms trace conditioning with paired tone-air, 2) pseudo-random tone-air, and 3) naive animals. Once the paired group was trained to criterion, the hippocampi from all the animals were removed. Synaptosomal fractions were prepared from each hippocampus and the protein concentrations were determined. Equal amounts of synaptosomal protein were subjected to SDS-PAGE and then transferred onto nitrocellulose. The filters were then incubated with antibodies (Chemicon) directed against specific AMPA receptor subunits GluR1-R4. Antibody against the 38 kD synaptophysin was also used as a control. The blots were scanned by HP ScanJet II and subjected to image analysis. Western blot analysis showed that in group 1 animals, there is a significant increase in the level of hippocampal GluR1 receptors as compared to those of the animals from groups 2 and 3. There is no significant difference in the level of synaptophysin among animals from the three groups. These results suggest that the increase in ^{3}H-AMPA binding in the hippocampus associated with trace eye-blink conditioning is due to an increase in the number, rather than affinity for the ligand, of AMPA receptor GluR1.

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479.5

NEURAL RESPONSES OF THE CEREBELLAR DEEP NUCLEI IN THE NAIVE AND WELL TRAINED RABBIT FOLLOWING NM CONDITIONING.

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The cerebellar deep nuclei appears to be necessary for classical conditioning of the rabbit nictitating membrane (NM) response. Reversible lesions of the anterior interpositus (IPA) prevent acquisition of the learned behavior in naive animals, and abolish the learned response in trained animals (Krupa et al., 1993). Previous studies have shown that lateral IPA and the medial dentate receive convergent projections from peripheral auditory and somatosensory stimuli and produce neural models of the learned NM response following conditioning to a tone conditioned stimulus (CS). (Tracy et al, 1991,1993, Neurosci Abs) This study replicates the same pattern of responses using a light stimuli, which is another effective CS.

Neural recordings were performed in the awake behaving rabbit. Naive rabbits received unpaired trials of light (350 msec) and air (100 msec, 3 psi). Trained rabbits were conditioned to a light (350 msec) that preceded and coterminated with an air puff to the eye (100 msec, 3 psi). Animals were trained to criteria with one day over-training. Recordings from the cerebellar deep nuclei in the naive rabbits show that the cerebellum receives projections from peripheral visual and somatosensory stimuli. Neurons throughout the deep nuclei respond to somatosensory information. The lateral IPA and the medial dentate respond to both visual and somatosensory information. In well trained animals, this same area that receives the convergence of visual and somatosensory information produces neural responses that precede and model the learned NM response. These neural models precede the learned behavior an average of 108 msec.

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479.6

INACTIVATION OF THE INTERPOSITUS NUCLEUS PREVENTS TRANSFER OF THE RABBIT'S CLASSICALLY CONDITIONED EYEBLINK RESPONSE FROM A LIGHT TO A TONE CS. B.D. Cipriano, D.J. Krupa, W. Almanza, and R.F. Thompson*. Neurosciences program, USC, Los Angeles, CA 90089-2520.

To study the role of transfer training from one CS to another , the following procedure was used. Three groups of rabbits were each implanted with a cannula in the left interpositus (IP). Groups I and II received 7 daily sessions of paired light-airpuff training (120 trials/session). Group III was restrained during sessions 1-7 but presented with no stimuli. Prior to session 8, group I was infused with 200ng muscimol to inactivate the IP. Groups II and III were infused with saline. Session 8 for all groups consisted 120 paired tone-airpuff trials (with 20 interpolated light-airpuff trials). Group I performed no CRs to the tone or light during session 8. Session 9 (48 hrs later) was the same as session 8 with the addition with the addition of 30 tone alone trials prior to any paired trials. No infusions were given. The results of the 30 tone alone trials indicate that group I did not learn during session 8 (IP inactivated), as evidenced by baseline responding. Groups II and III both showed learning during session 8. However, the percentage of CRs performed by Group II to the tone CS during session 8 and subsequent 30 tone alone trials (session 9) was significantly higher than the percentage performed by group III, demonstrating a strong transfer of learning between the 2 CS conditions. These results indicate that the cerebellum is essential for transfer training from one CS to a second, contradicting a recent report by Welsh & Harvey (J. of Physiol, 1991). [Support: NSF (IBN9215069), NIA (AFO5142), and Sankyo.]

479.7

A STUDY OF HABITUATION IN THE RABBIT TO A CORNEAL AIRPUFF AT DIFFERENT INTERSTIMULUS INTERVALS AND STIMULUS INTENSITIES. D. Ivkovich* and R.F. Thompson. Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520.

Peripheral airpuff stimulation is commonly used in classical conditioning of the defensive eyeblink response. Modulation of this response has been observed following both associative and non-associative training sessions. The present study addresses changes in reflexive eyeblink behavior to repeated airpuff stimulation in the absence of conditioned stimulus presentations.

Fifty New Zealand White rabbits were divided into 5 groups, each experiencing a different interstimulus interval (ISI): 1 s, 5 s, 10 s, 30 s, or 60 s. Each animal was given two consecutive days of unpaired airpuff alone stimulus exposure, a week of rest, then two more days of exposure. Within each group, half of the animals were presented in the first series with an airpuff intensity of 3 psi followed by 1 psi in the second series. The remaining animals experienced the reverse order. Each session consisted of 12 blocks of 9 trials (8 airpuff and 1 blank to test for anticipatory responding). Measures of response frequency and absolute and scaled (percentage change) amplitude were analyzed.

Animals responded above 90% to both stimulus intensities. Absolute response amplitudes to 3 psi were significantly higher overall. Significant response decrement (habituation) was observed within a session and the change on day 2 was greater than day 1. Scaled amplitude measures revealed an order effect for intensity: experiencing a strong stimulus facilitated later habituation to a weak one. There was no main effect of ISI on any measure, but there were complex interactions of ISI with intensity within and between days which suggest a balancing of sensitization and habituation processes described in previous electrophysiological preparations.

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479.8

SUPPRESSION OF RABBIT'S TRACE-CONDITIONING NICTITATING MEMBRANE RESPONSE BY SCOPOLAMINE. T. Kaneko* and R. F. Thompson, Neuroscience Program, Univ. of Southern California, Los Angeles, CA 90089-2520

It has been reported that scopolamine, a muscarinic acetylcholine receptor antagonist, delayed the rate of acquisition in classical conditioning of the rabbit nictitating membrane response on delay paradigm. However, no information is available concerning trace paradigm, even though an important role of the hippocampus, one of the main targets of cholinergic innervation in the brain, in trace conditioning has been suggested. In this study we have examined to what degree the cholinergic systems were involved in trace conditioning of rabbits by means of a simple pharmacological manipulation.

Rabbits were trained on a trace paradigm in which a 250 ms tone CS and a 100 ms airpuff US were presented with 500 ms i.s.i. Each training session a day was consisted of 10 tone alone, 10 airpuff alone and 80 paired trials. Scopolamine hydrobromide 0.03 or 0.1 mg/0.5 ml/kg, or saline 0.5 ml/kg was injected subcutaneously 15-20 min prior to daily training session.

In average, control animals having saline injections (n=5), showed more than 80% of CRs by training day 10. On the other hand, averaged CR percentage of rabbits having scopolamine 0.03 mg/kg (n=4) gained a plateau of about 60% after 12 days of training, and one of the animals failed to reach a criteria of learning (8 CRs out of consecutive 9 trials). None of 5 rabbits treated with the higher dose of scopolamine satisfied a criteria within 12 days of training. The averaged CR percentage of these animals remained about 10% through the period of training. On day 13, scopolamine 0.1 mg/kg, s.c. given to control animals decreased CRs, but still more than 50% of CRs was observed. CR percentage of these animals returned to the previous level on day 14 when rabbits treated with saline. In rabbits having treated with scopolamine 0.1 mg/kg, CR percentage gradually increased when scopolamine was switched to saline from day 13.

(Supported by NFS grant BNS-8718300 and a Sankyo grant to RFT)

479.9

INJECTIONS OF ANISOMYCIN INTO THE INTERPOSED NUCLEAR REGION AFFECT CONSOLIDATION OF THE CONDITIONED EYEBLINK RESPONSE. V. Bracha*, M.L. Webster, M.K. Stachowiak, J.R. Bloedel. Barrow Neurological Institute, Phoenix, AZ 85013.

The present experiments examined the effects of injecting anisomycin (ANI), a protein synthesis inhibitor, into the cerebellar interposed nucleus (IPN) on the acquisition and expression of the classically conditioned nictitating membrane response in the rabbit. Animals were conditioned for three days in a standard delay paradigm. Before each training session they were bilaterally injected into the IPN with 1 µl of an ANI solution. On the fourth day no drug was injected and the rabbits were tested for retention of conditioned responses (CR). All animals were further conditioned to test for any long-term effects of previous ANI applications.

The experimental group of rabbits injected with ANI during the three acquisition sessions exhibited a lower incidence of CRs than the control group of animals injected with vehicle. Importantly, animals in the experimental group exhibited a significantly lower incidence of CRs in the retention test. Animals showing ANI-affected retention of CRs acquired the CRs at a normal rate after the drug injections were discontinued. The ANI injections did not eliminate CR expression during either the initial training or in well trained animals. The results indicate that while anisomycin has few acute effects on the CR expression, it significantly reduces its retention, implying that the synthesis of new proteins in the IPN region is required for the process of consolidation of CRs between individual training sessions. NIH Grants NS 30013 and NS 21958.

479.10

STOCHASTIC SIMULATIONS OF CEREBELLAR-MEDIATED MOTOR ADAPTATION. Javier F. Medina and Michael D. Mauk* Department of Neurobiology and Anatomy, University of Texas Medical School at Houston, Houston, TX 77030.

Our lab has previously developed a linear mathematical model to study synaptic plasticity in the cerebellum. This model is based on the well characterized cerebellar synaptic organization and specific synaptic plasticity rules at Granule to Purkinje (Gr→Pkj) and Mossy fiber to Nucleus (MF→Nuc) synapses. We use stochastic computer simulations to study the possible influences of biologically relevant non-linearities on the predictions of the linear model. Empirical data from eyelid conditioning, a simple form of cerebellar-mediated motor learning, is then used to test the results from these simulations. During an eyelid conditioning training trial, an unconditioned stimulus (US), which is simulated as an increase in the excitatory drive to climbing fibers, is paired with a conditioned stimulus (CS), modeled as consistent changes in the activities of specific subsets of Gr→Pkj and MF→Nuc synapses from their background activity levels.

Simulations show that after acquisition, the model is capable of acquiring conditioned responses (CRs) as measured by increases in nuclear cell activity. Previous theoretical work suggests that during a conditioning trial only one subset of MF→Nuc but many different subsets of Gr→Pkj synapses are active. Using this assumption, simulations are capable of reproducing not only the net effect of training on the strength of CS-US associations but also capture key behavioral features of eyelid conditioning such as the ISI function and the form and timing of the conditioned response.

479.11

CEREBELLAR CORTEX IS NECESSARY FOR ACQUISITION OF PAVLOVIAN EYELID RESPONSES. Keith S. Garcia* and Michael D. Mauk. Department of Neurobiology and Anatomy, University of Texas Medical School at Houston, Houston, TX 77030.

Recent work has shown lesions of the cerebellar cortex that include the anterior lobe disrupt the timing and prevent the extinction of Pavlovian eyelid responses (CRs). These results have motivated a reexamination of the role of the cerebellar cortex in CR acquisition with emphasis on the anterior lobe. Aspiration lesions of the cerebellar cortex were performed on well-trained rabbits. By training subjects prior to surgery the effects of aspiration lesions on CR timing can be used as a functional indication that lesions have damaged the anterior lobe and that input and output pathways necessary for CR expression remain intact. Rabbits that showed disrupted CR timing following surgery were unable to acquire CRs when trained for 15 days with a novel conditioned stimulus (CS), although this CS supported acquisition in the opposite eye. These data suggest that Pavlovian eyelid conditioning is mediated by converging CS and unconditioned stimulus pathways in the cerebellar cortex and support the notion of two sites of plasticity in the cerebellum -- one site in the cerebellar cortex important for timing, and one site in the cerebellar nucleus that mediates the short-latency CRs. In addition, this work provides evidence that inhibitory input from the cerebellar cortex regulates or contributes to the induction of plasticity in the cerebellar nuclei.

479.12

TEMPORARY INACTIVATION OF THE CEREBELLUM PREVENTS THE EXTINCTION OF CONDITIONED NICTITATING MEMBRANE RESPONSES. Ramnani N*., Hardiman M.J., and Yeo C.H. Neuroscience and Behaviour Group, Department of Anatomy, University College London, London WC1E 6BT, U.K.

The classically conditioned nictitating membrane response (NMR) of the rabbit is associative learning of a simple motor response and is critically dependent upon the cerebellum. Destructive lesions of parts of the olivo-cerebellar system abolish or impair conditioned responses (CRs) (Yeo, Ann. N.Y Acad. Sci., *627*:292-304, 1991). Such lesions may impair CRs either because the cerebellum is actively engaged in learning, or because lesions interfere with the execution of learned responses. Reversible lesions can be used to distinguish between these possibilities. One such study shows that acquisition is impaired during muscimol inactivation of the interpositus nucleus of the cerebellum, but subjects acquire CRs normally after the blockade (Krupa et al. Science *260*:989-991, 1993). The cerebellum is therefore actively engaged in the acquisition of CRs. The same circuitry may also be essentially involved when there is extinction of NMR conditioning. Here we have examined the effects of muscimol blockade of the cerebellum during the extinction of previously acquired conditioning. Rabbits were implanted with cannula guides near the right interpositus nucleus of the cerebellum, and were given 4, daily sessions of NMR delay conditioning (tone CS, periocular electrical stimulation US; 100 trials per session, 90 CS-US, 10 CS alone). 4 daily sessions of extinction training (100 trials, CS alone) under muscimol then began. 1 µl of muscimol (1.54 nmoles) was infused over 1 min, 1 hour before each session. 4 more extinction sessions were then given without muscimol. Expression of CRs at the beginning of the first of these sessions was at the same maximal level as trained, unextinguished animals. These extinguished at the same rate as CRs of controls extinguishing for the first time. **Muscimol blockade of the interpositus nucleus prevented the extinction of conditioned NMRs, so normal cerebellar function is essential for extinction. The neural circuitry underlying acquisition and extinction of conditioned NMRs may be the same.**

479.13

REVERSIBLE INACTIVATION OF THE CEREBELLUM WITH MUSCIMOL PREVENTS THE ACQUISITION AND EXTINCTION OF CONDITIONED NICTITATING MEMBRANE RESPONSES IN RABBITS.

Hardiman M.J., Ramnani N., Gilbert P.F.C.*, Yeo C.H. Department of Anatomy, University College London, London WC1E 6BT, U.K.

We used muscimol to inactivate the cerebellum and test its involvement in acquisition and extinction of conditioned nictitating membrane responses. In 4 rabbits we implanted a guide cannula close to the interpositus nucleus of the cerebellum (Muscimol Group). In the acquisition phase of training each rabbit was injected with muscimol (1.54nmol in 1µl of PBS, pH7.4) 1 hour before delay conditioning. This procedure was repeated for 4 daily sessions of 100 trials (90 CS-US paired; 10 CS alone; CS - tone; US - periocular electrical stimulation). There were very few conditioned responses (CRs) in these training sessions. After 3 days subjects were then trained for 4 more sessions without injections of muscimol. There were no CRs on initial trials of the first session of retraining but all subjects produced CRs by the end of this session. Three of these subjects then underwent 4 daily sessions (100 trials, CS alone) of extinction training with muscimol and no CRs were produced. After 3 days there were 4 daily sessions of extinction training without muscimol. On the first of these sessions, all subjects immediately produced high levels of CRs. These responses then extinguished within and between sessions with characteristic beginning-of-session spontaneous recovery. Control subjects either remained in their home cages (Naive Group, n=4) or were placed in the experimental chambers but received no stimuli (Sit Group, n=4) during sessions equivalent to those of the Muscimol Group when the muscimol was delivered. Muscimol subjects neither acquired nor extinguished under muscimol blockade, but when trained after the blockade had lifted then did so at rates similar to those of the appropriate controls. We conclude that circuitry essential for both the acquisition and extinction of this simple form of motor learning includes the cerebellum.

479.14

PAIRING-SPECIFIC DEPRESSION OF PURKINJE CELL EPSPS RESULTS FROM A CLASSICAL CONDITIONING PROCEDURE IN THE RABBIT CEREBELLAR SLICE. B. G. Schreurs*, M. M. Oh and D. L. Alkon. Lab. of Adaptive Systems, NINDS, NIH, Bethesda, MD 20892.

We simulated classical conditioning in a rabbit cerebellar slice by stimulating parallel fiber inputs to Purkinje cells using a brief, high frequency train of eight constant-current pulses 80 ms before climbing fiber inputs were stimulated using a brief, lower frequency train of three constant-current pulses. Intradendritically recorded Purkinje cell excitatory post synaptic potentials (EPSPs) underwent a long-term reduction in amplitude following paired stimulation (30%, n=10, $p < .01$) but not following unpaired (n=11) or parallel fiber alone stimulation (n=10). We call this phenomenon pairing-specific depression (PSD). Facilitation of a second EPSP occurred both before and after paired stimulation, suggesting the locus of PSD was not presynaptic. Depression of a depolarizing response to focal application of glutamate following pairings added support to a postsynaptic locus for the PSD effect. Pairing-specific depression occurred both in the presence and absence of bicuculline. The potentiation of EPSPs by aniracetam (40%) was abolished after pairings. Removal of aniracetam from the bath 20 min following pairings revealed normal levels of PSD suggesting that the effect did not result from direct desensitization of AMPA receptors. Incubation in the protein kinase inhibitor H7 potentiated EPSPs (9%) but these values returned to baseline following pairings. The net reduction in EPSP amplitude of less than 10% following pairings suggested that H7 blocked PSD and that PSD may be mediated by protein kinases. Unlike LTD, PSD may be obtained using the sequencing and timing of stimulation appropriate for classical conditioning in the intact animal.

479.15

Effect of the Red Nucleus Lesion on the Hippocampal Multiple Unit Activity. J.W. Ryou*, S.Y. Cho, and H.T. Kim, Dept. of Psychology, Korea Univ., Seoul, 136-701, Korea.

Sears et al.(Behav. Neurosci.104.No.5.1990) showed that learning-related multiple unit activity (MUA) developing in the hippocampus during the classical conditioning of nictitating membrane response depends on the intactness of the cerebellar interpositus nucleus. The cerebellar interpositus nucleus is known to be an essential neural structure for this conditioning, and a place representing time-amplitude neural model of the conditioned response(CR). Lesioning the interpositus nucleus, therefore, obstructs the learning itself and as a result, the hippocampal MUA can not develop. In case the red nucleus receiving the projection from the interpositus nucleus is lesioned, the learning-related MUA in the interpositus nucleus can be recorded(Chapman et al., Brain Res,537.1990), which is possibly interpreted as the learning is established but the performance of CR is blocked. The present study was conducted to determine if the learning-related MUA could be developed in the hippocampus after lesioning the red nucleus in the output pathway of the interpositus nucleus. Rabbits were given unilateral electrolytic lesions of the red nucleus before the conditioning sessions (CS: 550msec tone, US: 100msec air puff, ISI: 450msec). They just showed 8% or less CRs by the end of the 8th session, whereas the control animals showed on an average 80% or more CRs by the 4th session($F(1,10)=187.84$, $p<0.001$). The control group showed typical increases of hippocampal MUA in the process of learning, while the group with the red nucleus lesion didnot showed the increase of hippocampal MUA in the CS period ($F(1,10) = 10.67$, $p<0.05$). These results suggest that the learning-related input from the interpositus nucleus via the red nucleus may be critical for the normal MUA development in the hippocampus.

479.16

Kainic acid Lesions of the Cerebellar Interpositus Nucleus Abolished the Conditioned Neural Activity in the Hippocampus. H.T. Kim*, K.J. Lee, and J.W. Ryou. Dept. of Psychology, Korea Univ., Seoul, 136-701, Korea.

The cerebellum and the hippocampus are known to be neural structures involved in the classical conditioning of nictitating membrane reponse of the rabbit. The neural activity related to learning, the conditioned multiple unit activity(MUA), can be observed in both the structures. It could be possible that the learning-related neural activity is established simultaneously in the interpositus nucleus and in the hippocampus, or hierarchically. The rabbits used in this study were chronically implanted with a 23 gauge of outer cannula in the region of the cerebellar interpositus nucleus and with a recording electrode(insulated insect pin #00) in the CA3 region of the hippocampus. The standard delay conditioning(CS: 400msec tone, US: 100msec air puff, ISI: 300msec) was performed. At the time when the learning rate reached 80%, kainic acid(0.3µL, 2mg/ml, Sigma) was injected into the interpositus nucleus of the experimental group while saline(0.9%, 0.3µL) the control group. And then training was resumed on the rabbit. Consequently, the injection of kainic acid abolished not only the conditioned behavioral response($F(1,11)=281.55$, $p<0.01$) but also the conditioned increase in neural activity of hippocampus($F(1,11)=17.85$, $p<0.01$), which had developed before the lesion. These results are consistent with those of Clark et al. study(Brain Res. 291. 1984), and suggest that the learning-related MUA in the hippocampus might depend on the input from the cerebellum.

479.17

TISSUE PLASMINOGEN ACTIVATOR INDUCTION DURING CEREBELLAR MOTOR LEARNING. N.W.Seeds*, B.L.Williams and P.C.Bickford Neuroscience Prog., MSTP, Depts. Biochem/B/Genetics and Pharmacology, Univ. Colorado H.S.C., Denver, CO 80262.

The cerebellar cortex is implicated in the learning of complex motor skills. Motor learning is thought to involve the modulation of mossy fiber input to cerebellar Purkinje neurons via climbing fiber activity. Studies suggest that this modulation may require synaptic remodeling of Purkinje cell inputs. The possibility that an extracellular serine protease, tissue plasminogen activator (tPA) which plays an important role in remodeling of various non-neural tissues and is associated with developing and regenerating neurons, may be activated during motor learning was explored. In situ hybridization shows that tPA mRNA expression is dramatically increased in Purkinje neurons within an hour of training rats for a complex motor task. By 4 hrs. after training tPA mRNA appears to spread into the molecular layer; however, this induction is transient and tPA mRNA levels are quite low by 24 hrs. after the learning task. Neither untrained animals nor exercised rats showed a specific increase in tPA mRNA expression. Similarly, antibody to tPA visualized the induction of tPA protein associated with cerebellar Purkinje cells. This induction of tPA mRNA and protein during motor learning may play a role in activity dependent synaptic plasticity and remodeling of Purkinje cell inputs. (Supported in part by grants from NIH, NSF, and NIMH)

479.18

CONDITIONED ENHANCEMENT OF THE EARLY COMPONENT OF THE RAT EYEBLINK REFLEX. Y.-W. Lam*, A. Wong, T. Canli and T. H. Brown. Depts. of Psychology and Cellular and Molecular Physiology, Yale University, New Haven, CT. 06520.

The orbicularis oculi (oo) muscle contributes the main force for the closing phase during an eyeblink in the rat. The EMG response in the oo muscle during an eyeblink consists of early (R1) and late (R2) components. The circuitry underlying the rat R1 response is relatively simple, consisting of only two central synapses. Here we explored the effects of fear conditioning on the rat R1 response.

Forty-two male Sprague-Dawley rats were randomly divided into three groups: a CS-US paired group, where subjects were trained with paired presentations of a tone CS and a footshock US; a CS-US unpaired group, in which subjects received the same number of CS and US presentations but they were explicitly unpaired; and a CS-alone group, where subjects were preexposed to the same number of CS presentations but had no experience with the US. In the subsequent testing session, eyeblinks were elicited by directly stimulating the 5th nerve. The magnitude of the integral of the full-wave-rectified R1 response was compared in the presence and in the absence of the CS.

A one-way ANOVA revealed a significant group effect on the percentage enhancement, $F(2,39) = 5.93$, $p < 0.01$. Planned contrasts showed significant CS-produced R1 enhancement in the CS-US paired group (61.1%) and the CS-alone group (16.7%) but not in the CS-US unpaired group (0.7%). Post hoc comparisons revealed significantly greater enhancement in the CS-US paired group than in the other two groups (Tukey HSD, $p<0.05$), indicating a pairing-specific component of CS-produced R1 modulation in rat. Supported by NIH.

479.19

Effects of Amygdala Lesions on Second-Order Pavlovian Conditioning. T. Hatfield*[1], J. S. Han[1], P. C. Holland[2] and M. Gallagher[1], [1]Department of Psychology, University of North Carolina, Chapel Hill, NC 27599, [2]Department of Psychology, Duke University, Durham, NC 27706.

Second-order conditioning is often used to measure whether a conditioned stimulus (CS) that had previously been paired with a unconditioned stimulus (US) can subsequently serve as a reinforcer. One function commonly attributed to the amygdala complex is learning the biological significance of events through which conditioned stimuli (CSs) acquire rewarding or aversive properties. As a further test of this idea, the present study examined the effects of neurotoxic amygdala lesions on appetitive second-order conditioning. In the second-order conditioning behavioral paradigm, following light-food pairings, rats are given tone-light pairings in the absence of food. Rats that received large neurotoxic lesions of the amygdala failed to acquire second-order conditioning. In a second experiment, rats with small neurotoxic lesions confined to the amygdala central nucleus acquired second-order conditioning indistinguishable from intact control rats. Currently, the effects of neurotoxic lesions confined to the amygdala basolateral complex are being examined. The results of this experiment in combination with the other studies should help to define whether a specific subsystem in the amygdala is critical for the acquisition of a CS's reinforcing properties in appetitive Pavlovian conditioning. Further, a differential behavioral effect of ABL and CN damage could support the idea that such reinforcing properties of a CS are mediated through amygdalo-ventral-striatal circuitry. Supported by an award from the Human Frontier Science Research Program and a Research Scientist Award (KO5-MH01149) to MG.

479.20

CONDITIONED ORIENTING BEHAVIOR IS MEDIATED BY THE AMYGDALO-NIGROSTRIATAL PATHWAY. J. S. Han*[1], R. W. McMahan[1], P. C. Holland[2] M. Gallagher[1,]1 Dept. of Psychology, Univ. of North Carolina, Chapel Hill, NC 27599, [2] Dept. of Psychology, Duke University, Durham, NC 27706.

Rats with neurotoxic lesions of the amygdala central nucleus (CN) fail to aquire conditioned orienting responses that are normally learned with conditioned stimulus (CS) -unconditioned stimulus (US) pairings (Gallagher et al. J. Neurosci. 10: 1906, 1990). The CN projects ipsilaterally to an area of the substantia nigra (SN) that innervates dorsolateral striatum. Based on evidence that orienting to sensory cues depends on SN input to dorsolateral striatum, a crossed-lesion model was employed to determine whether this pathway is used to express conditioned orienting responses. Unconditioned orienting to a light (that was later used as the CS) was first tested. Rats were then conditioned for 10 days with 5 trials per day consisting of a 10-sec light (CS) followed by 2 food pellets (US). There were no differences between the control group and the unilateral lesion groups - either an ibotenic acid lesion of CN or unilateral 6-OHDA lesion of the striatum - in the occurrence or habituation of unconditioned orienting (rear to light), in acquisition of the conditioned orienting response, or learning to approach the food cup during the CS. Relative to those groups, rats with the crossed lesion consisting of a unilateral CN lesion and contralateral striatal lesion showed deficits only in the acquisition of the conditioned orienting response. These results support our proposal that conditioned orienting behavior is mediated by the amygdalo-nigrostriatal pathway. Supported by an award from the Human Frontier Science Research Program and a Research Scientist Award (KO5-MH01149) to MG.

479.21

HYPEREXCITABILITY: EXAGGERATED FEAR-POTENTIATED STARTLE PRODUCED BY PARTIAL AMYGDALA KINDLING. J.B. Rosen*[1], E. Hamerman[2], M. Sitcoske[1], J.R. Glowa[3] and J. Schulkin[2]. [1]Biol. Psychiat. and [2]Clin. Neuroendocrinol. Brs., NIMH, and [3]Lab. Med. Chem., NIDDK, Bethesda, MD 20892.

The present study asked whether a hyperexcitable amygdala would have an effect on the expression of conditioned fear-potentiated startle. Rats were trained to be fearful of a light by pairing light and footshock together 5 times. During the following two days the amygdala or hippocampus was made hyperexcitable in half of the rats. Hyperexcitability was produced by delivering electrical stimulation bilaterally to the amygdala or hippocampus on two consecutive days which induced electrographic afterdischarges but no behavioral seizures (i.e., partial kindling). The following day, rats were tested for fear-potentiated startle. Amygdala kindled rats displayed more than twice the startle amplitude in the presence of the light compared to sham kindled rats, indicating exaggerated fear-potentiated startle in the amygdala kindled rats. Enhanced startle in the presence of the light was also found in the hippocampus kindled rats, but was no greater than the startle amplitude of sham kindled rats. Expression of c-fos mRNA, which was used as a marker for neuronal activation, was induced throughout the limbic and neocortices by partial amygdala kindling. In contrast, partial hippocampus kindling induced c-fos mRNA expression in the dentate gyrus, CA1, CA2 and CA3 only. These experiments demonstrate that following kindled-induced hyperexcitability of the amygdala and limbic cortices, rats exhibit exaggerated conditioned fear-potentiated startle. Hyperexcitability of amygdala fear circuits may be important for the evolution of fear states to pathological anxiety states.

479.22

THE ROLES OF THE AMYGDALA AND BED NUCLEUS OF THE STRIA TERMINALIS (BNST) IN THE ACQUISITION OF FEAR POTENTIATED STARTLE USING BOTH EXPLICIT AND CONTEXTUAL CUES. M. Davis*, J. C. Gewirtz, K. A. McNish, and M. Kim. Depts. of Psychiatry and Psychology, Yale Univ. Sch. of Med., New Haven, CT 06508.

The effects of lesions of the central nucleus of the amygdala and the BNST on the acquisition of fear-potentiated startle to both explicit and contextual cues were examined in two experiments. Over repeated experimental sessions rats were given two presentations of a 3.7-s light CS which terminated with a 0.5-s, 0.6-mA footshock US. To assess fear-potentiated startle, each session also included presentations of a 50-ms, 95-dB startle-eliciting stimulus alone, and of the same stimulus in the presence of the visual CS. We previously reported that bilateral electrolytic lesions of the central nucleus of the amygdala blocked acquisition of fear-potentiated startle to the visual CS (explicit cue conditioning, Kim and Davis, 1993). In Experiment 1, these same lesions also blocked acquisition of fear-potentiated startle to the startle-eliciting stimulus alone (context conditioning). In Experiment 2, bilateral electrolytic lesions of the BNST had no effect on the acquisition of fear-potentiated startle to the visual CS. However, the same lesions completely blocked the acquisition of fear-potentiated startle to the experimental context. These data suggest that the acquisition of fear to an explicit cue is dependent upon processes involving the amygdala, whereas the acquisition of fear to contextual cues is dependent upon both the amygdala and the BNST.

Interestingly, the BNST receives extensive projections from the hippocampus, and projects to similar hypothalamic and brainstem targets as the amygdala. The BNST may therefore be critical for the elicitation of autonomic and behavioral responses associated with contextual fear conditioning, just as the amygdala is critical for the elicitation of similar responses associated with explicit cue conditioning.

479.23

RELEASE OF CALCITONIN GENE-RELATED PEPTIDE IN THE LATERAL AMYGDALA MAY BE IMPORTANT FOR THE EXPRESSION OF AUDITORY FEAR CONDITIONING L.H. Poore* & F.J. Helmstetter

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Previous work has shown that the amygdala is critical for the acquisition and expression of Pavlovian fear conditioning in the rat. When auditory signals are used to predict the occurrence of a UCS this information reaches the lateral nucleus of the amygdala (LA) via the auditory cortex and via a direct projection to the amygdala from the medial geniculate nucleus (MG). This projection from MG to LA contains calcitonin gene-related peptide (CGRP) and we have recently shown that injections of CGRP into the LA of rats will produce behavioral effects which closely resemble normal fear responses. We have also shown that application of CGRP or the putative CGRP receptor antagonist $hCGRP_{8-37}$ to the LA prior to a training session in which auditory stimuli are paired with shock will affect the learning of fear responses measured 24 hr later. In the present study we investigated the effects of blocking CGRP receptors in the LA during the expression of fear-related CRs. Adult male rats were implanted with bilateral cannulae in the LA. During training rats received an injection of saline in LA and were exposed to 4 paired presentations of white noise (72dB/10sec) and foot shock (2mA/1sec) in a distinctive observation chamber. One the next day separate groups of rats were pretreated with $hCGRP_{8-37}$ (0.63nM, 0.63mM) or saline and observed for freezing behavior. One half of the animals were returned to the observation chamber in which training was conducted while the remaining rats were given a single white noise presentation in a distinctly different chamber. Blocking CGRP receptors selectively attenuated fear to the auditory CS. Since lesions of the lateral parabrachial nucleus (which provides the only other significant CGRP input to the amygdala) do not produce similar results these studies suggest that CGRP is released in LA by MG neurons during the expression of fear.

480.1

LTP IN RECURRENT NETWORKS GENERATES SEQUENCE LEARNING AND PREDICTION Kenneth I. Blum*[1] and L. F. Abbott[2]. [1]Dept. Brain and Cognitive Sciences, MIT, Cambridge, MA 02139, and [2]Center for Complex Systems, Brandeis Univ., Waltham, MA 02254.

Neuronal ensembles with recurrent feedback connections can store and recall sequences of population-coded inputs if NMDA-mediated LTP is active. In our model the ensemble activity of a network of neurons initially encodes the current value of a sensory input. We then train the network with repeated presentations of a given sequence of inputs. We assume that synapses between encoding neurons are modified during training by NMDA-mediated LTP with properties similar to those seen in slice experiments. After training, the population firing pattern of the network shifts so that the ensemble response to sensory input predicts future inputs in the training sequence. This effect arises automatically from the temporal asymmetry of LTP induction.

If the output of the network we study is fed to a motor system, the predictive power of the network can be used to generate motor sequences. The motion is executed by a feedback loop: the motor system chases the target provided by the coding network which responds to the arm position generated by motor activity.

In the conventional analysis of recurrent networks, feedback dominates inputs and a briefly presented input evolves quickly to a fixed-point. In contrast, the 'response-driven' memory we propose involves weaker recurrent feedback, so input about the current state of the system dominates network activity. This means that motor sequences can be generated at any desired speed and that the resulting movement is stable to perturbations.

480.2

WHAT THINKING OF NEURONS AS BINARY UNITS MISSES OUT A. Treves*[1], S. Panzeri[1], E.T. Rolls[2], K.M. Gothard[3] and W.E. Skaggs[3]. [1]SISSA, Cognitive Neuroscience, via Beirut 2-4, 34013 Trieste, Italy [2]Dept. of Experimental Psychology, Univ. of Oxford, OX1 3UD, UK [3]Neural Systems, Memory and Aging, Arizona Res. Labs, Tucson, USA.

The all-or-none nature of the action potential has made attractive the notion that CNS neurons may be modelled, to a first approximation, as binary elements, that at any moment in time are either firing or quiescent. Connectionists have related these two states to the truth or falsehood of underlying predicates, computer scientists have considered neuronal circuits in the context of digital processing, and statistical physicists have imported mathematical techniques developed for spin systems. The complex set of time scales associated with neuronal processing obviously makes neuronal dynamics much richer than could be described in binary terms. One consequence is that, even when the same computational mechanisms may operate for both binary units and real neurons, they operate with very different efficiency. We have quantified the effect in some cases. a) The amount of information conveyed by the firing rate of single cells in the primate temporal cortex about visual stimuli is compared with what could be conveyed by ideal binary units, and with what would be conveyed by binary units that responded in the same way as real neurons. b) The information conveyed by populations of rat hippocampal cells about the rat location is measured for different time windows, from very short ones in which neurons can be regarded as effectively binary, through intermediate ones related to the theta rythm, to windows as long as compatible with rat motion. c) The information that may be stored and retrieved with associative memories made of graded-response units is compared across different structures of memory representations, from binary to continuously graded.

These quantitative analyses yield insights on the differences, in general, between binary and neuronal processing.

480.3

PATTERN RECOGNITION BY A CIRCUIT MODEL OF DENDRITIC SPINES. K.T. Blackwell*[1], T.P. Vogl[1], D.L. Alkon[2]. [1]Environmental Research Institute of Michigan, Arlington, VA 22209; [2]Laboratory of Adaptive Systems, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD 20892

The properties of biological pattern matching were investigated in a circuit model of a piece of dendritic membrane with a number (1-10) of dendritic spines. Each spine head is modeled as a synaptic conductance in parallel with a calcium dependent potassium conductance. Each spine neck is modeled as an axial resistor and all the spines are attached to a common dendritic membrane compartment. In Hermissenda and in hippocampal CA1 cells, classical conditioning causes a decrease in conductance of the calcium dependent potassium channel (Alkon, Science, 1984; Sanchez-Andres and Alkon, J. Neurophysiology, 1991.) In the model, the effect of classical conditioning is represented by adaptation of the calcium dependent potassium channel to the intra-spine calcium concentration consequent to repeated stimulus presentations. Computer simulations show that the model's dendritic membrane depolarization is greater in response to a previously learned pattern of synaptic inputs than in response to a novel pattern of synaptic inputs. These simulations, in combination with analysis of the circuit equations, reveal that when the synaptic input pattern is similar to the learned pattern of synaptic inputs, total dendritic depolarization is a linear combination of dendritic depolarization contributed by individual spines. When as few as one synaptic input differs markedly from the learned value, dendritic depolarization is a non-linear combination of individual spine depolarizations. These properties are captured in a simplified set of equations which satisfies the computational requirements for a practical similarity measure for artificial neural networks.

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480.4

RECOGNITION AND RECALL IN DISTRIBUTED MEMORY MODELS. M. J. Kahana, & J. Lisman*. Volen National Center for Complex Systems, Brandeis University, Waltham, MA 02254.

Distributed memory models have been able to provide a unified account of data on recognition and recall as well as numerous other effects in the human memory literature. These models are now challenged with accounting for data on the contingency relations between different memory tasks. Data from successive testing experiments yield moderate correlations (~ 0.5) when item recognition is followed by cued recall. In contrast, associative recognition followed by cued recall yield very high correlations (~ 0.9). A theoretical analysis demonstrated that models which assume separate representations for item-specific and relational information (e.g., Metcalfe, 1989; Murdock, 1989) predict independence between item recognition and cued recall and perfect dependence between associative recognition and cued recall. These models generally fail to consider the effects of encoding variability in storage and retrieval processes. Once included, these sources of variability have opposing effects on the correlations. Encoding variability increases the correlation whereas retrieval variability decreases it. Using a mixed-list / pure-list design we obtained experimental evidence supporting the claim that encoding variability actually increases the observed correlation between item recognition and cued recall.

480.5

MASSIVELY PARALLEL COMPUTER SIMULATIONS OF LARGE-SCALE NEURAL NETWORKS: APPLICATION TO GRANULE-GOLGI CELL DYNAMICS DURING EYELID CONDITIONING. G.T. Kenyon* and M.D. Mauk Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77225.

We have implemented a general purpose neural simulator on a massively parallel computer (CM-5). Since many biological neural networks consist of a large number of neurons drawn from a limited number of types, massively parallel computers represent a natural platform for such systems. A considerable increase in computational performance is obtained since the massively parallel architecture allows the internal dynamical variables of all neurons of a given type to be updated simultaneously. Because synaptic interactions require general communication between processors, this step might be expected to be rate limiting. However, when multiple synapses converge on the same target, the associated impulses are summed linearly, which effectively addresses the problem of collisions. Membrane potentials are updated using a parallel version of the Hines algorithm for solving the multi-compartment diffusion equations and the threshold dynamics is modeled as an integrate-and-fire process. The simulator allows for the arbitrary specification of conductance kinetics, dendritic morphology, and connectivity patterns for each neuron type. The simulator additionally allows the user to configure arbitrary patterns of sensory input corresponding to different experimental protocols. An analysis package, fully optimized for the massively parallel architecture, allows the results of the simulation to be displayed using a variety of electrophysiological measures, including raster plots, intracellular records, and massed post-stimulus time and cross-correlation histograms. The simulator is currently being used to study the dynamics of granule-Golgi cell interactions in the cerebellar cortex during simulated eyelid conditioning. Preliminary results have better characterized the physiological factors that influence the response of the granule cell population vector to changes in the mossy fiber input elicited by the CS.

480.6

A MODEL NETWORK THAT PERFORMS BOTH SHORT-TERM AND LONG-TERM MEMORY. O. Jensen, M.A.P. Idiart* and J.E. Lisman. Center for Complex Systems, Brandeis University, Waltham MA 02254-9110

In a previous model we showed that the continued firing that maintains short-term memory might be due to a known intrinsic cell property, the depolarizing afterpotential that is triggered by action potentials. We showed that when neurons with this property are embedded in an oscillatory network, the network could maintain 7 different memories, each in a different ("40 Hz") subcycle of a theta-frequency oscillation (*Science* **267**:1512, 1995). Here we extend this model by incorporating excitatory recurrent collaterals among neurons (as in Hopfield nets), with each synapse modified according to a Hebb rule. Our simulations show that this hybrid model preserves both the short-term memory capability of our original model and the long-term memory capability typical of Hopfield nets. Moreover, the repetition of firing patterns caused by the short-term mechanism causes a natural transition of information into the slowly modifiable synapses that encode long-term memory. Interestingly, the build up of long-term memory provides an error correction mechanism for the firing associated with short-term memory.

480.7

MAXIMAL INFORMATION TRANSFER IN SPIKING NEURONS. B.A. Pearlmutter*and L.C. Parra. Siemens Corporate Research, 755 College Road East, Princeton, NJ 08540.

It is known that an exponential distribution of inter-spike intervals maximizes the rate of information transmitted by a spiking neuron. We first show that this holds even in the presence of a refractory period Δ, giving a "front porch exponential" density

$$p(s) = \begin{cases} s<\Delta & 0 \\ s\geq\Delta & (1/\tau)\exp-(s-\Delta)/\tau \end{cases}$$

We then derive a relationship among the time constant τ, the refractory period Δ, and the standard deviation of the noise in spike timing σ, assuming $\sigma \ll \Delta$,

$$\frac{\Delta}{\sigma} = \frac{\tau}{\sigma}\left(\log\frac{\tau}{\sigma} - \frac{1}{2}\log(2\pi e)\right)$$

If neurons truly maximize only their coding efficiency, the predicted relationship between the optimal time constant τ and the physiological characteristics of the neuron should be observable. Somewhat surprisingly, if the neuron maximizes its bit rate under a limited energy budget, the ISI distribution retains its exponential form. An extra parameter α that trades off energy against bits is introduced,

$$\frac{\Delta}{\tau} = \log\frac{\tau}{\sigma} - \frac{1}{2}\log(2\pi e) - \alpha\left(\frac{\tau}{\sigma} + \frac{\Delta}{\sigma}\right)^2$$

We are currently searching for a system sufficiently controlled and biophysically characterized to allow these relations to be tested.

480.8

A COMPUTATIONAL MODEL OF HIPPOCAMPAL REGION CA3 FOR FLEXIBLE LEARNING OF TEMPORAL SPATIAL ASSOCIATIONS. W. B Levy*, X. B. Wu, & R. A. Baxter Univ. of Virginia Health Sciences Ctr., Dept. of Neurosurgery, Charlottesville, VA 22908.

We have been studying an extremely simple and biologically motivated model of hippocampal region CA3. This model is distinguished by its sparse connectivity and local associative modification. This model is capable of a variety of forms of sequence learning which would be useful for solving problems in cognitive mapping. The problems solved by the network include sequence completion, goal finding without search, finding shortcuts, and piecing together separately learned subsequences. In finding goals without search, this model essentially imagines the path to the goal without the step-by-step changes of sensory inputs typical of other approaches that model an animal walking through an environment. The most notable aspect of neuronal firing in this network is the formation of a place cell analog. That is, different cells learn to recognize specific parts of the sequenced patterns that correspond to specific places. The cell firing patterns form spontaneously based on local associative modification. The firing of these cells is interleaved with one another such that the ability to predict sequences or to find goals exceeds the firing time of individual place cells. Thus, the network is able to solve the prediction problems mentioned above by virtue of one set of place cells passing off activity to another set of place cells.

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480.9

INCORPORATING SYNAPTIC FAILURES INTO A CA3 NEURAL NETWORK. D A August*and W B Levy. Dept. of Neurosurgery, Univ. of Virginia, Charlottesville, VA 22908.

Some brain areas may operate with a high rate of synaptic failure. We have investigated the effect of synaptic transmission failures on the sequence-learning and sequence-prediction ability of a simplified network model of hippocampal area CA3. The network consisted of input (EC) and output (CA3) layers, as well as feedforward and feedback inhibitory units. The CA3 layer had a sparse (10%) recurrent connectivity. During learning, a sequence of slowly-shifting input patterns was presented to the EC layer. After learning, the network was tested with the first pattern of this sequence, and then allowed to relax.

We performed two simulations to study the effects of quantal noise on this network. In the first experiment, synaptic weights were modified by a Hebbian rule, and transmission failures occurred stochastically with a fixed probability. Synaptic failure probability (at recurrent CA3-CA3 synapses, and CA3-inhibitory synapses) were varied between $p = 0.125$ and $p = 0.5$. In the second experiment, the synaptic weights were fixed, and the probability of reliable synaptic transmission was modified with a Hebbian rule. Two properties were observed in both experiments. First, the noisy networks required less inhibition to properly recall a sequence than the noise-free networks. Second, for certain levels of recurrent inhibition (less than the amount needed for recall), the network performed sequence prediction. That is, network replayed the sequence faster than it was learned. Thus, the presence of quantal failures modulated the effect of inhibition. Supported by NIH grants MH10702 to DAA and MH00622 to WBL.

480.10

THE TRANSIENT 40-Hz RESPONSE AND MISMATCH NEGATIVITY AS INDICES OF ATTENTIONAL PROCESSES. H. Tiitinen*, Cognitive Psychophysiology Research Unit, Dept. Psychology, FIN-00014 Univ. Helsinki, Finland.

The results of five recent EEG and MEG studies on the neurophysiological basis of selective attention and auditory sensory memory are reviewed. The results demonstrate that the transient 40-Hz response is enhanced by selective attention, attenuated in the course of long-term stimulation, and is not affected by qualitative changes in auditory stimuli. Therefore, the 40-Hz response seems to be closely related to selective and sustained attention whereas it can be dissociated from memory mechanisms. Changes in the auditory environment are registered by a pre-attentive sensory memory, as indexed by mismatch negativity (MMN). By this time, the 40-Hz oscillations have terminated or asynchronized. According to the present results, sensory memory for tone frequency is located in tonotopically organized auditory cortex and, more importantly, this pre-attentive memory mechanism governs attentive detection of changes in the auditory environment.

480.11

A DYNAMICAL NEURAL NETWORK MODEL OF MISMATCH NEGATIVITY AND THE UNDERLYING MEMORY TRACE. P. May[1], J. G. Taylor[1], H. Tiitinen[2], I. Winkler[2]*, and R. Näätänen[2]. (1) Dept. Mathematics, King's College London, WC2R 2LS, UK. (2) Cognitive Psychophysiology Research Unit, Dept. Psychology, FIN-00014 Univ. Helsinki, Finland.

Mismatch negativity (MMN), a component of the event-related potential elicited by changes in the auditory environment and indexing auditory sensory memory, was replicated for frequency and temporal changes in stimulation in a neural network model describing columnar cortical structure. Analyses of the non-linear dynamics of the system revealed that MMN can be explained as a transient state of the system due to a switch in the location of the attractor associated with the input. An experimentally measurable quantity is suggested for the strength of the underlying memory trace.

480.12

CORRELATION-BASED INVERTIBLE ENCODING IN A MODEL OF HIPPOCAMPAL MEMORY. N.H. Goddard, J.L. McClelland*, R.C. O'Reilly. Dept. of Psychology, Carnegie Mellon Univ., and Center for the Neural Basis of Cognition, Carnegie Mellon Univ. and Univ. of Pittsburgh, Pittsburgh, PA 15213.

The extended temporal role of the hippocampal formation in memory suggests it forms stable traces of cortical activity lasting up to several years. We explore the role of EC-CA1-EC pathways in a model designed to meet this severe constraint [1]. In the model, a new experience is encoded twice, as independent orthogonalized sparse patterns in CA3 and in CA1. The sparsity and orthogonalization minimize interference between memories. Synaptic plasticity stores the experience in the EC-CA3 pathway and the recurrent CA3 collaterals. The bi-directional pathways between EC and CA1 implement a fixed encoder/decoder: The EC-CA1 connections map an EC input to a pattern in CA1 that returns the corresponding EC pattern via the CA1-EC connections. The CA1 and CA3 patterns are associated via plasticity in the Schaeffer collaterals. During retrieval, a partial cue arising in EC retrieves the stored CA3 pattern. The CA3 pattern then retrieves the associated CA1 pattern, which in turn completes the EC pattern.

Our simulations investigate the exploitation of correlations in the recovery of EC inputs from the CA1 patterns they produce. With symmetric, random EC-CA1 connections the model recovered only 67% of the EC cells active in the input pattern, using biologically realistic parameters, but performance improved dramatically when correlated activity between EC neurons was used in setting a subset of the connection weights. Recovery of the EC input increased to 96% for the optimal number of correlated connections. The results support the view that the EC-CA1-EC pathways could implement the proposed invertible encoding operation.

[1] J.L. McClelland *et al.*, *Neuroscience Abstracts*, 18(2):1216, 1992.

480.13

MEAN-FIELD APPROACH TO MODELING INTERACTING NEURONS J. C. Lin and D. M Durand* Department of Biomedical Engineering, Applied Neural Control Laboratory, Case Western Reserve University, Cleveland, OH 44106

A mean-field theory is developed to describe the temporal behavior of interacting neurons. A 2-dimensional iterative map is obtained to estimate the membrane potentials of both excitatory and inhibitory neurons in a large neural network. This mapping function is derived using a mean-field approach that represents the average behavior of excitatory and inhibitory neurons. The threshold for excitation, the connectivity number, and the synaptic conductance are random variables with mean values and known distributions. By averaging over all the cells, the behavior of the network is captured by two nonlinear equations which give the membrane potentials of excitatory and inhibitory cells. This simple 2-dimensional iterative model can generate potential wave forms such as resting (no action potentials), spontaneous quasi periodic and chaotic pattern of neuronal firing, and spontaneous bursting similar to epileptiform activity. These various wave forms can be generated by adjusting the values of the model parameters such as the neural connectivity, the synaptic conductance, the threshold, and their fluctuations, etc. The ability of the model to simulate neuronal activity similar to what is observed physiologically will be helpful in determining the mechanisms underlying normal and abnormal neuronal network behaviors. Supported by NSF grant # IBN 9319591.

480.14

ASSOCIATIVE PLASTICITY OF POLYSYNAPTIC INPUTS TO HIPPOCAMPAL CA1 NEURONS: IMPLICATIONS FOR TEMPORAL INFORMATION PROCESSING. D. Buonomano*, P. Hickmott, M. Merzenich. Keck Center, UCSF, San Francisco, CA 94143-0732.

Previous theoretical work (Buonomano and Merzenich, 1995) suggests that temporal information can be transformed into a spatial code as a result of the interaction between time-varying neuronal properties such as paired-pulse facilitation and slow inhibition, and local circuit properties. As an initial experimental test of this hypothesis we analyzed the plasticity of polysynaptic EPSPs (poly-EPSPs) in rat hippocampal slices. Our objectives were twofold: (1) To determine whether different subpopulations of neurons are activated by the first and second of two stimuli delivered 100 ms apart;. (2) To determine whether it is possible to obtain order-sensitive synaptic plasticity.

Poly-EPSPs, elicited by stimulation of the hilus or molecular layer of the dentate gyrus, were recorded intracellularly in CA1 neurons. Paired-pulses were delivered with an interstimulus interval of 100 ms. Our hypothesis was that the first and second pulses might activate overlapping but different subpopulations of neurons in the CA3 region. Rather than record simultaneously from a large population of CA3 neurons, we recorded poly-EPSPs from a single CA1 neuron, thus effectively sampling from a population of CA3 neurons. To determine whether the first and second poly-EPSPs were from different subpopulations of CA3 neurons we used associative LTP to "tag" the synapses. By depolarizing the postsynaptic cell during either the first or second pulse we analyzed whether it was possible to produce differential facilitation, that is, a change in the EPSP2/EPSP1 ratio. After stable poly-EPSPs were established and a 5 min baseline was obtained, either the first or second pulse was paired with depolarization for 20 - 40 trials. Synaptic changes were analyzed both 5 min and 20 min after training. Our results show that on average EPSP2/EPSP1 decreased when the first pulse was paired with depolarization (Δ=0.858 and 0.873, at 5 and 20 min respectively) and increased when the second pulse was paired (Δ=1.1, 1.22). These differences were significant at both 5 and 20 min posttraining ($t[20]=3.3$ $p<0.01$; $t[17]=3.1$ $p<0.01$). These results support the hypothesis that as information flows through a neuronal layer temporal information can be converted into a spatial code. This mechanism could function as a basis for the formation of temporal combination-sensitive cells. [Supported by NIH grants NS10414 and MH10431, and HRI].

480.15

THE ROLE OF GENETIC BACKGROUND IN ASSESSING THE BEHAVIOUR OF TRANSGENIC MICE. D.P. Wolfer*, Marijana Bozicevic-Stagliar, H.-P. Lipp. Department of Anatomy, University of Zürich, CH-8057 Zürich, Switzerland

Swimming navigation learning was studied in 743 mice using a standardised schedule. The paths of all mice were recorded digitally and analysed using an identical set of parameters. Large differences in performance were found among the inbred strains and crosses that have been studied so far: C57BL/6, DBA/2, CBA, BALB/c, I/Ln, 129/J, 129/Sv, Swiss Albino. 129/Sv, 129/J and BALB/c typically are the donor strains for embryonic stem cell lines used for gene targeting. In our hands, they are among the worst navigators and show peculiarities such as passive floating and behavioural instability. I addition, they suffer from dysgenesis of the corpus callosum and the 129/J strain also carries the *pink-eye dilute* mutation.

392 mice with total or partial gene knockouts (prion protein, tissue plasminogen activator, urokinase, myelin-associated glycoprotein, nectadrin, amyloid precursor protein) were tested in our setup. Most test samples were F2 generations of crosses between 129/Sv chimeras and C57BL/6 mice. Their genetic background contained variable and unknown proportions of C57BL/6 and 129/Sv alleles. Typically, behavioural performance in both mutants and wildtype controls varied on a large scale. In comparison, mutation effects, if present, were often subtle or even lacked complete penetrance.

In order to avoid false-positive results when studying small samples of knockout mice, the anatomical and behavioural peculiarities of the background strains must be evaluated. Ideally, behavioural effects of gene targeting should be tested against a constant genetic background with decent performance, e.g. by backcrossing to C57BL/6. A pure 129/Sv or 129/J background should be avoided. Negative results must be interpreted cautiously as well. Our group is currently establishing new approaches to verify behavioural profiles obtained in the laboratory by natural selection studies in large outdoor pens. Swiss NF 31-37497, 31-42347.94.

480.16

IDEAL GENETIC BACKGROUNDS FOR TRANSGENIC ANIMALS DEVELOPED FOR ASSESSMENT OF COGNITIVE FUNCTION. E.W. Hanebuth, S.F. Logue, D. L. Rasmussen, and J.M. Wehner*. Instit. for Behav. Genetics, Dept. of Psychology, University of Colorado, Boulder, CO, 80309-0447.

Recently, many inbred mouse strains have been used to create transgenic animals. Because complex learning is regulated by polygenic systems, the genetic background of these parental strains is important in evaluating the performance of transgenics. In an effort to look at learning performance in the context of genetic backgrounds, fourteen inbred mouse strains and seven F_1 crosses have been examined in multiple behavioral tasks. The tasks used in this study include: Morris Water task, conditioned fear responses, open field activity, Y-maze activity, startle response, and pre-pulse inhibition. The Morris task and contextual conditioned fear test an animal's ability to use complex information in a learning task, while the other tasks assess sensory and motor function.

Various strains were significantly different in their performance during these tasks. To highlight a few strains, C57/6J mice were capable of learning the Morris task and contextual conditioned fear. However, 129/SvJ mice, who are not visually impaired, only learn contextual conditioned fear. A visually impaired strain, FVB, was not capable of learning either task. Understanding the cognitive capabilities of the parental strains will aid in the selection of an appropriate parental strain for the transgenic animals modelling cognitive function.(Supported by MH-16880, MH-48663).

480.17

GENETIC VARIATION IN THE SEPTOHIPPOCAMPAL CHOLINERGIC SYSTEM IN THE MOUSE: A POSSIBLE EXPLANATION FOR DIFFERENCES IN SPATIAL LEARNING AND MEMORY? [1]H. Schwegler, [1]R. Linke, [1]M. Boldyreva, and [2]H.-P. Lipp*. [1]Inst. Anatomy, University of Magdeburg, D-39120 Magdeburg, Germany, and [2]Inst. Anatomy, University of Zürich, CH-8057 Zürich, Switzerland.

Lesion studies, pharmacological and electrophysiological experiments have shown that the septohippocampal cholinergic system is involved in learning and memory processes. In contrast to these invasive approachs, we were interested in genetic variation within the septohippocampal cholinergic system and correlations between cholinergic markers with behavioral data obtained in a water maze and different radial mazes. The following cholinergic variables were studied in eight different inbred mouse strains: i) the number of cholinergic neurons as revealed by choline acetyltransferase (ChAT) histochemistry, ii) the number of ChAT-positiv, septohippocampal projection neurons (after retrograde labeling), and iii) the density of cholinergic fibers in different hippocampal subregions as revealed by acetylcholinesterase (AChE) histochemistry. For all cholinergic and behavioral variables studied significant strain differences were found. Furthermore, we also obtained significant morpho-behavioral correlations: i) the density of AChE-positive fibers in the dentate gyrus was positively correlated with activity-dependent learning in the water maze, ii) the number of septohippocampal cholinergic projection neurons was positively correlated with spatial working and reference memory capacities in an eight arm radial maze.

From these studies we conclude that the cholinergic system is essential for learning and memory processes and that strain differences in learning performance might be explained by variation within this system.
(Supported by the DFG, SFB 325)

480.18

THE DIFFERENT REACTIVITY OF C57BL/6 AND DBA/2 INBRED MICE TO SPATIAL AND NON SPATIAL CHANGE IS SUBSERVED BY A STRAIN DEPENDENT HIPPOCAMPAL AND POSTERIOR PARIETAL CORTEX INTERPLAY. M. Ammassari-Teule, E. Save, C. Rossi-Arnaud and C. Thinus-Blanc*. Ist. Psychobiology and Psychopharmacology, Rome , Italy and Lab. Neurosciences Cognitives, Marseille , France.

Inbred mice such as C57BL/6 (C57) and DBA/2 (DBA) differently perform in a variety of spatial tasks with C57 doing better at such learning. The low spatial performance of DBA could, therefore, reflect defects of acquisition or, alternatively, deficits yet in the processing of spatial information. In order to test these hypotheses, mice from both strains were placed in an open field containing five objects and their reactivity to the displacement (spatial change) or the substitution (non spatial change) of some of these objects was examined. The results show that C57 mice react to spatial change by exploring more the displaced than the non displaced objects while DBA mice do not show any reaction. Conversely the two strains strongly react to the substituted object. Dorsal hippocampal lesions abolish the reactivity to spatial change in C57 and stress the desinterest towards the five objects in DBA. Posterior parietal cortex lesions also abolish selectively the reactivity to spatial change in C57 but do not produce any effect in DBA .Taken together, these results indicate that the high spatial learning performance of C57 mice could be based upon (1) the capability of perceiving and updating spatial relationships between objects and (2) the involvement of both the hippocampus and the posterior parietal cortex in such processes. Data concerning DBA mice suggest (1) a genetic defect in encoding of spatial relationships between objects (2) a non specific involvement of the hippocampus and (3) no involvement of the posterior parietal cortex in the exploration of object configurations.
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480.19

QUANTITATIVE TRAIT LOCUS MAPPING OF CONTEXTUAL AND CUED FEAR-CONDITIONED FREEZING IN C57BL/6J X DBA/2J RECOMBINANT INBRED STRAINS OF MICE. S.C. Christensen*, E.W. Hanebuth, S.F. Logue and J.M. Wehner. Institute for Behavioral Genetics, Dept. of Psychology, and School of Pharmacy, University of Colorado, Boulder, CO, 80309-0447.

C57BL/6J (B6) and DBA/2J (D2) inbred strains have been shown to differ in conditioned fear responses with respect to contextual stimuli but not an auditory CS following a single, 0.35 mA shock (Paylor *et al.*, Behav. Neurosci., 108(4):1-8, 1994). B6 mice freeze to contextual stimuli significantly more than D2 mice. Research from this laboratory supports the hypothesis that the decreased contextual freezing observed in D2 mice is the result of functional deficiencies of the hippocampus, where integration of spatial information and other complex cues involved in learning and memory processes are believed to take place.

Putative chromosomal loci responsible for the observed differences in conditioned fear responses between B6 and D2 parental strains have been mapped by quantitative trait locus (QTL) analyses of mean contextual, altered context and auditory CS freezing scores in 22 B6XD2 recombinant inbred (RI) strains. The analyses indicate a polygenic mode of inheritance for all measures, and heritability estimates are 0.25, 0.16 and 0.24 for the three freezing scores, respectively. Estimates of the minimum number of loci involved are 2.6 for context, 2.5 for altered context, and 4.5 for the auditory cue. QTLs were detected on mouse chromosomes 1, 9, 11, 13, 14 and 17. These data may be used in future studies to screen loci on these chromosomes for candidate genes that may be implicated in different fear-conditioned responses (Supported by MH-16880, AA-00141 and MH-48663).

480.20

LEARNED HELPLESSNESS IN SHUTTLEBOX AVOIDANCE LEARNING IN AUTOIMMUNE BXSB MICE? L. A. Hyde and V. H. Denenberg* Biobehavioral Sciences Graduate Degree Program, University of Connecticut, Storrs, CT 06269.

Autoimmune mice do not learn shuttlebox avoidance well. Due to the aversive nature of the avoidance task, it is always the last test administered in our battery of behavioral tests. We investigated whether learned helplessness was a possible explanation for the observed learning deficits. One group of mice was tested in avoidance first, a water version of the Lashley maze second, and water escape last, while a second group was given these tests in the reverse order. If the animals were experiencing learned helplessness, we would expect that this would transfer and the animals would show poorer learning in other tests following avoidance. Contrary to the predictions of this theory, animals who were exposed to avoidance first did not demonstrate any learning deficits in subsequent tests. This would suggest that the poor performance in shuttlebox learning demonstrated by autoimmune mice is not due to learned helplessness. Supported in part by NIH grant H-20806.

LEARNING AND MEMORY: PHARMACOLOGY V

481.1

THE EFFECT OF ACUTE FLUOXETINE AND BUSPIRONE ADMINISTRATION ON PERFORMANCE IN A LATENT INHIBITION (LI) TASK. A. Jakob* and J. Rochford. Douglas Hospital Research Center, Dept. Psychiatry, McGill University, Montreal, Quebec

LI reflects the decrement in the acquisition of Pavlovian conditioning evoked by extensive conditioned stimulus (CS) preexposure. LI has been suggested to be an animal model of selective attention in that it assesses the ability to ignore irrelevant stimuli. It has been shown that lesioning the mesolimbic serotonergic (5-HT) system abolishes LI. As yet, few studies have been performed to examine the effects of pharmacological manipulation of 5-HT transmission on LI. In the present experiments we assessed the effects of acute administration of the 5-HT reuptake inhibitor fluoxetine and the 5-HT1A receptor agonist buspirone in an LI task. Following lever press training, different groups of animals were preexposed to 0 (nonpreexposed, NPE),10 or 40 presentations of a tone (the to be conditioned stimulus; CS). Over the following two conditioning days all animals were exposed to two tone-shock (0.6 mA, 0.5 sec) pairings and the acquisition of conditioned suppression was measured. Different groups received either 5 mg/kg fluoxetine, 5 mg/kg buspirone or vehicle (i.p.) prior to the preexposure day and the conditioning days or on the conditioning days only. The LI phenomenon occured in that untreated animals preexposed to 40 tones learned the tone-shock association less rapidly than those not preexposed to the tone. Fluoxetine-treated animals preexposed to 40 tones performed similarly to vehicle-treated animals exposed to 40 tones. However, fluoxetine induced an LI-like effect in animals preexposed to 10 tones. Preexposure to 10 tones did not induce LI in untreated animals. Buspirone was without effect in animals administered 10 or 40 tones. These findings suggest that acute 5-HT reuptake blockade, but not acute 5-HT1A receptor activation, can augment LI. (Funded by the FRSQ).

481.2

PHARMACOLOGICAL ANALYSIS OF SEROTONERGIC RECEPTORS ACTIVATION OR BLOCKADE ON LEARNING. A. Meneses* & E. Hong Terapéutica Experimental, Depto. Farmacología y Toxicologia, CINVESTAV-IPN. Ap. Postal 22026, México, D.F., 14000. MEXICO.

We have tested several 5-HT selective agonists and antagonists ($5\text{-HT}_{1A/1B}$, $5\text{-HT}_{2A/2B/2C}$, 5-HT_3 or 5-HT_4), an uptake inhibitor and 5-HT depletors in the autoshaping learning task. The present works deals with the receptors which stimulation increases or decreases learning, as well as possible explanations for the coexistence of the two effects. Impaired consolidation of learning was observed after the presynaptic activation of 5-HT_{1B}, 5-HT_3 or 5-HT_4 or the blockade of postsynaptic $5\text{-HT}_{2C/2B}$ receptors. On the other hand, improvement occurred after the presynaptic activation of 5-HT_{1A}, 5-HT_{2C}, and the blockade of presynaptic 5-HT_{2A}, 5-HT_{2C} and 5-HT_3 receptors. The blockade of postsynaptic 5-HT_{1A}, 5-HT_{1B}, 5-HT_3 or 5-HT_4 receptors and 5-HT inhibition of synthesis and depletion did no alter learning by themselves. The present data suggest that multiple pre- and postsynaptic serotonergic receptors are involved in the consolidation of learning. Stimulation of most 5-HT receptors increase learning, however, some of 5-HT subtypes seem to limit the data storage. Furthermore, the role of 5-HT receptors in learning seem to require an interaction with glutamatergic, GABAergic and cholinergic neurotransmission systems. Supported by CONACYT grant 4367-M9406

481.3

THE INCREASE IN LEARNING INDUCED BY INDORENATE AND 8-OH-DPAT WAS BLOCKED BY SILENT 5-HT_{1A} ANTAGONISTS. E. Hong* & A. Meneses Terapéutica Experimental, Depto. Farmacología y Toxicología, CINVESTAV-IPN. Ap. Postal 22026, México, D.F., 14000. MEXICO.

We have previously reported that the 5-HT_{1A} agonist 8-OH-DPAT (D) enhanced consolidation of learning and such effect was reversed by PCPA pretreatment. In the present work, the effect of post-training i.p. injection of WAY 100135 (W) or S-UH-301 (S) plus D or indorenate (I) was determined in autoshaping. Animals were individually trained to find 30 pellets, the unconditioned stimulus (US) in the food magazine. Each session had 20 trials and a trial consisted of illumination of a retractable lever for 8 sec (conditioned stimulus, CS) followed by the delivery of a US after the 8 sec period that were carried out every 60 sec. If the animal pressed the lever (conditioned response, CR) the trial was shortened and the lever was retracted, the light was turned off and the US was delivered immediately. The results showed that D and I increased the CR. W (5.0-20.0 mg/Kg) or S (0.3-3.0 mg/Kg) did not alter CR by themselves, but antagonized the effect induced by D and I. PCA neither induced changes in learning, nor modified the silent characteristic of W and S. At behavioral active doses, ketanserin or ondansetron reversed the effect of D. The present data suggest that D and I are involved on learning through activation of 5-HT_{1A} receptors. However, integrity of 5-HT_2 and 5-HT_3 receptors seem to be required in order that the stimulation of 5-HT_{1A} receptors be able to enhance learning. Supported by CONACYT grant 4367-M9406

481.4

STIMULATION OF 5-HT_{1A} RECEPTORS INDUCES ANXIOGENIC-LIKE EFFECTS AND FACILITATES ACQUISITION OF A SPATIAL DISCRIMINATION TASK IN MICE. I. Micheau*, B. Van Marrewijk and R. Jaffard, Lab. de Neurosciences Comportementales et Cognitives URA CNRS 339, Université de Bordeaux I, Ave des Facultés, 33405 TALENCE cedex, FRANCE.

Behavioural and pharmacological studies indicate that central 5-HT systems may mediate coping responses to acute and chronic stress. Consequently, the widespread action of 5-HT in anxiety related behaviour makes difficult to investigate the role of this transmitter on learning and memory. The aim of the present study was to investigate a possible relationship between the anxiolytic- or anxiogenic-like effects of 8-OH-DPAT, a 5-HT_{1A} agonist, and its influence on memory processes.

Mice were tested in an elevated plus maze before being submitted to a spatial discrimination task in a 8-arm radial maze. The 8-OH-DPAT (1 mg/kg, ip) administred 30 mn prior to the test of anxiety, induced anxiogenic-like effects. In addition, 8-OH-DPAT improved the acquisition of the spatial discrimination ($p<0.05$), probably by facilitating retrieval processes. Moreover, a regression analysis showed that the index of anxiety was positively correlated to the performance level reached at the forth day of training. Because very high concentrations of 5-HT_{1A} receptors are localized in the hippocampus, a possible interaction with the cholinergic septo-hippocampal system was investigated in the following experiment. Immediately after testing in the elevated plus maze, the animals were killed to measure the high-affinity sodium-dependent choline uptake (SDHACU) in hippocampal synaptosomes. The testing-induced activation of hippocampal SDHACU was reduced by the 8-OH-DPAT treatment (1 mg/kg) ($p<0.05$).

These results suggest that anxiogenic-like effects induced by the stimulation of 5-HT_{1A} receptors may have a positive influence on the acquisition of a hippocampal-dependent learning task.

481.5

EFFECTS OF ONDANSETRON, AN ANTAGONIST OF THE 5-HT$_3$ RECEPTOR, ON HIPPOCAMPAL CELLULAR ACTIVITY *IN VIVO*.

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Serotonin has been shown to block hippocampal LTP in a dose-dependent fashion, and antagonists of the 5-HT$_3$ receptor have been demonstrated to enhance learning and memory in rodents and primates. Ondansetron (ON), a potent and specific 5-HT$_3$ receptor antagonist, has recently been found to facilitate LTP, to significantly increase the frequency of the EEG ('theta rhythm'), and to enhance performance on olfactory and spatial memory tasks in rats. The present study sought to characterize the mechanisms responsible for these changes. Male Long-Evans rats (250-300 g) were surgically implanted with chronic microelectrode bundles into the CA1 region of the hippocampus bilaterally, and extracellular single-unit activity and EEG were recorded. Pyramidal cells and interneurons ('theta cells') were identified based upon their action potential waveform and firing properties with respect to the EEG during open field, exploratory and wheel running activities. The exact locations of the recorded units within hippocampal strata were verified histologically upon termination of the study. Following baseline measurement of average firing rate, ON (0.5 and 1.0 mg/kg) was administered intraperitoneally fifteen minutes subsequent to an injection of an equal volume of physiological saline. Enhanced firing of pyramidal cells (4/8, $p<0.01$) was observed following ON injection when compared to both control and saline conditions, accompanied by a decrease (10/20, $p<0.01$) or increase (1/20, $p<0.01$) in the firing rate of interneurons. The peak effect occurred between 15 and 30 minutes post-injection, and is consistent with an inhibitory action of ondansetron on 5-HT$_3$ receptors present on interneurons and subsequent disinhibition of pyramidal cells, thereby promoting activity patterns appropriate for the occurrence of long-lasting synaptic changes thought to underlie certain forms of memory.

481.6

HALOPERIDOL- INDUCED CHANGES IN NEUROTRANSMISSION IN CHICK BRAIN: A MICRODIALYSIS STUDY. M. GRUSS[1], G. POEGGEL*[2] and K.BRAUN[1]. [1]Federal Institute for Neurobiology, 39118 Magdeburg, Germany [2]University of Leipzig, 04103 Leipzig, Germany

We use auditory filial imprinting in chicks as an experimental model to study the mechanisms which underly juvenile learning processes. We have previously reported an increase of extracellular glutamate in response to the acoustic imprinting stimulus in the associative forebrain region medio-rostral neostriatum/hyperstriatum (MNH) and to a lesser extent also in the basal ganglia. Anatomical investigations revealed a dopaminergic input into the MNH and basal ganglia and we speculate that dopaminergic modulation of the glutamatergic pathway may be critically involved in synaptic changes during auditory filial imprinting. Thus we tested the effect of acute application of the dopamine- antagonist haloperidol on the extracellular concentrations of catecholamines and amino acids. A CMA11 microdialysis probe was stereotaxically implanted into the right MNH or the basal ganglia of 2 to 4 day old chicks. Haloperidol (10^{-5} - 10^{-3} M) was infused locally through reverse microdialysis. In both regions haloperidol induced a concentration-dependent increase of dopamine, dihydroxyphenylacetic acid, homovanillic acid and hydroxyindoleacetic acid. In addition the extracellular levels of glutamate, aspartate and GABA were increased in a concentration-dependent manner. The increase of catecholamine metabolites was higher in the basal ganglia than in the MNH, which correlates with the higher densities of dopamine receptors in the basal ganglia.

We suggest that the blockade of D2-autoreceptors leads to a local increase of dopamine release and that this increased dopamine level enhances the release of amino acids via activation of presynaptic D1-receptors. These findings further support the hypothesis of a modulatory influence of dopamine on the glutamatergic transmission system. *Supported by BMBF, GSF 07 NBL 06*

481.7

INVOLVEMENT OF DOPAMINE IN THE CHICK STRIATUM IN ONE-TRIAL PASSIVE AVOIDANCE TRAINING. E. Harrison, M.G. Stewart, P. Kabai, A. Csillag and G. Swanson*. Dept. of Biology, Open University, Milton Keynes, MK7 6AA,; Semmelweis University, Budapest IX, Hungary; TINS, Hills Road, Cambridge, CB2 1LA., UK.

One-trial passive avoidance training utilizes the ability of 1-day old domestic chicks (*Gallus domesticus*), to learn to distinguish between pleasant and unpleasant tasting substances such as methyl anthranilate (MeA). Major cellular changes following avoidance training occur in two forebrain regions, part of the hyperstriatum ventrale (IMHV), and the lobus parolfactorius (LPO). The LPO is a major component of the avian paleostriatal complex, equivalent to the medial striatum (caudate-putamen) of mammals, and is rich in dopamine receptors in pigeons. Dopamine has been implicated as a modulator of motivational behaviour, and contributes to the maintenance of avoidance responding in mammals. In birds, the striatum plays an important role in stereotyped behaviour, and alterations in dopamine binding may therefore be involved in modification of the pecking response in avoidance training.

The present study has used quantitative receptor autoradiography to investigate the binding of [^{3}H]SCH 23390 to D_1, and [^{3}H]spiroperone to D_2 receptors in chick forebrain. Our data show that D_1 and D_2 ligands bind at high levels to the striatum and pallidum of the 2-day old chick, as reported in the pigeon, turtle and rat. In addition, as in these species, the proportions of D_1 and D_2 receptors in the chick are relatively similar in the striatum and pallidum, apart from the paleostriatum augmentatum where D_2 receptors outnumber those of D_1 by a factor of two. In the passive avoidance experiments the data show a large and highly significant bilateral increase in binding to D_1 (but not D_2) receptors in part of the striatum, the LPO.

481.8

SENSITIZATION AND CONDITIONING WITH INTRASTRIATAL DOPAMINE AND DIBUTYRYL CYCLIC AMP IN RATS WITH NIGRAL LESIONS. P.B. Silverman* Dept. of Psychiatry, Univ. of Texas HSC, Houston, TX 77030.

Previous work in this laboratory has shown that systemic D1 and D2 selective dopamine receptor agonists both induce rotation in unilateral nigral rats which sensitizes with repeated administration, but that only D1 agonists result in rotation conditioned to the test environment which can be demonstrated weeks later. Here intrastriatal administration of dopamine and dibutyryl CAMP were tested for acute rotation, sensitization and conditioned rotation. Under pentobarbital anesthesia, female S-D rats were lesioned with 6-hydroxydopamine in one substantia nigra. At the same time, an indwelling cannula was implanted in the ipsilateral striatum. Two to three weeks later, they were injected intrastiatally on 3 consecutive days with dopamine HCl or with dibutyryl cyclic AMP. Both dopamine and dibutyryl cyclic AMP induced rapid contralateral circling which increased significantly with repeated administration. Neither cyclic AMP sodium salt, which reputedly has limited intracellular penetration, nor butyrate induced circling. When tested, undrugged, in the rotation environment 2 or more weeks after administration of dopamine or dibutyryl cyclic AMP, rats exhibited a brief burst of conditioned rotation. The results show that acute rotation, sensitization to repeated administration of dopamine or dibutyryl CAMP and conditioned circling can all be demonstrated with intrastriatal administration alone. It is apparently possible to bypass stimulation of the D1 receptor in this preparation and obtain classically conditioned behavior by direct administration of second messenger.

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481.9

DOPAMINERGIC BEHAVIORAL DRUG SENSITIZATION AS A DUAL PROCESS PHENOMENON: MOTORIC ACTIVATION AND RESPONSE REORGANIZATION. Ernest N. Damianopoulos* Huiliang Dai and Robert J. Carey. Psychiatry, SUNY Health Science Center and Research and Development Service - 151, VA Medical Center, Syracuse, NY 13210

Previous apomorphine behavioral sensitization studies have shown that locomotor activation is enhanced by repeated apomorphine treatment. This effect, however, is not a simple enhancement of locomotor behavior but actually entails both locomotor stimulation and locomotor response reorganization. We have proposed that the reorganization component is mediated by a Pavlovian conditioning process and, therefore, it represents a quasi-permanent change in the behavioral action of the drug treatment. To further assess this issue and to determine whether the two drug sensitization components; i.e., motoric activation and response reorganization, are associated or dissociated processes, a time-course study was carried out to assess the relative durability of the locomotor reorganization vs. the locomotor activation effect resulting from the repeated apomorphine treatment. Using Sprague-Dawley rats matched into three sets of paired - unpaired treatment groups, the animals were administered 7 once/day saline/apomorphine (2.0 mg/kg sc) treatments paired or unpaired to a 20 min placement into the drug test environment. The three sets of animals were then tested separately for behavioral sensitization after 1, 7, and 28 days of withdrawal/non testing. The results showed that locomotor activation enhancement was retained after 1 and 7 but not after 28 days. In contrast, the acquired locomotor pattern changes were retained at all test intervals suggesting that the response pattern reorganization effect is dissociated from locomotor activation enhancement and displayed the relatively-permanent characteristics of a learned response uniquely marking the drug effect.

481.10

A SMALL DOSE OF APOMORPHINE IMPROVES MEMORY ON A DIFFICULT SPATIAL TASK. A.G. Gittis*, C. Rudy and M. Esch. Psychology Dept., Westminster College, New Wilmington, PA 16172.

Gittis and Falleroni *(SN Abs, 1993)* demonstrated that accuracy of conditioned alternation (CA) is closely related to the geometric arrangement of the choices. A model was developed that sees CA as a manifestation of a higher order motor program which permits maintenance of orientation. Neurally, this implies that CA is mediated by frontal-corpus striatal circuits rather than temporal/parietal-limbic circuits. Therefore, an injection of a dopaminergic agonist should accentuate the alternation motor pattern which would have the cost of interfering with CA in geometric configurations that are not congruent with the motor pattern.

Rats were trained in a CA paradigm in a **X** - shaped maze in which the choice between two arms was systematically varied angularly and relative to midline. After training the animals received an injection of the dopaminergic agonist apomorphine (.05 mg/kg) or a vehicle injection and then tested. It was predicted that apomorphine injections would interfere with performance particularly when an arm incompatible with the alternation motor pattern had to be selected, e.g., when both arms are positioned on the same side lateral to midline.

As observed in previous studies, pre-injection alternation between arms laterally positioned was poorer than when midline had to be crossed. Contrary to the prediction, injections of apomorphine ***improved*** CA performance in the laterally positioned arms. This pattern of improvement confirms a report of memory enhancing effects of low doses of apomorphine *(White et.al., 1993)*. These authors suggest that the memory improvement is mediated by the activation of presynaptic autoreceptors which functionally reduce dopaminergic transmission. Hence the improvement in performance on the most difficult CA choices may have arisen from a reduction of dopaminergic transmission.

481.11

DIFFERENTIAL EFFECTS OF BROMOCRIPTINE AND 7-OH-DPAT ON RESPONDING FOR CONDITIONED REWARD IN RATS. M.A.Sutton, P.L.Nakonechny, N.G.Rolfe and R.J.Beninger*. Dept. Psychol., Queen's Univ., Kingston, K7L 3N6, Canada.

Previous studies have shown that receptor subtype-specific dopamine agonists differentially affect responding for conditioned reward; D1 agonists impair whereas D2 agonists selectively enhance responding. To compare the effects of the D2 agonist bromocriptine to the D3 agonist 7-OH-DPAT, food deprived rats (N=58) were pre-exposed to a chamber with two levers, one producing a tone (3 s) and the other lights-off (3 s), for 5 40-min sessions. Then, in 4 60-min sessions, with the levers removed, the lights-off stimulus was paired with food (80 pairings per session). During two 40-min test sessions, lever press responses again produced the tone and lights-off stimuli. (+)-Bromocriptine (5.0 mg/kg ip 30 min before) selectively enhanced responding on the lights-off lever. Animals treated with 7-OH-DPAT (0,1.0,2.5,5.0 mg/kg 30 min before), although responding preferentially on the lights-off lever, did not show an enhancement. Results suggest that D2 and D3 receptors are differentially involved in the control of responding by conditioned rewards.

481.12

"SUPER" LATENT INHIBITION (LI) WITH HIGH DOSE OF AMPHETAMINE J. Feldon[√#], U. Shalev[√], I. Weiner[√#]. [√]Dept. Psychology, Tel-Aviv University, Tel-Aviv, **Israel**. [#]Lab. Behav. Biol. Funct. Toxicol., Instit. Toxicol., Schorenstrasse 16, Schwerzenbach 8603, **Switzerland**. (ENA)

LI is a two stage paradigm: in preexposure, the subject receives a series of stimuli (e.g. tones); in conditioning, this stimulus is followed by reinforcement. LI consists of slower conditioning in the preexposed as compared to nonpreexposed subjects, and is considered to measure organisms' ability to ignore irrelevant stimuli. In both rats and humans, LI is disrupted with low but not high doses of amphetamine (AMPH). We attributed the former effect to enhanced behavioral switching, whereby animals switch rapidly to respond according to the stimulus-reinforcement contingency in conditioning. Since at high doses, AMPH produces behavioral perseveration, we tested the hypothesis that in LI this will be reflected in perseveration in ignoring irrelevant stimuli. One way to demonstrate such "super-LI" is to increase the number of stimulus-reinforcement pairings in conditioning to a level at which normal animals switch to respond according to the new contingency, and thus cease to show LI; Under these conditions, AMPH treated animals should persevere in ignoring the stimulus, and continue to show LI. Two experiments were conducted, using a conditioned emotional response procedure, in which animals received 20 nonreinforced tone preexposures followed by 5 tone-shock pairings. In Exp.1, vehicle controls, as expected, did not show LI, whereas animals treated with 4mg/kg AMPH showed LI. In Exp.2, we showed that AMPH-produced "super LI" is identical to that produced by 0.2 mg/kg haloperidol. These results may have implications for the LI model of schizophrenia. Until now, the model has been based on the parallel between LI disruption in rats by low AMPH doses and in acute schizophrenics. However, LI reappears in schizophrenics as a function of chronicity of illness.The "reappearance" of LI under high doses of AMPH may provide an animal parallel to this puzzling clinical phenomenon.

481.13

EFFECTS OF INTRA-CAUDATE NUCLEUS INJECTIONS OF PLATELET-ACTIVATING FACTOR AND BN52021 ON MEMORY. L. A. Teather*, M. G. Packard, & N. G. Bazan. Louisiana State Univ. Medical Center & Dept. of Psychology, Univ. of New Orleans, N. O., LA 70148.

Platelet-activating factor (PAF; 1-0-alkyl-2-acetyl-*sn*-glycero-3-phosphoryl-choline) is a biologically active lipid metabolite derived from phospholipase A_2, and is present in mammalian brain. PAF has recently been proposed as a retrograde messenger critical for the generation of long-term potentiation, and is involved in the formation of hippocampal-dependent and amygdala-dependent memory. The present experiments were designed to extend these findings by examining the effects of post-training intra-caudate nucleus injections of methylcarbamyl-PAF (mc-PAF) and the PAF antagonist BN52021 on memory using a caudate-dependent visible platform water maze task.

Male Long-Evans rats received an eight trial (30 second ITI) training session in which a visibly cued escape platform was located in a different quadrant of the maze on each trial. Following trial 8, the rats received a unilateral post-training intra-caudate injection of mc-PAF (1 ug/0.5 ul), BN52021 (0.5 ug/0.5 ul), or vehicle. On a retention test 24 hours later, latency to mount the escape platform was used as a measure of memory. The retention test escape latencies of rats receiving mc-PAF were significantly lower than those of vehicle-injected controls, indicating an enhancement of memory. The retention test escape latencies of rats receiving the PAF antagonist BN52021 were significantly higher than those of vehicle-injected controls, indicating an impairment of memory. Injections of mc-PAF or BN52021 did not affect retention when the injections were administered 2 hours post-training, indicating a time-dependent effect of the drugs on memory storage processes. The findings suggest that endogenous PAF plays a role in caudate nucleus dependent memory processes.

481.14

NOREPINEPHRINE RELEASE IN THE AMYGDALA IN RESPONSE TO FOOTSHOCK STIMULATION USED IN INHIBITORY AVOIDANCE TRAINING. **J.L. McGaugh*, R. Galvez and M.H. Mesches. Center for the Neurobiology of Learning and Memory and Department of Psychobiology, University of California, Irvine, CA 92717-3800.**

Extensive evidence indicates that the amygdala plays a major role in modulating memory storage induced by emotionally arousing stimulation such as footshock used in inhibitory avoidance training. Pharmacological evidence suggests that such memory modulating effects may be mediated by the release of norepinephrine (NE) in the amygdala. The present study examined NE levels released in the amygdala of freely moving rats in response to a single footshock comparable to that typically administered in inhibitory avoidance training.

One week prior to the experiment, male Sprague-Dawley rats were implanted bilaterally with guide cannula aimed at the dorsal side of the central nucleus of the amygdala. The microdialysis probe was inserted into the guide cannula 2 hours prior to collecting the first sample. One hour and thirty minutes after inserting the probe the rat was placed into a 30 X 30 cm box where footshock could be delivered through a grid floor. The flow of the artificial cerebrospinal fluid was 1 µl/min, and samples were collected every 15 minutes. After establishing a stable baseline, the rat was given a 0.55 mA / 1 sec shock. Samples were collected until NE levels returned to baseline. NE levels, as quantified by HPLC, increased significantly after the footshock. These results are consistent with previous pharmacological findings suggesting that NE release in the amygdala is involved in modulating memory storage.

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481.15

ENHANCEMENT OF MEMORY PROCESSING IN A Y-MAZE DISCRIMINATION TASK BY POSTTRAINING INFUSION OF CLENBUTEROL INTO THE NTS. C.L. Williams* & J.L. McGaugh. Center for the Neurobiology of Learning & Memory and Dept. of Psychobiology, University of Califiornia, Irvine, CA 92717-3800.

It is well established that administration of noradrenergic agonists peripherally or into limbic brain regions improve retention in memory tasks. There is evidence to suggest that neurons in the nucleus of the solitary tract (NTS) may mediate some of the effects of norepinephrine (NE) on memory. Peripheral afferent nerve fibers containing (NE) innervate several regions of the NTS. In addition, NE neurons in the A2 region of the NTS send nerve terminals to brain structures such as the amygdala, where NE infusion is known to facilitate retention. This experiment examined whether memory processes are affected by NE activation of the NTS. Male Sprague-Dawley rats (225-275 grams) implanted with cannulae tips placed above the NTS were trained to obtain food pellets placed in 2 arms of a Y-maze and then given a footshock in one arm of the maze. Immediately after the footshock, the rats received bilateral injections of 0.5 *u*l of phosphate buffer or the NE agonist clenbuterol (10, 50, or 100ng) into the NTS. On the retention tests given 24 and 48 hours after training, the number of seconds to enter either alley of the maze and the time to consume all food in the alley where footshock was received served as indices of retention. The latencies of animals given posttraining injections of clenbuterol (100ng/0.5 *u*l) were significantly longer on these measures relative to phosphate buffer-treated controls. These findings suggest that NE activation of NTS neurons is one mechanism by which memory storage processes are regulated.

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481.16

THE EFFECTS OF CONTINUOUS INFUSION OF ATIPAMEZOLE ON BEHAVIOR AND CNS NEUROCHEMISTRY IN RATS A. Haapalinna, T. Viitamaa, E. MacDonald, J.S. Salonen, J. Sirviö* and E. Heinonen. Orion Corporation, Orion-Farmos, P.O.Box 425, FIN 20101, Turku, Finland. Dept. of Pharmacology and Toxicology and A.I. Virtanen Institute , University of Kuopio, P.O.Box. 1627, FIN 70211, Finland.

Atipamezole (ATI) is a selective α2-adrenoceptor antagonist that, after acute dosing (0.3 mg/kg s.c.), stimulates release of central norepinephrine, potentiates neophobia, enhances maze learning, but impairs active (electric shock) avoidance learning. In the present work the effect of continuous ATI (100 µg/kg s.c./h) 6 -10 days infusion was tested on behavior and central monoamine turnover and compared with the effect of 24 h infusion. Also central α2-antagonism against detomidine in a rat mydriasis model and ATI concentration in serum and brain was measured. ATI 24 h infusion had no effect on motor activity, but decreased exploratory behavior in a staircase test. ATI after 6 days infusion had no effect on motor activity nor exploratory behavior. Active avoidance learning test was carried out at days 7, 8 and 9 of infusion and ATI treated animals made significantly more correct avoidance responses than control animals at day 9. Central α2-antagonism measurement and sacrifice of the animals were done after 24 h or 10 days of infusion. ATI 24 h infusion caused a marked increase in central norepinephrine, dopamine and serotonin turnover rate. ATI 10 days infusion had only a slight effect on central monoamine turnover rates, but shifted detomidine dose response curve to left with same potency than 24 h ATI infusion, indicating the same degree of α2-antagonism level. The mean ATI concentrations in serum and brain were 47 ng/ml and 84 ng/g after 24 h infusion and 47 ng/ml and 118 ng/g after 10 days infusion, respectively. These results indicate that after subchronic treatment with ATI, there was not longer potentiation of neophobia nor impairment, instead improvement in stressful learning test and only a slight stimulation of central monoamine turnover rate. However, the α2-antagonism potency was maintained and the concentration of ATI in brain was slightly increased.

481.17

ODOR PREFERENCES OF CONTROL RATS ARE ALTERED BY THE NUMBER OF DSP-4 TREATED RATS IN THE HOME CAGE. H.G.McFarlane, S.Henry, T.H.Dewey AND C.A.Cornwell*. Dept. of Psychology, Syracuse University, Syracuse, NY 13244.

In a previous study, control rats showed an abnormal aversion to a new home cage odor when they were housed with an equal number of cagemates treated with the NE neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4). The present study examined the effects of altering the ratio of DSP-4 treated rats to controls caged together. Male Sprague-Dawley rats were injected subcutaneously two days after birth with 50mg/kg of DSP-4 or vehicle (distilled water). After weaning, they were placed in one of three housing conditions: eight vehicle-injected controls; four vehicle-injected rats and four DSP-4-injected animals, or one vehicle-injected and seven DSP-4-injected animals. On postnatal day thirty-two, the bedding was switched from pine to cedar. Odor preferences for cedar versus pine were tested on postnatal day thirty-five. Social housing influenced odor preferences for the controls, but not for the DSP-4-injected rats. Controls housed with zero (0) or four (4) DSP-4-treated cagemates showed a significant aversion to cedar. In contrast, controls housed with 7 DSP-4-treated animals showed no preference between odors and had scores significantly higher than the other controls. Odor preferences for DSP-4-treated rats showed no trend toward a social effect. These data are consistent with the hypothesis and existing evidence that NE depletion reduces the effects of the social environment on odor preferences in rats. Since cage density was held constant, environmental effects on controls were not due to crowding.

481.18

SYMPATHETIC AFFERENTS DO NOT APPEAR TO PARTICIPATE IN THE MODULATION OF MEMORY STORAGE PROCESSES. M. Noyes♣, L.L. Murphy♣, and R.A. Jensen*♣. Departments of Psychology♣and Physiology♣, Southern Illinois University at Carbondale, Carbondale, IL 62901.

Research from this laboratory indicate that vagotomy significantly attenuates the memory-enhancing effects of peripherally acting substances such as 4-OH amphetamine (Williams & Jensen, 1991, *Peripheral Signaling of the Brain*), and vagotomy also attenuates the memory-impairing effects of peripherally administered leu-enkephalin (Williams & Jensen, 1993, *Behav. and Neural Biol.*). These findings suggest that peripheral events, often associated with arousal, send messages to the brain via the vagus nerve to modulate the storage of memories. However, as vagotomy only attenuated and did not block these memory modulatory effects it is possible that other neural pathways from the periphery, such as those associated with the sympathetic system, may also participate in the modulation of memory consolidation. To test this idea, three groups of male Long-Evans rats -- nonoperated controls, sham-operated, and splanchnicectomized animals -- were prepared. The splanchnic nerves were cut bilaterally below the adrenal branch, leaving the innervation to the adrenal glands intact. This was done so that the release of adrenal epinephrine and norepinephrine in response to arousal would be unaffected. Following a seven-day recovery period, the animals were trained in an inhibitory avoidance task where they received a 0.75 mA footshock. Immediately after the training, either saline or 1.0 mg/kg 4-OH amphetamine was administered intraperitoneally, and a retention test was given 24 hours later. To verify the effectiveness of the surgery, only those rats that showed an increased rate of stomach emptying compared to controls were retained in the splanchnicectomy group. In each experimental group, median entrance latencies were significantly higher in the 4-OH amphetamine-treated groups than in the saline-treated groups. These data strongly suggest that splanchnic afferents do not participate in a significant way in the modulation of memory storage processes following peripheral arousal and that afferents of the vagus appear to be the primary route by which peripherally acting substances influence memory storage.

481.19

SEXUALLY DIMORPHIC DSP-4 EFFECTS ON ODOR PREFERENCES AND WEIGHT LOSS IN MICE. T.H. Dewey*, S. Henry, R. Desai, A. Fijman, P. Hancock-Abel, M.Zavod, and C.A. Cornwell. Dept. of Psychology, Syracuse University, Syracuse, NY 13244

Odor preferences were investigated in NE-depleted mice and their control cagemates. Same-sex living groups of 8 mice per cage were housed together from weaning over hardwood shavings. At 60 days of age, each group was subdivided into cohorts of 4 mice each, which were then housed in modified plastic cages (30 square inches) over either hardwood or pine shavings for the duration of the three day experimental period. 2 of the mice in each experimental cage were injected i.p. with 50 mg/kg of the NE neurotoxin, N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4). Their cagemates and mice in control cages received injections of water vehicle. Odor preferences were tested 3 days after injection. Mice that were continuously housed over hardwood exhibited significant preferences for that odor. Exposure to a new odor, pine, reduced preferences for the hardwood odor in all groups with the exception of the DSP-4-treated females. An unexpected finding was that female mice in all groups demonstrated a significant reduction in weight during the 4 days between injection and testing while males did not show any significant weight changes. The data support previous evidence that norepinephrine facilitates olfactory learning in female mice but also suggest that the effect may be sexually dimorphic. The sex differences in weight changes imply that separation from their cagemates or the crowding conditions may have been more stressful to the female mice.

481.20

EFFECT OF IDAZOXAN (IDZ) IN CONTROL AND LEAD-EXPOSED RATS: EVIDENCE FOR THE ROLE OF NOREPINEPHRINE (NE) IN ATTENTION. L.E. Bayer* & B. J. Strupp. Developmental Neuroscience Group & Div. Nutr. Sci., Cornell University, Ithaca, NY 14853.

The present study was conducted with a dual objective: (1) to assess adrenergic receptor involvement in Pb-induced cognitive dysfunction; & (2) to examine the role of noradrenergic activity in selective attention and distractibility. Pb-exposed and control rats were administered the α-2 adrenergic antagonist IDZ (0, .1, .5, 1 mg/kg) and tested on a distraction task. This task assessed the ability of the S's to monitor an unpredictable light cue of either .5 or 1 sec duration, and maintain performance when presented with olfactory distractors. Preliminary analysis did not provide evidence for a differential effect of IDZ in Pb-exposed and control rats but did reveal specific attentional effects of both Pb exposure and IDZ. Pb-exposed rats made significantly more omission errors than control rats, specifically in the short light cue condition. IDZ (1 mg/ml) improved accuracy, specifically in the most attentionally demanding conditions: the short light cue and distraction conditions. This pattern of results rules out nonspecific alterations in performance as the basis of both the Pb and IDZ effects. These results provide evidence for the hypothesized role of the coeruleocortical NE pathway in attentional function.

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481.21

ADMINISTRATION OF L-TYROSINE PREVENTS COLD-INDUCED MEMORY DEFICITS IN NAVAL SPECIAL WARFARE PERSONNEL. J. Schrot*, J.R. Thomas, and D. Shurtleff Naval Medical Research Institute, Bethesda, MD 20889-5607.

Administration of l-tyrosine has been shown to prevent cold-induced memory deficits in a laboratory environment. The present study extended those findings to a military field setting. Thirty-six active duty Naval Special Warfare Personnel volunteered to serve as subjects while conducting Arctic and Mountain Warfare Training in Alaska. The subjects were first trained on a computerized performance assessment test battery in Virginia approximately one month prior to deploying to Alaska. Once in Alaska the subjects were divided into two equivalent groups based on baseline test scores. Both groups were then exposed, on at least two occasions, to outdoor ambient cold temperatures, which ranged from -22°C to -3°C (-10°F to 28°F), for one hour subsequent to consuming either 6.0 g of tyrosine or an equal amount of placebo. At the end of the cold exposure the subjects were brought inside and were administered the test battery. On average the subjects who consumed placebo showed performance decrements following cold exposure while the subjects who consumed tyrosine had test scores comparable to those obtained under baseline conditions. The results indicate that consumption of a large dose of tyrosine can prevent cold-induced memory deficits.

482.1

ASSESSMENT OF HABITUATION TO A NOVEL ENVIRONMENT AS A MEMORY MODEL. H. Dai*, E.N. Damianopoulos and R.J. Carey. Dept. of Psychiatry, SUNY HSC and VA Medical Center, Syracuse, NY 13210.

Habituation to a novel environment is a basic learning process manifested throughout the animal kingdom. Although widely used as an index of behavioral adaptation in many animal model studies, relatively, few attempts have been made to systematically evaluate habituation as an index of memory. Toward this end, a series of studies were conducted in which rats were exposed to a novel environment for 10 minutes. Twenty four hours later the rats exhibited a decrease in locomotor behavior typical for an habituation effect. In order to develop this response to be relevant to memory, other sets of animals were exposed to the test environment for variable intervals of 2.5, 5, 7.5 or 10 min and then tested twenty four hours later for habituation. In addition, other groups were given a 10 min exposure but were not tested for habituation for 7 or 14 days. The results showed that habituation was positively related to exposure time but negatively related to the length of the inter-test interval. These manipulations provided an experimental means to enhance or attenuate habituation and thereby provide a context in which to assess other memory enhancement or amnestic properties of drugs. Consistent with this analysis, sets of animals were given their first test exposure with drugs known to induce amnestic effects scopolamine (0.5 mg/kg) or MK-801 (0.05-0.3 mg/kg). Both scopolamine and non-motoric doses of MK-801 (less than 0.2 mg/kg) induced deficits in habituation consistent with amnestic effects. Interestingly, higher doses of MK-801 (0.2-0.3 mg/kg) induced both hyperlocomotion and seemingly normative habituation and memory 24 hours later. These studies demonstrate the importance of monitoring the direct behavioral effects of a drug in any attempt to evaluate subsequent effects in memory.

482.2

THE EFFECTS OF MK-801, A NON-COMPETITIVE ANTAGONIST OF THE NMDA RECEPTOR, ON THE PARTIAL REINFORCEMENT EXTINCTION EFFECT. S. Bland* and A. Amsel. Dept. of Psychology and Inst. for Neuroscience, University of Texas at Austin, Austin, TX, 78712, USA.

The partial reinforcement extinction effect (PREE) is a paradoxical reward-schedule effect in which resistance to extinction (learned persistence) is increased after partial (PRF) but not continuous (CRF) reinforcement training. Hippocampal insults such as prenatal and early postnatal alcohol, x-irradiation, and electrolytic lesions attenuate the PREE, suggesting that this type of learning depends on the integrity of the hippocampal formation. The NMDA receptor, a subtype of glutamate receptors which is densely concentrated in areas CA1 and dentate gyrus of the hippocampus, has been implicated in a variety of learning and memory tasks as well as long-term potentiation (LTP), an experimental model of synaptic plasticity. To assess the effects of NMDA receptor blockade on the PREE, rat pups (15 days old) were injected with either MK-801 (dizocilpine maleate, .05 ml/kg i.p.), a non-competitive antagonist of the NMDA receptor, or saline 30 minutes before either PRF or CRF training (two acquisition sessions and one extinction session) on a straight runway. An additional group was injected with saline before each aquisition session and with MK-801 before the extinction session. The PREE was attenuated in animals treated with MK-801; this difference was greatest in animals receiving MK-801 before both acquisition and extinction. These results suggest a role for the NMDA receptor in learned persistence. (Supported by NIAAA grant AA07052.)

482.3

EFFECTS OF THE NMDA ANTAGONIST MK-801 ON OLFACTORY DISCRIMINATION LEARNING AND VICARIOUS TRIAL-AND-ERROR. G. Griesbach*, D. Hu & A. Amsel. Dept. of Psychology & Inst. for Neuroscience, University of Texas, Austin, TX 78712.

MK-801 (a noncompetitive NMDA receptor antagonist) has been shown to interfere with learning. MK-801 induced learning impairments appear to affect acquisition but not retention. The effects of MK-801 on simultaneous olfactory discrimination learning and vicarious trial-and-error (VTE) were observed. The term VTE was used by Tolman (Psychol Rev, 1939), who described it as a conflict-like behavior at a choice point in discrimnation learning. VTE takes the form of head movements from one stimulus to the other. It has been proposed as related to hippocampal function during learning (Amsel, Hippocampus, 1993). Twenty-two day-old Sprague-Dawley rats received systemic MK-801 that was injected intraperitoneally 30 min before the onset of training. Subjects were trained for a total of 9 sessions of 20 trials, 3 sessions per day. In the 10th session the odors were reversed. Reversal training was terminated after the 18th session. The present results were that the MK-801 treated animals needed significantly more sessions to reach a criterion of 18 out of 20 rewards per session, and that the control group obtained significantly more rewards per session than the MK-801 treated animals. Control animals also showed a significantly larger number of VTE's. In reversal learning there was no difference between the MK-801 and the control groups. There was also no difference for VTE between the 2 groups. The lack of significance in reversal learning suggests that the effects of MK-801 on olfactory discrimination learning and VTE are dependent on task familiarity obtained in the initial training. Beacause MK-801 acts on the NMDA receptors, the implication is that the NMDA receptor is more likely to be involved in attention or encoding rather than retention and retrieval. Supported by NIAAA grant AA07052.

482.4

EFFECTS OF MK-801 ON ACQUISITION AND RETENTION OF AN ACTIVE AVOIDANCE RESPONSE IN RATS. E.R. Delay*, Dept. Psychology, Regis University, Denver, CO 80221.

Depending upon the task, MK-801, an NMDA noncompetitive antagonist, can either improve or disrupt learning and memory (e.g., Mondadori et al, Exp. Brain Res., 75: 449-456, 1989). This study examined the effects of MK-801 on the acquisition and retention of rats trained to avoid shock cued by either a light or a noise intensity CS in a 4-way shuttle. In Experiment 1, four groups of rats were given 30 trials on each of 4 consecutive days with the noise CS. Each group of rats was injected with MK-801 (0.1 mg/kg, IP) 35-40 min prior to one of the 4 sessions. The drug blocked acquisition of the avoidance task only when administered before the first training session. In Experiment 2, rats received the drug within 1 min after completing the training session. No drug effects were found. In Experiment 3, rats were trained with the light CS in Session 1, and with the noise CS in Sessions 2 and 3. MK-801 (0.05 & 0.1 mg/kg) blocked learning in Session 1 and retarded performance in Session 2 but not in Session 3. Drug-induced increases in activity were not related to changes in avoidance behavior. MK-801 blocked acquisition (but not retention) of the active avoidance task and disrupted cross-modal transfer of learning.

482.5

RECONSOLIDATION AFTER REACTIVATION OF MEMORY S.J. Sara J. Przysbyslawsky and T. Alexinsky (SPON: Euorpean Brain and Behaviour Society) Institut des Neurosciences, Université P & M Curie, Paris, France.

Rats were trained in a radial maze to choose 3 fixed arms/8, a task requiring integration of distal spatial information contained in cues strategically placed around the maze. Pretrial injection of the noncompetitive NMDA receptor antagonist, MK-801, at a dose which had no effect on overt behavior, markedly disrupted the well-trained performance of the task. Surprisingly, the behavioral deficit persisted on subsequent drug-free trials, 24h and even 48h later. Posttrial injections produced the same proactive effects on performance on one or two subsequent daily trials. There was a temporal gradient of efficacy of tdrug treatment: delay of injection up to 2h post trial induced significant amnesia, when the rats were tested 24h later. A more difficult version of the task yielded a still longer temporal gradient. Thus it appears that activation of a well-established memory circuit triggers cellular events which depend upon NMDA receptors for more than 2h. To what extent the entire postacquisition cascade of intracellular events associated with long term memory consolidation is recapitulated each time a memory is activated and reorganised is probably a function of the age and complexity of the memory and the amount of new information to be integrated into the circuit. These results provide physiological evidence that memory, as a dynamic process, undergoes continual reorganisation as a function of the ongoing experience of the organism.

482.6

SYSTEMIC AND INTRA-CAUDATE NUCLEUS INJECTION OF NMDA RECEPTOR ANTAGONISTS IMPAIR MEMORY IN A VISIBLE PLATFORM WATER MAZE TASK. R.J. Bennett* & M.G. Packard. Dept. of Psychology, University of New Orleans, N.O., LA, 70148.

Lesion and post-training treatment studies demonstrating dissociations between the effects of lesions and post-training treatments of the hippocampus and caudate nucleus indicate that these two structures are parts of independent memory systems. The caudate nucleus receives glutamatergic input via corticostriatal projections, and it is hypothesized that such input provides sensory information critical to the role of the caudate in "stimulus-response" habit formation. Previous findings indicate a role for the glutamatergic NMDA receptor in hippocampal-based memory. The present study was designed to extend these findings by examining the role of NMDA receptors in the acquisition of a caudate-nucleus dependent cued water maze task.

In Exp. 1 rats received a single 8 trial (30 sec inter-trial interval) training session with a visibly cued escape platform located in a different quadrant of the maze on each trial. Following trial 8, rats received a post-training systemic injection of the non-competitive NMDA receptor antagonist MK-801 (0.1 mg/kg), or saline. On a retention test 24 hours later, latency to mount the escape platform was used as a measure of memory. The retention test escape latencies of rats receiving MK-801 were significantly higher than those of saline treated animals, indicating an impairment of memory. When injections of MK-801 were delayed 2 hours post-training, no effect on retention was observed, indicating a time-dependent effect of MK-801 on memory storage processes.

In Exp. 2, different groups of rats given identical training in the cued task received bilateral post-training intra-caudate nucleus injection of the competitive NMDA receptor antagonist AP5 (2.0, 5.0, 10.0 ug/0.5 ul), or saline. Intra-caudate injection of AP5 at a dose of 2.0 ug significantly impaired memory relative to saline injected controls. The findings suggest that the role of NMDA receptors in memory extends to caudate-nucleus dependent memory processes.

482.7

MK 801 BUT NOT HALOPERIDOL SPECIFICALLY ALTERS THE REACTIVITY OF MICE TO SPATIAL CHANGE. P. Roullet, A. Mele and M. Ammassari-Teule*. Ist. of Psychobiology and Psychopharmacology, Dip. Gen. e Biol. Mol. Università Roma I, Rome , Italy and Lab. of Psychophysiology, Univ. Tours, France.

NMDA as well as dopaminergic antagonists produce specific place but not cue learning deficits. in the Morris water maze. The present work was, therefore, aimed at examining if the blockade of NMDA and dopaminergic receptors interfere with spatial information processing even in non associative tasks i.e. in tasks which do not involve an explicit reinforcing procedure. CD1 mice injected with MK801 (.1 and .25 mg/kg), haloperidol (.04 and .08 mg/kg) or saline were placed in an open field containing five objects and their reactivity to the displacement (spatial change) or the substitution (non spatial change) of some of these objects was examined. The results show that saline injected mice react to spatial as well as to non spatial change. by increasing the time spent exploring the displaced objects or the substituted one. Both doses of MK 801 prevented the mice to discriminate between displaced and non displaced objects while their reactivity to the novel object was unchanged. Both doses of haloperidol did not affect the reactivity of mice to spatial or to non spatial change. These results indicate that NMDA antagonists specifically disrupt the capability to perceive and/or to update spatial relationships since they alter performance in associative as well as in non associative spatial tasks. Conversely, the fact that haloperidol produces deficits of performance limited to associative spatial tasks suggests that dopaminergic antagonists can interfere with spatial information processing only in the presence of a reinforcing event.

482.8

CLASS I METABOTROPIC GLUTAMATE RECEPTOR AGONIST tADA FACILTATES LTP BUT BLOCKS MEMORY FORMATION IN RATS. G. Riedel*[1], W. Wetzel[2], D. Manahan-Vaughan[2], M. Reiser[2], S. Vieweg[2], G. Behnisch[2], A.P. Kozikowski[3] and K.G. Reymann[2], Dept of Psychol., Univ. of York, York, YO1 5DD, United Kingdom, [2]Fed.Inst. for Neurobiol., Dept. Neurophysiol., P.O.Box 1860, D--39008 Magdeburg, Germany and [3]Neurochem.Res., 12 Mershon Drive, Princetown, NJ 08540, USA

As previously shown metabotropic glutamate receptors (mGluRs) are involved in LTP *in vitro* and *in vivo* as well as memory formation. In order to classify which subtypes may participate in such forms of plasticity, we invented the effects of trans-azetidine-2,4-dicarboxylic acid (tADA) in both LTP in the dentate gyrus and spatial alternation learning versus brightness discrimination. tADA selectively acts on class I mGluRs in hippocampal slice preparations, and had no effect on forskolin-stimulated cAMP formation.

Weak tetanization (3 bursts, 200 Hz, 75 ms) applied to chronically implanted and saline i.c.v. injected rats (bipolar stimulation electrodes in medial perforant path, monopolar recording electrode in granular cell layer of dentate gyrus, guide cannula in lateral ventricle) caused a 2 hr potentiation, which was significantly prolonged when tADA (20 mM/5 μl) was i.c.v. injected 30 min. pretetanus.

The same tADA concentration injected 30 min pretraining had no effect on acquisition performance in a shock-reinforced spatial alternation task in the Y-maze. When tested for retention 24 hr later tADA treated rats were amnesic. State-dependent effects could be excluded. No effect of tADA was observed in brightness discrimination learning.

These data suggest that mGluR activation, although it facilitates LTP maintenance, blocks spatial memory formation, possibly via an unspecific saturated activation of mGluRs. This saturation may in turn decrease the signal-to-noise ratio of synaptic transmission and thus block memory formation.

482.9

KETAMINE BLOCKS A CONDITIONED TASTE AVERSION (CTA) IN NEONATAL RATS. **G.A. Mickley*, M.A. Schaldach, K.J. Snyder, T. Len and S.A. Balogh. Department of Psychology, Baldwin-Wallace College, Berea, OH 44017-2088.**

We have been exploring the effects of glutamate, NMDA receptor blockade on the formation and retention of CTA memories in young rats. Previous data from our laboratory (Mickley et al., Dev. Brain Res. 85, 1995, 119-127) suggested that ketamine administration potentiates the CTA of E18 rat fetuses. The current studies investigated this phenomenon in neonates.

On their day of birth, Sprague-Dawley rat pups received injections of 0.1 or 10 mg/kg ketamine, i.p. or a saline control injection. Pups were then injected orally with either saccharin (SAC; 10μl of 0.3%) or water followed by an injection of either Lithium Chloride (LiCl; 81mg/kg) or saline (i.p.). The CTA was evaluated in 2 different tests. Two weeks after conditioning, the dam was anesthetized and we measured the frequency with which pups attached to SAC-painted nipples *vs* nipples painted with water (nipple test). After weaning, we also evaluated the CTA by measuring the amount of SAC (0.3%) or water consumed during a 2-bottle test.

The data suggest that neonates receiving saline control injections can acquire CTAs. However, the ketamine-injected pups did not exhibit CTAs on either the nipple or bottle tests. Rats injected with ketamine and then SAC+LiCl drank more SAC than pups receiving saline before SAC+LiCl treatments.

These data reflecting a ketamine-induced blockade of neonatal CTAs are in contrast with findings in which ketamine potentiated fetal CTAs. This difference could reflect distinct dosing procedures employed in fetal *vs* neonatal rats. Alternatively, NMDA receptor blockade may shape memory formation in a manner that is dependent on the stage of brain development.

482.10

ADVERSE EFFECTS OF DEXTROMETHORPHAN ON THE SPATIAL LEARNING OF RATS IN THE MORRIS WATER MAZE. D. C. Rojas*, A. J. Bane, K.I. Indermauer, T. L. Bennett, and D. D. Avery. Dept. of Psychology, Colorado State Univ., Ft. Collins, CO 80523.

The effects of dextromethorphan (DX), a common over-the-counter antitussive, on spatial learning were assessed using the Morris Water Maze procedure. DX was administered IP to 4 groups of rats in 10, 20, 30, 40 mg/kg doses, as well as a saline vehicle control group. DX, a non-competetive n-methyl-d-aspartate (NMDA) receptor antagonist, impaired learning dose-dependently in the initial training phase of the experiment. During the probe trial conducted after initial training, in which the animals swam for 60 seconds in the maze with no escape platform, dose-dependent performance deficits were noted in the first 15 seconds of the trial only. Strategy differences between the lowest and highest dose group were also observed during the probe trial, with the saline control group spending more time in the target quadrant than the 40 mg/kg group during the first 15 seconds, then searching other quadrants; the opposite pattern was observed in the 40 mg/kg group. During the reversal training phase, when the quadrant was moved to a location 180 degrees from the original location, the dose-dependent impairment was seen again, but the 40 mg/kg group perseverated to the former location longer than the other groups. A cued control trial, in which the platform could be seen by the animals, indicated that in addition to the learning impairment produced, the highest dose of DX may also impair motor coordination.

482.11

NEUROPEPTIDE Y AND CALCITONIN GENE-RELATED PEPTIDE ATTENUATE MEMORY IMPAIRMENT INDUCED BY MK-801 LIKELY VIA A SIGMA RECEPTOR-RELATED MECHANISM. [1]P.Bouchard*, [2]T.Maurice, [3]S.St-Pierre, [2]A.Privat and [1]R.Quirion. [1]Douglas Hospital Research Center and McGill University, Montréal, Québec, Canada [2]I.N.S.E.R.M. U.336, Montpellier, France, and [3]Institut National de la Recherche Scientifique, Pointe-Claire, Québec, Canada.

It was recently shown that high affinity sigma (σ) ligands (DTG, (+)NANM and (+)pentazocine) significantly attenuated the amnesia induced in mice by the systemic administration of the non-competitive NMDA receptor antagonist MK-801. The purported σ receptor antagonists BMY-14802 and NE-100 blocked the effect of σ ligands, suggesting the involvement of σ receptors in mnemonic processes mediated by the NMDA receptor (Maurice et al., 1994). It was also shown, using *in vivo* binding (Bouchard et al; 1993; 1994) and electrophysiological (Monnet et al., 1992; Bouchard et al., 1994) paradigms that neuropeptide Y (NPY) and calcitonin gene-related peptide (CGRP) modulate σ sites in the rat and mouse brain, *in vivo*. Taking into consideration these various results, we investigated the possible modulatory actions of NPY and CGRP on MK-801 -induced amnesia in mice, using a well-established step-down passive avoidance test (Maurice et al., 1994). At time 0, male swiss mice were injected i.p. with either saline, MK-801 (200 µg/kg; i.p.) or MK-801 (200 µg/kg, i.p.) in combination with BMY-14802 (5 mg/kg; i.p.) as σ blocker. Immediately thereafter, mice were injected i.c.v. (3µl) with either saline, NPY or CGRP. Ten min later, animals received their first training session. Step-down latency (SDL) and the number of flinching actions and vocalizations were recorded. The second training period was carried out 90 min after the first, with an upper limit of 60 sec. The retention test was carried out 24 hr after the second training, with an upper limit of 300 sec. Compared to control mice, SDL was substantially reduced following the preadministration of MK-801 suggesting a memory impairment. NPY (1000 pmol; i.c.v.) and CGRP (1000 and 1500 pmol; i.c.v.) while having no significant effect by themselves, attenuated the MK-801 -induced amnesia as shown by increased SDL. Furthermore, when co-administered with MK-801, BMY-14802 prevented the effect of NPY and CGRP in the behavioral task suggesting the involvement of σ sites. Hence, while *in vitro* evidence for direct interaction between NPY or CGRP and σ sites have failed to be obtained thus far, various *in vivo* models have shown that these peptides can modulate σ receptor systems in the brain. (Supported by the NSERC and Jouveinal Laboratories)

482.12

BEHAVIOURAL EVIDENCE FOR A MODULATING ROLE OF THE SIGMA LIGANDS JO 1784 AND 2-DTG IN MEMORY PROCESSES IN THE RAT.
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The nature and extent of the behavioural changes induced by the non-competitive NMDA antagonist, dizocilpine (MK-801), were assessed in the eight arm radial maze task. Male Sprague-Dawley rats were trained on the eight-arm radial maze task for food reinforcement. The effects of various doses of 1,3-di(2-tolyl)guanidine (2-DTG) (1 and 3 mg/kg s.c.) and JO 1784 (0.1, 0.3, 1.0 and 3.0 mg/kg s.c.) were determined alone and in combination with MK-801 (0.25 mg/kg s.c.). Relative to vehicle controls, JO 1784 and 2-DTG alone did not affect performance in the radial arm maze. MK-801 alone decreased the number of correct responses in the first eight arm choices, while increasing both the number of errors and the time necessary to complete the maze. 2-DTG produced a slight attenuation of MK-801 induced amnesia. Moreover, JO 1784 attenuated MK-801 induced amnesia only after the administration of 2-DTG. These results suggest that sigma ligands are capable of modulating learning and memory processes.

482.13

DIFFERENTIAL EFFECTS ON SPATIAL LEARNING AND MEMORY BY THE NMDA RECEPTOR ANTAGONIST CGP 37849. L. Jeu, J. Baruch, and V. Luine*. Department of Psychology, Hunter College of CUNY, New York, NY 10021

Using a spatial memory task, we assessed the behavioral effects of the NMDA receptor antagonist CGP 37849. CGP 37849 enhances cell birth in the dentate gyrus of the rat hippocampus and causes a 24% increase in the number of granule neurons one month post-treatment (Cameron et al., in press). Twelve adult male rats were administered one i.p. injection of 7 mg/kg CGP 37849 or saline. Two months after treatment, rats were trained and tested using the 8-arm radial maze (RAM). Overall, the CGP-treated rats did not perform as well as control rats. During regular trials, they made mistakes sooner and more frequently. During delay trials, CGP-treated subjects also exhibited impaired performance with 3, 4, 5, and 6 h delay intervals between the 4th and 5th choices.

In an attempt to determine whether impairments were due to learning or memory function, rats were tested with four arms baited. Surprisingly, in the first 10 of 25 trials, CGP-treated rats made fewer reference errors (entries into unbaited arms which represent acquisition of the procedural aspect of the maze), suggesting enhanced learning. In contrast, in the last 15 of 25 trials CGP-treated rats made more working errors (re-entries into baited arms which represent short term memory), suggesting impairments in working memory. These results show that a single injection of an NMDA receptor antagonist causes a long term effect on spatial memory performance. Moreover, CGP 37849 appears to differentially affect learning and memory, enhancing the former and impairing the latter.

(NIMH MH16705 to H.C.)

482.14

NMDA RECEPTORS ARE INVOLVED IN LEARNING A SPATIAL TASK BUT MAY NOT BE NECESSARY FOR ENCODING A SPATIAL REPRESENTATION OF A SPECIFIC ENVIRONMENT. D.M. Bannerman, M.A. Good, M. Ramsay, S.P. Butcher, S.D. Iversen*, R.G.M. Morris, Centre for Neuroscience and Dept of Pharmacology, Univ of Edinburgh, Edinburgh EH8 9JZ, Scotland.

Morris et al., (*Nature*, 1986, 319:774-776) found that chronic i.c.v infusion of the competitive NMDA antagonist, D,L-2-amino-5-phosphopentanoate (AP5) impairs the acquisition of spatial learning in the watermaze and blocks the induction of LTP in the dentate gyrus *in vivo*. In contrast, both the retention of previously acquired spatial information and performance on a non-spatial visual discrimination task are unaffected by AP5. These results support the hypothesis that NMDA receptor-dependent changes in synaptic efficacy, similar to LTP, may contribute towards the neural mechanisms underlying spatial learning. However, in a subsequent study we found that, although a 30 mM dose of D-AP5 reliably impairs spatial learning in naive animals, the behavioural deficit is greatly reduced if spatial training in a different environment is given prior to the drug phase of the experiment. This outcome is not because of generalisation between the 2 laboratories, occurs despite the learning of the second task remaining dependent on the integrity of the hippocampus, and with intracerebral levels of D-AP5 well in excess of those required to block LTP *in vivo* in the same animals as tested behaviourally. In contrast, if rats are pretrained as normal animals in the first watermaze, under conditions that minimise spatial learning, the D-AP5 induced deficit in the second watermaze re-appears. These results suggest that spatial learning in the watermaze may involve NMDA receptor-dependent and -independent components, and that while NMDA receptors are required while learning a spatial task, they are not necessary for encoding the spatial representation of a specific environment.

482.15

THE EFFECTS OF BASAL FOREBRAIN LESIONS ON EXPECTANCY IN THE RAT. J.D. Stoehr* and G.L. Wenk. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

A two-choice operant task was used to measure the effect of lesions of the basal forebrain on a task that requires shifting of attention between two visual stimuli. Discriminibility between these two stimuli and response bias were determined in rats given injections of N-methyl-D-aspartate (NMDA) or a selective cholinergic toxin, IgG-192 saporin, into the basal forebrain. NMDA injections significantly reduced ChAT activity, a measure of basal forebrain cholinergic cell loss, in the frontal and parietal cortex, but not in the hippocampus or olfactory bulbs. IgG-192 saporin injections significantly reduced ChAT activity in all of these brain areas. Both toxins produced a significant shift in response bias that correlated with the degree of cholinergic cell loss in cortical and hippocampal areas. IgG-192 saporin injections produced a significantly greater impairment in choice accuracy and response bias compared to injections of NMDA. The results suggest that the cholinergic basal forebrain may influence the selection of response strategies in simple operant tasks involving motor responses to visuospatial stimuli.

482.16

EFFECTS OF A COMPETITIVE NMDA ANTAGONIST, CGP-39551, ON HIPPOCAMPAL LTP AND PLACE LEARNING J. M. Hoesing*, A.D. Barnes, R.I. McDonald, and R.J. Sutherland, Dept. of Psychol. & Phsyiol., Univ. of New Mexico, Albuquerque, NM 87131-1161.

Manipulations which affect hippocampal LTP, such as blockade of NMDA receptors, have been shown to block place learning. In general, these results support the idea that NMDA-dependent hippocampal synaptic plasticity is necessary for the acquisition of certian types of information. In particular, the effects of CGP-39551, a competitive NMDA antagonist, on induction of hippocampal LTP and on the acquisition of spatial information in the Morris water task were examined. LTP was induced in urethane-anesthetized rats by electrical stimulation of perforant path-dentate gyrus synapses. CGP-39551 produced a dose-dependent deficit in LTP induction - at 12 mg/kg LTP was blocked. Experimentally naive rats treated with 12 mg/kg did not learn to swim to the fixed location of a hidden platform in the Morris water task. Experimentally sophisticated rats, trained in a place learning-set procedure, were able to acquire accurate place navigation in a novel room after having been treated with the same dose, 12 mg/kg. This suggests that acquisition of spatial information *per se* does not require NMDA-dependent synaptic processes.

BIOLOGICAL RHYTHMS AND SLEEP: AGING

483.1

PATHOLOGICAL CORRELATES OF CIRCADIAN RHYTHM DISTURBANCES IN LATE-STAGE ALZHEIMER'S DISEASE. A. Satlin*, E.G. Stopa, D. G. Harper, V. Kuo-LeBlanc, M. Rodriguez-Wolf, L. Volicer. Dept. of Psychiatry, McLean Hospital, Belmont, MA 02178; E.N. Rogers Memorial Veterans Hospital, Bedford, MA 01730; Dept. of Pathology, Brown Univ. Sch. of Med., Providence, RI 02903.

We previously reported a phase delay in the circadian rhythms of locomotor activity and core-body temperature in patients with late-stage Alzheimer's disease, and a decreased amplitude of the locomotor activity rhythm. Neuropathologic examination of the suprachiasmatic nucleus in patients with advanced AD has revealed a significant decrease in vasopressin-containing neurons and an increase in the glia/neuron ratio compared to age-matched controls. The present study sought correlations between disturbances in circadian rhythmicity and pathologic changes in the brains of 16 late-stage AD patients. Vasopressinergic neuronal loss was associated with decreased mean diurnal ($r=0.81$; $p<0.001$) and nocturnal ($r=0.74$; $p<0.01$) core-body temperature and with increased fragmentation of the locomotor activity rhythm (intradaily variability; $r=0.62$; $p<0.01$). Loss of neurotensin-containing neurons was associated with lower circadian temperature rhythm amplitude ($r=0.57$; $p<0.05$). There were no significant associations between loss of either cell population and the phase of the temperature or activity cycles. However, later phase of the temperature cycle was associated with axonal degeneration in the optic nerve ($r=0.56$; $p<0.05$). These findings confirm an association between degeneration in brain structures thought to mediate circadian rhythmicity and objective measures of circadian function in late-stage AD patients. They also suggest that differential degeneration in neuronal populations and structures in AD may have variable effects on circadian function. Supported by AG09301.

483.2

CIRCADIAN RHYTHMS AND AGING: A LONGITUDINAL STUDY OF FREERUNNING PERIOD AND ACTIVITY LEVEL IN SYRIAN HAMSTERS. N. Viswanathan* and F.C. Davis. Dept. of Biology, Northeastern Univ., Boston, MA 02115.

Age-related changes in the regulation of circadian rhythms have been reported in humans and other animals. In Syrian hamsters the freerunning period of the activity/rest rhythm has been reported to shorten with age. Although this has been observed under a variety of experimental conditions (including blinded hamsters), continuous measurements of freerunning period over the life of animal have not been made in intact hamsters. In the present study, the wheel running activity/rest rhythm was continuously measured in male hamsters (*Mesocricetus auratus*) in dim constant light (< 1 lux) from 8 to 90 weeks of age. Freerunning period and activity level were analyzed beginning at 30 weeks of age to ensure that after-effects of the initial light/dark cycle had decayed and that the reproductive state of the hamsters had stabilized. Fifteen hamsters (28% of initial number) survived to at least 90 weeks. The average freerunning periods of these hamsters at 30, 50, 70 and 90 weeks of age were 24.08, 24.13, 24.11, and 24.10 respectively, and there was not a significant shortening of period ($P= 0.38$, repeated measures ANOVA). Some individual hamsters showed a shortening of period while others showed no change or a lengthening. In contrast to freerunning period, average activity level showed a 60% decrease over the course of the study ($P<0.001$). To determine whether period changed prior to death, the freerunning periods of hamsters that were at least a year old were measured at 2 weeks, 3 months, and 6 months prior to death ($n=18$). There was also no change in average period in this group. These results demonstrate that the average freerunning period of the circadian activity/rest rhythm does not change with age in intact hamsters continuously maintained under constant conditions. Previous observations of a shortening of freerunning period with age may be related to the particular conditions of those studies.
Supported by PHS grant P01 AG09975.

483.3

THE ROLE OF SUPRACHIASMATIC NUCLEUS IN AGE-RELATED CHANGES OF CIRCADIAN RHYTHM. S. Yamaoka*, N. Koibuchi, and M. Sakai. Dept. of Physiology, Dokkyo Univ. Sch. of Med., Mibu, Tochigi, 321-02 Japan

Age-related changes in various circadian rhythms have been documented. However aging mechanisms in circadian system is still unknown. Previously we reported that the aging changes of circadian sleep rhythm in female rat correlated with the aging changes in sexual cycle. To clarify the role of suprachiasmatic nucleus (SCN) in correlation between aging mechanism and circadian system, we adapted the analyses of circadian locomotor activity and the quantitative immunohistochemistry. 1). Male rats (Sprague Dawley, SD) were divided into three groups: 2-5 months young rats, 12-16 middle aged rats, 18-30 months old rats. Zitter rat (zi; a mutant rat), which shows spongy degeneration of the central nervous system and a generalized body tremor, divided two groups (1-3 months and 7-10 months). The circadian locomotor activity (LMA) was measured by infra-red area sensor. The free-running period under constant dark shortened with age in both strains. Three hours light pulse caused the phase shift depending on the applied circadian time in young SD rat, but not in old SD and 7-10 months zi rat. In old SD rats, the re-entraining days to reverse lighting schedule also postponed with age. These aging effects of 7-10 months zi rat were equivalent with over 24 months SD rats. 2). The Fos-like immunoreactive (Fos-IR) cells was diffusely located in SCN. Greater amount of Fos-IR cells were seen in light phase than dark phase under light-dark condition. This change was disrupted in old rat. Fos-IR cells were confined in the ventrolateral part of SCN by the light pulse during constant dark. This change did not show any difference with the age or the phase of activity. these results suggest that a part of aging mechanism in circadian rhythm due to the disruption of signal transduction from circadian oscillator, and the signal transduction from retina is not affected by aging.

483.4

BASAL AND PHASE-SHIFTED NEURONAL RHYTHMS IN THE AGED SCN IN VITRO. V. H. Cao, D. M. Edgar, H. C. Heller, W. C. Dement and J. D. Miller*. Ctr. Sleep and Circadian Neurobiology, Stanford University, Stanford, CA 94305.

The mammallian suprachiasmatic nuclei (SCN) contain a circadian pacemaker that produces a 24hr rhythm in spontaneous neuronal activity *in vitro*. This *in vitro* rhythm can be phase-advanced by application of the 5HT1A/5HT7 receptor agonist, 8-OH-DPAT. Although numerous investigators have noted a decline in behavioral circadian rhythmicity *in vivo* with aging, it is not known whether this decline represents an alteration in afferent input to the SCN, intrinsic function of the SCN pacemaker, or SCN efferents. To address these questions we have examined basal and 8-OH-DPAT-phase-shifted single unit rhythms in SCN slices from behaviorally characterized (locomotor activity) aged Fischer 344 or Wistar male rats. Brain slices prepared during lights-on from 4-32 month old male rats, housed in 12:12 LD were maintained *in vitro*. In some experiments basal single unit rhythms were recorded. In other experiments slices were treated for one hr with 8-OH-DPAT (5 uM) at CT6 on the first day *in vitro*. Neuronal activity was recorded on day 2. In nearly all baseline studies (n=35 at present, including 20 rats from 12-32 mo old) the usual unimodal circadian rhythm was observed. In 8-OH-DPAT studies, phase advances of about 150% the magnitude of the usual advance in young rats (~4 hr, 2-4 mo old) were noted in the aged rats (~6.3 hr, 17-32 mo old, n=5). Robust phase advances and the usual unimodal single unit rhythms were observed, even from rats with very low amplitude behavioral rhythms. Our preliminary data suggest that the intrinsic circadian pacemaker in the SCN is relatively resistant to aging. The large phase advances to 8-OH-DPAT in aged slices suggest development of 5HT7 receptor supersensitivity. Frequently observed low amplitude behavioral rhythms in these studies further suggest that age-related degeneration in rhythmicity may largely reflect deficits in SCN efferents or downstream clock effector mechanisms. Supported by AG11084.

483.5

AGE-RELATED CHANGES IN THE EXPRESSION OF BRAIN-DERIVED NEUROTROPHIC FACTOR mRNA IN THE RAT SCN. D. Earnest*, B. Earnest and F. Sohrabji,. Dept. of Human Anatomy and Medical Neurobiology, Texas A&M Univ., College of Medicine, College Station, TX 77843.

Aging of the circadian pacemaker in the rodent suprachiasmatic nucleus (SCN) is characterized by changes in period length and in responses to environmental stimuli. While the physiological or biochemical changes underlying this age-related deterioration of SCN circadian function are unknown, such changes may be associated with age-related alterations in specific growth factors that mediate structural or synaptic plasticity. Since recent data from our lab indicates that brain-derived neurotrophic factor (BDNF) mRNA is expressed within the rat SCN, the present study examined whether aging is associated with changes in SCN expression of this neurotrophin gene.

Total RNA from the SCN of 4-month and 21-month old male Fisher 344 rats was DNased and then assayed by semi-quantitative RT-PCR for BDNF mRNA. Template cDNA was formed using reverse primers specific for the rat BDNF gene (Exon V) and cyclophilin and then amplified by the addition of gene-specific, ^{32}P-labelled forward primers. Amplified product was size-fractionated and visualized by film autoradiography. The PCR product of BDNF primers was normalized to cyclophilin mRNA that had been concurrently reverse-transcribed and amplified. SCN expression of BDNF mRNA showed signs of age-dependent regulation, suggesting that aging may alter SCN plasticity in response to environmental stimuli. Continuing work will explore the relationship between this age-related alteration in BDNF expression and deterioration of SCN circadian function.

483.6

REDUCED CIRCADIAN RESPONSES TO LIGHT IN AGED C3H/HeN MICE. S. Benloucif,* M.I. Masana, and M. L. Dubocovich, Dept. Mol. Pharmacol. Biol. Chem., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Light-induced immediate early gene expression in the SCN of aged rodents is attenuated, suggesting decreased responsiveness by the circadian pacemaker (Neurobiol. Aging, 1993,14:441). The present study examined the effect of age on phase shifting responses to light in C3H/HeN mice. Young and old male C3H/HeN mice (4 or 16 months at the beginning of the experiment, n = 9) were housed in constant darkness with circadian wheel running activity rhythms monitored by an online computer. Shifts in phase were assessed by comparing the steady state activity onset before (10 days) and after (14 days) the treatment. Light pulses of 15 min duration (30, 100, 300 or 1000 lux) were delivered every 3 weeks over a 6 month period and the magnitude of the phase shifts compared. Aging reduced the phase delaying effect of light at CT 14 at all light intensities ($p < 0.001$), with an interaction of age and intensity that approached significance ($p = 0.06$). A light pulse of 30 lux was saturating in the young mice, resulting in delays in phase of -2.79 $\pm$ 0.22 h (n = 6). In contrast, only one of 5 old mice shifted phase at this intensity ($p < 0.001$). In the old mice, light pulses of 100, 300, and 1000 lux induced phase delays of lower magnitude than young controls [1000 lux, young: -2.28 $\pm$ 0.13 (n = 5); old: -0.98 $\pm$ 0.47, $p < 0.05$ (n = 6)]. These results support reduced responses to light by the circadian pacemaker or its effector systems in aging. The molecular responsiveness of the pacemaker will be determined at the conclusion of behavioral testing by assessing the photic induction of immediate early genes (c-*fos* and *jun*-B) in the SCN. Supported by a Glaxo grant and NIH F32-AG05608.

BIOLOGICAL RHYTHMS AND SLEEP: DISORDERS AND CLINICAL STUDIES

484.1

EVIDENCE FOR LOCALIZED NEURONAL DEGENERATION IN THE NARCOLEPTIC DOG J. M. Siegel*, R. Nienhuis, H. Fahringer, S. Gulyani, †E. Mignot and R. C. Switzer III. Neurobiology Research Sepulveda VAMC, Dept of Psychiatry, UCLA School of Medicine, North Hills CA 91343 and †Dept of Psychiatry, Stanford University, Stanford CA

Narcolepsy develops postnatally in both humans and animals. While changes in receptor levels have been reported, no underlying pathology responsible for these changes has been identified. We hypothesized that narcolepsy is caused by a transient degenerative process occurring at the age of symptom onset. We used the cupric silver technique to search for axonal degeneration shortly after symptom onset in four narcoleptic dobermans and four age and breed matched normal canines. All narcoleptic animals showed extensive degeneration in the medial septal nucleus and diagonal band region. None of the controls showed such degeneration ($p < .001$, 2 way ANOVA). The areas of degeneration are adjacent to basal forebrain neuronal systems known to be of primary importance in sleep initiation and motor control. Therefore this damage can explain the principle symptoms of narcolepsy.

484.2

BEHAVIOR DISRUPTION AND RECOVERY IN PORTACAVAL SHUNTED RATS

P. A. Hawkins, M. R. DeJoseph and R. A. Hawkins*, Department of Physiology and Biophysics, Finch University of Health Sciences/The Chicago Medical School, North Chicago, IL 60064

Portacaval shunting causes liver atrophy and encephalopathy as evidenced by a variety of metabolic and behavioral abnormalities including a disruption of the normal circadian rhythm. Whether the changes associated with liver dysfunction are completely reversible has been hypothetical. We developed a rat model that allows blood circulation to be restored to the liver resulting in its re-growth to normal size and the elimination of many of the metabolic signs of liver dysfunction. The focus of this study was on behavioral activity before, during and after a period of portacaval shunting to determine whether the normal pattern of activity could be restored after a sustained period of liver dysfunction. Behavioral activity was monitored in a specially designed cage for 2 weeks to establish pre-shunt activity. Rats were then given side-to-side shunts and activity was monitored for 6 weeks. Finally, blood circulation was restored to the liver and behavioral activity was recorded for at least an additional 6 weeks. Portacaval shunted rats showed a depression in nighttime activity and the night-to-day ratio within 1 week. Redirecting blood flow to the liver resulted in restoration of normal behavior in all but 1 rat (n=10). The ability to reverse behavioral changes in rats with portacaval shunts of 6 weeks duration suggests a non-permanent nature of hepatic encephalopathy in this time period. Monitoring activity seems to be an efficient way to test potential therapies for chronic hepatic encephalopathy. Supported by NIH grant NS 16389.

484.3

EFFECTS OF OLFACTORY BULBECTOMY ON RUNNING-WHEEL ACTIVITY IN ADULT MALE RATS. M. E. Vagell, M. Y. McGinnis*, B. Possidente, and A. R. Lumia. Depts. of Psych. & Bio., Skidmore College, Saratoga Springs, NY 12866. Dept. of Cell Bio./Anat., Mount Sinai School of Medicine, NY, NY 10029.

Bilateral olfactory bulbectomy (OBX) in rodents results in several behavioral and biochemical alterations leading to its development as a rodent model for depression. OBX mice entrained to 12:12 LD schedules increase both amplitude and mean running-wheel activity levels. The present study tests whether OBX affects running-wheel activity in adult male Long Evans rats as it does in mice. Wheel-running activity was measured with an automated system for counting revolutions per ten minute interval. Activity was measured for two weeks before OBX surgery, followed by two weeks of surgical recovery, and then measured for two final weeks. The data were analyzed using a linear COSINOR model with the frequency set for 1 cycle/day to determine amplitude and mean activity levels. Statistics were performed using the percent change from pre-surgical activity levels for each rat because of high inter-animal variability. We found that OBX rats showed significant increases in both amplitude and mean activity levels when compared to sham operated controls (Mann-Whitney U test). The data from the present study on OBX rats corresponds to other studies involving OBX mice. We conclude that in spite of the large individual differences in wheel-running activity, Long Evans rats are suitable for studying the chronobiological effects of OBX.

484.4

AMPLITUDE OF WHEELRUNNING RHYTHMS IS DECREASED IN HAMSTERS TREATED WITH SCOPOLAMINE AS NEONATES H. Klemfuss*, P. R. Ramos and J. C. Gillin Veterans Affairs Medical Center, San Diego, CA 92161 and Department of Psychiatry, University of California, San Diego.

Cholinergic-aminergic imbalance may be involved in mood and sleep disorders. Treatment of neonatal rats with monoamine uptake inhibitors can produce a depression-like syndrome in adults that includes REM sleep disturbances and alterations in the circadian rhythm amplitude. We tested effects of neonatal treatment with scopolamine HCl (SCOP), a muscarinic antagonist, on circadian running rhythms in the golden hamster.

Neonatal hamsters were housed with the mother on an LD14:10 cycle, with lights on at 6 am. From age 8-16 d, 3 ♂ and 3 ♀ were given sc injections of 1 mg/kg SCOP at 7 am and 7 pm, and 9 control hamsters (5 ♂, 4 ♀) received saline injections. Hamsters were weaned at d 16. 10 mg/L SCOP was added to the water of experimental hamsters from d 16-21. Starting on d 28 each hamster was housed in a running wheel cage and given water to drink.

There were no differences between groups in weight gain or appearance. Neonatal SCOP decreased the total harmonic amplitude of the wheelrunning rhythm in juvenile hamsters ($p < .001$) and decreased goodness-of-fit by several methods ($p < .001$). Harmonic amplitude was also increased when expressed as % of mesor ($p < .01$). Circadian phase was not significantly altered (saline-control 20:44 ± .2 hr; SCOP-treated 19:89 ± .4 h; lights off 20:00)($p = .11$). This preliminary work indicates that transient disturbance of muscarinic metabolism in developing hamsters can produce lasting effects on circadian oscillator amplitude.

Supported by the Dept. of Veterans Affairs

484.5

QUANTITATIVE ELECTROENCEPHALOGRAPHIC AND LOCOMOTOR ACTIVITIES IN THE GRAVID RAT. G. Livezey* and C. Smith, Dept. of OB/GYN, University of Nebraska College of Medicine, Omaha, NE 68198-3255

In developing an animal model for the study of prenatal exposure to centrally acting drugs, we compared the electroencephalographic (EEG) and locomotor activities in the pregnant and nonpregnant rat. Mature female Sprague-Dawley rats were implanted with telemetry transmitters. EEG and locomotor activities were sampled every 10 minutes around the clock for 5 weeks prior to breeding, throughout their 21 day gestation and 3 weeks postpartum (weaning period). Contemporary controls consisted of those not bred. The EEG data was converted to the power spectra and averaged over 24 hours. We divided the spectra into delta (1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), middle beta (12-16 & 16-20 Hz), and fast beta (20-24, 24-28, & 28-32 Hz) frequency bands. Each band was converted to a percentage of the total spectra. Locomotor activity was analyzed by ANOVA and power spectral frequency bands by MANOVA. Data are presented as effects of circadian rhythm, menses cycle, progression of pregnancy and maternal behavior.

484.6

EFFECT OF ZINC MALNOURISHMENT ON DIURNAL PHASE SHIFT IN ADOLESCENT RHESUS MONKEYS. M.S. Golub*, L. A. Buck, M.E. Gershwin, and C.L.Keen. California Regional Primate Research Center, Univ. California, Davis, CA 95616.

Diurnal activity cycles were determined in female rhesus monkeys (22-39 months of age, premenarchal to sexually mature) as part of an evaluation of the effects of zinc deprivation on adolescence. An extension of the active period of the diurnal rest-activity cycle occurs in adolescence as reflected in longer nocturnal activity periods in rodents and later bedtimes in children. Monkeys were housed in indoor cage rooms with a 12:12 (lights on at 7 AM) light cycle. Activity was recorded for 2 consecutive 24-hr periods biweekly using a small commercial monitor consisting of a mercury switch with associated micro circuitry for data recording. The monitor was worn in a harness on the animal's back and could be removed to transfer data to a computer with custom software which generated plots of activity counts (switch closures) in 1 min intervals. Moderately zinc deprived (ZD) monkeys (n=4, 2 μg Zn/g diet) were compared to controls (n=4, 50 μg Zn/g diet). The extension of the daily active period during the 17 mo study period was 79 ±15 min for controls and 21 ± 20 min for ZD monkeys ($p = .059$). The increase in delay of phase shift (time to onset of nighttime rest period) was 132±14 min for controls and 51±24 min for ZD monkeys ($p = .028$). ZD monkeys also exhibited growth retardation and one ZD monkey had delayed sexual development. The data suggest that dietary zinc deprivation can influence functional brain maturation in adolescent monkeys. Supported by HD14388.

484.7

ONGOING AND INDUCED BRAIN RHYTHMS: MANIPULATION BY INVASIVE MAGNETIC PULSEFORMS. H. Stowell*, HRT Brain & Behavior, Milledgeville GA 31061-3420.

In 1994 a new biomedical instrument arrived on the USA market from South Wales U.K. It claims unprecedented control of a magnetic field, by frequency and amplitude modulation of magnetic pulses peaking at 30 MW, entrained as 8 pulses from a single coil or 4 from a pair of coils independently timed; the coils are designed to sit bilaterally over human & other primate heads. Interpulse spacing permits entrainment of up to 8 pulses within a bandpass of 10 Hz to 2 KHz, by steps of 0.0001 Hz, which includes all but the lowest frequencies of human ongoing & induced rhythms in the brain (OIRB) or EEG & event related brain potential (EEG-ERBP). Its makers say that an FDA-IDE is necessary for "commercial use", but in 1994 the FDA claimed never to have heard of either makers or instrument; neuroscientists familiar with basic research on OIRB, earlier claims for behavioral modification by weak UHF-carrier, low-frequency modulated irradiation of nonhuman brains, traditional ECT, and the morally indignant response of AAAS to the 1989 report of magnetic stimulation for epileptic relief [1], may be concerned at the claims and likely uses of this Magstim Quadropulse Magnetic Pulse Train Workstation.

Reference: Stowell H. Int J Neurosci 32, 34 (1987a,b) 861-874 & 117-122; ibid. 50 (1990a) 121-126; Epilepsy, electronics & entertainment. ibid. (1990b); SN Abstrs 15, 16 (1989 & 1990) 314.9 & 503.15.

484.8

DIURNAL VARIATION IN CEREBROSPINAL FLUID (CSF) NOREPINEPHRINE (NE) CONCENTRATIONS IN HEALTHY HUMANS. M.A. Kling*, M.D. De Bellis, D. Hu, T.D. Geracioti, D.S. Goldstein, E.H. Oldfield, and P.W. Gold. Clin. Neuroendo. Br., NIMH; Clin. Neurosci. and Surgical Neurology Brs., NINDS, Bethesda, MD 20892.

CSF concentrations of NE have been utilized extensively in studies of the pathophysiology of neurologic and psychiatric disorders. However, nearly all data are based on single time point measures of CSF NE obtained by lumbar puncture (LP). We report here the results of a study in which CSF NE concentrations were determined at hourly intervals over a 30-hour period in 7 healthy volunteers (4 male; ages 23-52 yr) undergoing continuous CSF sampling through indwelling lumbar catheters. In these volunteers, a 20-g epidural catheter was inserted in a low lumbar interspace through an 18-g Tuohy spinal needle under local anesthesia at 0900-1000 h. CSF was collected at a constant rate of 6 ml/h using a mini-roller pump; subjects remained flat in bed throughout the study. NE was measured by HPLC-EC in hourly aliquots (1 ml) for 30 hours beginning at 1100 h. A significant ($p < 0.05$) diurnal rhythm in CSF NE was observed in 6 of the 7 volunteers by cosinor analysis; the mean CSF NE level in the 7 volunteers also showed a sigificant diurnal rhythm ($p < 0.001$), with a fitted mean level of 99.1 pg/ml, a half-scale amplitude of 18.1 pg/ml, and acrophase at 11:55 am. These data are consistent with previous findings in patients who underwent LPs at different times of day. The finding of a diurnal variation in CSF NE has implications, not only for single time point studies of CSF NE, but also for the possible physiologic significance of CSF NE measurements in human subjects in states of health and disease.

484.9

RELATIONSHIP BETWEEN AROUSAL LEVEL AT DAYTIME AND SLOW WAVE SLEEP IN HEALTHY YOUNG ADULTS.

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It's reported that the long arousal state makes Slow Wave Sleep (SWS) increasing and that physical and mental stress don't change the total time of SWS but move SWS to the first period. The background activity (BgA) in electroencephalogram (EEG) is important to decide the arousal level. In the relationship between the amplitude of EEG and the arousal level, it is reported that alpha amplitude is low in excited or stimulated state and alpha amplitude is high in relaxed state. This time, we studied polysomnographic characteristics at night and BgA of EEG at daytime.

Subjects: After approval of the study protocol, five male subjects between 20 and 25 years of age were recruited. They were in good, mental and physical health as determined by medical history, psychiatric evaluation, physical examination, clinical laboratory tests, electrocardiograph and chest X-p.

Procedures: A subject slept over 3-nights in the laboratory. The first and second nights are adaptation. The third night polysomnograph (PSG) is used for the basic PSG. The EEG at 5:00 PM on third day is used for the BgA. PSG was analyzed according to the roles of Rechtschffen and Kales. We used the power spectrum program to analyze BgA. We examined Total sleep time, every stage latencies and every stage time. About the BgA, the peek of power spectrum, the mean alpha amplitude, the alpha 2/alpha 1 ratio (alpha 1; 8-10 Hz, alpha 2; 10-13 Hz) and % theta in frontal were examined.

Results: The amount of SWS related significantly to the mean alpha amplitude. The high alpha amplitude makes SWS increasing. These results suggest that relaxed state makes SWS increasing.

484.10

EFFECTS OF CL 284,846 AND TRIAZOLAM ON SLEEP EEG POWER SPECTRA IN SUBJECTS WITH PRIMARY INSOMNIA.

Y. Kim[1,2], H. Tachibana[2], K. Emori[2], G. Nino-Murcia[1] and C. Guilleminault[3]. [1]Sleep Medicine and Neuroscience Institute, Palo Alto, CA 94303; [2]Dept. of Neuropsychiatry, Toyama Med. & Pharmaceut. Univ., Toyama 930-01, Japan; [3]Sleep Disorders Clinic and Research Center, Stanford University School of Medicine, Stanford, CA 94305.

CL 284,846 is a non-benzodiazepine compound for N-[3-(3-cyanopyrazolo[1,5-a]pyrimidin-7-yl)phenyl]-N-ethyl acetamide which was developed by the American Cyanamid Company. In this study, we investigated, with polysomnographic recordings, the effect of two dose levels of a non-benzodiazepine, CL 284,846 10 mg (CL-10) and CL 284,846 40 mg (CL-40), and one dose of triazolam 0.25 mg (TRZ) versus placebo on the results of visual scoring and FFT analysis of sleep EEG in subjects with primary insomnia.

Eight patients with primary insomnia (ages 40-59 years) were randomized in a double-blind design and the effects of 0.25-mg triazolam (TRZ), CL-10 and CL-40 on the sleep EEG were examined. In comparison of visual scoring, the significant differences between 3 drug nights were sleep onset latency and total wake time. FFT analysis revealed that 3 drugs had significant effects on sleep EEG power spectra. CL-10 significantly increased mean power density per NREM epoch (mean NREM power) for 2.0-8.0 Hz band than TRZ. CL-40 significantly increased mean NREM power for 0.0-6.0 Hz band than TRZ, and significantly decreased mean NREM power for 6.0-10.0 Hz than CL-10. Quantitative electroencephalography aids in differentiating specific effects of hypnotics on EEG power spectrum.

This study was supported in part by a grant from American Cyanamid Company, Pearl River, NY, USA.

484.11

19-YEAR CHRONOBIOLOGIC STUDY OF A MIDDLE-AGED CYCLOTHYMIC MALE SUBJECT.

D.A. Goodman*, Newport Neuroscience Center, San Marcos, CA 92079-0803.

The long-term longitudinal study of single subjects is essential for human chronobiology. Generally, the n-of-1 study lasting decades is critical for the identification of long-duration infradian rhythms that might otherwise be undetected. In the present study, a white male, age 37, diagnosed as cyclothymic, in January 1977 agreed to keep a daily psychobiological journal of mental and physical states, also physiological health factors that could impact on biological rhythms. The subject agreed to restrictions in daily life that might increase the applicability of the findings, meaning that he lived alone, worked out of his own facilities, and avoided medications capable of altering biological rhythms. As of May 1, 1995, the subject has kept daily records for 6,694 consecutive days and nights, and has preserved archival data from more than 12,000 dreams.

Among the major findings from the 19-year study is the discovery that the subject's circatrigintan (about 28-day) rhythm observed during his 37th year over time increased monotonically in frequency until by the 55th year it easily overlapped the circadian. This shifting of a monthly rhythm to a daily rhythm (not the circadian) is of interest for two reasons: (1) Its use as a temporal reference makes possible statistical inference of future states; and (2) it establishes the value of longitudinal design, wherein data are collected serially to identify a novel infradian rhythm

484.12

RECOGNITION OF REM SLEEP WITH ARTIFICIAL NEURAL NETWORKS BASED ON PREPROCESSED PERIODS OF 2.5 SECONDS OF EEG ACTIVITY.

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For different scientific and clinical applications in psychiatry we have been working on a robust automatic online algorithm to detect Rapid Eye Movement (REM) sleep from single channel EEG data without using EMG or EOG information. The continous signal was analysed in time periods of 20 seconds in order to be compatible with the manual evaluation, which is based on time periods of at least this length. For data preprocessing every EEG segment was digitally filtered in 6 frequency bands (0.5-45, 0.5-3.5, 3.5-7.5, 7.5-15, 15-35 and 35-45 Hz) and the RMS values of the filtered signals were calculated. This vector of 6 real numbers served as input to an artificial neural network. One output neuron was to indicate the presence of REM sleep. In 2 groups of 5 resp. 8 healthy volunteers this system was able to correctly classify close to 90% of the time intervals not belonging to the training set.

Unfortunately this kind of data preprocessing is dissipating information about short events like sleep spindles, because the calculation of the RMS values is averaging the signals over 20 seconds. The use of a shorter time basis of 2.5 seconds was considered to improve the results. The alpha band was divided by adding a 7th filter in the range of 7.5-11 Hz. Additionally the 3 layer backpropagation neural network had to be extended 8 fold to 56 input neurons for the time periods to remain compatible with the manual evaluation. 6 hidden neurons and 1 output neuron together with all possible feedforward connections completed the architecture. As compared to the 20 second time basis the percentage of misclassified time periods could be reduced by more than 20% for data not belonging to the training set.

484.13

THE ANS ACTIVITIES IN ASSOCIATION WITH EEG SLEEP : Power spectrum components of heart rate variability highly correlated with delta-wave activity during sleep

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Several characteristics of the changes in sympathetic and parasympathetic activities during sleep have been indicated in relation to EEG based on a method of power spectral analysis of heart rate variability (HRV), suggesting a possibility of a simple (non-invasive) and objective method other than EEG to evaluate sleep quality, which is often needed especially in epidemiologic studies on sleep disturbance. Therefore, investigated was the most suitable index to reflect sleep quality indicated especially by deep sleep component out of various parameters obtained from spectral analysis of HRV. Data on EEG at parietal region (bipolar) and R-R intervals (bipolar chest leads) were collected from six healthy subjects aged 24-29 yrs. during sleep in an insulated experimental room with mostly light off from 23:00 to 8:00. Spectral power of HRV as well as EEG were calculated using fast Fourier transformation method, with which three major powers in the ranges of 0.005-0.05 Hz (VLF), 0.05-0.15 Hz (LF) and 0.15-0.50 Hz (HF) for HRV and one in the range of δ wave (0.5-4.0 Hz) for EEG were calculated. When correlations between amounts of δ component of EEG and each of the three components of HRV were examined for a series of 300 sec-divided records, the strongest correlation (r=0.59 for all subjects) was found between δ wave component and spectral power in the VLF range of HRV, although there was rather large individual variation. Moreover, a tendency that correlation is stronger in the lower frequency even among the VLF was also found, which might be related to the decrease in the slope of the regression line relating $1/f^t$ plots (log amplitude vs. log frequency) of the spectra of single neuronal spontaneous activity in variaous sites of cat's brain during δ wave sleep (Yamamoto et al in Brain Res. 366,1986).

485.1

ANGIOTENSIN II ANTAGONISTS BLOCK ETHANOL EFFECTS ON THE AERIAL RIGHTING REFLEX. H. Tracy, M.J. Wayner, D.L. Armstrong, and J. Polan-Curtain*. Division of Life Sciences, The University of Texas at San Antonio, San Antonio, TX 78249.

The purpose of the present study was to determine the effects of an angiotensin II (AII) AT_1 antagonist, losartan 20 mg/kg i.p., and the AII AT_2 antagonist, PD 123319, 20 mg/kg i.p. on ethanol intoxication as measured by the aerial righting reflex in male rats. 25% ethanol, 2.0 g/kg, was administered by stomach tube under mild metaphane anesthesia and the aerial righting reflex was determined at 30 min intervals for 3.5 hours. The AII antagonists were administered intraperitoneally 2 hours before the ethanol. There were 4 groups of 10 rats each, 235 to 335 g in weight; ethanol alone, losartan plus ethanol, losartan plus PD 123319, and losartan alone. Data were analyzed by a two-way ANOVA with repeated measures on one factor. Results show a clear intoxicating effect on the aerial righting reflex that was blocked significantly by losartan and to a greater degree by both losartan and PD 123319. Losartan alone had no observable effect. The administration of both antagonists completely blocked the ethanol effect on the aerial righting reflex. The involvement of AII in the mediation of ethanol intoxication effects on the aerial righting reflex supports results of our earlier studies on the effects of ethanol on open field behavior, retention of an inhibitory shock avoidance response, and on hippocampal granule cell long term potentiation; all of which can be blocked by losartan.

485.2

MODULATION OF ETHANOL-INDUCED MOTOR INCOORDINATION (EIMI) BY ADENOSINE (ADO) IN THE RAT MOTOR CORTEX. V. S. Barwick and M. S. Dar*. Department of Pharmacology, School of Medicine, East Carolina University, Greenville, NC 27858.

Previous studies in our lab showed that ADO uptake-blockers and antagonists enhanced and attenuated, respectively, EIMI. It was later found that EIMI was significantly enhanced and attenuated when ADO agonists and antagonists, respectively, were microinfused into the cerebral ventricles, striatum and cerebellum of rats and mice. The purpose of this study was to determine if ADO in the motor cortex would modulate EIMI. Using male rats, a unilateral guide cannula was implanted into the motor cortex. Each rat received a microinfusion of drug or artificial cerebrospinal fluid (ACSF) in a volume of 200 nl and ethanol (1.5 g/kg) or saline by i.p. injection. Microinfusion of the ADO A_1- and the A_2-selective agonists, CHA and CGS-21680, respectively, revealed significant and dose-dependent accentuation of EIMI when compared to the control group. CHA was 194-fold more potent in enhancing EIMI than CGS-21680 suggesting that motor cortical ADOergic modulation of EIMI may be mediated by the A_1 receptor. The A_1-selective antagonist, DPCPX, significantly blocked the effect of CHA on EIMI. The non-selective ADO agonist, NECA, also significantly accentuated EIMI with an ED_{50} higher than CHA but markedly lower than CGS-21680. Microinfusion of the ADO agonists followed by i.p. injection of saline had no effect on motor coordination suggesting a selective interaction between ethanol and ADO. To obtain further evidence for the role of the A_1 receptor in the modulation by ADO of EIMI, the effect of the R- and S-stereoisomers of PIA on EIMI were compared. R-PIA, the A_1-selective stereoisomer, was significantly more potent in modulating EIMI than S-PIA. From these data, it may be inferred that ADOergic modulation of EIMI in the rat motor cortex may be via the A_1 receptor. This receptor is known to be negatively coupled to adenylyl cyclase via a pertussis toxin (PT)-sensitive G-protein (G_i). Pretreatment of the motor cortex with PT significantly attenuated the effect of CHA on EIMI affirming a role for G_i and indirectly supporting the supposition that ADO modulates EIMI through the A_1 receptor.

485.3

STRESS CONTROLLABILITY BUT NOT PREDICTABILITY ALTERS THE ATAXIC POTENCY OF BOTH MIDAZOLAM AND ETHANOL IN THE RAT.
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Last year we reported that exposure to escapable but not inescapable shock is associated with the release of a benzodiazepine-like compound in brain. Other research has indicated that a B-carboline-like substance may be released following uncontrollable stress. The present work examines the functional significnace of these endogenous changes as they may influence subsequent drug reactivity at the benzodiazepine/GABA receptor complex (BGRC). Using a rotarod treadmill to assess motor ataxia, we find that uncontrollable but not controllable stress potentiates the motor incoordinating effects of both midazolam and ethanol in comparison to naive controls. Stressor predictability shows little effect on these indices We are initiating in vitro binding analyses to complement these behavioral findings. These findings suggest that stress control and prediction (safety signals) may be working through different mechanisms in brain. Finally, psychological dynamics of stress markedly alters subsequent CNS depressant drug reactivity which may have implications for drug abuse and dependence. Research supported by PHS grant MH 45475 to RCD.

485.4

ETHANOL SELF-ADMINISTRATION PRODUCES CHANGES IN CIRCADIAN AND ULTRADIAN RHYTHMS. D.G. Harper*, W. Tornatzky, J.C. Cole, and K. A. Miczek. Dept of Psychology, Tufts University, Medford, MA 02155.

Many physiological and behavioral effects of ethanol may be due to disturbed rhythms of homeostatic functioning. It is the purpose of the present experiment to quantify the ability of ethanol to shift circadian and ultradian rhythms in 8, adult male Long-Evans Rats. Animals were studied in a reversed 12:12 D:L (lights off at 08:00) environment and implanted with a telemetry sender (Data-Sciences) which monitored heart rate and temperature. Following recovery from surgery, animals were food restricted to maintain stable body weight and induced to drink ethanol via the sucrose fading technique. Animals self-administered 10% ethanol at 10:30am for 30 min/day for 3 weeks and were tested for entrainment effects. Animals were then offered ethanol at differing concentrations (5%, 10%, and 20% counterbalanced) each for 5 days. Finally, animals were withdrawn from ethanol for 3 weeks. Preliminary results indicate discrete effects for both food restriction and ethanol. Food restriction caused an increase in the amplitudes of circadian and 2 cycle/day rhythms as well as an adjustment in the phase of the 2 cycle/day rhythm of heart rate and temperature. Ethanol self-administration caused an entrainment effect on the phase of the 5 cycle/day rhythm of heart rate but not of temperature. Goodness of fit measures indicate a shift in heart rate rhythms away from circadian control and towards ultradian rhythms. Manipulating ethanol concentrations induced a dose dependent diminution of amplitude and dose dependent phase advance of the circadian temperature rhythm. These results demonstrate that animals can adjust their rhythms to accommodate salient events such as daily access to ethanol.

485.5

TASTE REACTIVITY TO ALCOHOL AND BASIC TASTES IN OUTBRED MICE. S.W. Kiefer* and K.G. Hill. Dept. of Psychology, Kansas State Univ., Manhattan, KS 66506-5302.

The taste reactivity test was adapted for mice to determine their gustofacial responses to alcohol and basic taste solutions. Outbred mice (n=10) were implanted with an intraoral fistula and then tested for taste reactivity to distilled water, 0.1 M sucrose, 0.1 M sodium chloride, 0.00001 M quinine hydrochloride, 0.01 M. hydrochloric acid, and to four concentrations of alcohol (3%, 6%, 9%, and 12%, v/v). Mice were given daily 1 min trials with a single solution at an infusion rate of 0.2 ml/min. Taste responses were scored as either ingestive or aversive. Results showed that the response profiles to the various solutions were quite similar. Mice made a substantial number of aversive responses to all solutions; the sucrose solution produced the lowest amount of aversive responding (X=13.5) whereas the other solutions elicited approximately 30 aversive responses. Ingestive responding was relatively consistent across solutions; there was a slightly higher rate of ingestive responding to sucrose and hydrochloric acid. The initial data from outbred mice suggest that these subjects do not show differential taste reactivity responses to a variety of taste solutions or to a range of alcohol concentrations. Supported by NIAAA grant RO3 AA09335.

485.6

OPERANT RESPONDING FOR ALCOHOL CHANGES ITS PALATABILITY IN RATS. K.G. Hill*, D.R. Remmers-Roeber, S.W. Kiefer, and J. Frieman. Department of Psychology, Kansas State University, Manhattan, Kansas, 66506-5302.

Male Wistar rats were tested for initial taste reactivity responses to water and a 10% alcohol solution. Subjects were then trained in an operant box to press a lever for 10% alcohol that was delivered through an intraoral cannula. Subjects were tested for taste reactivity responses to 10% alcohol after they reached a fixed-ration 5 (FR5) and fixed-ratio 10 (FR10) schedule. Ingestive responding to 10% alcohol was significantly higher after operant training. The average number of ingestive responses after operant training was nearly double those responses made prior to conditioning. There was no statistically significant difference between taste reactivity responding at FR5 and FR10. Aversive responding to alcohol was unchanged by the training procedure. Subsequent extinction trials failed to reduce ingestive responding. These data suggest that operant responding for 10% alcohol may alter its palatability by increasing its hedonic value.

485.7

BLOOD VERSUS BRAIN ETHANOL LEVELS AND ETHANOL-INDUCED LOCOMOTOR ACTIVITY IN DBA/2Abg AND C57BL/6Abg MOUSE STRAINS. T. Tritto*, J.A. Clarke, and B.C. Dudek. Dept. of Psychology, Univ. at Albany, SUNY, Albany, NY 12222

Dose-response studies have shown that while most mouse strains, such as the DBA/2 (D2) strain, show a large degree of locomotor activation in response to 0-1.5 g/kg of ethanol, other strains, such as the C57BL/6 (B6) strain, show little or no activation (Dudek & Tritto, 1994). Genetic studies suggest that this variation is under simple genetic control with only a few genes responsible. Although CNS sites of ethanol action may be important for such differences, brain and blood absorption profiles of ethanol require examination in the D2 and B6 strains. Locomotor activity was measured for 15 min following an i.p. injection of either 0 or 1.5 g/kg of ethanol using a computerized activity monitoring system, and blood and brain ethanol levels were measured immediately afterward. The D2 strain showed the typical large degree of behavioral activation at the 1.5 g/kg dose, while the B6 strain showed no activation. Blood and brain ethanol levels were nearly identical for both strains, with the brain ethanol levels being approximately 80% of those of the blood levels. These results demonstrate that the differences between these strains do not arise from variations in alcohol absorption rates. [This work was supported by NIAAA grants K02-AA00170 and R01-AA09038.]

485.8

ANALYSIS OF THE BIPHASIC LOCOMOTOR RESPONSE TO ETHANOL IN HIGH (HR) AND LOW (LR) RESPONDERS TO NOVELTY: A STUDY IN WISTAR RATS. M.A. Gingras * and A.R. Cools. Psychoneuropharmacology, Univ. of Nijmegen, P.O. 9101, 6500HB Nijmegen, The Netherlands.

The purpose of the study was to investigate the biphasic locomotor response to ethanol in rats. Given the recent finding that HR and LR differ in ethanol intake and preference (1), it was initially investigated to what extent HR and LR differ in locomotor responses to ethanol. A dose-response curve (0.2-2.0 g/kg,ip) was established using standardized activity cages. HR showed a significant increase at 0.5 g/kg, followed by a significant decrease at doses 1.0-2.0 g/kg; LR showed only a decrease at doses 1.0-2.0 g/kg. Secondly, it was investigated to what extent stress altered the ethanol-induced increase and decrease, respectively. For that purpose, the ethanol-induced locomotor effects (0.5 & 1.0 g/kg) were analyzed in habituated and non-habituated (stressed) HR. Stress significantly enhanced the excitatory effects in HR, but had no effect on the sedative effects in HR and LR. Finally, the locomotor effects of a subchronic treatment (7 days) with an excitatory (0.5g/kg) or sedative (1.0 g/kg) dose were analyzed in HR and LR. The excitatory effect of 0.5 g/kg disappeared throughout this treatment in HR, whereas the sedative effects of 1.0 g/kg did not change in HR and LR. Apparently, the mechanisms underlying the ethanol-induced locomotor increase fully differ from those underlying the ethanol-induced locomotor decrease. Given the known differences in the make-up of the brain and endocrine system between HR and LR (2), HR and LR are suggested to be good models for studying the mechanisms underlying the biphasic locomotor response to ethanol.

1) Gingras, M.A. and Cools, A.R. (1994) Society for Neuroscience Abstracts. pp. 1031. 2) Cools, A.R. et al. (1993) Neuropsychobiology. 28, 100-105.

485.9

OPERANT RESPONDING FOR ETHANOL IN DEPENDENT VS. NON-DEPENDENT RATS: A. J. Roberts and G. F. Koob*, Dept. Neuropharmacology, The Scripps Research Inst. La Jolla, CA. 92037.

Tolerance and dependence are potentially very important factors in the continued use of alcohol by alcoholics. Recently, our laboratory has been interested in producing models of ethanol (EtOH) intake in dependent animals. In this set of experiments, rats previously trained to lever press for oral EtOH and made dependent in alcohol vapor chambers were allowed to respond for EtOH over an extended period following withdrawal. In this way patterned operant responding for EtOH could be compared between dependent and non-dependent animals. Male Wistar rats were trained to lever press for 10% EtOH or water using a sweetened solution fading procedure. Rats were then housed for 2 weeks in EtOH vapor chambers with average blood alcohol levels of 133 ± 9 mg%. Control animals were simultaneously exposed to air. Every 4 days rats were taken out of the vapor chambers and placed in operant boxes for their entire 12 hr dark (active) phase. Each box contained a full food dish and levers equipped to deliver water and 10% EtOH. In each of 4 trials, EtOH vapor exposed (dependent) rats responded more than air exposed (non-dependent) controls between 6 and 8 hr following withdrawal. This corresponds to the expected time of peak withdrawal symptomology. In order to further examine responding in this time period, rats were withdrawn a 5th time, but left out of the operant boxes until hr 7. Dependent rats displayed visible signs of withdrawal at this time and again responded more than non-dependent rats for EtOH. Rats were then left out of the EtOH for 1 week and retested in order to determine whether response differences still existed in the absence of visible withdrawal signs. Again, rats previously exposed to EtOH vapor responded for EtOH to a greater degree than air exposed rats, however there was no obvious temporal pattern of responding. The results of this set of experiments suggest that rats experiencing acute withdrawal respond in a pattern consistent with the avoidance of withdrawal symptoms and that enhanced, but unpatterned, responding persists following abstinence. These two types of responding may represent models of EtOH intake associated with dependence and tolerance, respectively. Supported by grants AA08459 and AA06420.

485.10

CONCURRENT COCAINE (COC) AND ETHANOL (EtOH) EFFECTS: MICRODIALYSIS AND BEHAVIORAL STUDIES.
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The concurrent use of EtOH and COC has become a common abuse pattern. While both compounds administered alone produce reinforcing effects and increasing extracellular dopamine concentrations, little is known about their effects when administered together. The goal of this study was to investigate the effects in rats given IP injections of EtOH (0.1-0.5g/kg), COC (0.1-0.5mg/kg), or both given in combination, on concentration of monoamines in the nucleus accumbens and locomotor behavior. Monoamine levels were determined using high pressure liquid chromatography and electrochemical detection (HPLC-ECD). Open-field locomotor activity was measured using a computerized video system. Activity levels and monoamines were measured every ten minutes for 60 minutes. While EtOH and COC each elevated locomotor activity during the first 10 minute period, concomitant administration increased locomotion over the effects of either agent during the 10-30 minute time periods. Little effect was observed during the final 30 minutes of testing. Microdialysis measurement of dopamine levels in the nucleus accumbens showed increases consistent with behavioral changes. (NIDA #DA07237).

485.11

EFFECT OF ETHANOL ON SPATIAL AND NON-SPATIAL LEARNING IN AN 8-ARM RADIAL ARM MAZE: COMPARISONS WITH DIAZEPAM AND MK-801. J.L. Vandergriff, D. B. Matthews, P.J. Best and P.E. Simson. Department of Psychology, Miami University, Oxford, OH 45056.

In addition to slowing reaction times and impairing coordination, ethanol (ETOH) can also impair performance on a number of learning and memory paradigms. For example, acute administration of ETOH impairs the use of spatial memory (Matthews et al., 1995). Additionally, chronic administration of ETOH impairs learning of a spatial task (Ardent et al., 1988) that is sensitive to hippocampal damage (Olton and Papas, 1979). More recently, our laboratory has reported that ETOH selectively impairs the learning of spatial, but not non-spatial, tasks (Vandergriff et al., 1995).

In the present study, we compared the effect of ETOH on spatial and non-spatial learning to those of diazepam and MK-801, two drugs that have been likened to ETOH on a number of behavioral and electrophysiological measures. The paradigm utilized in the present work does not require progressive training techniques, and has been shown to be dependent on an intact hippocampus (Matthews and Best, 1995). Ethanol (0.75 - 2.5 g/kg, i.p.) impaired learning of the spatial task to a greater extent than the non-spatial task. Diazepam (0.5 - 2.0 mg/kg, i.p.) impaired learning of the spatial task, while slightly facilitating learning of the non-spatial task. The effect of diazepam on spatial and non-spatial learning thus bears remarkable resemblance to the effect of lesions of the fimbria-fornix on these learning tasks (Matthews and Best, 1995). Finally, MK-801 (0.1 - 3.0 mg/kg, i.p.) markedly impaired learning of both the spatial and non-spatial tasks at all doses tested. These results suggest that ETOH shares characteristics of both diazepam and MK-801 on spatial and non-spatial learning.

485.12

CHRONIC ETHANOL EXPOSURE ENHANCES PERFORMANCE OF ADULT RATS IN RADIAL ARM MAZE TASKS. E.S. Steigerwald and M.W. Miller*. Res. Serv., V.A.M.C., Iowa City IA 52242, and Depts. of Psychiatry and Pharmacology, Univ. of Iowa Coll. of Med., Iowa City IA 52242.

The effect of chronic exposure to ethanol on learning and memory was assessed by examining the ability of adult male rats to learn radial arm maze tasks that depended upon either extra-maze cues (visual-spatial) or intra-maze cues (various odors) for solution. Rats were fed *ad libitum* with a liquid diet containing 6.7% (v/v) ethanol (Et) or pair-fed an isocaloric diet (Ct). Eight subjects per treatment group were tested after being on the diet for 14 or 20 wk. Rats were maintained on this diet throughout the behavioral testing, but limited to a 2 hr feeding period. Rats were tested in both the visual-spatial and odor cue conditions twice daily for 26 days. The latency required to successfully navigate the maze, and the number of reference memory (RM) and working memory (WM) errors was recorded. Et-fed rats performed significantly better than Ct-treated rats in both testing conditions by making fewer RM errors and requiring a shorter latency to navigate the maze successfully. The Et-fed rats initially made fewer WM errors, but this pattern disappeared with repeated testing. The results were obtained in rats fed Et for 14 or 20 wk. Thus, moderate amounts of ethanol consumption appear to improve performance in some learning and memory tasks. Supported by the V.A. and N.I.H. (DE07734, AA06916, and AA07568).

485.13

ETHANOL IMPAIRS PERFORMANCE IN A NON-SPATIAL WORKING MEMORY TASK. K. McMahon*, T. Wallace, M. Ostrander and B. Givens. Department of Psychology, The Ohio State University, Columbus, OH, 43210.

Ethanol impairs spatial working memory in rats but its effects on non-spatial working memory have been difficult to assess. The difficulty in developing an operant model of non-spatial working memory has been in devising a task that does not allow any mediating behaviors during the delay. Trials began with either a signal (0.5 or 1.0 s center panel light), or a non-signal (absence of light). They were then required to match-to-sample (DMTS) in a choice phase, where one of two lights was illuminated. A lever was located beneath each light. Choice accuracy was significantly affected by delay (1 or 4 sec), but not by stimulus length (500 or 1000 msec). Following ethanol administration (0.25, 0.5, 0.75, and 1.0 g/kg), there was a dose-dependent change in choice accuracy and an interaction between dose and delay. Ethanol affected choice accuracy primarily at the 1 sec delay, with an increase at lower doses and a decrease at higher doses. These results demonstrate that performance in this task is both sensitive to the demands of working memory and is impaired by ethanol. A more detailed examination into the role of the prefrontal cortex in this task will be presented.

485.14

THE BEHAVIORAL AND NEUROPHYSIOLOGICAL EFFECTS OF ETHANOL ON SUSTAINED ATTENTION IN RATS. B. Givens*, K. Hutchinson,& K. McMahon. Dept. Psychology and Neuroscience Program, The Ohio State University, Columbus, OH 43210.

Investigations into the effects of acute alcohol intoxication on human performance consistently show deficits in continuous performance tasks that require sustained attention. Long-Evans rats were trained on an operantly-conditioned vigilance task that measures attentional abilities via manipulation of signal length (25, 50, and 500 msec) intertrial interval duration (9 ± 3 sec), and signal salience. Each trial began with the presence or absence of a signal (central panel light) followed 1 sec later with a tone which signaled lever activation. A correct response was a left lever press on a signal trial and a right lever press on a non-signal trial, and was rewarded with water. Ethanol (0.0, 0.5, 1.0, and 1.25 g/kg i.p.) increased reaction time, in a time- and stimulus length-dependent fashion. Reaction time for correct responses was substantially increased during the first half of the session, and were greatest at the shortest (25 ms) stimulus length. The only effect of ethanol on choice accuracy was a facilitation on signal trials but not on non-signal trials at the lowest dose (0.5 g/kg). The higher doses of ethanol (1.0 & 1.25 g/kg) increased omissions but this effect did not interact with signal length. Following ethanol testing, rats were surgically implanted with movable, fine-wire electrodes into the medial prefrontal cortex for single unit recording. Preliminary electrophysiological data suggest that only a small fraction of prefrontal neurons are engaged in performance aspects of the task. Neurons with evoked-activity were time-locked to the stimulus, and increased their activity during decreased signal salience, correlating with decreased choice accuracy. The results suggest that the prefrontal cortex may be involved in ethanol-induced changes in sustained attention.

485.15

GENETIC ANALYSIS OF ETHANOL-INDUCED HYPERGLYCEMIA USING RECOMBINANT INBRED BXD MICE. F. O. Risinger* and M. M. Brown. Oregon Health Sciences University, Portland, Oregon 97201-3098.

Sensitivity to ethanol's glycemic effect is influenced by genotype and may be related to the thermic and motivational effects of ethanol (Risinger and Cunningham, Alcoholism Clin Exp Res. 15, 1991; Life Sci. 50, 1992). In the present study, 16 recombinant inbred strains of the BXD/Ty series, derived from C57BL/6J (B6) and DBA/2J (D2) inbred mice, were acutely exposed to either 4 g/kg ethanol (IP) or saline (order of exposure counterbalanced). Tail blood samples were obtained immediately before injection and 120 min after injection. As expected, strains differed in the magnitude of ethanol-induced hyperglycemia (i.e., Strain x Drug Treatment x Time; $F(18,222)=3.1, p<0.01$). Further, a continuous range of hyperglycemia was noted in the BXD strains, including levels above and below those seen in the progenitor B6 and D2 mice. Comparison of strain means with mapped genetic markers (Quantitative Trait Loci analysis) revealed associations with a number of regions located on chromosomes 3,17 ($ps<0.01$) and 4 ($p<0.02$). This pattern of strain differences suggests polygenic mediation of sensitivity to ethanol's glycemic effects.

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485.16

SEX DIFFERENCE IN ETHANOL INTOXICATION IN FAWN HOODED RATS. C.M. Harris* and D.S. Ritchel. N.Y. Col. Osteo. Med., Old Westbury, NY 11568.

Male and female Fawn Hooded (FH) rats voluntarily consume ethanol (1.15 g/kg/day in our colony) whereas male and female Long Evans Hooded (LEH) rats do not. We gave these four groups ethanol, 15% w/v (or control) solution by gavage and rated intoxication severity on a 7-point scale. In two independent tests with ethanol, 2.6 g/kg (8/group) and 3.0 g/kg (5-7/group) scores differed significantly between groups (both p values <.005). FH females were least drunk and scores for FH males were intermediate between those of the two LEH groups consistently throughout 2 hrs of observations. In a 3rd test blood ethanol was measured and rank order for intoxication was replicated. Blood ethanol concentrations at 40 min after 3 g/kg (4/ group) were lower in the FH strain (M+/-SEM mg/dl: FH =113+/-15; LEH=177+/-10; p=.005), but did not differ between sexes for either strain. Results suggest that sex difference for intoxication in FH rats was due not to pharmacokinetic factors, but rather to different neural responsiveness. These results are significant because FH males drink voluntarily despite high sensitivity to blood ethanol. Supported by DA06873 (NIDA).

485.17

ACETALDEHYDE LEVELS IN ASIAN MEN WITH DIFFERENT ALDH2 GENOTYPES. T.L. Wall*, C.M. Peterson, K.P. Braun, M.L. Johnson, H.R. Thomasson and C.L. Ehlers. The Scripps Research Institute, La Jolla, CA 92037.

About half of Asian men and women have a genetically defined deficiency in the aldehyde dehydrogenase (ALDH2) isoenzyme responsible for metabolizing acetaldehyde, which results from the oxidation of alcohol. ALDH2 deficiency results from the inheritance of the mutant ALDH2*2 allele, a dominant mutation that disrupts the function of the subunit polypeptide. Asians possessing one or both alleles of ALDH2*2 drink less alcohol, are less likely to be alcoholic, and have more intense responses to alcohol, including a flushing reaction, than Asians homozygous for the ALDH2*1 allele, which encodes the active subunit polypeptide. It is hypothesized that acetaldehyde mediates the more intense alcohol response. Few studies have measured acetaldehyde levels in Asians who have been genotyped for ALDH2 and past studies have not controlled for recent use of alcohol and tobacco. This study assessed healthy 21-25 year Asian American men following oral administration of placebo and 0.75 ml/kg alcohol. Subjects with ALDH2*1/*1 genotype (n=12) were matched to subjects with ALDH2*1/*2 genotype (n=8) on demographics and recent drinking and smoking history. Blood alcohol and blood acetaldehyde levels were measured before and 15, 30, 45, 60, 90, 120, and 150 minutes following beverage administration. The group with ALDH2*1/*2 genotype had significantly higher blood acetaldehyde levels at 45, 60, 90, 120, and 150 minutes after drinking alcohol compared to the group with ALDH2*1/*1 genotype, despite equivalent blood alcohol levels. These results suggest that post-alcohol acetaldehyde levels may, in part, mediate enhanced sensitivity to alcohol and may contribute to the decreased likelihood of alcohol intake and protection from alcoholism among Asians. Supported by grants AA00098, AA00155, AA06420, and RR00833.

485.18

EVENT-RELATED POTENTIALS IN NATIVE AMERICAN CHILDREN C.Garcia-Andrade, E.Phillips, E.Bisson, E.L.Battenburg*, T.L.Wall and C.L.Ehlers. Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

The P300 component of the event-related potential (ERP) has been suggested to have predictive value as an index of vulnerability for alcoholism. Native Americans, as a group, are at higher risk for this disorder. ERP studies, however, have not been accomplished in this population. This study evaluated Native American children (NACh) between the ages of 10 and 13. Subjects were recruited from the Luiseño and Diegueño tribes in southern California. Data were available from 41 children, twenty-five (mean of age= 11.3 years) who were identified as Family History Positive (FHP) for alcoholism and 16 (mean of age= 11.2 years) as Family History Negative (FHN). None of the subjects had borderline IQs, abnormal EEGs, or first-degree relatives with major Axis I DSM-III-R disorders. FHP children had a father who met criteria for alcohol dependence. FHN children had no alcoholic relatives. Subjects were also evaluated by their percentage of Native American heritage (NAH). Fifteen subjects were ≥50% NAH (mean=68%) and 10 <50% NAH (mean=35%). ERPs were elicited using an auditory "oddball plus difficult discrimination" paradigm. EEG was analyzed from three derivations (Fz, Cz and Pz). Preliminary results showed a tendency for FHP children to have decreased P300 amplitudes [$F=3.16$, $<.08$] in the parietal lead (Pz) in response to the non-difficult target tone. When data were evaluated based on NAH (n=25), there was a significant difference observed between groups in several late ERP components. Longer latency N2 components, elicited by the difficult to discriminate tone, were found at Fz and Cz in the≥50% NAH group compared to the <50% NAH group ($F=6.6$ $p<.02$ and $F=11.9$ $p<.002$). Lower amplitude P300 components to the difficult to discriminate tone were also observed at Pz ($F=10.5$ $p<.004$) in the ≥50% NAH group compared to the <50% NAH group. These studies suggest that ERPs might provide measures that along with other biological and psychological factors may be coupled with heritability to alcoholism. (Supported by NIAAA, 00098, 00155, 06420, 10201, and RR00833).

485.19

QUANTITATIVE MICRODIALYSIS OF ETHANOL IN RAT BRAIN - COMPARISON OF THEORY TO EXPERIMENT. R.A. Gonzales*, T.L. Ripley, J. McNabb, and P.M. Bungay[1]. Univ. of Texas, Inst. of Neuroscience, Austin, TX 78712, and [1]Biomed. Eng. & Instr. Prog., NCRR/NIH, Bethesda, MD 20892-5766

Microdialysis is commonly used to study effects of drugs, including ethanol (EtOH), on levels of extracellular neurotransmitters *in vivo*. However, due to diffusional resistance, concentration gradients exist within the membrane and the tissue surrounding the probe, thus making tissue concentrations of specific analytes unknown. We have applied the steady-state theory of quantitative microdialysis (Bungay *et al.* Life Sci 46: 105, 1990) to simulate the EtOH concentration profiles when the probe is perfused with an EtOH solution. Microdialysis probes (3 mm long and 270 µm o.d.) were perfused *in vitro* at 1.65 µl/min in a stirred solution at 37°C. The measured steady-state extraction fraction (recovery), 0.41±0.05 (mean±SD, N=3), agreed with the predicted value of 0.41. *In vivo* experiments were conducted by placing probes into the striatum of rats which had been implanted with a guide cannula at least one week before. After perfusing overnight with artificial cerebrospinal fluid, the perfusing solution was changed to one that contained 1% EtOH (v/v) and samples were collected every 15 min for 1 hr. Following this period the rats were decapitated within 30 s and the heads frozen in liquid N_2. Dialysate EtOH and brain tissue EtOH content was determined by GC headspace analysis. The *in vivo* EtOH extraction fraction, 0.25±0.07 (N=5), together with the *in vitro* value, were used to make several predictions: EtOH concentration at the probe-tissue interface was 40-50% of the inflow concentration, the extent of diffusion into the tissue was less than 1 mm from the probe, and the diffusivity of EtOH in striatum is 8.3×10^{-6} cm^2/s. These predictions incorporate the effect of EtOH clearance by efflux to blood. The diffusivity estimate was then used to predict a value of 2.4 µg for the tissue content of EtOH in the region surrounding the probe, including correction for washout of EtOH during the post-perfusion delay. A comparable experimental value of 0.94±0.40 µg (N=3) was obtained. This work suggests that transcellular EtOH diffusion contributes substantially to rapid movement of EtOH through tissue. Work supported by NIAAA (AA08484 and AA00147) and the Alc. Bev. Med. Res. Found.

485.20

RAPID TOLERANCE AS A MODEL FOR CHRONIC TOLERANCE. J.M. Khanna* and H. Kalant. Dept. of Pharmacology, Univ. of Toronto, Addiction Research Foundation, Toronto, Canada M5S 1A8.

Development of tolerance is believed to increase alcohol consumption by reducing the aversive effects of alcohol relative to its rewarding effects. The most commonly studied form, called chronic tolerance, increases gradually over days or weeks of alcohol administration, and such studies are therefore slow and costly. Another form, called rapid tolerance, is seen in response to a second dose of alcohol given 8-24 hr after the effect of the first dose has disappeared. Rapid tolerance has been known for over 15 years, but its relation to chronic tolerance has not been studied systematically until recently (Khanna et al, Pharmacol. Biochem. Behav., 41:355-360, 1992). In the present work, we have extended these investigations by examining the effects of various pharmacological interventions (i.e. protein synthesis inhibition with cycloheximide, 5-HT modification with p-chlorophenylalanine and L-tryptophan, modification of NMDA receptor system and use of calcium channel blockers) on rapid tolerance development. All these interventions, which are known to affect chronic tolerance development, affected rapid tolerance in exactly the same way. These findings suggest that rapid and chronic tolerance are two expressions of the same alterations in brain function, and that experiments on rapid tolerance can therefore be used to predict the effects of drug therapies on chronic tolerance, at a great saving in time and costs.

485.21

Induction and maintenance of moderate blood alcohol levels by atraumatic pulsatile gastric infusions in "experienced" rats: transient effects on hypothalamo-pituitary-adrenal (HPA) activation. Rasmussen,D.D.*, Levin,N.°, Lawson,P.T., Wilkinson,C.W. Univ. of Washington and Seattle/American Lake V.A. Med. Center, Tacoma WA, 98493; °Genentech,Inc., South San Francisco CA, 94080.

Ethanol administration can stimulate HPA activity. However, paradigms commonly used to investigate this activation may elicit confounding non-specific stress responses. We have developed a model in which "experienced" rats (previously introduced to ethanol administration, so that the subjective response is not a novel stimulus) receive pulsatile intragastric ethanol infusions during the dark (active) photophase to produce and sustain (for 3 h) moderate (0, 50, 90-100), or 120-150 mg/dl) blood alcohol levels (BALs) in a range which has been demonstrated to be reinforcing. The lowest ethanol dosage (BAL: approx. 50 mg/dl) did not significantly alter plasma ACTH, corticosterone (CORT), or β-endorphin (βEND) concentrations at any time point evaluated (30, 60, 180, 360 min). BALs of 90-100 mg/dl increased plasma ACTH, CORT, and βEND concentrations at 30 min; these declined to control levels at 60 and 180 min, even though the BALs had been maintained. BALs of 120-150 mg/dl increased ACTH, CORT and βEND levels at both 30 and 60 min; ACTH and CORT declined to control levels by 180 min, whereas βEND levels remained moderately elevated. At 360 min (3 h after cessation of infusions), ACTH, CORT, and βEND levels for all treatments were comparable to controls. We are also assaying paraventricular nucleus corticotropin-releasing factor (CRF) mRNA concentrations in these same rats as an index of CRF neurosecretion. These results demonstrate that, under relatively non-stressful conditions, induction of moderate BALs acutely activates the HPA axis, but that this activation is transient, diminishing even when BALs are maintained. (NIH/NIAAA AA10567)

DRUGS OF ABUSE: ALCOHOL III

486.1

DIFFERENTIAL NEURONAL SENSITIVITY TO ETHANOL IN THE CEREBELLUM AND HIPPOCAMPUS, BUT NOT NEOCORTEX OF HAS AND LAS RATS. **R.K. Freund, B.J. Pearson, and M.R. Palmer*. Dept. of Pharmacology, Univ. of Colorado Health Sci. Ctr., Denver, CO 80262.**

We previously reported that cerebellar Purkinje neurons of low alcohol sensitive (LAS) and high alcohol sensitive (HAS) rats are differentially sensitive to the depressant effects of locally applied ethanol. In this study we determined if neurons in other brain areas also show a similar differential ethanol sensitivity. We studied electrophysiologically identified single neurons from layers V and VI of prefrontal neocortex, the CA1 region of the hippocampus and Purkinje neurons in the cerebellum from urethane-anesthetized LAS and HAS rats. Single neurons were recorded from one barrel of two-barrel micropipettes, and ethanol was locally applied by pressure ejection from the other barrel. We found higher ethanol sensitivity to the depressant effects of local ethanol applications in HAS rats than in LAS rats for cerebellar Purkinje neurons as well as both pyramidal and theta cells from hippocampus. The difference in the ethanol sensitivity of hippocampal pyramidal neurons between LAS and HAS was similar to that observed between cerebellar neurons in those two rat lines, whereas there was a much larger difference in the ethanol sensitivity of hippocampal theta cells. Neurons in prefrontal cortex, on the other hand showed a similar sensitivity to ethanol-induced depressions between the two rat lines. These data suggest that the neuronal ethanol sensitivity differences observed between LAS and HAS rats is not limited to the cerebellum, but is not found in all brain regions.

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486.2

ETHANOL ALTERS THE RESPONSE PROPERTIES OF NEURONS IN THE STRIATUM OF THE AWAKE, BEHAVING RAT. P.H. Janak*, M.G. Laubach and D.J. Woodward. Dept. of Physiology & Pharmacology, Bowman Gray School of Medicine, Wake Forest Univ., Winston-Salem NC 27157

Ethanol intoxication is known to impair the execution of sensorimotor behavior. To understand the underlying neuronal processes that are altered by ethanol, we studied the effects of ethanol on the spike activity of single neurons in the striatum of freely-moving rats during 1) open field behavior, 2) treadmill locomotion, and 3) reaction-time performance. Simultaneous recordings of the spike activity of 5-20 single units per subject were obtained from microwire electrode arrays chronically implanted into the striatum of adult male Long-Evans rats. Multiple measures of neuronal activity were compared following systemic ethanol or saline adminstration. Alterations in neuronal activity that accompanied the behavioral effects of systemic ethanol included dose-dependent changes in the firing rate and the firing mode (i.e., bursts versus single spikes) of striatal neurons, and alterations in the spatiotemporal patterns of firing within pairs and groups of neurons. In the drug-free animal, striatal neurons exhibit altered firing rates during one or two behaviors of the sequence that comprise task performance. A striking effect of ethanol was the emergence of responses by neurons during additional behaviors for which the neurons previously were quiescent. These observations suggest the hypothesis that ethanol may impair sensorimotor behavior by altering cortically-driven response properties of neurons in the striatum. *Supported by AA 07565 to PHJ, AA 05396 to MGL, AA 3901 to DJW. W.W.W. server: http://biogfx.bgsm.wfu.edu.*

486.3

EFFECT OF CHRONIC ALCOHOL CONSUMPTION ON SPECIFIC STRESS-AXIS RESPONSIVE REGIONS IN THE CENTRAL NERVOUS SYSTEM OF THE MALE RAT. C. Hamelink, R. Daoud, T. Torda, J. Presson*, T. Castonguay, and R. Eskay. Lab. of Clinical Studies, NIAAA, NIH, Bethesda, MD 20892

Chronic alcohol consumption (CAC) leads to degeneration of site-specific regions of the brain. Previous morphological analysis has revealed the hippocampus to be preferentially sensitive to the neurotoxic consequences of CAC. Preferential hippocampal damage also occurs over time in the presence of elevated glucocorticoids, which energetically weaken hippocampal neurons through a toxic cascade involving glutamate and calcium ions. Using the intragastric ethanol infusion model, blood alcohol levels were continuously maintained between 150-350 mg/dl for up to 4 weeks with a daily 2-10 fold ethanol-induced elevation of plasma corticosterone (CS) at the nadir of the CS rhythm. Considering that a change in corticosteriod receptor activity may reflect early neuronal degeneration in the hippocampus, we have analyzed steady-state glucocorticoid (GC) and mineralocorticoid (MC) receptor mRNA levels in specific subfields of the hippocampus. Initial findings in the dorsal hippocampus with in situ hybridization using antisense probes have revealed a 20-30% reduction of GC mRNA levels in the CA1, DG or CA3 subfield regions following 10 days of CAC. Similar reductions were observed in MC receptor mRNA levels in the hippocampal subfields. The effects of CAC appear not to preferentially endanger the CA3 subfield region of the hippocampus but additional analysis of prolonged (4 weeks) alcohol exposed brains will clarify this issue.

486.4

NEUROTOXICITY OF NITROPRUSSIDE AND ITS POTENTIATION BY CHRONIC ETHANOL IN BRAIN ORGANOTYPIC SLICES AND CEREBELLAR GRANULE CELL CULTURES. J.Y. Zou*, E.J. Neafsey and M.A. Collins. Neuroscience Program, Loyola University of Chicago Medical Center, Maywood, IL 60153.

Our *in vivo* study demonstrates that chronic ethanol-induced selective neuronal degeneration in entorhinal cortex and dentate gyrus in adult rats is not prevented by blockade of endogenous nitric oxide (NO) production using nitric oxide synthase inhibitors. The present study uses *in vitro* models to examine the relationship between chronic ethanol and excessive NO neurotoxicity. Entorhinal-hippocampal complex slices were prepared from 8-10 day old neonatal rats and cultured on interface membranes (4-6 slices per membrane) for 2 weeks. Cerebellar granule cell cultures were prepared from 8 day old neonatal rats and maintained in cultures for 8 days. Neurotoxic effects of NO generator sodium nitroprusside (SNP) in these cultures were assessed by measuring LDH release 24 hr after treatment. In controls, SNP (300μM) treatment for 30 min produced obvious neurotoxicity in these cultures as indicated by increased LDH release. Ethanol (100mM) exposure for 4 days significantly increased SNP-induced LDH release compared to the SNP group. SNP neurotoxicity in both groups was partially blocked by poly (ADP-Ribose) synthase (PARS) inhibitor dihydroxyisoquinoline, but not by benzamide. Concurrent measurements indicated that SNP-induced neurotoxicity was accompanied by a dramatic increase in cGMP production. Chronic ethanol exposure significantly increased basal cGMP level and SNP-stimulated cGMP production compared to corresponding controls in both brain slice and cerebellar granule cell cultures, indicating that chronic ethanol may up-regulate guanylate cyclase. These results suggest the possible involvement of excessive NO in chronic alcohol-related neuronal damage. Supported by Loyola NSAI Postdoctoral Fellowship.

486.5

ETHANOL INDUCES APOPTOTIC CELL DEATH IN SCHWANN CELLS COCULTURED WITH SYMPATHETIC NEURONS. M.I.Johnson*, J. Gilmartin, M. Collier, and M. Thompson. Dept. of Pediatr., Steele Mem. Childr. Res. Cntr., Univ. of Arizona, Tucson, AZ 85724-0001.

Fetal alcohol exposure may result in striking craniofacial abnormalities as well as microcephaly and CNS dysfunction. The microcephaly may be secondary to reduced numbers of both glia and neurons. In prior studies we have shown that ethanol enhances axonal elongation but reduces the number of both total and dividing Schwann cells even after exposure times of less than 24 hours and levels of ethanol as low as 9 mg%. In these experiments we utilize the TUNEL procedure to identify nicked DNA in the nuclei of Schwann cells undergoing apoptotic cell death. Rat sympathetic ganglia were cultured for 4-5 days in vitro and treated 19-22 hours with ethanol before fixation and application of the TUNEL procedure. Increased numbers of apoptotic Schwann cells were seen with ethanol exposure, particularly at distances greater than 1 mm from the explant edge. In one experiment the percent of total Schwann cells that were TUNEL positive at 1.25 mm was 2.7 ± 1.1 for controls and 4.7 ± 1.7, 19.3 ± 4.9, and 19.3 ± 5.5 for 50, 300, and 750 mg% ethanol respectively. (Mean ± SEM; $p < .004$ for both 300 and 750 mg%). The positive cells were often in clusters and just proximal to the tips of the axons. They frequently showed a very discrete positive area that appeared confined to the nucleus. In others the TUNEL positive material appeared to spill out of the nuclear area. In summary, preliminary experiments indicate that ethanol increases the number of apoptotic Schwann cells and this effect is dependent on both the concentration of ethanol and the distance from the edge of the explant. (Supported by ADC 82-2689, USPHS-NIH R01 AA08950, and Howard Hughes Medical Institute 71109-52130).

486.6

DENDRITIC MICROTUBULES IN THE DENTATE FASCIA ARE AFFECTED BY CHRONIC ETHANOL EXPOSURE D. Lang*, E. Fifková, M. Beno. Dept. of Psychology, University of Colorado, Boulder, CO 80309.

Ethanol-sensitive LS_{IBG} and ethanol-insensitive SS_{IBG} mice were exposed to ethanol diet (23.5% ethanol-derived calories) for 4 months. Half of the animals were sacrificed at this time and the other half was withdrawn from the ethanol diet for 1 month. Electron microscopy was used to estimate the density of dendritic microtubules in the dentate molecular layer (DML). In the LS line, the density of microtubules was significantly decreased in the middle and distal thirds of the DML during ethanol exposure. This change was normalized during withdrawal. In the SS line the microtubule density was decreased during ethanol exposure in the distal third only and was normalized during withdrawal. However, during withdrawal in the proximal and middle thirds a decreased and increased microtubule density, respectively was observed in the SS mice. The present observation on ethanol-related loss of neuronal microtubules is supported by ethanol-induced loss of microtubules in hepatocytes. Here acetaldehyde, the first metabolite of ethanol oxidation, forms stable adducts with tubulin dimers which markedly impairs microtubule assembly leading to a reduced number of microtubules (Tuma et al., Ann. NY Acad. Sci., 625:786,1991). In vitro acetaldehyde reacts with neuronal tubulin in a way similar to that of hepatic tubulin. The presence of acetaldehyde in the brain has been previously debated, however, recently acetaldehyde has been observed in the cerebrospinal fluid. This can be accounted for by alcohol metabolism that occurs directly in the brain (Anandatherthavarada et al., Brain Res., 601:279, 1993) as cytochrome P-450IIE1 oxidizing ethanol into acetaldehyde can be induced by chronic ethanol intake, both in liver and in brain. Thus, acetaldehyde could be responsible for the loss of microtubules observed in the reported experiments. This loss is in agreement with the ethanol-related loss of tubulin in the brain. Moreover decreased density of microtubules may account for the decreased dendritic arborization found following chronic ethanol treatment (Paula-Barbosa et al., Addiction, 88:237, 1993). Supported by grants AA061196 and AA00130.

486.7

CHANGES IN BRAIN ACTIN AND TUBULIN FOLLOWING CHRONIC ETHANOL TREATMENT E. Fifková*, H. Eason, K. Bueltman, J. Jóo, M. Morales. Dept. of Psychology, University of Colorado, Boulder, CO 80309 and Dept. of Neuropharmacology, Scripps Research Institute, La Jolla, CA 92037.

Ethanol-sensitive LS_{IBG} mice (15) treated for 4 months with ethanol diet were compared to control mice (9). Pools of actin and tubulin were prepared by Triton-X to solubilization of brain homogenates prepared under microtubule-stabilizing conditions (Black et al., Brain Res., 295:255, 1984). After centrifugation of the homogenate (H) the Triton-soluble fraction containing unpolymerized cytoskeletal component was recovered in the supernatant (S-1) and the Triton-insoluble fraction containing polymerized cytoskeletal component was recovered in the pellet (P-1). The P-1 fraction was resuspended in calcium - containing buffer and incubated at 4° C and centrifugation yielded a calcium/cold-insoluble cytoskeletal component in the pellet (P-2). In each fraction the total protein was established. Following protein separation on SDS-PAGE, the amount of α-tubulin and actin in each fraction was determined with a quantitative immunoblotting procedure (Black et al., J. Neurosci, 9:358, 1989). In the P-1 fraction both α-tubulin and actin were significantly reduced following ethanol treatment. In addition α-tubulin was also significantly reduced in the homogenate. Thus, both major components of the neuronal cytoskeleton are affected by chronic ethanol exposure. The loss of tubulin in the fraction containing polymerized components of the cytoskeleton corroborates our results showing a loss of microtubules following chronic ethanol treatment. Given that microtubules are essential for axonal transport, this loss could be implicated in the decreased axonal transport observed after chronic ethanol treatment (McLain, Alcohol, 4:385, 1987). In addition, the ethanol-related loss of actin an ubiguitous neuronal proteins implicated in cytoskeletal functions as well as in synaptic plasticity suggests that chronic ethanol significantly impairs basic neuronal functions. Supported by grants AA06196 and AA00130.

486.8

ETHANOL EFFECTS ON MEMBRANE RESPONSE PROPERTIES OF LAYER II-III NEURONS OF RAT SOMATOSENSORY CORTEX. **F.-C. Hsu, J. Zhai, T.N. Felder, R.C.-S. Lin, A.E.K. Kosobud, S.J. Wieland, M.C. Kennedy* and F.M. Sessler. Dept. of Anat. & Neurobiol., Med. Col. of Pennsylvania and Hahnemann Univ. Philadelphia, PA 19102, and Dept. of Psychol., Indiana Univ. IN 47405.**

In a previous report we described acute and chronic actions of ethanol (EtOH) on membrane function of layer V somatosensory cortical pyramidal neurons. These experiments demonstrated various actions of EtOH on neocortical circuits that may underlie its effects on information-processing. The present experiments were conducted to examine the effects of EtOH on another cell population, i.e., layer II-III pyramidal neurons, which is known: 1) to express high levels of NMDA receptors and 2) to be involved in corticocortical functions. Experiments were done using an in vitro brain slice preparation from rat somatosensory cortex. Microelectrodes containing either neurobiotin (2-3%) or lucifer yellow (5%) were used for intracellular recording and morphological characterization of individual layer II-III neurons. Postsynaptic potentials (EPSP and IPSP) were evoked by electrical stimulation of either layer IV or the underlying white matter. Bath application of EtOH (1-100mM) produced dose dependent suppressant effects on EPSP amplitude, EPSP area and decreased the probability of spiking in response to threshold and suprathreshold synaptic stimulation. Basic membrane parameters were not affected to the same extent. Some cells were more sensitive than others to low concentrations of EtOH (1-10mM) suggesting a differential susceptibility to alcohol effects within the neocortical cell population. These data indicate that EtOH can affect signal processing capability and corticocortical transfert mechanisms through a direct action on layer II-III vulnerable neurons. (Supported by NIDA DA08405 and Allegheny-Singer Research Institute Award to FMS).

486.9

LONG-TERM ETHANOL EXPOSURE OR GAMMA-HYDROXYBUTYRATE EACH REDUCE LACTATE DEHYDROGENASE RELEASE FROM RAT ORGANOTYPIC HIPPOCAMPAL SLICES. King, M.A., Carlan, D.E., and Walker, D.W.*, Department of Neuroscience and Brain Institute, University of Florida College of Medicine, and Veterans Administration Medical Center, Gainesville, FL 32610-0244.

Rats experiencing withdrawal from chronic ethanol demonstrate EEG hyperexcitability and transient increases in brain c-fos expression. These indices may reflect the occurrence of excitotoxic processes in the absence of overt seizures that could account for some of the brain pathology associated with chronic alcoholism. We used an organotypic interface slice culture model to assess cellular damage resulting from prolonged ethanol exposure or subsequent withdrawal, as reflected in the levels of lactate dehydrogenase (LDH) released into culture media.

Gammahydroxybutyrate (GHB) has recently been shown to ameliorate acute ethanol withdrawal symptoms in humans and rats. We also attempted to mimic whole animal and clinical effects of GHB on withdrawal. Slices from P7 Long-Evans rat pups, plated on Millipore culture plate inserts, were fed fresh media with or without 600 mg% ethanol (ETOH) daily for 7 days. Control and ETOH plates were then treated with fresh media containing ETOH, 20 mM GHB, both, or neither. Media were collected at 6 hrs for LDH assay. Ethanol and GHB alone each reduced LDH but were not additive. LDH in plates withdrawn from ETOH did not rise above control levels. GHB reduced LDH release in plates withdrawn from ethanol to levels observed in nonwithdrawal plates. This model system may be an efficient paradigm for the study of phenomena related to long-term ethanol exposure.

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486.10

CALCIUM HOMEOSTASIS IN CULTURED NGF-TREATED HIPPOCAMPAL NEURONS IS ALTERED BY ETHANOL. B.Webb*, S.S. Suarez, M.B. Heaton, and D.W. Walker. University of Florida, V.A. Medical Center, Gainesville, Fl 32610.

Acute ethanol treatment (AET) can cause changes in basal levels of intracellular calcium $[Ca2+]_i$ in hippocampal neurons. Nerve growth factor (NGF) may alter the effect of AET on calcium (Ca2+) homeostasis in hippocampal neurons. We cultured embryonic (E18) rat hippocampal neurons for 2 weeks. The cultures were treated with NGF (20 ng/ml) for 24 h and loaded with 2μm indo-1. Baseline $[Ca2+]_i$ measurements were taken. The buffer for each group was exchanged for buffer containing NGF and control (no ethanol (EtOH)) or 100,400, or 800 mg% EtOH. Fast responses were recorded for the first 6.4 sec and then at 10,30,45,60 min. Overall, $[Ca2+]_i$ increased in hippocampal neurons treated with 100 or 800 mg% EtOH ($p<0.0001$). No change in $[Ca2+]_i$ was observed with 400 mg% EtOH. When EtOH-treated cultures were compared to control, 100 mg% EtOH increased resting $[Ca2+]_i$ from 3.2 sec until 10 min while the increase in $[Ca2+]_i$ persisted for 60 min in neurons treated with 800 mg% EtOH. We compared this data with previous data from hippocampal neurons treated with similar levels of EtOH but not with NGF. NGF treatment did not alter $[Ca2+]_i$. NGF-treated neurons responded to 100 mg % ($p<0.0037$) or 800 mg% ($p<0.0001$) EtOH with greater increases in $[Ca2+]_i$ than did similarly treated non NGF-treated neurons. These results indicate that NGF enhanced the response of hippocampal neurons to AET, causing greater changes in $[Ca2+]_i$. Both NGF and EtOH affect cellular mechanisms involved in the regulation of Ca2+ homeostasis. EtOH may alter the ability of NGF to regulate $[Ca2+]_i$ or NGF may alter the sensitivity of hippocampal neurons to EtOH. These alterations in $[Ca2+]_i$ may be an underlying mechanism of EtOH neurotoxicity. Supported by NIAAA grants AA00200, AA09128, and Fellowship #1F31AA05372-01; and Medical Research Service Department of Veterans Affairs.

486.11

A CUT-OFF PHENOMENA FOR INHIBITION OF GLIAL INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) BY PRIMARY ALCOHOLS. P.J. Syapin* and J.D. Militante. Department of Pharmacology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Ethanol (C2) has been shown to dose-dependently inhibit glial iNOS expression in C6 glioma cells induced by treatment with phorbol ester + lipopolysaccharide. The inhibition appears transcriptional, but little is known about the underlying mechanism of action. The present study examined other primary alcohols for effects on iNOS activity. C6 glioma cells were exposed to primary alcohols (C1 to C8) during a 24 h induction period, and iNOS activity was estimated by nitrite accumulation, as described previously for C2 (Syapin, Alcohol Clin Exp Res, 19:262-267, 1995). Alcohols ≤ C7 inhibit nitrite accumulation in proportion to measures of their hydrophobicity (e.g., carbon chain length and membrane/buffer partition coefficient), however, no inhibition is observe with C8 (1-octanol), a more hydrophobic alcohol. Experiments with C10 are under way to confirm this cut-off phenomena. Although significant correlations between potency and hydrophobicity suggest that alcohols ≤ C7 inhibit iNOS in a manner similar to ethanol, experiments are being done to rule out a possible direct inhibition of enzymatic activity or indirect inhibition as a consequence of cytotoxicity. It is increasingly proposed that the actions of ethanol and other intoxicant-anesthetics are due to effects on hydrophobic regions of proteins, as opposed to membrane disordering effects on lipids. Our data supports the hypothesis that primary alcohols can interact directly with proteins to have biologically significant effects. (Supported by a Seed Research Grant and the Southwest Institute for Addictive Diseases)

486.12

ETHANOL ENHANCES RESTING AND STIMULATED SUPEROXIDE ANION PRODUCTION IN CULTURED MICROGLIA. C. Colton *, O. Chernyshev and J. Snell. Dept. Physiology, Georgetown University Medical School, Washington DC 20007.

Increased production of reactive oxygen species (ROS), i.e., superoxide anion, hydrogen peroxide, the hydroxyl free radical (OH•) and/or nitric oxide (NO), has been implicated as a causative factor in the tissue damage associated with ethanol-induced toxicity. Although a similar mechanism has been proposed for CNS damage, the source of ROS has not been clarified. Microglia, the CNS macrophages, are known to produce large quantities of ROS and are critical to normal development of the brain as well as the response of the brain to injury and infection. Using cultured neonatal hamster microglia, we have examined the effect of ethanol on microglial superoxide anion and NO production. Microglia were treated with 20 mM ethanol for 30 min, 3 hours, 24 hours and 48 hours and both resting (non-stimulated) and phorbol myristate acetate (PMA) -stimulated superoxide anion production examined for each time point. A significant increase in non-stimulated superoxide anion production was seen at 24 hours but not at the other time points studied. PMA-stimulated production was also significantly increased but only after 48 hours of continual treatment with 20 mM ethanol. Ethanol also altered the response of the microglia to lypopolysaccharide (LPS), a known inflammatory mediator. Treatment with both LPS and 20 mM ethanol for 24 hours produced a significantly grater resting release of superoxide anion than either LPS or ethanol alone. PMA stimulated release in ethanol and LPS treated microglia, however was not significantly greater. NO production was not induced by ethanol at any time point or concentration studied. These results suggest that microglia may be a significant factor in the production of oxydative stress induced by chronic exposure to ethanol.

486.13

INDUCTION OF RAT BRAIN CYTOCHROME P450 BY NICOTINE AND ETHANOL. R.B. Newton, R.M. France, L. Wecker*. Department of Pharmacology and Therapeutics, University of South Florida, College of Medicine, Tampa, FL 33612.

The ability of nicotine and ethanol to induce cytochrome P450 in the rat brain was investigated. Twenty-four rats were assigned to four groups of six animals each and treated with nicotine alone, ethanol alone, nicotine and ethanol, or saline and water. Nicotine (1.76 mg/kg) was administered subcutaneously twice daily for ten days using an equivalent dose of saline as control. Ethanol (0.5 ml of 50% solution) was administered as a single dose by gastric gavage 18 hours prior to decapitation with normal drinking water administered as a control. Total P450 levels were determined by spectroscopic analysis. Administration of nicotine alone for ten days twice daily resulted in a 2-fold increase in total brain P450 levels. Administration of a single dose of ethanol 18 hours prior to decapitation resulted in a 2-fold increase in brain cytochrome P450 as well. Chronic subcutaneous administration of nicotine twice daily for ten days followed by a single administration of ethanol by gastric gavage resulted in a 3-fold increase in total brain cytochrome P450. Immunoblotting of pooled samples indicated that both nicotine and ethanol increased cytochrome P4502B immunoreactive protein, and the combination treatment was equal to or greater than either treatment alone. These data indicate that treatment of rats with nicotine and ethanol alters brain cytochrome P450 levels and cytochrome P4502B immunoreactivity, effects that may alter the metabolism of other psychoactive compounds.

486.14

BASAL AND PHORBOL ESTER - STIMULATED PHOSPHORYLATION OF MARCKS IN SITU ARE INCREASED IN ASTROCYTES CHRONICALLY EXPOSED TO ETHANOL. T.L. Smith* and M.S. Bitrick, Research Service (151), Dept. of Veterans Affairs Med. Ctr., Tucson, AZ 85723

Chronic ethanol (E) exposure selectively inhibits metabotropic-glutamate receptor-stimulated polyphosphoinositide hydrolysis in astrocytes (Smith, Alcohol, 11:405, 1994). Because this receptor system is highly sensitive to modulation by protein kinase C (PKC), the aim of the present study was to determine whether chronic E affected PKC activity. PKC was monitored indirectly by determining the phosphorylation of MARCKS in situ. Type I astrocytes were grown in 96 well plates and exposed to either control media or media containing either 50 mM E or 100 mM E for 4 days. Cell wells were then rinsed with PBS and incubated at 25° for 12 mins. with a HEPES-buffered physiological salt solution containing: 50 μ/ml digitonin; 5mM EGTA: 100 μM MARCKS; 100 μM [^{32}P] ATP (10^5 CPM/pmol). Reactions were stopped with TCA. Aliquots were spotted on phosphocellulose paper and counted for radioactivity. Phosphorylation of MARCKS expressed as pmoles P/mg protein/12 mins were: control, 633 ± 18; 50 mM E, 909 ± 39; 100 mM E, 983 ± 53. Cells preincubated with 100 nM PMA for 15 mins: control, 2956 ± 280; 50 mM E, 4948 ± 677; 100 mM E, 5719 ± 152. The results suggest that the ability of PKC to phosphorylate MARCKS in situ is significantly increased in a concentration-dependent manner by chronic E exposure employed in the present study. (supported by a research grant from the Dept. of Vet. Affairs, Washington, DC).

486.15

ALCOHOL REGULATION OF HYPOTHALAMIC-PITUITARY-ADRENAL ACTIVITY AND EXPRESSION OF ARGININE VASOPRESSIN MRNA IN MALE RATS BEARING LESIONS OF THE PARAVENTRICULAR NUCLEUS. K. M. Ogilvie* and C. Rivier. Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037.

Exposure to alcohol results in profound perturbations of homeostasis accompanied by activation of the hypothalamic-pituitary-adrenal (HPA) axis. The hypothalamic peptide corticotropin-releasing factor (CRF) is an essential modulator of this response. Previous work in this laboratory shows that in male rats, electrolytic lesions of the paraventricular nucleus of the hypothalamus (PVN) attenuate, but do not abolish, ACTH secretion in response to acute alcohol administration. Since the PVN is the major site of CRF neurons that project to the median eminence, this observation suggests that other brain areas, and perhaps ACTH secretagogues other than CRF (e.g. arginine vasopressin (AVP)), mediate HPA activation by alcohol.

In a preliminary experiment, injection of AVP antiserum (0.4 ml, iv) prior to injection of AVP (1.2 µg/kg BW, iv) completely blocked ACTH secretion. Use of the same antiserum attenuated the ACTH response to alcohol (3 g/kg BW, ip) in sham-lesioned animals. Immunoneutralization of AVP also diminished alcohol-evoked ACTH secretion in PVN-lesioned animals, indicating that endogenous AVP from outside of the PVN partially mediates the HPA response to alcohol. Because of the role of AVP in alcohol-stimulated ACTH secretion, we sought to determine whether alcohol also regulates AVP mRNA levels in the supraoptic nucleus (SON) and PVN using standard *in situ* hybridization techniques. Sham-lesioned animals sacrificed 3 hours after administration of alcohol (3g/kg BW, ip) had increased PVN levels of AVP mRNA in comparison to saline injected controls. In the SON, alcohol decreased levels of AVP mRNA in PVN-lesioned animals but had no effect in sham-lesioned animals.

The results demonstrate that in addition to CRF of PVN origin, the mechanisms by which alcohol elicits ACTH secretion involve AVP secretion from brain areas outside of the PVN. However, the precise manner in which alcohol stimulates the AVP response, especially in PVN-lesioned animals, has yet to be established.

486.16

INFLUENCE OF EXERCISE AND ETHANOL ON ACETYLCHOLINESTERASE ACTIVITY AND LIPID PEROXIDATION IN BRAIN REGIONS OF RAT. K. Husain, L.P. Rybak, S.M. Somani, R.J. Elble*. Department of Pharmacology and Surgery, Southern Illinois University School of Medicine, Springfield, IL 62794-9230.

We have recently shown that exercise and ethanol increase lipid peroxidation (malondialdehyde-MDA) in the brain. AChE is lipid-dependent membrane bound enzyme. This study was sought to determine the effect of exercise and ethanol (Et) on AChE activity in specific brain regions. Male Fisher-344 rats were sacrificed after acute exercise (AE) (100% VO_{2max}), Et 20% (1.6 gm/kg po), AE+Et and sedentary control (SC). Brain regions were isolated and AChE and MDA were determined. The results indicate that AE decreased AChE activity in corpus striatum (CS) (40%), medulla (M) (23%) and cerebral cortex (CC) (17%) compared to SC, and MDA increased 112%, 13% and 57% respectively. Ethanol increased AChE activity in CC (24%), and M (20%) and MDA increased 73% and 36% respectively compared to SC. However, the combined effect of AE+Et resulted in an increase activity in CC (26%), and decrease in CS (24%) with increased level of MDA 56% and 53% respectively. These results suggest that AE, Et and combination of both modulate AChE activity differentially in specific brain regions with enhanced lipid peroxidation.

DEGENERATIVE DISEASE: PARKINSON'S—OTHER MODELS

487.1

CHARACTERIZATION OF A RAT MUTANT WITH SIMILARITIES TO PARKINSON'S DISEASE. R.W. Davies*, R.G. Sutcliffe, P. Shiels, M. Duran Alonso, D.J. Clarke†, D. Gilmore and A.P. Payne. †Department of Human Anatomy, Oxford University, Oxford. Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G11 6NU, Scotland, U.K.

A mutant strain of rat (AS/AGU; Albino Swiss/Anatomy Glasgow University) with many phenotypic features similar to human Parkinson's disease is being characterized genetically and biochemically. AS/AGU rats show marked and progressive movement disorder and a loss, at one year, of 60% of tyrosine hydroxylase positive (dopaminergic) cells in the substantia nigra pars compacta (SNc). Dopamine levels are reduced by 45% in the SNc and by 26% in the dorsal caudate nucleus (DCN). HPLC measurements of dopamine levels in various regions of the caudate-putamen (CPu) show regional localization of dopamine depletion, and a precise correlation with the inheritance pattern of the mutation. Neurotransmitter level differences in the raphé nucleus (5HT) and locus coeruleus (NA) have also been analysed. An extensive programme of genetic mapping is underway.

487.2

PARTIAL-DEPLETION RODENT MODEL OF PARKINSON'S DISEASE. M.D. Lindner*, D.F. Emerich, M.A. Plone, J.M.Francis, J.D. Salamone, and M.S. Hooks CytoTherapeutics Inc., Two Richmond Square, Providence, RI 02906

The discovery and development of new treatments with potential clinical efficacy in Parkinson's disease could be facilitated with a rodent model which simulates the clinical disorder more closely than the models currently available. Previous reports by Salamone et al. have demonstrated a pattern and degree of dopamine depletion analogous to the clinical disorder, and they have demonstrated a behavioral deficit with apparent clinical relevance (e.g. analogous to tapping speed). The present work replicates and extends their findings using rats with partial bilateral dopamine depletions by demonstrating that: (1) rats can be maintained following these partial bilateral dopamine depletions with low (0%) mortality, (2) the need for labor-intensive, time-consuming, manual forced-feedings following surgery is precluded by the use of sipper tubes and liquid diet, (3) clinically-relevant behavioral deficits can be quantified with simple, automated, fixed-ratio operant conditioning tasks, (4) these behavioral deficits are reliable, robust and long-lasting (>3mo). This model may prove useful in testing the effects of potential treatments including Sinemet and apomorphine.

487.3

FOREHAND ADJUSTMENT STEPPING IN DOPAMINE-DENERVATED RATS AS A MODEL FOR THE AKINESIA OF PARKINSON'S DISEASE. S. R. Wachtel*, D. Young and U. J. Kang. Dept. of Neurology, University of Chicago, Chicago, IL 60637.

A paradigm characterizing non-drug-induced forelimb movements as a model for the akinesia of Parkinson's disease has recently been introduced by Norton et al. (NS Abs 1992) and Olsson et al.(NS Abs 1993). Of the parameters monitored, contralateral forehand adjusting steps in rats with unilateral 6-hydroxydopamine-induced denervation demonstrated the most consistent and robust deficit. In the present study, forehand steps over a fixed distance was used as simplified measure to characterize this behavior as a function of striatal dopamine (DA) depletion. Forehand steps showed two distinct levels as a function of the degree of DA loss. Whereas subjects with DA depletion <80% did not display any significant change in the number of contralateral forehand steps (11.1±3.0), stepping was essentially eliminated (0.3±0.4) in all rats with DA depletion >80% ($p < 0.0001$). The stepping deficits were evident by one week and remained stable up to 2 months after the lesion. Furthermore, the decrease in steps was partially restored by a low dose of L-DOPA (6.25 mg/kg i.p.). A similar biphasic response was noted by a comparison of lesion size with contralateral rotations induced by apomorphine (0.1 mg/kg); however, the appearance of apomorphine-induced rotation required greater (>95%) DA depletion than the loss of forehand stepping. Thus, reduced stepping behavior identified more animals with partial lesions than drug-induced behavior. In summary, forehand stepping changes provide a very consistent and reliable indicator of DA depletion, and reverses with pharmacological restoration of DA.

487.4

EFFECTS OF COMPLETE AND PARTIAL LESIONS OF THE DOPAMINERGIC MESOTELENCEPHALIC SYSTEM ON SKILLED FORELIMB USE IN THE RAT. S. Parmentier, M. Mazadier, J.M. Miquet, A. Boireau, P. Dubédat, J.-C. Blanchard, and P. Barnéoud*. Rhône-Poulenc Rorer S.A., CRVA, Biology Department, 13 quai Jules Guesde, F-94403, Vitry-sur-Seine Cedex, France

This study compares certain behavioral consequences of partial and complete unilateral lesions of the dopaminergic mesotelencephalic system. We investigated skilled forelimb use, rotations induced by apomorphine and amphetamine, and dopaminergic metabolism of the nigrostriatal system of rats that had received a unilateral injection of 6-hydroxy-dopamine (2x6µg) into the medial forebrain bundle. The rats classified Apo(+), which rotated after the administration of apomorphine, had a complete lesion of the nigrostriatal system, whereas those classified Apo(-), which did not rotate after the administration of apomorphine, had a partial lesion of the nigrostriatal system. In the Apo(+) rats, 99.8% of the dopamine in the striatum was depleted, as was 85% of that in the substantia nigra. For the Apo(-) rats, 72% of the dopamine in the striatum was depleted as was 56% of that in the substantia nigra. When investigated with the staircase test, the animals with the most severe dopamine depletions were those most impaired in the paw reaching task. Complete and partial unilateral depletions of the dopaminergic mesotelencephalic system impaired the hierarchic phases of the paw reaching differently. A complete dopamine depletion, but not a partial one, decreased the number of attempts made with the contralateral paw, and induced a bias towards the ipsilateral paw. A partial dopamine lesion impaired the sensorimotor coordination of both paws, whereas the complete dopamine lesion had a greater effect on the contralateral paw than on the ipsilateral paw. The mild paw reaching impairments observed in animals with moderate depletions of dopamine are proposed as model of the early symptoms of Parkinson's disease that may be useful for the development of protective or restorative therapies.

487.5

DECREASES IN DOPAMINE RELEASE AND UPTAKE RATES IN THE PARTIALLY DENERVATED STRIATUM ARE PROPORTINAL TO THE LOSS OF DOPAMINE TERMINALS. P. A. Garris*, Q. D. Walker and R. M. Wightman. Department of Chemistry and Curriculum in Neurobiology, University of North Carolina, Chapel Hill, NC 27599-3290.

The present study tested the hypothesis that normal concentrations of extracellular dopamine can be generated in the partially denervated striatum without compensatory changes in dopamine uptake or release rates. One to four weeks after adult rats were unilaterally lesioned with 6-hydroxydopamine, fast-scan cyclic voltammetry at Nafion-coated, carbon-fiber microelectrodes was used to monitor extracellular dopamine levels in vivo, under urethane anesthesia. Simultaneous voltammetric recordings were collected in the lesioned striatum and contralateral control. Extracellular dopamine was elicited by bilateral electrical stimulation of the medial forebrain bundle. A 20 Hz stimulation evoked similar concentrations of extracellular dopamine in both lesioned and control striata, although tissue dopamine was decreased 30 to 70% in lesioned striata, as determined subsequently by HPLC-EC. However, kinetic analysis of the voltammetric recordings revealed that the concentration of dopamine released per stimulus pulse and V_{max} for dopamine uptake decreased in proportion to the magnitude of the lesion. These data support the hypothesis that normal extracellular dopamine levels can be generated in the partially lesioned striatum in the absence of active neuronal compensation. These results also suggest that passive mechanisms involved in the regulation of extracellular dopamine might play an important role in maintaining function during the preclinical phase of Parkinson's disease.

487.6

REGIONAL STUDY OF STRIATAL LEVELS OF DA UPTAKE SITES, DA D_1 AND D_2 RECEPTORS, DA D_2 AND PREPROENKEPHALIN mRNAs AND FOS IMMUNOREACTIVITY AFTER PARTIAL DOPAMINERGIC DEGENERATION IN THE RAT BRAIN. M. Savasta[1*], V. Blanchard[2], F. Javoy-Agid[2], R. Raisman[2], C. Feuerstein[1] and M. Chritin[1]. 1. INSERM-LAPSEN U.318, Pavillon de Neurologie, CHU de Grenoble, Grenoble, France ; 2. INSERM U.289, Hôpital Salpêtrière, Paris, France.

The presymptomatic situation of Parkinsonian patient has been reproduced in the rat by partially lesioning the nigrostriatal dopaminergic pathway. In this animal model, only the lateral part of the substantia nigra was destroyed by unilateral stereotaxic injection of 6-hydroxydopamine. Striatal levels of DA uptake sites, DA D_1 and D_2 receptors, DA D_2 and preproenkephalin (PPE) mRNAs and Fos immunoreactivity were studied one month after lesion by quantitative autoradiography, *in situ* hybridization and immunohistochemistry. Using an image analyser system, two striatal regions were differentiated: one corresponding to the denervated area and the other one being non affected by the lesion. Topographically, the disappearance of DA terminals as revealed by the ^{3}H-GBR 12935 (a marker of DA uptake sites) labelling, affects preferentially the lateral part of the striatum and concerns 30 to 70% of the total surface of this structure. Our results showed that in the denervated striatal subregion, no modification in D1 and D2 receptor densities or in D2 receptor and PPE mRNA levels was detected, comparatively to ipsilateral control values. On the contrary, a differential Fos immunoreactivity has been evidenced according to the location of the striatal cells: the number of Fos reactive neurons induced by amphetamine was significantly reduced (-25%) in the deafferented part of the striatum whereas this number remained unchanged in the intact one. This last observation suggests that striatal cells located in the denervated part of the striatum are sensitized to the partial denervation. In conclusion, the fact that we did not observe any hypersensitivity of DA parameters suggests the existence of compensatory mechanisms induced by remaining DA neurons. Such mechanisms allow the maintenance of striatal dopaminergic transmission at post-synaptic level and could probably contribute to delay the appearance of neurological symptoms in Parkinsonian patients.

487.7

ELEVATED STRIATAL FOS IMMUNOREACTIVITY FOLLOWING 6-HYDROXYDOPAMINE LESIONS IS MEDIATED BY EXCITATORY AMINO ACID TRANSMISSION.

A.J. Cooper*, S. Wooller and I.J.Mitchell School of Psychology, University of Birmingham, Edgbaston, Birmingham, U.K., B15 2TT

Pharmacological depletion of dopaminergic neurotransmission can result in an elevation in striatal Fos levels. This elevation may occur as a direct result of decreased dopaminergic neurotransmission or indirectly via elevated cortico-striatal glutamatergic neurotransmission which occurs secondary to dopamine depletion. To test the hypothesis that elevated N-Methyl-D-Aspartate-mediated corticostriatal transmission may underlie the increase in striatal Fos levels upon dopamine depletion, rats were unilaterally 6-hydroxydopamine lesioned and either allowed to recover for 6 hours or maintained under anaesthesia for this period induced by the NMDA antagonist, ketamine. This was followed by sacrifice and quantification of striatal Fos immunoreactivity. The results demonstrate that dopamine depletion following 6-hydroxydopamine lesioning can result in elevated striatal Fos levels which can be attenuated by contiguous treatment with an NMDA antagonist. This suggests that the increase in striatal Fos levels observed following dopamine depletion may occur as a result of elevated cytoplasmic calcium levels in the striatal cells.

487.8

EFFECT OF LEVODOPA TREATMENT ON STRIATAL TYROSINE HYDROXYLASE FOLLOWING PARTIAL LESION OF THE SUBSTANTIA NIGRA. G. Dziewczapolski[1], O. Gershanic[1], S. Vyas*[2], Y. Agid[2] and R. Raisman-Vozari[2]. (1) Instituto de Investigaciones Farmacologicas,CONICET, Buenos Aires, Argentina; (2) INSERM U 289, 75013 Paris, France.

It is controversial whether levodopa (L-DOPA) therapy influences the progression of Parkinson's disease (PD) because of its toxic potential to dopaminergic neurons. This is also a reason for delay in L-DOPA administration at least in the early stages of PD.

Partial lesions of the substantia nigra (SN) in rat provide a model of the early stages of PD. We have recently demonstrated a spontaneous recovery of striatal tyrosine hydroxylase (TH) six months after partial lesions of the SN with 6-hydroxy dopamine (6-OHDA) (Blanchard et al., J. Neurochem. 64, 1669, 1995). In the present study, we investigated the effects of chronic treatment with L-DOPA on TH on partially lesioned rats. Using immunoautoradiographic methods (Raisman-Vozari et al., J. Neurochem. 57,1212,1991)we quantified TH protein in the striatum (ST) from rats given L-DOPA + Carbidopa (170 and 17 mg/kg/day) for 7 months after partial (40-60% cell loss) unilateral 6-OHDA lesions of the SN.

In untreated lesioned rats (n=8), the decrease in striatal TH was 45.7 ± 4 % compared to the unlesioned side. Seven months after L-DOPA treatment, in lesioned rats (n= 7) the decrease of TH in the striatum was 15 ± 3 % compared to the unlesioned side. The exact mechanisms by which chronic treatment with L-DOPA increases TH in the ST in this animal model of PD remains to be clarified. However, our results demonstrate an absence of L-DOPA toxicity in the remaining dopaminergic neurons.

487.9

THE EFFECTS OF STRIATAL DOPAMINE DEPLETION ON CYTOCHROME OXIDASE ENZYME ACTIVITY IN THE RAT.

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Recent studies have demonstrated changes in mitochondrial enzyme activity and protein levels of constituents of the electron transport chain in selected brain areas in Parkinson's disease. Accordingly, we examined the activity of the mitochondrial enzyme cytochrome oxidase (CO) dorsolateral striatum and substantia nigra of rats and mice following treatment with MPTP, 3-acetylpyridine (3-AP), or 6-hydroxydopamine (6-OHDA)-induced striatal dopamine depletion. Our preliminary data suggest that 3-AP-treated rats sacrificed 7 days or 21 days after treatment did not show significant changes in CO activity in the striatum (CP) or substantia nigra (SN). Likewise, MPTP did not cause a significant change in CO activity in the CP or SN of C57BL6 mice sacrificed 21 days after treatment. Rats subjected to unilateral SN injections of 6-OHDA and sacrificed 21 days after treatment did not show a significant change in CO activity between the striata ipsilateral and contralateral to the lesion. These results contrast with our previous data demonstrating an increase in mitochondrial mRNAs for subunits of the electron transport chain following treatment with 6-OHDA, MPTP, or 3-AP. These data suggest that neurotoxin-induced alterations in mitochondrial gene transcription may not always be accompanied by a corresponding change in enzyme activity.

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487.10

NOREPINEPHRINE LOSS ENHANCES METHAMPHETAMINE-INDUCED STRIATAL DOPAMINE DEPLETION IN MICE AND RATS. F. Fornai*, M.T. Torracca, L. Bassi, F. Vaglini, R. Maggio, and G.U. Corsini. Institute of Pharmacology, University of Pisa, 56126 Pisa, Italy

Evidence is accumulating that norepinephrine depletion enhances the neurotoxic effect of the parkinsonism inducing neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). In this study we investigated whether norepinephrine loss potentiates methamphetamine-induced striatal dopamine depletion.

Injection of C57Bl/6N mice with methamphetamine (2 X 5 mg/Kg i.p., at two hour intervals) produced only a partial (50%) striatal dopamine depletion 7 days after drug administration. Pretreatment with the selective noradrenergic neurotoxin DSP-4 (50 mg/Kg, i.p.) enhanced methamphetamine-induced striatal dopamine depletion to 86%, without decreasing striatal dopamine levels when injected alone. Pretreatment of Sprague-Dawley rats with DSP-4 (50 mg/Kg, i.p.) did not decrease striatal dopamine levels when injected alone but produced a significant decrease in striatal dopamine levels when combined to a dose of methamphetamine (3 X 5 mg/Kg i.p., at two hour intervals) which did not change striatal DA levels when injected alone. There was a direct correlation between the degree of norepinephrine depletion in specific brain areas and the extent of enhancement of methamphetamine toxicity produced by DSP-4. Our results extend previous findings obtained with the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in DSP-4-pre-treated mice and confirm that norepinephrine loss might enhance neurotoxic damage to nigrostriatal dopaminergic neurons.

487.11

THE DOPAMINERGIC NEUROTOXICITY OF METHAMPHETAMINE IS PREVENTED BY NITRONE SPIN TRAPPING AGENTS. C. J. Schmidt* and V.L. Taylor Marion Merrell Dow Research Institute, 2110 E. Galbraith Rd., Cincinnati, OH 45215.

A large body of indirect evidence suggests that oxidative damage may play a major role in the degeneration of dopaminergic nerve terminals observed following high doses of methamphetamine (METH). When coadministered with iprindole, a single of METH (20 mg/kg, s.c.) reduced striatal dopamine concentrations in the rat by 40 to 50% after one week. Pretreatment with the nitrone spin trap, α-phenyl-*tert*-butylnitrone (PBN) or the novel cyclic nitrone, MDL 101,002, prevented the METH-induced decline in dopamine concentrations. Previous research has demonstrated that neuroprotection in this model may be achieved by agents which interfere with METH-induced hyperthermia. Temperature studies were therefore conducted to determine if such a mechanism could account for the protective effects of the nitrones. Acute administration of METH (20 mg/kg, s.c.) resulted in a significant hyperthermia of 2 to 2.5 °C above baseline which lasted greater than 6 h. PBN alone (100 mg/kg, i.p.) produced a modest hypothermia (-1 to -1.5 °C) of 4 to 5 h duration but did not affect the hyperthermic response to METH. Although not neuroprotective, the same dose of MDL 101,002 produced a hypothermic response of similar duration to PBN but of greater magnitude (-2.5°C). The neuroprotective dose of MDL 101,002 (300 mg/kg, i.p.) produced a profound hypothermia (-3.5 to -4 °C) but like PBN, did not alter the effect of METH. The results support the hypothesis that free radical damage is an important component of METH-induced neurotoxicity and further indicate that nitrone spin trapping agents are centrally effective antioxidants.

487.12

ANIMAL MODELS OF PARKINSONIAN SYMPTOMS: THE ANTICHOLINESTERASE TACRINE INDUCES TREMULOUS JAW MOVEMENTS IN RATS. A.J. Mayorga*, D. Carriero, G. Gianutsos and J.D. Salamone. Dept. of Psychology, U. of Connecticut, Storrs, CT 06269-1020 USA

Tacrine (Cognex) is an acetylcholinesterase inhibitor that is being widely used as a treatment for Alzheimer's disease. Some of the reported side effects of tacrine include parkinsonian symptoms such as tremor, akinesia, rigidity and gait disturbance. For these reasons, the effects of tacrine on tremulous jaw movements in rats were investigated. Intraperitoneal injections of tacrine induced tremulous jaw movements in the dose range of 1.25-10.0 mg/kg. These movements, like those produced by physostimine and pilocarpine, tended to occur in rapid, repetitive bursts of jaw movement characterized by vertical deflections of the lower jaw. The jaw movements induced by 5.0 mg/kg tacrine were blocked by injections of the muscarinic antagonist scopolamine. These results indicate that tacrine can produce signs of extrapyramidal motor disturbance, which is consistent with some of the reported side effects of this drug. In addition, these results are consistent with the extensive literature indicating that muscarinic cholinergic stimulation can lead to parkinsonian symptoms, including tremor. Investigations of tremulous jaw movements in rats may be useful as a tool for investigating the neurochemical basis of the motor side effects of tacrine.

487.13

ANIMAL MODELS OF PARKINSONIAN SYMPTOMS: EFFECTS OF ACUTE AND REPEATED CLOZAPINE INJECTIONS ON CHOLINOMIMETIC-INDUCED TREMULOUS JAW MOVEMENTS. J.D. Salamone*, and E. Chesler, Dept. of Psychology, U. of Connecticut, Storrs, CT 06269-1020 USA

Three studies were undertaken to investigate the effects of the atypical neuroleptic clozapine on the tremulous jaw movements induced by cholinergic stimulation in rats. In the first 2 studies, acute clozapine injections (2.0-16.0 mg/kg) produced a dose-related suppression of the tremulous jaw movements induced by either 0.4 mg/kg physostigmine or 4.0 mg/kg pilocarpine. The third study compared the effects of acute and repeated clozapine injections. Either acute or repeated injections of 16.0 mg/kg clozapine reduced tremulous jaw movements relative to rats that received pilocarpine alone, and the two clozapine-treated groups did not differ from each other. Sedation ratings indicated that acute injections of clozapine produced substantial drowsiness and sedation, whereas rats that had received clozapine for 14 days did not show substantial sedation. These results indicate that clozapine can suppress cholinomimetic-induced tremulous jaw movements, and that the suppression of pilocarpine-induced jaw movements is not merely an artifact of clozapine-induced sedation. Because pilocarpine-induced tremulous jaw movements share some characteristics with human parkinsonian symptoms, the present results are consistent with reports indicating that repeated injections of clozapine produce anti-parkinsonian effects.

487.14

ANIMAL MODELS OF PARKINSONIAN SYMPTOMS: L-DOPA IMPROVES SKILLED MOTOR FUNCTION IN RATS WITH VENTROLATERAL STRIATAL DOPAMINE DEPLETIONS. M.S. Cousins* and J.D. Salamone, Dept. of Psychology, U. of Connecticut, Storrs, CT 06269-1020 USA

Previous studies indicated that ventrolateral striatal (VLS) dopamine (DA) depletions produced profound motor deficits that interfered with lever pressing. DA depletions induced by VLS injections of 6-OHDA significantly reduced lever pressing on a fixed ratio 5 (FR5) operant schedule. This deficit was largely due to a dramatic increase in the average response initiation time, which was defined as the time from offset of one lever press to the onset of the next one. Analysis of the distribution of response initiation times indicated that VLS DA-depleted rats made relatively few responses with fast initiation times, and also that VLS DA depletions led to a dramatic increase in the number of pauses in responding. The observed lever pressing deficits produced by VLS DA depletions were partially reversible with L-DOPA. Systemic administration of 40.0 mg/kg L-DOPA significantly increased the number of responses in DA-depleted rats, whereas both 20.0 and 40.0 mg/kg L-DOPA partially reversed the response initiation deficits. The deficits in response initiation and execution produced by VLS DA depletions in rats could be related to the impairments in skilled motor usage that are characteristic of patients with Parkinson's disease. This paradigm may be useful for examining other potential antiparkinsonian agents such as D1 and D2 agonists, or cholinergic and excitatory amino acid antagonists.

487.15

ANIMAL MODELS OF PARKINSONIAN SYMPTOMS: FURTHER CHARACTERIZATION OF TREMULOUS JAW MOVEMENTS IN RATS. M. Finn*, A. Jassen, P. Baskin, E. Chesler, M. Cousins and J.D. Salamone, Dept. of Psychology, U. of Connecticut, Storrs, CT 06269-1020 USA

Vacuous jaw movements are vertical deflections of the lower jaw that resemble chewing but are not directed at any stimulus. Because vacuous jaw movements in rats can be produced by dopamine (DA) antagonism, striatal DA depletion, and muscarinic stimulation, it has been suggested that vacuous jaw movements share some characteristics with parkinsonian symptoms. In order to determine if vacuous jaw movements are tremulous in nature, rats were videotaped in Plexiglas tubes for observation of jaw movements. The videotapes were observed at 1/6 normal speed, and the observed jaw movements were recorded by a computer program that measured the interresponse time of each jaw movement. These methods were used to study the jaw movements induced by 2.0 and 4.0 mg/kg pilocarpine, as well as the movements induced by depletion of DA. Analysis of interresponse times indicated that most of the jaw movements occur in the same frequency range as parkinsonian tremor (3-7 Hz). Additional studies showed that vacuous jaw movements can be induced by ventrolateral striatal (VLS) DA depletions, and that DA depletions in the ventral portion of the VLS were correlated with both jaw movements and feeding deficits. Haloperidol facilitated jaw movements in DA depleted rats on days 5 and 15 after surgery. The "vacuous" vertical jaw movements induced by cholinomimetics or DA depletion may be useful as a model of parkinsonian tremor.

487.16

RETROGRADE NEURONAL DAMAGE CAUSED BY LYSO-PHOSPHOTIDYLCHOLINE: ROLE OF METHYLATION IN NEURODEGENERATION. C. G. Charlton*. Florida A&M University, College of Pharmacy, Tallahassee, Florida 32307.

The CNS injection of S-adenosyl-methionine (SAM) causes Parkinson's disease-like changes. The movement disorders may be caused by dopamine (DA) loss. The cause of SN damaged, however, is unknown. Products of methylation, like methyl-beta-carbolines were indicated, but other agents, like methylated phosphalipids (PL), may be involved. Lyso-phosphatidylcholine (lyso-PTC) is a likely candidate, because it is a potent cytotoxic secondary product of SAM, that damages cell membranes and fibers and reduces DA receptor binding. To know if lyso-PTC may be involved in SAM-induced cell damage, the PL was injected into the brain of rats. Injections into the ventricle cause tremor, teeth chattering, hypokinesia, and dilation of the ventricles. Following injection into the caudate nucleus, neuronal membrane damage, gliosis, axonal swelling, tangle-like debris and filamentous remains of blood microvessels were seen close to the injection sites. Degeneration was traced, retrogradely, to the substantia nigrs (SN), where Nissl staining showed degenerated neurons and silver staining identified damaged axonal stumps. PL methylation increases membrane solubility, and lyso-PTC was reported to be involved in ischemic cell death, therefore, methylated PL, which represent about 19% of dry cell mass, may play a role in neuronal damage and in cerebrovascular accidents. Supported by NIH RR 03020 and RO1 28432 and 31177.

487.17

PARAQUAT ELICITED PARKINSON-LIKE EFFECT IN RATS: BEHAVIOR, NEUROCHEMISTRY AND HISTOLOGY. H.H. Liou[1]*, M.C. Tsai[2], Y.F. Tsai[3], W.P. Chen[1], Y.C. Chang[1] and R.C. Chen[1]. [1]Department of Neurology, [2]Pharmacology, [3]Physiology and [1]Anatomony, National Taiwan University, Taipei 100, Taiwan, ROC.

Epidemiologic studies point to a connection between paraquat exposure and parkinsonism. To evaluate a potential role for the paraquat, male Wista rats were unilateral intranigral injection of paraquat (1-5 μg). This study showed: (1) behaviorally, apomorphine induced dose-related rotational behavior in rats contralaterally to the lesioned side 2 weeks after paraquat treatment. (2) neurochemically, ipsilateral striatal dopamine level was decreased to 91.5 % after 3 μg paraquat-treated. Paraquat also produced a dose-dependent depletion of dopamine in the ipsilateral striatum of rats 2 weeks following treatment. The decreased striatal dopamine is long-lasting and irreversible. (3) morphologically, marked decreased Nissl bodies and prominent gliosis in the substantia nigra were observed 2 weeks after paraquat injection. Whereas 3 μg paraquat caused a severe necrotic lesion in the injected area. The behavioral, neurochemical and histological results are identical to those observed following intranigral injection of 1-methyl-4-phenylpyridinium ion (MPP+). The structure resemblance of paraquat to MPP+ makes the possible correlation between paraquat and Parkinson's disease more intriguing. The present results indicated that intranigral injected paraquat could possess a marked neurotoxicity and induce nigrostriatal dopaminergic system degeneration in rats. These findings suggested that paraquat should be investigated as a potential etiological agent of parkinsonism. (Supported by NSC-84-2331-B002-280, Taiwan)

487.18

TRANSGENIC MICE EXPRESSING HUMAN MONOAMINE OXIDASE B DRIVEN BY THE NEURON-SPECIFIC ENOLASE PROMOTER. P. Malherbe*, J. Gottowik, E. Borroni, J.G. Richards, A. Cesura, C. Kühn, R. Pink, H. Blüthmann and M. Da Prada. Pharma Division, Preclinical Research, F. Hoffmann-La Roche Ltd, CH-4002 Basel, Switzerland

To gain insight into the possible role of monoamine oxidase B (MAO-B) in dopaminergic cell death in Parkinson's disease, we have generated lines of transgenic mice in which neurons overexpress the human MAO-B protein under the control of the neuron-specific enolase promoter. The transgenic mice express 4-6 fold higher MAO-B activity in brain as compared to non-transgenic littermates, whereas the activity of MAO-B in the liver was equal to that of control animals. Analysis of brain monoamines in 5-8 weeks old transgenic mice showed a 30-40% increase in the basal levels of the dopamine metabolites DOPAC and HVA, whereas no changes were observed in the basal levels of dopamine (DA). Further analysis of brain regions showed an increase in the levels of DOPAC and HVA in the substantia nigra and striatum of transgenic mice, whereas no changes in neurotransmitter level were seen in the locus coeruleus and frontal cortex. The transgenic mice metabolise approximately 20% more DA than control animals under basal conditions. Thus, the increased rate of dopamine metabolism observed in mice overexpressing human MAO-B is likely to result in an increased oxidant stress since the conversion of DA to DOPAC, catalysed by MAO, is stoichiometrically linked to the production of hydrogen peroxide. Therefore, these animals could serve as an invaluable tool to study the ethiology of Parkinson's neuropathologies in brain.

487.19

TYROSINE HYDROXYLASE IMMUNOREACTIVITY IS PRESERVED IN TWO SUBSETS OF NIGRAL NEURONS IN CULTURED RAT MIDBRAIN SLICES T. R. Mhyre*, S. N. Haber, and J. Holliday. Dept. of Neurobiology & Anatomy, Univ. of Rochester Medical Center, Rochester, NY 14642

The complexity of the nervous system presents unique problems in studying normal and pathologic processes in both *in vivo* and standard *in vitro* systems. A technique combining the relative ease of manipulation of culture experiments with the more physiologically relevant aspects of whole animal studies, therefore, is a highly desirable research tool. We wanted to devise a method that would allow midbrain slices to remain viable in culture for at least 3 days and at the same time maintain the appropriate nigral neuronal phenotypes.

Adult female Sprague Dawley rats received bilateral, intra-striatal injections of the retrograde tracer, dextran-conjugated lucifer yellow (LY). The animals were sacrificed after 7 to 8 days and 200 μm thick midbrain vibratome sections were cut. Slices containing the substantia nigra (SN) were then cultured on polycarbonate filters in depolarizing medium. Following fixation on 0, 1, 2, and 3 days *in vitro* (DIV), the slices were resectioned (25 μm thickness) and stained for tyrosine hydroxylase (TH) or calbindin D (Cal D) immunoreactivity (IR). Fluorescence microscopy demonstrated that the retrogradely labeled neurons of the SN retained the LY through 3 DIV and were also double labeled for TH IR. However, only a subset of retrogradely labeled neurons double labeled with Cal D IR, a marker for dorsal tier neurons of the SN.

These results demonstrate that midbrain slices 1) survive in culture for at least 3 days and, more importantly, 2) appropriate dorsal tier and ventral tier neuronal pools are maintained in these cultures. Thus, this system provides a unique model for studying mature, subset-specific neurons in the SN without the inherent problems of standard *in vitro* and *in vivo* experiments. Supported by funds from the Patterson Trust and Rochester Area Pepper Center.

487.20

HEMIPARKINSON RATS DISPLAY ACTIVE SUPPORT IN THE GOOD LIMBS VS PASSIVE SUPPORT IN THE BAD LIMBS ON A SKILLED REACHING TASK OF VARIABLE HEIGHT. E. I. Miklyaeva* and I. Q. Whishaw. Dep. of Psychology, Univ. of Lethbridge, Lethbridge, AB, Canada, T1K 3M4

Control rats and rats with unilateral DA-depletion, produced by 6-hydroxydopamine (6-OHDA) injected into the nigrostriatal bundle, were tested in a skilled reaching task. The height to be reached was varied in order to force the rats to use their supporting limbs to assist them in reaching. Control rats used the contralateral-to-reaching rear limb to stretch their body at high heights and the contralateral-to-reaching forelimb to lower their body at low heights. The DA-depleted rats failed to use their bad hind limb to extend their reach at high heights and failed to lower their body with their bad forelimb in order to reach at low heights. Consequently, they failed to obtain a reward at the height extremes. At intermediate heights, for which use of the bad limbs was not essential, their reaching performance approximated that of the control rats. It is suggested that extensive DA-depletion may result in the loss of the ability to apply forces with affected limbs for both voluntary movements and for postural adjustments.

DEGENERATIVE DISEASE: PARKINSON'S—CLINICAL

488.1

PARKINSON STUDY GROUP DNA REPOSITORY. C.M. Tanner♠, C.W. Shults★, P. Chan*♠, J.S. Fink♦, M. Kurth+, K.L. Marek▾, L.W. Elmer# and Parkinson Study Group. ♠ The Parkinson's Inst., Sunnyvale, CA; ★ Univ. Cal. San Diego, La Jolla, CA; ♦ Mass. Gen. Hosp., Boston, MA; + Barrow Neurol. Inst., Phoenix, AZ; ▾ Yale Univ., New Haven, CT; # Univ. Mich., Ann Arbor, MI.

The Parkinson Study Group (PSG), a consortium of 34 centers across the United States and Canada has established a DNA repository with samples from over 400 persons who were clinically diagnosed as having typical early Parkinson's disease (PD) and enrolled in the DATATOP (Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism) clinical trial in 1987-88 (Arch. Neurol. 1989; 46:1052-1060). Evaluations of the patients were performed through 1995 and included measures of neurological function, severity of PD (including the Unified Parkinson's Disease Rating Scale, Schwab and England scale and Hoehn and Yahr scale), cognition, mood, drug treatment and drug-associated adverse events. Additional information includes gender, race and age of onset for PD. Investigators interested in use of this repository should contact Dr. C.W.Shults (Fax: 619-552-7513, e-mail: cwshults@vapop.ucsd.edu). Access to this resource will be determined by the PSG based on the scientific merit and appropriateness of the proposal. To our knowledge, this represents the largest collection of well characterized, uniformly followed PD patients from whom DNA has been collected. DNA from this repository is likely to be useful in investigations of genes that may be associated with PD.

488.2

NIGRAL INFECTION BY INFLUENZA A VIRUS-A POSSIBLE CAUSE OF POSTENCEPHALITIC PARKINSONISM. M. Takahashi, T. Yamada*, S. Nakajima, K. Nakajima, T. Yamamoto and H. Okada. Choju Medical Institute, Noyori Fukushimura Hospital, Toyohashi 441, Japan.

Clinical and immunohistochemical studies were done for 3-39 d on mice after intracerebral inoculation with the neurovirulent A/WSN/33 (H1N1; WSN) strain of influenza A virus, the nonneurovirulent A/Aichi/2/68 (H3N2; Aichi) strain, and two reassortant viruses (R96 and R404BP) between them. Both theWSN and R404BP strains, which contain the WSN gene segments coding for neuraminidase and matrix protein, were clearly neurovirulent both clinically and pathologically. On day 3 after inoculation, WSN-antigen was detected in meningeal areas and neurons of the substantia nigra zona compacta. On day 7, meningeal reactions as well as neuronal staining was still seen, and advanced accumulation of the viral antigen was evident in the substantia nigra zona compacta. Double immunostaining demonstrated that the WSN antigen was only seen in neurons. Immunostaining for a lectin, maackia amurensis agglutinin, that recognizes the Neu5Ac α2, 3 Gal sequence which serves as a binding site for influenza A virus on target cell membranes, showed that positive staining was localized in the ventral substantia nigra. These results suggest that neurovirulent influenza A viruses could be one of the causative agents for postencephalitic parkinsonism.

488.3

IS FAMILIAL PARKINSONISM ASSOCIATED WITH THE EXPANSION OF TRINUCLEOTIDE REPEATS? K. Markopoulou[1], R.F. Pfeiffer[2], Z.K. Wszolek[1] and B.A. Chase*[3]. [1]Section of Neurology, University of Nebraska Medical Center, Omaha NE 68198-2045, [2]Dept. of Neurology, University of Tennessee, Memphis TN 38163, [3]Dept. of Biology, University of Nebraska at Omaha, Omaha NE 68182-0040.

Interest is increasing concerning the role of genetic factors in the etiology of Parkinson's disease. We report the analysis of a Greek-American kindred with levodopa responsive parkinsonism. Of the 98 individuals present in six generations of this pedigree, 16 individuals in three successive generations have developed parkinsonism. The phenotype of this pedigree is that of idiopathic Parkinson's disease and appears to be inherited in an autosomal dominant manner. Anticipation is present in this pedigree. The age of disease onset decreases, while the phenotypic severity appears to increase in successive generations. The affected members in the third generation developed symptoms at ages 50-71, in the fourth at ages 40-55 and in the fifth at age 31. This is another example of a neurodegenerative disease with autosomal dominant inheritance and anticipation. Diseases displaying anticipation have been associated with trinucleotide repeat expansion in their genes. The method of repeat expansion detection (RED) has been used to determine whether this pedigree is associated with trinucleotide repeat expansion. Preliminary findings will be presented.

488.4

PATTERN OF BRAIN ATROPHY IN DIFFERENT PARKINSONIAN SYNDROMES. D.A. McRitchie, K.L. Double, G.M. Halliday and E.M. McLachlan*. Prince of Wales Medical Research Institute, Randwick, 2031, Australia.

There have been no previous studies of regional brain volumes in Parkinsonian syndromes, despite many patients having significant cortical signs in life. The present study analysed atrophy in brains collected from prospectively followed donors and controls with the clinical diagnosis of Parkinson's disease (PD) confirmed postmortem. The study was approved by the Human Ethics Committee. Four patients had idiopathic PD (with no cortical pathology), 9 patients had PD with dementia (4 with cortical plaque pathology; 5 with diffuse Lewy body disease), and 10 were age- and sex-matched controls. The brains were weighed and volumes calculated before and after fixation. The cerebrum volume was remeasured prior to cutting into 3mm coronal slices. Each brain slice was photographed and printed. Frontal, temporal and parieto/occipital cortices, amygdala/hippocampus, basal ganglia and white matter were delineated. The volume of each region was calculated in each slice by point counting (each point=0.05ml). Repeated measures gave a 1% maximum error with an inter-rater error of less than 1%. There was no atrophy of white matter or basal ganglia in any case. Patients with idiopathic PD had no atrophy of cortical structures but a significant volume loss in the amygdala/hippocampus (24%). In contrast, PD patients with dementia had significant losses of frontal (16%), temporal (17%) and parieto/occipital (11%) cortices. In addition, volume reduction of the amygdala/hippocampus was more marked (33% loss) compared to PD patients without dementia. The results show that PD patients with dementia have significant cortical atrophy, without concomitant Alzheimer's disease. Surprisingly, significant volume changes occur in deep temporal lobe structures without overt memory deficits.

488.5

CO-LOCALIZATION OF NEUROFILAMENT AND UBIQUITIN IN LEWY BODIES. W.D. Hill* and G. Oblak. Dept. of Cellular Biology and Anatomy, Medical College of Georgia, Augusta, GA 30912.

Lewy bodies (LBs) are the pathological hallmarks of Lewy body associated disorders including Parkinson's disease and diffuse Lewy body disease. These filamentous neuronal inclusions characteristically label with antibodies to ubiquitin and with antibodies to neurofilament (NF) triplet proteins. Neurofilament proteins have been proposed as the major structural components of the filaments in Lewy bodies. However, several reports of NF antibody labeling of LBs suggest that not all LBs are labeled by NF antibodies. Additionally, the NF antibodies generally labeled only the outer portion of the LB. These observations have raised the possibility that NFs are not consistent features of LB filaments and therefore are not universal components of LBs.

In this study we provide further evidence that the Lewy body fibrils are composed of neurofilament proteins. Confocal microscopy was used in conjunction with immunoprobes to the neurofilament-M subunit and ubiquitin. Substantia nigra pars compacta neurons from 5 Parkinson's disease cases, 6 Alzheimer's/Parkinson's disease cases and 4 diffuse Lewy body disease cases were examined. The RMO32 neurofilament-M epitope is distributed throughout the Lewy bodies, wherever the filaments existed. Additionally, RMO32 labeling co-localized with anti-ubiquitin labeling. This demonstrates that NF containing Lewy bodies are not distinct from ubiquitinated Lewy bodies. Reported differences in NF labeling may be due to disease associated modifications to the NF epitopes and differences in antibody specificity and sensitivity.

488.6

UBIQUITIN AND MAP5 OCCUR SPECIFICALLY IN DEGENERATING NEURITES AND LEWY BODIES IN PARKINSON'S DISEASE AND DIFFUSE LEWY BODY DISEASE. W.P. Gai*, P.C. Blumbergs, and W.W. Blessing. Depts. of Physiology and Medicine, Centre for Neuroscience, Flinders University, Bedford Park, SA 5042, Australia.

We examined degenerating neurites in serial sections of brainstem and forebrain regions from Parkinson's disease (PD, n=14), diffuse Lewy body disease (DLBD, n=7), Alzheimer's disease (n=6), multisystem atrophy (n=4), motor neuron disease (n=5), vascular parkinsonism (n=2), and neuropathologically normal aged controls (n=11). Sections were stained routinely for histopathological examination or immunocytochemically with antibodies to ubiquitin, tau, bA4, and other cytoskeletal proteins, including MAP5 and neurofilaments. Doubly immunostained sections were examined with a confocal microscope. Ubiquitin-positive, tau-negative degenerating neurites were found in PD and DLBD, largely confined to brain regions where Lewy bodies were present. Corresponding neurites were absent in normal controls, and in non-Lewy body degenerative conditions. In PD, density of degenerating neurites in the dorsal motor vagal nucleus inversely correlated with the duration of symptoms.

MAP5 was consistently present in Lewy bodies and degenerating neurites (identified by ubiquitin double staining) throughout the brain, including the neurites in hippocampal CA2 area. Confocal microscopy showed MAP5 and ubiquitin reactivities partially overlapped and both were located in the Lewy body core and in the central region of degenerating neurites. Neurofilamentswere located in the periphery of the Lewy bodies and degenerating neurites in which they occurred. Our results suggest support the view that degenerating neurites and Lewy bodies may derive from the same pathogenic process.

488.7

PLASMA CATECHOLAMINE LEVELS OF ELDERLY MEN DURING EXERCISE: COMPARISON OF NORMALS WITH PARKINSONIAN PATIENTS. Susan L. Stoddard, Todd D. Miller, Gertrude M. Tyce, J. Eric Ahlskog, Alan R. Zinsmeister, and Stephen W. Carmichael*, Mayo Medical Ventures, Departments of Cardiology, Physiology, Neurology, Statistics, and Anatomy, Mayo Clinic/Foundation, Rochester, MN 55905.

Plasma levels of epinephrine (EPI), norepinephrine (NE), and dopamine (DA) were measured by HPLC with EC in 17 normal men (mean age 61 years; range 52 to 75) before, during, and 3 minutes after exercise to exhaustion on a cycle ergometer. The same measurements were made on a group of 8 men (mean age 48.3 years; range 38 to 58) with Parkinson's disease (PD), before and one year after autologous transplantation of the adrenal medulla to the caudate nucleus.

PLASMA CATECHOLAMINES (CA) IN PG/ML - Median Values									
	EPI			NE			DA		
	Norm	Pre	Post	Norm	Pre	Post	Norm	Pre	Post
Rest	29*	54	60	253	268	436	26*	1043	1192
Sub-Max	63	43	70	871*	302	529	30*	895	842
Max	127	78	86	1648*	548	708	43*	880	831
3 Min	72	72	75	1451*	599	582	42*	1252	854

EPI in normals was significantly (*) less than either PD group at rest. NE in normals was significantly greater than both PD groups during exercise, most likely due to the greater intensity of exercise possible in this group. DA was significantly lower in normals than either PD group for all measures, most likely due to the administration of L-dopa and carbidopa to PD patients. Pre-op and post-op PD patients had no differences in any CA for any measure, suggesting that the remaining adrenal medulla compensates following unilateral adrenalectomy.

488.8

A DEVICE FOR THE QUANTIFICATION OF PARKINSONIAN RIGIDITY S.K. Patrick*, S.C. Naaman, M.J.A. Gauthier, A. Prochazka Division of Neuroscience, University of Alberta, Edmonton, Alberta, Canada T6G 2S7

The subjective nature by which Parkinsonian rigidity is measured clinically makes inter- and perhaps intra-clinician comparison of rigidity scores difficult and unreliable. A device currently under development aims to overcome these problems by quantifying the rigidity elicited during the clinical exam. Portable, lightweight sensors are used to record the forces the clinician evokes as he manipulates the patient's arm about the elbow. In this way, the procedure retains the advantages of the conventional clinical exam, yet a more reliable, standardized rating of the rigidity is achieved (Prochazka, A., Bennett, D.J., Stephens, M.J., Sears-Duru, R., Roberts, T., Jhamandas, J.H., Movement Disorders (submitted)).

Recently, the device has undergone substantial improvement. Notably, a Windows™-based user interface and modifications to the hardware make use of the device straightforward and uncomplicated, while increasing its portability. A Motorola 68HC11 microprocessor is now used to sample the data. This replaces larger, more cumbersome equipment and allows the user easy adjustment of signal gain and offset. The computer interface displays the incoming data in real-time, manages all data files, and invokes Matlab® to perform the analysis. Results of the analysis, and information regarding the patients, examinations, and individual tests are easily accessible to the user for future reference.

Calculated stiffness scores correlate well with clinicians' ratings, and the rigidity is described as it changes with time, facilitating detection of drug effects. Preliminary investigations show that this device may also be applicable to measurement of spasticity; calculated stiffness scores agree better with clinicians' ratings than do those of the standard pendulum test.

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488.9

ABILITY TO PERFORM TWO MOTOR ACTS IS PRESERVED IN PROGRESSIVE SUPRANUCLEAR PALSY

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Progressive supranuclear palsy (PSP) is a sporadic neurodegenerative disorder characterized clinically by parkinsonism, supranuclear ophthalmoplegia, and dementia. In its earlier stages, PSP can be misdiagnosed as Parkinson's disease (PD). We tried to find a clinical sign to distinguish PSP from PD.

The subjects were 9 patients with PSP and 20 patients with idiopathic PD. All of them were clinically diagnosed under the consideration of neurological signs, imaging of the brain, and response to levodopa. Neurological symptoms of each patient were evaluated by modified united Parkinson's disease rating scale. Test battery consists of diadocokinesis and tapping of the hands. First, diadocokinesis and tapping were performed separately (single motor act). Second, diadocokinesis was performed in one hand and tapping was performed in the other hand (two motor acts). All movements were recorded by a video system. In some patients, finger tapping was recorded by a transducer and diadocokinesis was recorded by an X-Y recorder. The amplitude, frequency, and rhythm of the movement were evaluated.

Of these two groups, neurological symptoms were more strongly affected and ability of daily life was more limited in PD than PSP. The patients with rigidity in their wrist could not perform diadocokinesis well even in single motor act; 2 out of 9 with PSP and 4 out of 20 with PD could not perform it. Two motor acts were more difficult than single motor act. All 7 with PSP could perform two motor acts well also, while 15 out of 16 with PD failed to perform them. In their earlier stages, two motor acts seem to be one of a useful clinical sign to distinguish PSP from PD. The mechanism of the difference is difficult to explain. One possibility is a topographical difference of afferent dopaminergic neurons within the striatum between PSP and PD.

488.10

MOVEMENT PREPARATION IN INITIATING GAIT IN PARKINSON'S DISEASE. E. Roy[1,2*], T. Sora[1], J. Frank[1], A. Patla[1], J. Saint-Cyr[1], A. Lang[2]. [1]Dept. of Kinesiology, University of Waterloo, Waterloo, Ontario and [2]Movement Disorders Clinic, Toronto Hospital, Toronto, Ontario

Reaction time in gait initiation was examined in patients with Parkinson's disease (PD, N=10) and in ten healthy elderly adults. The task involved a single step (SN) or a sequence of two steps (SEQ) initiated from a standing position to four targets placed on the floor directly ahead of the subject. The SN task involved two reaction time conditions, simple (SRT) and choice (CRT), in which the subject knew the stepping target in advance in the SRT but not the CRT condition. In the SEQ task the subject initiated one of three two step sequences in a CRT paradigm. Reaction time (the time to initiate the step), movement time (time from initiation to stepping on the target) and fractionated early and late components of reaction time, weight transfer to stepping leg (WT) and step initiation (SI), respectively, were the dependent variables. Analyses revealed no group differences in movement time, but the PD patients exhibited significantly longer reaction time. While the slowing in reaction time in the PD patients was of equal magnitude across tasks, slowing in the components of reaction time was not. The PD patients were slower in both components when initiating single movements but only in the late component when initiating movement sequences. These findings suggest that PD effects a general slowing in movement initiation but not movement execution. This slowing in movement initiation is unaffected by task complexity defined either by event uncertainty (SRT vs CRT tasks) or sequence length (SEQ tasks). Task demands, however, mediate the effects of PD on the components of reaction time.

488.11

LARYNGEAL ELECTROMYOGRAPHIC FINDINGS IN PARKINSON'S DISEASE C. Gracco*, K.L. Marek, Walter Naito, Nancy Bryant. Depts Neurology, Surgery (ENT), Yale Univ/Haskins Laboratories, New Haven CT

The role of the intrinsic laryngeal musculature in voice production is well documented in normal human subjects. In particular, the thyroarytenoid(TA) and crycothyroid(CT) muscles are known to function in laryngeal adduction during modal phonation and pitch adjustment respectively. This investigation characterized laryngeal manifestations of Parkinson's Disease (PD) using complimentary instrumental techniques. Six subjects, 4 PD and 4 controls were evaluated. Simultaneous EMG recordings were made from TA and CT during speech and non speech maneuvers using hooked wire electrodes after the method by Hirano and Ohala (1970) Acoustic and aerodynamic measures were made in conjunction with EMG recordings from intrinsic laryngeal muscles to characterize vibratory cycles.

In patients with demonstrated vocal fold vibrational deficits, laryngeal EMG suggested the tendency for antagonistic co-contraction, reduced amplitude of vibration, and longer vibratory cycles in this population. In particular, the timing of activity in CT and Voc muscles differed for normal subjects and Parkinson's patients. Glottal closure and aperture for context based speech tasks showed reduction in activity of vocalis and increase in CT for voiceless tasks. The PD patients showed no depression of vocalis and a constant level of activity for CT. Further, these patients showed a premature increase in vocalis and CT activity which was sustained for longer periods during the consonant gesture.These findings support the notion that PD may affect the laryngeal musculature and the limbs similarly.

488.12

IMPROVEMENT IN ACCURACY WITH PRACTICE: EFFECTS OF AGE AND PARKINSON'S DISEASE. W. A. Hening[1,2], M. Rolleri[1], and J. Gordon*[3]. Neurology Depts., VAMC[1], Lyons, NJ & UMDNJ-RWJohnson Med Sch[2], New Brunswick, NJ; and Ctr for Neurobiology & Behavior[3], Columbia Univ, NY, NY

We previously reported that practiced, but not unpracticed, Parkinsonian subjects show normal accuracy in a simple planar pointing task when deprived of task information (Neurosci Abstr 18:935; 20:1464). We have now examined performance in unpracticed normals (N=15), aged 20 to 79, and Parkinsonians (N=4), aged 63 to 71. Subjects made movements to match four randomized computer screen targets requiring 8 to 32 cm excursions of the draped arm over a digitizing tablet. In the No Information task, the subjects were shown a target which was blanked, together with a cursor indicating hand position, at a "Go" signal. No feedback about performance was provided. Subjects were studied in 2 sessions; the No Information task followed by trial blocks with task information. We analyzed accuracy and movement features (reaction time, movement duration, linearity), comparing the two sessions, subject age and normal vs. Parkinsonian performance. In the entire subject group, accuracy, but not movement features, improved with practice. Performance did not vary with age. Parkinsonian subjects were less accurate than normals in the first, but not the second, session. Their movement features were comparable to age matched controls. We conclude that all subjects, including Parkinsonians, can increase accuracy with practice when performing without task information, but show only modest changes in movement features. Parkinsonian subjects are less accurate only before practice.

This research supported by the Dept. of Veterans Affairs Medical Research Council.

488.13

HOW PARKINSONIANS POINT IN 3-DIMENSIONAL SPACE. Berkinblit, Michael, Fookson, Olga, Hening, Wayne*, Poizner, Howard. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102; Institute for Problems of Information Transmission, Russian Acad. of Sci., Moscow, Russia; Department of Neurology, Lyons Veterans Administration Medical Center, Lyons, NJ 07939

It is known that Parkinson's disease patients (PDPs) perform planar goal directed movements without visual control less accurately than healthy subjects. However it is not known whether PDPs are accurate in 3-dimensional space for unconstrained movements. We studied the accuracy and kinematics of pointing movements to memorized targets in three-dimensions for five PDPs (stage II and III) and five normal control subjects. Five target locations were presented in 3D space by a programmable robot arm. The subjects were required to memorize the target location, close their eyes, and point to the remembered location upon an auditory signal. Movements of multiple joints of the arm were recorded in three dimensions. Errors in azimuth, elevation, radial distance, and in absolute 3D distance of finger from target were measured as were kinematic parameters of the trajectories.

Although PDPs differed from controls in elevation, absolute 3D errors and did not differ significantly between the two groups. Likewise, peak velocities, velocity symmetry, and movement duration were not significantly different. Thus, under conditions of unconstrained, 3D pointing movements executed at a comfortable speed, PDPs were able to accurately control the final arm position in 3D space. PDPs thus appear to be able to plan one entire "ballistic" movement without vision of their moving arm or of the target.

488.14

VISUAL MOTION CUES AND PARADOXICAL MOVEMENTS IN PARKINSON'S DISEASE PATIENTS. M.J.Majsak, A.M. Gentile, J.R. Flanagan, and M.E. Boyd-Topolski*. Teachers College, Columbia Univ., NYC, NY 10027 and Wheeling Jesuit College, Wheeling, WV 26003.

Individuals with Parkinson's disease (PD) frequently display bradykinesia (slowness in movement execution). However, paradoxical fast movements have been reported, sometimes anecdotally, when the environmental context provides visual cues of object- or self-motion (Purdon-Martin, 1967; Forssberg et al, 1984). Glickstein & Stein (1991) have described neural pathways that relay information about direction and velocity of rapidly moving targets from motion-detection cells in cortical visual areas to visual mossy fibers of the cerebellum via pontine projections. They proposed that PD patients can execute rapid movements when visual motion cues provide access to this cerebellar circuit. Thus, the intact cerebellar circuit permits an alternate mode of motor control less dependent on basal ganglia function. We tested these proposals in a task involving reaching to grasp a stationary or moving ball within a designated contact zone. The moving ball rolled down an inclined ramp (2.09m/sec) from left to right, in a plane parallel to the frontal plane of subjects at a distance equal to 80% of arm length. Six seated PD and 6 age/gender-matched, healthy subjects were tested under 4 conditions: (a) stationary ball at subject's preferred speed, (b) stationary ball at subject's maximum speed, (c) ball in motion, (d) retest of stationary/maximum speed. Kinematic analysis (2D) of hand trajectories showed that PD patients were slower than controls under all conditions. For PD patients ball motion produced faster movement times, higher peak velocities (PV), and faster time-to-PV than under stationary conditions. Indeed, these measures for PD patients approached those of controls under ball-motion conditions. Controls had sped up across testing conditions and showed carryover of speed set with retesting under stationary/maximum. In contrast, following tests with ball-motion, PD patients returned to prior levels observed under the stationary/maximum condition. These results offer support for Glickstein & Stein's proposals and implicate an alternative, cerebellar pathway for motor control in tasks involving visual motion cues.

488.15

A COMPARISON OF SENSORY PERCEPTION BETWEEN PATIENTS WITH PARKINSON'S DISEASE (PD) AND MATCHED CONTROLS. E. E. Jobst, M. E. Melnick*, N. N. Byl, G. A. Dowling, M. J. Aminoff, Grad Program in Physical Therapy, UCSF/SFSU, San Francisco, CA, 94143.

The accurate interpretation of kinesthetic and proprioceptive information is critical to the control of voluntary movement. Recent evidence suggests that there is decreased sensation of the position and movement of the limbs and trunk in space in patients with PD. This may contribute to the motor disturbances. We compared sensory discrimination skills of patients with PD to age and gender-matched controls to further define the sensory disturbances. Ten subjects with PD and 10 age/gender-matched control subjects were tested on four subtests of the Sensory Integration and Praxis Test (SIPT): Finger Identification (FI), Graphesthesia (GRA), Localization of Tactile Stimuli (LTS) and Kinesthesia (KIN). Data were analyzed using paired t-test for ratio data and the paired Wilcoxon test for ordinal data. Patients with PD performed significantly worse (p_{KIN} = 0.0014) on the test of kinesthesia than did the controls. There were no significant differences on the other tests. Thus, PD patients had no more difficulty than controls in identifying tactile input to the fingers, discriminating designs drawn on the hands or in making movements to a target located on the body surface. However, PD patients have significant difficulty in localizing a target outside the body, a task in which they must rely on the sensation of the movement itself (kinesthetic feedback). These results support the role of the basal ganglia in sensory processing and integration of sensory input into a motor task.

488.16

WORKING MEMORY IN PATIENTS AT DIFFERENT STAGES OF PARKINSON'S DISEASE. J. L. Iddon[1], A. M. Owen[1], J. R. Hodges[2] & T. W. Robbins[1]* Departments of Experimental Psychology[1] and Neurology[2], University of Cambridge, Downing Street, Cambridge, CB2 3EB, UK.

Groups of patients with Parkinson's disease (PD), either unmedicated and early in the course (n=8), medicated (mild) (n=7) or medicated (severe) (n=7), together with age- and IQ-matched control subjects (n=35) were tested on three analogous self ordered computerised searching tasks used to compare spatial, visual and verbal working memory.

In the spatial condition subjects were required to systematically search through a number of boxes presented on the screen to find 'tokens' whilst avoiding boxes in which a 'token' had previously been found. In the visual and verbal conditions, subjects were required to search in exactly the same manner, but through a number of abstract 'designs' and 'surnames', avoiding designs or names in which a token had previously been found without respect to their spatial location.

Results, (as analysed using MANOVA with between group effects tested using orthonomal contrast analysis) showed that the unmedicated PD patients were unimpaired on all three tests. Medicated (mild) PD patients were impaired only in terms of the number of returns to boxes in which a token had already been found ('between search' errors) on the spatial working memory task. However, the medicated (severe) patients had an impaired 'between search' errors score on all three tests of working memory. In addition on the test of visual working memory this group also made more returns to boxes previously unopened and shown to be empty ('within search' errors). The working memory deficits in patients with medicated Parkinson's disease could not be attributed to the inefficient use of a particular searching strategy in any of the three test conditions.

These results reveal (i) the progressive nature of the working memory impairment observed at different stages of Parkinson's disease. (ii) This impairment cannot be attributed to a deficit in the use of a strategy but (iii) can be contrasted with the lack of effect of frontal lesions on the visual and verbal memory tasks.

DEGENERATIVE DISEASE: PARKINSON'S—MECHANISMS

489.1

NO-SYNTHASE AND NEURONAL VULNERABILITY IN PARKINSON'S DISEASE S. Hunot, B. Faucheux*, F. Boissière, A. Mouat-Prigent, Y. Agid and E.C. Hirsch. INSERM U289, Salpêtrière hospital, 47 Bd de l'Hôpital, 75013 Paris, France.

Parkinson's disease is characterized by a loss of dopaminergic neurons in the substantia nigra. Yet, not all dopaminergic neurons of the mesencephalon degenerate during the disease. Indeed, the neuronal loss is severe in the substantia nigra pars compacta, intermediate in the ventral tegmental area and catecholaminergic cell group A8 and almost nil in the central grey substance. The mechanism of nerve cell death in Parkinson's disease is not known but oxidative stress may participate in the cascade of events leading to neuronal death. Because nitric oxide (NO) may be involved in the increased production of oxygen free radicals, we have analyzed the production mechanisms of NO in the mesencephalon of 3 patients with idiopathic Parkinson's disease and 3 matched control subjects.

Using histoenzymology, we have analyzed the NADPH-diaphorase (NADPH-D) activity of NO-synthase (NOS), the enzyme involved in the synthesis of NO. NADPH-D activity has been detected in glial cells and neurons of the human mesencephalon. Using specific antibodies directed against the constitutive isoform of NOS and immunohistochemical techniques, we have evidenced that this isoform of NOS is solely present in neurons. By contrast, immunohistochemical analysis performed with an antibody against the inducible isoform of NOS solely revealed a glial staining. Quantitative analysis showed: 1) an increased density of NADPH-D positive glial cells in the dopaminergic regions characterized by a neuronal loss in Parkinson's disease, 2) an increased proportion of NADPH-D positive neurons among catecholaminergic neurons in the parkisonian patients.

These data suggest a potential deleterious role of glial cells producing NO in Parkinson's disease on the one hand and the existence of subpopulations of dopaminergic neurons expressing NADPH-D which seem to be relatively preserved during the course of the disease on the other hand. The exact role of NO in this phenomenon remains to be analyzed.

489.2

PATTERN OF CELL LOSS IN THE SUBSTANTIA NIGRA IN PARKINSON'S DISEASE P. Damier[1,2]*, E.C. Hirsch[2], Y. Agid[2] and A.M. Graybiel[1]. [1]Dept. Brain & Cogn. Sci., MIT, Cambridge, MA 02139; [2]INSERM U289, Hôpital de la Salpêtrière, Paris, France.

To investigate the pattern of cell loss in the substantia nigra pars compacta (SNpc) in Parkinson's disease (PD), we developed a calbindin immunohistochemistry protocol. We showed that calbindin D_{28K}-immunostaining is intense in the ventral midbrain and forms compartmental patterns with prominent bands and pockets of low staining where most of the dopaminergic neurons are densely packed. Moreover, these calbindin fibers survive in PD and thus can be used as landmarks both in control and parkinsonian midbrains.

Dopamine-containing neurons, identified by their tyrosine hydroxylase (TH) content, were mapped on regularly spaced sections (every 360 μm) from three control and three parkinsonian midbrains, using an image analysis system (HistoRag, Biocom, France). Sets of adjacent sections were stained for calbindin immunoreactivity and were used to delineate the outlines of the calbindin-poor and -rich zones on the maps of TH-positive neurons. The outlines were added to each computerized chart of TH-positive neurons, and the number of TH-positive neurons in each subgroup defined by calbindin patterns was calculated. The total number of dopaminergic neurons per subgroup was obtained by integrating the number of neurons per section over the entire length of the SNpc. The percent cell loss in PD was calculated from these values.

The loss of dopaminergic neurons was significantly higher in calbindin-poor zones (92%) than in calbindin-rich areas (71%) ($p<0.02$). Moreover, analysis of the three-dimensional labyrinths formed by calbindin-poor zones allowed five calbindin-poor pockets to be defined. The cell loss appears to vary from one pocket to another: i.e. 97% in the main pocket, located in the caudal and mediolateral part of SNpc, and only 56% in the rostral and mediolateral pocket. This pattern of cell loss was consistent from one parkinsonian SNpc to another.

Compartmental patterns of calbindin immunochemistry allowed the loss of dopaminergic neurons to be defined with great accuracy in the SNpc and thus should permit investigations of the differences between nigral subgroups underlying the differential cell loss observed.

Supported by NIH Javits Award NS25529 and National Parkinson Foundation.

489.3

APOPTOTIC DEGENERATION OF NIGRAL DOPAMINERGIC NEURONS IN PARKINSON'S DISEASE. P. Anglade, S. Vyas, F. Javoy-Agid, M. T. Herrero, P. P. Michel, J. Marquez, A. Mouatt-Prigent, M. Ruberg, E. C. Hirsch*, Y. Agid. INSERM U289, Hôpital de la Salpêtrière, Paris, France.

Parkinson's disease (PD) is a degenerative disorder of the central nervous system characterized by a selective and progressive loss of dopaminergic neurons in the substantia nigra. Oxidative stress and decreased activity of the complexe I of the respiratory chain are probably involved in the process of degeneration. However, the mechanisms leading to neuronal death are still unknown. In this work, we searched to elucidate whether, in PD, dopaminergic neurons die by apoptosis (programmed cell death), already described during development and in experimental models of cell death. Nigral dopaminergic neurons were analyzed at the ultrastructural level in the substantia nigra of 3 PD patients and 3 control subjects. In PD patients, characteristics of apoptosis, such as chromatin condensation and cell shrinkage, were observed in 6 neurons on a total of 160 analyzed. Signs of autophagic degeneration were detected in 3 neurons, in only 1 patient. Neither apoptotic nor autophagic cell was observed in controls (90 cells examined). Factors known to intervene in programmed cell death, such as the apoptosis-preventing gene *bcl-2* in dopaminergic neurons and the cytokine TNF-α in glial cells, were detected in the substantia nigra of PD patients. These results suggest that, in PD, dopaminergic neurons die through molecular mechanisms anologous to those described in programmed cell death.

489.4

DIFFERENTIAL CELL DEATH IN MITOCHONDRIAL TRANSFORMED HUMAN NEUROBLASTOMA CELLS J.P. Sheehan, J.B. Tuttle*, P.A. Trimmer, R.Swerdlow, S. Miller, R.L. Davis, and W.D. Parker. Departments of Neurosurgery, Neuroscience, Urology, and Neurology, University of Virginia, Charlottesville, VA 22908 and Applied Genetics, San Diego, CA.

Significant evidence links Parkinson's disease and other neurodegenerative disorders to defects in mitochondrial DNA (mitDNA). However, the exact role, if any, of mitDNA in disease etiology is not yet defined. Starting with the human neuroblastoma cell line SY5Y, Rho^0 118/5 cells (Applied Genetics, San Diego, CA) devoid of mitDNA were developed. Then, mitDNA from either control or Parkinson's patients' tissues was inserted into the Rho^0 118/5 cells. Using these mitDNA transformed cells as an in vitro model, vulnerability to the mitochondrial complex I toxin 1-methyl-4-phenylpyridinium (MPP^+) was examined. Both cell types were exposed to selected concentrations of MPP^+ for 24 and 48 hours and, subsequently, stained with bisbenzimide. The ratio of pyknotic to total nuclei was determined for each experimental condition, and the results are as follows:

MitDNA Type	Incubation Time	[MPP^+]	Ratio of Pyknotic:Total Nuclei
Parkinson's	24 hours	80 µM	0.59
		40	0.13
	48	80	0.52
		40	0.80
Control	24	80	0.034
		40	0.018
	48	80	0.037
		40	0.43

Cells with mitDNA from Parkinson's patients exhibited substantially increased cell death when exposed to MPP^+. These data suggest that the mitDNA from Parkinson's patients confers a predilection for cell death when the cells containing such DNA are exposed to the neurotoxin MPP^+.

489.5

SOMATOSTATIN mRNA EXPRESSION IN PARKINSONIAN BASAL GANGLIA: A QUANTITATIVE IN SITU HYBRIDISATION STUDY

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United Kingdom Parkinson's Disease Society Brain Bank, 1 Wakefield Street, London WC1N 1PJ, UK

Levels of the neurotransmitter somatostatin (SS) have previously been shown to be reduced in the cortex and hippocampus of demented parkinsonian patients and patients with Alzheimer's disease. In the basal ganglia, SS has been demonstrated within striatal interneurons, colocalised with nitric oxide synthase (NOS) and neuropeptide Y, and in the medial medullary lamina of the globus pallidus (MML). *In situ* hybridisation histochemistry (ISHH) was used to examine SS mRNA expression within the striatum and MML in Parkinson's disease (PD) and in matched controls. ISHH was performed with an ^{35}S tail-labelled oligonucleotide DNA probe to human SS mRNA on 12μ coronal sections cryostat cut from flash-frozen human basal ganglia blocks (at approximately mamillary body level). Quantitation of SS mRNA expression from emulsion dipped sections revealed a significant increase of 82% in the MML of the globus pallidus in PD (56.5μ^2 of silver grain / cell, n=9 cases) compared to controls (26.3μ^2 / cell, n=13 cases, $p<0.01$, Student's *t*-test). No difference was detected in SS mRNA expression in putamen of PD cases (n=10) compared with controls (n=14). Upregulation o, NOS mRNA has previously been demonstrated in MML neurons in PD cases and these results suggest that SS mRNA expression within MML cells is upregulated in parallel. Somatostatinergic neurons of the MML are thought to project to the striatum, and SS has been shown to enhance dopamine release in striatal slice preparations. The reported changes in MML SS mRNA expression in PD could therefore play a part in compensating for striatal dopamine depletion in this disorder.

489.6

ALTERED STRIATAL DOPAMINE TRANSPORTER IMMUNO-REACTIVITY IN PARKINSON'S DISEASE.

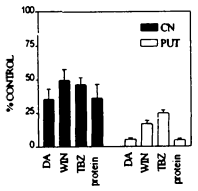
G.W. Miller, C.J. Heilman, J.T. Perez, J.K. Staley, D.C. Mash, D.B. Rye, A.I. Levey* Department of Neurology, Emory University School of Medicine, Atlanta, GA 30322.

Ligand binding studies have demonstrated a selective loss of striatal dopamine transporter (DAT) in patients with Parkinson's disease (PD). Determination, however, of DAT protein expression in PD has awaited production of specific antibodies to DAT. In this study we describe the production and characterization of a highly specific rat monoclonal antibody to the N-terminus of human DAT (mDAT-Nt), and its use in Western blot analysis and immunocytochemistry. Homogenates of microdissected samples of caudate (Ca), putamen (Pu), and nucleus accumbens (NA), from control (n=3) and PD brains (n=6), were separated by SDS-PAGE, and analyzed by Western blotting and enhanced chemiluminescence (Amersham). DAT immunoreactivity was observed at high levels in Ca, Pu and NA of control brains, with marked reductions in Ca and Pu, and mild reductions in NA of PD brains. Immunocytochemistry performed on several of the same brains showed similar results. DAT immunoreactive fibers displayed puncta and were dense throughout the striatum of control brains, but were virtually non-existent in Pu in PD brains. Caudate showed modest densities of terminals, mostly along the border of the ventricle and appeared largely preserved in NA. Thus, the monoclonal antibody mDAT-Nt provides a novel means for characterizing DAT protein in PD and other disorders with improved sensitivity, specificity, and subcellular resolution, and also offers the potential for quantitation.
Supported by APDA, DA9484 and NS31937.

489.7

STRIATAL PROTEIN CONCENTRATION OF THE DOPAMINE TRANSPORTER IS MARKEDLY REDUCED IN IDIOPATHIC PARKINSON'S DISEASE. S.J. Kish*, A.I. Levey, O. Hornykiewicz and J.M. Wilson. Human Neurochemical Pathology Lab., Clarke Institute of Psychiatry, Toronto, Ontario and Department of Neurology, Emory University, Atlanta, Georgia.

We have recently developed a specific monoclonal antibody (McAb) directed to the N-terminus of the human dopamine (DA) transporter (hDAT) (Miller, et al., Soc. Neurosci. 1995). In order to determine whether hDAT McAb might be useful for the quantitative estimation of dopamine nerve terminal concentration in human brain disorders, we compared, in striata (caudate and putamen) of 12 patients with idiopathic Parkinson's disease (PD) and 10 matched controls, levels of DAT protein immunoreactivity (SDS-PAGE with rat hDAT McAb) vs. levels of three established markers of striatal DA nerve terminal integrity, namely, [^{3}H]WIN 35,428 (WIN, 0.25-100nM) and [^{3}H]dihydrotetrabenazine (TBZ, 0.25-30nM) binding and DA levels (HPLC). As compared with the controls, striatal concentrations of all four DAergic markers were markedly reduced in the PD patients, with DAT protein and DA levels being reduced similarly and to the greatest extent (see Fig). As expected, levels of the four DAergic markers were reduced to a greater extent in putamen vs. caudate.



Statistically significant ($p<0.05$) correlations were observed between striatal protein levels of DAT vs. WIN (r=0.46) and TBZ (r=0.43) binding. We conclude that blot immunolabelling analysis of hDAT protein in postmortem brain may provide a useful quantitative estimate of DA nerve terminal integrity in human disorders which involve DAergic systems. (Supported US NIDA DA 7182, DA 9484, NS 26034, NS 31937 and APDA).

489.8

MODIFICATION OF PROTEIN FUNCTION BY DOPAMINE OXIDATION: EFFECT ON DOPAMINE TRANSPORT. S.B. Berman*, M.J. Zigmond and T.G. Hastings. Departments of Neuroscience and Neurology, University of Pittsburgh, Pittsburgh, PA 15260.

Dopamine (DA) can oxidize to form reactive oxygen species (ROS) and DA quinones, and we have previously shown that quinones formed from DA and its metabolite, dihydroxyphenylacetic acid (DOPAC) covalently bind to cysteinyl residues on proteins in striatum (Hastings and Zigmond, 1994; Hastings et al, 1994). It is possible that striatal proteins with a higher affinity for DA may be at greater risk for this modification. One such protein is the DA transporter, which contains cysteinyl residues that are critical to its function and which may be oxidized by $O_2^{\cdot}$, OH, or quinones. In these studies, we tested whether DA transport in rat striatal synaptosomes could be modified by generators of ROS, including DA. We found that ^{3}H-DA uptake was reduced in the presence of ascorbate (0.85 mM), which can function as a proxidant in the presence of iron, while the antioxidant, glutathione (1 mM), had no effect. This inhibition by ascorbate was prevented by DETAPAC (1 mM), an iron chelator. To examine this effect further, synaptosomes were *preincubated* under various oxidative conditions and washed, and then uptake of ^{3}H-DA (250 nM) was measured. Preincubation with ascorbate inhibited subsequent ^{3}H-DA uptake by 45%, and this effect was again prevented by DETAPAC. Xanthine (500 μM) + xanthine oxidase (50 mU/ml) reduced subsequent uptake of ^{3}H-DA by 80%. Preincubation with DA (100 μM) caused a 60% inhibition of subsequent ^{3}H-DA uptake, an inhibition attenuated by DETAPAC (1mM), which as an iron chelator prevents Fe-catalyzed oxidation of DA during the preincubation. These findings suggest that DA oxidation and ROS can modify DA transport function. Further experimentation is required to determine whether this transport inactivation might function to protect DA terminals or to act destructively by further promoting extracellular DA oxidation (Supported in part by USPHS grant NH19608).

489.9

INTRASTRIATALLY ADMINISTERED DOPAMINE, DOPA AND METH-AMPHETAMINE: EVIDENCE OF NEUROTOXICITY. T.G. Hastings*, A.D. Rabinovic, M.J. Zigmond and D.A. Lewis, Neurology, Neuroscience and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA

Dopamine (DA) neurons of the nigrostriatal pathway are selectively vulnerable to degeneration in Parkinson's disease. We have shown in vivo that intrastriatal DA resulted in a central region of nonspecific cytotoxicity surrounded by a region of selective decrease in tyrosine hydroxylase (TH) immunoreactivity. Toxicity was accompanied by DA oxidation as shown by the presence of protein-bound cysteinyl-DA and cysteinyl-DOPAC. The administration of exogenous antioxidants reduced the amount of cysteinyl-catechol formation and blocked the selective toxicity to DA terminals associated with exogenous DA exposure. The present study further explored the neurotoxic actions of DA by examining the time course of TH loss and by utilizing agents known to increase the availability of DA. DA (0.4 μmol), DOPA (0.4 μmol), and methamphetamine (MA; 1.0 μmol) were stereotaxically injected into rat striatum. At 24 and 48 h following DA injections, TH labeled axons and terminals were enlarged and dystrophic. At later time points, the density of these dystrophic processes decreased in association with a general loss of TH immunoreactivity, suggesting the loss of DA terminals and not merely a decrease in TH immunoreactivity. At 7 d, intrastriatal injection of either DOPA or MA, like DA, resulted in nonspecific damage as indicated by staining for Nissl substance. However, unlike DA, neither DOPA nor MA showed a region of selective loss of TH immunoreactivity. These findings suggest that exogenous DA produces a degeneration of striatal DA axons, whereas exposure to agents that increase the availability of DA in vivo can cause generalized cytotoxicity. (Supported in part by USPHS grants NS19608 and AG05133.)

489.10

DOPAL: A POTENTIAL NEUROTOXIN FOR DOPAMINERGIC NEURONS IN PARKINSON'S DISEASE M. B. Mattammal, H. D. Chung*, J.H. Haring, S. Wulff, K-W. P. Yoon and R. Strong. Departments of Medicine, Neurology and Anatomy, St. Louis University and GRECC, VAMC, St. Louis, MO and Departments of Cellular and Structural Biology and Pharmacology, University of Texas Health Science Center and GRECC, Audie L. Murphy Memorial Veterans Hospital, San Antonio, TX, 78284.

It has been suggested that aberrant metabolism of dopamine plays a role in the pathogenesis of Parkinson's disease. The metabolism of dopamine by MAO-B liberates 3,4-dihydroxy-phenylacetaldehyde (DOPAL). We found that DOPAL is present in substantia nigra of individuals who had pathologically confirmed PD and is undetectable in SN of control cases. We hypothesized that DOPAL is a selective dopaminergic neurotoxin whose specific effects are mediated by the dopamine transporter. *In Vitro*, DOPAL selectively destroyed dopamine containing synaptosomes, while 5HT and glutamate containing synaptosomes were unaffected. The specific uptake of [^{3}H]DOPAL in neostriatal synaptosomes was inhibited by mazindol, a specific dopamine uptake inhibitor. Moreover, exposure of cultured mesencephalic neurons to DOPAL caused a selective disappearance of TH-positive neurons. *In vivo*, DOPAL was delivered to the SN of rats. Immunocytochemistry and cell counts through the extent of the SN revealed that the number of TH-positive neurons in the SN receiving DOPAL injection was significantly reduced to 27 ± 4% of the control. GABA neurons in the SN were not affected. These data suggest that DOPAL is a selective dopaminergic neurotoxin that may have relevance to the neuronal degeneration observed in Parkinson's disease.

489.11

ISCHEMIC DAMAGE TO THE NIGROSTRIATAL TRACT: RELATIONSHIP TO INCREASED DOPAMINE RELEASE AND OXYGEN RADICAL GENERATION AND PROTECTION BY THE DOPAMINE AGONIST PRAMIPEXOLE. P.K.Andrus*, T.J.Fleck, J.A.Oostveen, J.S.Althaus, G.J. Fici, P.F.VonVoigtlander and E.D.Hall. CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49007

We examined progressive retrograde degeneration of the dopaminergic nigrostriatal (NS) tract over 28 days in gerbils subjected to a 10-min period of forebrain ischemia. There was a 39% loss of NS cell bodies in the zona compacta (TH immunocytochemistry) over that time period. To explore the mechanism of this degeneration, we measured striatal levels of dopamine (DA) and DA metabolites (DOPAC, HVA, 3-MT) via HPLC-ECD and hydroxyl radical production (•OH) via the salicylate trapping method during the first hr after reperfusion. DA levels did not change after reperfusion, but there was a significant increase in DOPAC (+537% at 5 min), 3-MT (+1300% at 1 min), and HVA (+185% at 5 min), indicative of an increased DA turnover. It is proposed that this post-reperfusion increase in striatal DA release/metabolism and the associated elevation in •OH production (i.e., increased MAO activity) are responsible for oxidative damage to the NS dopaminergic nerve terminals and subsequent retrograde degeneration of the NS neurons. Pramipexole at 1 mg/kg was able to significantly reduce this increased dopamine turnover. Consistent with the acute attenuation of DA turnover, daily post-ischemic oral dosing (1 mg/kg p.o. BID beginning at 1 hr after insult) decreased 28-day post-ischemic loss of NS DA neurons by 36% ($p<0.01$ vs vehicle treated).

489.12

S-NITROSOTHIOLS: SNAP AND GSNO INHIBIT PEROXIDATION OF BRAIN LIPIDS IN VIVO AND IN VITRO. P. Rauhala, I. Sziraki & C.C. Chiueh*. Unit on Neurotoxicology & Neuroprotection, Laboratory of Clinical Science, NIMH, NIH, 10/3D-41, Bethesda, MD 20892-1264

We previously reported that sodium nitroprusside or nitroferricyanide (SNP) increases, while S-nitroso-N-acetyl-penicillamine (SNAP) decreases •OH formation induced by ferrous citrate iron-complexes. We compared further the effects of these two NO• donors as well as S-nitrosoglutathione (GSNO) on the peroxidation of rat brain lipids (assayed by a fluorometric microassay procedure) both in vivo and in vitro.

The present results demonstrated that SNP is a prooxidant while SNAP is antioxidant based upon their opposite effects on brain lipid peroxidation in the rat substantia nigra. Similar to SNAP, GSNO is a S-nitrosothiol compound and it also inhibited brain lipid peroxidation. However, SNP significantly increased brain lipid peroxidation since it is a nitroferricyanide complex leading to an increase in generation of •OH (> 24 hr) and a brief release of NO• (<30 min). These effects of GSNO and SNAP but not SNP were blocked by light exposure.

In conclusion, S-nitrosothiols such as SNAP and GSNO, through the release of NO•, may protect brain neurons against oxidative injury caused by •OH radicals. Furthermore, these results raise a new hypothesis that NO• and its S-nitrosothiol derivatives may be a new class of neuroprotective antioxidants.

489.13

PROTECTION BY MANGANESE AGAINST FERROUS IRON-INDUCED NEUROTOXICITY IN THE BRAIN IN VIVO. I. Sziráki*, P. Rauhala, D. L. Murphy & C. C. Chiueh. Unit on Neurotoxicology and Neuroprotection, Laboratory of Clinical Science, NIMH, NIH 10/3D41, Bethesda, MD 20892-1264.

It has been proposed that transition metals such as Fe and Mn can induce nigrostriatal degeneration through dopamine autooxidation and OH radical generation. However, ours and others' results indicate that Fe but not Mn can induce peroxidation of brain lipids. In fact, Mn inhibits peroxidation of brain lipids. Thus, we re-investigated the possible prooxidant and antioxidant roles of Fe and Mn in the brain in vivo.

Intranigral infusion of 4.2 nmol of Fe, but not Mn increased brain lipid peroxidation in the substantia nigra assayed by a fluorometric microassay procedure. Fe decreased striatal dopamine levels by 75%, while Mn caused no change. Mn completely blocked the Fe-induced lipid peroxidation and nigral injury as indicated by the absence of dopamine depletion in the nerve terminal region.

In summary, the present in vivo results demonstrated that manganese protects against oxidative injury of nigrostriatal neurons induced by ferrous iron complex. In conclusion, physiological levels of manganese may be a neuroprotective antioxidant in the brain.

489.14

SELECTIVE INDUCTION AND EXPRESSION OF HIPPOCAMPAL METALLOTHIONEIN ISOFORMS. M. Ebadi* and M.P. Leuschen. Departments of Pharmacology and Cell Biology and Anatomy, Univ. Nebraska Coll. Med., 600 South 42nd Street, Omaha, NE 68198-6260.

Zinc induces the synthesis of metallothionein (MT) isoforms in hippocampal neurons in primary culture in a dose- and time-dependent manner. Moreover, the hippocampal MT becomes induced by agents causing oxidative stress, such as 6-hydroxydopamine (6-OHDA), and/or involved in inflammatory processes, such as interleukin-1. In addition, the *in situ* hybridization of ^{35}S-labeled cDNA showed that MT-I mRNA is heavily localized in select areas of brain, including hippocampal formation. The induction of MT by zinc or neurotoxin is selective in that it occurs in some brain areas such as hippocampus, choroid plexus, and granular layer of cerebellum, but not in the caudate putamen.

The lack of induction of striatal MT by either zinc or 6-OHDA is of extreme clinical significance in Parkinson's disease and other movement disorders. In view of the fact that striatal tissue in patients with Parkinson's disease have a high concentration of iron (prooxidant) and a low concentration of ferritin (iron-binding protein which, in part, prevents the toxicity of excess iron), makes the striatum especially susceptible to oxidative stress. The evidence provided here suggests that the lack of induction of striatal metallothionein (antioxidant) by either zinc or 6-OHDA, along with high levels of iron, render the striatum especially susceptible to a high degree of oxidative stress. (Supported in part by a grant provided by USPHS NS 34566.)

489.15

PRELIMINARY CHARACTERIZATION OF ß-CARBOLINE-2-N-METHYLTRANSFERASE FROM MAMMALIAN BRAIN. D.A. Gearhart*, M.A. Collins and E.J. Neafsey#. Neuroscience Program and Dept. of Biochemistry and Dept. of #Anatomy, Loyola University Medical School, Maywood, IL 60153.

Brain ß-carboline-2-N-methyltransferase (ßC-2-NMT) catalyzes the S-adenosylmethionine (SAM)-dependent N-methylation of environmental ß-carbolines (ßCs) to yield 2-N-methylated ß-carbolinium (MBC$^+$) derivatives which are structural analogs of 1-methyl-4-phenyl-pyridinium ion (MPP$^+$). The activity of this enzyme leads to the formation of cationic products which may be trapped within the brain. Evaluation of ßC-2-NMT is necessary since it generates potential neurotoxins that may play a role in idiopathic Parkinson's disease (Collins et al., **Brain Res.**, 1993). Our studies indicate that ßC-2-NMT activity resides primarily in the cytosolic fraction of brain homogenates, but that nuclear activity is notable. As expected for SAM-dependent methyltransferases, the enzyme is inhibited by S-adenosyl-homocysteine (K_i=10 uM). ßC-2-NMT activity in bovine brain cytosol exhibits a broad pH optimum ranging from pH 8-8.5 in potassium phosphate-bicine buffer. Apparent kinetic constants for ßC-2-NMT with respect to SAM are: K_m=137 uM and V_{max}=100 pmoles/mg protein/hr when 9-MeNh substrate is fixed at 540 uM. Apparent kinetic constants for ßC-2-NMT in regard to 9-MeNh are: K_m=66 uM; V_{max}=87 pmoles/mg protein/hr with SAM fixed at 500 uM. Preliminary characterization of ßC-2-NMT is fundamental to assay optimization in preparation for enzyme purification as well as measurement of enzyme activity in normal and Parkinsonian brain. Research supported by NIH (NS23891).

489.16

ESTROGEN MAY PROTECT FEMALES FROM PARKINSON'S DISEASE. X.L. Chen, R. Li and M. Gupta*. Dept. of Anat. Sci. & Neurobiology, U of Louisville Sch. of Med., Louisville, KY 40292, USA

Most studies have found that Parkinson's disease (PD) is more common in men than in women. Furthermore, striatal neurons have been shown physiologically to respond to direct applications of estrogen. In postnatal day 10 female rat, scattered striatal neurons have been shown to co-localize estrogen receptor mRNA and estrogen binding. Using the mouse Parkinsonian model, present studies were undertaken to determine if treatment of ovarectomized female mice with 17ß estradiol, prior to MPTP treatment, would prevent MPTP-induced damage of the nigrostriatal system. Young adult female ovarectomized mice were treated with : (A) 17ß estradiol (15µg/ mouse over 2d, s.c.), (B) vehicle, (C) 17ß estradiol followed by MPTP, (D) vehicle followed by MPTP. Three days later, all the animals were sacrificed by decapitaion to cause minimal stress. Brains were quickly removed. Striata were dissected and frozen until neurochemical analysis to determine the levels of dopamine and its metabolites using LCEC. Remaining brain pieces were immerse-fixed in 4% paraformaldehyde in 0.1M PO_4. Frozen 40µm thick adjacent sections through the substantia nigra (SN) were stained immunocytochemically for tyrosine hydroxylase (TH) and the number of TH-positive neurons were quantitated. The data shows that treatment of ovarectomized mice with 17ß estradiol, prior to MPTP, can reduce the MPTP-induced decrease in the number of dopaminergic neurons in the substantia nigra as well as dopamine levels in the striatum, thereby suggesting that estrogen may play a protective role in females against Parkinson's disease.

489.17

RAT AND HUMAN LIVER ARE RICH SOURCES OF A NOVEL DOPAMINE RELEASING PROTEIN. S. Kuhananthan*, J. Hyeon-Lee, C. Pullins, and V.D. Ramirez. Dept of Molecular and Integrative Physiology, Univ of Illinois, Urbana, IL

A dopamine releasing protein (DARP) was first purified from rat adrenal gland and subsequently characterized as a multiunit glycoprotein of 200K. Monoclonal antibodies produced against this protein reduce brain dopamine levels when injected into newborn rats. These monoclonal antibodies immunostain specific areas in the rat adrenal and rat and human brain. DARP was also demonstrated to be present in human serum when tested on a direct ELISA assay. Interestingly, Parkinson's disease patients had higher concentrations of serum DARP when compared to age-matched controls. The first 36 N-terminal amino acids of DARP are already microsequenced and a synthetic peptide (DARP-36) is available.

We studied the concentrations of DARP in crude extracts of different rat tissues using a direct ELISA assay. Highest concentrations of DARP were found in liver and brain. Skeletal muscle did not contain significant amounts of DARP. Crude liver extract also competes with DARP-36 to bind anti-DARP in a competitive ELISA assay. Western blot analysis of crude liver extract (50 µg protein) under reducing and non reducing conditions reveal a strong band of 60K detected by anti-DARP. However, several other tissues including skeletal muscle did not elicit any immunostaining. Crude liver DARP was also biologically active in releasing dopamine from superfused rat corpus striatal fragments in a dose dependent manner. Human liver extract gives a strong ELISA response between 125-2000ng protein and also reveals the same 60K band in western blot analysis with monoclonal anti-DARP.

The above data suggests that liver could be the site of production of serum DARP.

489.18

CHARACTERIZATION OF CATECHOLAMINE ABSORBING PROTEINS USING A NOVEL HIGH SPECIFIC ACTIVITY AFFINITY LIGAND. R.K. Mishra[1], B.E. McCarry[2], V. Nair[1], H. Niznik[3], R.J. Riopelle[4]* and G.M. Ross[4]. [1]Department of Biomed. Sci. and [2]Chemistry, McMaster Univ., Hamilton, Ontario, [3]Clark Inst. of Psychiatry, Toronto, Ontario and [4]Department of Medicine, Queen's Univ., Kingston, Ontario.

Catecholamine Absorbing Proteins (CATNAP's) are a family of CNS specific proteins which appear to play a role in oxidative processes within the brain. CATNAP's may represent a scavenging mechanism for highly reactive products of aberrant catecholamine metabolism which may arise from free radical mediated mechanisms. Such mechanisms have been implicated in the pathophysiology of several neurodegenerative diseases including Parkinsonism. We have synthesized a high specific activity CATNAP probe ([^{125}I]-DATN) for pharmacological and biochemical characterization of these proteins, which had previously been identified using the ligand [^{3}H]-N-propylnorapomorphine (Ross et al., 1993, J. Mol. Neurosci. 4:3 141). The ligand [^{125}I]-DATN labelled CATNAP proteins with the same tissue distribution, molecular weight distribution and pharmacological profile as the tritiated compound. Purification of these proteins was facilitated by the radioiodinated ligand, which has also allowed the generation of polyclonal antisera against purified CATNAP's. Antibodies specific to each of the various molecular weight CATNAP's have been used to characterize changes in expression of these proteins under a variety of pharmacological manipulations known to modulate free radical mediated mechanisms, including administration of MPTP and 6-OHDA.

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489.19

DOPAMINERGIC-INDUCED DYSKINESIA IN THE MPTP MONKEY MODEL IS ASSOCIATED WITH AN INCREASE IN ΔFosB IN THE STIATUM. P.J. Bédard[1], P.J. Blanchet[1], J.-P. Doucet[2], R. Grondin[1], Y. Nakapeppu[3], M. Filion[1]* and G.S. Robertson[2], Neurobiology Research Center[1] Laval Univ. 1401 18th St., Quebec City, Que, Canada G1J 1Z4, Dpt of Pharmacology, Univ. of Ottawa, Ottawa, Ont., Canada, K1H 8M5, and Dpt of Biochemistry[3], Medical Institute of Bioregulation, Kuyshu University 69, Fukuoka 812, Japan.

Dyskinesia is a frequent complication of treatment of Parkinson's disease with dopaminergic agents. Its appearance requires denervation and chronic DAergic treatment. Once established it is persistent, suggesting a learning phenomenon the nature of which has eluded us so far. We have previously shown, by Western Blot, using an antibody raised again st the DNA binding region of Fos, that in rats, DA denervation by 6-OHDA is followed by a long-lasting increase in Fos-like immunoreactivity in striato-pallidal neurons and that it corresponds to a 38 kD Fos protein called ΔFosB. An even more important and persistent increase in ΔFosB is seen in striato-nigral neurons, in denervated rats treated chronically with a D_1 agonist. More recently, we examined the effects of chronic (one month) daily administration of D_1-like and D_2-like dopamine receptor agonists on striatal ΔFosB expression in the MPTP-primate model of Parkinson's disease. The animals were sacrificed four days after the last dose of DAergic agent. In monkeys rendered parkinsonian by MPTP, but left untreated, there was a modest increase of ΔFosB-like protein(s). Chronic administration of the D_1-like agonist SKF-82958 by multiple daily injections, induced the development of dyskinesia and was accompanied by large increases of ΔFosB-like protein(s). Interestingly, the same total daily dose of the same D_1 agonist administered by minipump did not induce dyskinesia nor an increase in ΔFosB. In contrast, administration of the long acting D_2-like agonist cabergoline which alleviated parkinsonian symptoms without producing dyskinesia reduced ΔFosB levels to near normal. Taken together, these results indicate that a persistent enhancement of ΔFosB-like proteins(s) in striatopallidal and striatonigral neurons is associated with the development of parkinsonism and dyskinesia, respectively. ΔFosB-like proteins(s) may therefore contribute to the development of these movement disorders by participating in gene signalling pathways activated by prolonged alterations in D_1- and D_2- like receptor stimulation.(Supported by the MRC of Canada).

489.20

BEHAVIORAL AND BIOCHEMICAL EFFECTS OF CHRONIC TREATMENT WITH THE LONG-ACTING DOPAMINE D2 AGONIST CABERGOLINE IN PARKINSONIAN DRUG-NAIVE MONKEYS. R. Grondin*[1], M. Goulet[2], T. DiPaolo[2] and P.J. Bédard[1]. [1]Neurobiol. Res. Center, Enfant-Jesus Hosp., Laval University and [2]Dept. of Molecular Endocrinol., CHUL, Quebec, Canada.

Continuous dopaminergic stimulation is now considered as an interesting approach for the control of motor complications such as dyskinesias often seen in parkinsonian patients treated chronically with levodopa. There is some evidence that dopamine (DA) D1 receptors are more prone to tolerance than the DA D2 receptors under such continuous stimulation. A few clinical studies using cabergoline (CBG) which is a long-acting DA D2 receptor agonist have been completed so far with good results in parkinsonian patients who have been previously exposed to other drugs and in whom the current levodopa-therapy was maintained during the CBG treatment.

Therefore, the purpose of the present study was to test the effects of repeated s.c administration of CBG at 0.25 mg/kg every 48h during a month in 3 drug-naive monkeys (*Macaca fascicularis*) rendered parkinsonian by MPTP to see whether or not CBG would have a sustained antiparkinsonian effect and would induce dyskinesias. The animals were rated to quantify the antiparkinsonian as well as the dyskinetic response and the motor activity was monitored by photocells. The locomotor response, initially greatly stimulated, decreased somewhat, but was thereafter maintained at a high level (more or less 5 times higher than control values). The parkinsonian features were improved in a sustained manner and transient dyskinesias were present in 2 monkeys. Receptor binding assays were then performed on striatal and pallidal tissues homogenates with tritiated selective antagonists and were compared with those of 3 healthy and 3 MPTP-exposed monkeys otherwise untreated. A decrease in striatal DA D2 receptor density (Bmax) and a reversal of the supersensitivity of $GABA_A$ receptors in the GPi were seen which may explain the behavioral effects mentioned above. Thus, continuous DA D2 receptor stimulation appears to be an interesting strategy of treatment especially in parkinsonian patients who are experiencing motor complications under levodopa-therapy. [Supported by Parkinson Foundation and MRC of Canada].

DEGENERATIVE DISEASE: PARKINSON'S—PHARMACOLOGY

490.1

CHARACTERIZATION OF AROMATIC L-AMINO ACID DECARBOXYLASE IN RAT STRIATUM. M. Ahmed and U. J. Kang*. Dept. of Neurology, Univ. of Chicago, Chicago, IL 60637.

L-Dihydroxyphenylalanine (L-DOPA) is the most effective treatment for Parkinson's Disease (PD). L-DOPA must be converted to dopamine by aromatic L-amino acid decarboxylase (AADC) to be effective, but the source of AADC in these patients and dopamine-depleted animal models are not known. The major source of AADC is terminals of dopaminergic neurons which are lost with dopaminergic lesions. This suggests that there are important sources of AADC outside dopaminergic neurons. Previous investigators have suggested intrinsic striatal neurons or striatal glial cells as the sources of AADC, but immunohistochemistry and *in situ* hybridization showed no evidence of AADC expressing cells (Kang et al., 1992). Recently, two different splicing variants of AADC messages have been found in neuronal and non-neuronal tissues (Hahn et al., 1993). Therefore, the present investigation attempted to examine the sources of AADC by characterizing the type of AADC mRNAs in the striatum. cDNA was synthesized from total RNA extracted from different areas of brain by using antisense primer specific to AADC axonal sequences common to neuronal and nonneuronal forms. The cDNA was amplified by Polymerase Chain reaction (PCR) using specific neuronal and nonneuronal primers respectively. Striatal tissue contained neuronal mRNA for AADC. Adrenal tissue and substantia nigra also contained exclusively neuronal RNA. In contrast, liver had only the nonneuronal mRNA of AADC. Our data suggest that intrinsic striatal neurons rather than glia may be the source of AADC that catalyze the conversion of L-DOPA to dopamine in the denervated striatum.

490.2

L-DOPA IS CONVERTED TO DOPAMINE IN SEROTONERGIC TERMINALS IN THE RAT STRIATUM. R. Arai*, N. Karasawa, M. Geffard[1] and I. Nagatsu. Dept. of Anatomy, Fujita Health Univ. Sch. of Med. Toyoake, Aichi 470-11, Japan, [1]IDRPHT and EPHE, 33400 Talence, France.

L-DOPA administration is the most effective drug treatment for the Parkinson's disease. The administered L-DOPA may be converted to dopamine by enzymatic decarboxylation and restore the dopamine depletion in the striatum. Serotonergic cell bodies in the raphe nuclei project their nerve terminals to the striatum. These serotonergic neurons contain aromatic L-amino acid decarboxylase. In the present study, we used a double immunofluorescence staining method and examined the possibility that a portion of the administered L-DOPA is converted to dopamine in serotonergic terminals of the striatum. After i.p. injection of L-DOPA into the rat, dopamine immunoreactivity was found in serotonergic cell bodies of the raphe nuclei and in their terminals of the striatum. This suggests that, in the striatum of parkinsonian patients, the serotonergic terminals are involved in the conversion of L-DOPA to dopamine.

490.3

EFFECT OF CHRONIC LEVODOPA TREATMENT ON mRNA LEVELS OF D1 AND D2 DOPAMINE RECEPTORS IN THE BASAL GANGLIA OF MPTP-MONKEYS. M. Morissette[*1], M. Goulet[2], P.J. Blanchet[1], P.J. Bédard[1], and T. Di Paolo[2]. [1]Dept of Pharmacology, Faculty of Medecine, Laval Univ., Québec, G1K 7P4, and Neurobiology Res. Center, Enfant-Jésus Hospital; [2]Sch. of Pharmacy, Laval Univ., and Dept of Molecular Endocrinology, CHUL, Québec, G1V 4G2, CANADA.

The effect of levodopa on dopamine receptor gene expression in the basal ganglia of MPTP-monkeys was investigated using quantitative *in situ* hybridization. Seven ovariectomized female *macaca fascicularis* monkeys were rendered parkinsonian after subcutaneous administration of the toxin MPTP. Three MPTP-monkeys were treated with levodopa (100 mg) and the peripheral DOPA decarboxylase inhibitor Benserazide (25 mg) once daily for one month. Naive controls and untreated MPTP animals were also included in the experiment. Levodopa treatment produced very good motor recovery but caused progressive sensitization to treatment and, as expected, induced choreic dyskinesia. In MPTP-treated monkeys, a decrease of D1 receptor mRNAs was observed in the rostral part of the caudate and the putamen compared to control animals (-20% and -17% respectively). Chronic treatment of MPTP-monkeys with levodopa returned D1 receptors mRNA levels near to values for control monkeys. No such changes were observed in the more caudal parts of the striatum. A decrease of D1 receptor mRNA levels was observed in the olfactory tubercule (-21%) in MPTP-monkeys compared to control animals while no change was seen in the nucleus accumbens. An apparent increase in the levels of D2 receptor mRNAs was observed in the caudal part of the caudate and putamen (+24% and +23% respectively). Chronic levodopa treatment returned these levels to control values. No variation of D2 receptor mRNA levels was seen in the more rostral parts of the striatum. Our results show for the first time that levodopa can influence gene expression of D1 and D2 receptors in MPTP-monkeys. Supported by the MRC of Canada (to P.J.B.) and the Parkinson Foundation of Canada (to T.D.P. and P.J.B.).

490.4

CHRONIC L-DOPA TREATMENT PREVENTS THE DERANGEMENT OF THE STRIATAL SYNAPTIC TERMINALS INDUCED BY 6-OHDA. M.T. Pacheco-Cano , L. Colín-Barenque, M.R. Avila-Costa, V. Anaya-Martinez, J. Espinosa- Villanueva, E. Montiel-Flores, N. Manzano-León and F. García-Hernández*Neuroscience, ENEP Iztacala UNAM. A.P. 314. Tlalnepanla, México.

Unilateral lesioning of the nigrostriatal pathway with 6-hidroxydopamine (6-OHDA) produces a profound derangement of the striatal synaptic terminals. In the present study we analyzed the ultrastructural changes in the striatum of unilaterally 6-OHDA lesioned rats after chronic L- dopa treatment. Fourteen male Wistar rats were stereotaxically injected with 8 µg of 6-OHDA into the left medial forebrain bundle. Control rats (n=10) received vehicle sham lesions. All 6-OHDA lesioned rats showed more than 200 turns /30 minutes when tested with 0.25 mg/kg of apomorphine. Also all rats were continuously tested in the narrow uphill beam test. The sham lesioned group received a daily oral dose of 0.9% NaCl for 30 days, the 6-OHDA lesioned rats were then divided into two groups, one of which remained untreated (n=5), while the second group (n=9) received a daily oral dose of L-dopa (50mg/Kg) for 30 days. Rats were then sacrificed and the brain fragments obtained from the right (unlesion) and left (lesion) caudates were conventionally processed for electron microscopy. The ultrastructural analysis consisted of a) synaptic endings diameter, b) synaptic vesicles distribution and c) synaptic contact type. Our results confirm our previous observations about the dramatic derangement caused in the striatal synaptology by 6-OHDA, nevertheless in the 6-OHDA lesioned L-dopa treated group, almost all of the evaluated features remained not different from the values obtained from the sham lesioned group. The only difference between this to groups was a significant decrease of axospinous contacts made with the head of the spines. However, L-dopa treatment did not ameliorated the motor disturbances evaluated. These results indicate that L-dopa prevents the ultrastructural alterations that 6-OHDA produces.

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490.5

ASSESSMENT OF THE EFFECTS OF A D2-SPECIFIC DOPAMINE AGONIST (PRAMIPEXOLE) ON MOTOR BEHAVIOR OF PARKINSON'S DISEASE (PD) PATIENTS. N.J. Greenberg*, D.B.Willingham and J.P. Bennett. Dept. of Psychology, Univ. of Virginia, Charlottesville, VA 22903.

Treatment of Parkinson's Disease by drugs such as L-dihydroxypheylalanine (L-DOPA) have helped alleviate bradykinesia and performance deficits in initiation and planning of motor movements. L-DOPA, however, affects both D1 or D2 receptor subtypes which restricts conclusions that can be drawn from these studies. The present study assessed the effects of a D2-specific agonist, Pramipexole. Comparisons of performance on motor tasks were designed to selectively investigate the effects of D2 on speed, planning , and execution of motor responses. Twelve patients in the early stages of PD participated in a drug trial with half randomly assigned to a placebo group and half to an active medication group. The study was double-blind. Dosage in the active group was ramped over the course of the study. Patients were tested on fifteen visits spanning three months on tests of tapping speed, simple (SRT) and choice (CRT) response time , and sequencing. Results indicate that the D2-specific agonist is effective in alleviating motor symptoms of PD, but it's effectiveness varies by task.

490.6

***IN SITU* HYBRIDIZATION OF D1 AND D2 DOPAMINE RECEPTOR mRNAs IN THE CAUDATE-PUTAMEN OF MPTP MONKEYS: EFFECT OF CHRONIC TREATMENT WITH THE D2 AGONIST U91356A.** M. Goulet[1], M. Morissette[2], P. J. Blanchet[2], P.J. Bédard*[2] and T. Di Paolo[1] [1]Sch. of Pharmacy, Laval Univ., and Dept of Molecular Endocrinology, CHUL, Québec, G1V 4G2, [2]Dept of Pharmacology, Faculty of Medecine, Laval Univ., Québec, G1K 7P4, and Neurobiology Res. Center, Enfant-Jésus Hospital; CANADA.

Quantitative *in situ* hybridization histochemistry (ISH) was used to analyze the effect of a chronic D2 receptor (D2R) agonist (U91356A) treatment on D1 and D2 dopamine receptor mRNAs. U91356A was administered to ovariectomized female *macaca fascicularis* MPTP-monkeys for 27 days using an intermittent (n=3) or continuous (n=3) schedule. Untreated MPTP as well as naive control animals were also studied. U91356A relieved parkinsonian symptoms and stimulated locomotion in all animals. Animals treated in a pulsatile mode with U91356A showed progressive sensitization and had dyskinesias whereas those receiving U91356A continuously developed behavioral tolerance and no dyskinesia. Density of D1 and D2 receptor mRNAs were examined by ISH using human-specific cRNA probes. Densitometric analysis of X-ray film demonstrated that in general D2R mRNA levels varied rostro-caudally with lower values in the posterior striatum. In addition higher D2R mRNA levels were observed in the lateral versus the medial caudate-putamen. D1 receptor mRNA in anterior caudate and putamen were lower in MPTP monkeys compared to control animals whereas D2R mRNA in the posterior striatum was increased. U91356A treatment whether continuous or pulsatile did not affect D1 receptor mRNA. Pulsatile administration of U91356A did no significantly decrease striatal D2R mRNA versus MPTP. Continuous administration of U91356A decreased D2R mRNA in the posterior striatum, more specifically in the putamen versus MPTP. These findings are consistent with our previous results on D1 and D2 receptor densities. The present results suggest that continuous but not intermittent administration of D2 agonist leads to a decrease of D2R expression in the putamen, that may be implicated in behavioral tolerance. Supported by the MRC, the Parkinson Foundation of Canada and the Upjohn Co., Kalamazoo, USA.

490.7

U-95666A. A POTENTIAL ANTI-PARKINSON DRUG. V.H. Sethy*, B.R.Ellerbrock and H.Wu, CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001

U-95666A (U) is very selective for its binding to D_2 dopamine (DA) receptors. This compound has been investigated to determine its effect on striatal acetylcholine (ACh) and was compared with other DA agonists and antagonists. U, quinpirole, pergolide and bromocriptine significantly ($p<0.01$) increased, whereas haloperidol significantly ($p<0.01$) decreased striatal ACh levels. In rats with unilateral lesion of the substantia nigral (SN), U produced significantly ($p<0.02$) higher elevation in ACh on the lesioned side as compared to the intact side. Chronic treatment with U had no significant effects on U-induced increases in ACh either on the lesioned or intact striatum. U and diazepam decreased cerebellar cGMP and attenuated stress-induced elevation in this nucleotide. In conclusion, U is both a dopamine agonist and an anxiolytic, and would be useful for the treatment of Parkinson's disease.

490.8

D_1 DOPAMINE RECEPTOR ACTIVITY OF ANTI-PARKINSON DRUGS. G.J. Fici, H. Wu, P.F. VonVoigtlander* and V.H. Sethy. CNS Diseases Research, Upjohn Laboratories, Kalamazoo, MI 49001.

Clinical and preclinical investigations suggest that stimulation of D_1 dopamine receptors may be responsible for dyskinesias induced by dopamine agonist treatment of Parkinson's Disease (PD) and that these dyskinesias may be decreased by treatment with a D_1 antagonist (clozapine). We have examined the effects of dopamine agonists and antagonists in a primary cerebellar granule cell model of cAMP formation that seems to be highly responsive and discerning for the D_1 receptors. A ~9-fold increase in cAMP was observed with the D_1 agonist SKF 38393 that was completely blocked by the D_1 antagonist SCH 23390 but there was no significant cAMP change with the D_2 agonist U-95666A. SKF 38393, lisuride, pergolide, dopamine, bromocriptine and 7-OH-DPAT showed concentration dependent increases in cAMP with EC_{50} values of 0.013 µM, 0.053 µM, 1.04 µM, 2.18 µM, 50.9 µM and 54.4 µM, respectively. SKF 38393, dopamine and pergolide had similiar intrinsic activity (100%) while the intrinsic activity of 7-OH-DPAT, bromocriptine and lisuride were 28.0%, 20.7% and 17.2%, respectively. The D_2 agonist U-95666A showed no significant effect (1-1000 µM), while the D_3 agonist pramipexole (0.1-100 µM) showed a slight but significant decrease in cAMP formation. Clozapine concentration dependently blocked pergolide-induced increases in cAMP and was ~1700-fold less potent than SCH 23390 (IC_{50} values 0.97 µM and 0.56 nM, respectively). These data suggest that D_1-induced cAMP formation may be predictive of dopamine agonist-induced dyskinesias in PD. We hypothesize that a D_2 or D_3 agonist with either low D_1 agonist activity or D_1 antagonist activity would be an effective anti-Parkinsons drug.

490.9

PHARMACOKINETICS OF THE FULL EFFICACY D_1 AGONIST DIHYDREXIDINE IN RATS AND MONKEYS. K.W. Locke* Interneuron Pharmaceuticals Inc., Lexington, MA 02173.

The pharmacokinetic profile of dihydrexidine (DHX), a selective, full efficacy dopamine D_1 agonist undergoing clinical evaluation for the treatment of Parkinson's disease, was evaluated in rats and monkeys. In Sprague-Dawley rats administered a single 3.0 mg/kg 15 min iv infusion of ^{14}C-DHX, plasma radioactivity decreased in a biphasic manner, rapidly from maximal levels at 0 hr post-infusion and more slowly 3 hrs after the infusion. The elimination half-life of radioactivity from plasma was 6.8 hrs. Radioactivity distributed rapidly into all tissues, including brain, with significant concentrations observed 0 hr post-infusion. In general, clearance of radioactivity from tissues was greater than that from plasma. Radioactivity was excreted predominately by the urinary route, however, 18% of ^{14}C-DHX-derived radioactivity appeared to undergo biliary excretion. Plasma samples were collected on the last day from Cynomolgous monkeys administered 2.5, 7.5 or 15 mg/kg of DHX by 15 min iv infusion for 28 days. DHX was extracted from plasma using methyl-t-butyl ether and analyzed by HPLC-EC. DHX was rapidly eliminated from plasma by first order kinetics; the half-life of DHX ranged from 1.6 to 8.4 min. AUC values increased in proportion to the administered dose. As with other catechols, DHX appears to be rapidly metabolized by catechol-O-methyltransferase (COMT); this hypothesis is supported by mass spectrometry and by *in vivo* interaction experiments with COMT inhibitors. Pharmacodynamic studies demonstrate a much longer duration of action, than would be predicted from its relatively short plasma half-life.

490.10

L-DEPRENYL DOES NOT AFFECT DOPAMINE DEPLETION WHEN ADMINISTERED AFTER MPTP. L.E. DeLanney*, D.A. Di Monte, A.M. Janson[1], P. Chan, I. Irwin and J.W. Langston. The Parkinson's Institute, Sunnyvale, CA 94089, [1]Dept. of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden.

Although it is known primarily as an inhibitor of monoamine oxidase (MAO) B, deprenyl possesses a number of additional properties which may contribute to its therapeutic effects in Parkinson's disease. In particular, it has recently been shown to rescue dopaminergic neurons from death caused by the parkinsonism-inducing neurotoxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) without affecting the MAO-mediated MPTP metabolic activation. This rescue effect was demonstrated solely by counting neuronal cells in the substantia nigra of mice and has yet to be replicated by other research groups. Therefore, the levels of dopamine and dopamine metabolites were measured in this study as potential additional markers of the neuroprotective action of deprenyl in the same experimental conditions. C57BL/6 mice were first injected i.p. with 30 mg/kg/day MPTP for 5 consecutive days. Then, starting 72 hrs after the last MPTP injection, mice were treated with 10 mg/kg/day i.p. deprenyl three times per week for 3 consecutive weeks. Animals were killed and levels of dopamine and dopamine metabolites were measured in the striatum and ventral mesencephalon (v. m.). There were no differences between dopamine concentrations in the v. m. of mice treated with saline, deprenyl, MPTP or MPTP/deprenyl. In the striatum, dopamine levels were 20-30% higher in mice treated with deprenyl alone as compared with saline controls; MPTP alone decreased dopamine concentrations by 50% and dopamine values were 20-30% higher in the deprenyl/MPTP group as compared to the MPTP alone group. Thus, deprenyl did not affect MPTP-induced striatal dopamine depletion when values were expressed as per cent of the corresponding control groups. Neurochemical measurements do not reflect therefore the reported rescue effect of deprenyl on MPTP-induced neuronal death. Cell counting of dopaminergic neurons in the v. m. using an unbiased stereologic method is in progress.

490.11

CHRONIC ADMINISTRATION OF L-DEPRENYL INHIBITS MONOAMINE OXIDASE A ACTIVITY. D.A. Di Monte*, I. Irwin, P. Chan, L.E. DeLanney and J.W. Langston. The Parkinson's Institute, Sunnyvale, CA 94089.

The monoamine oxidase (MAO) B inhibitor deprenyl has attracted considerable attention as an adjunctive drug and a possible neuroprotective agent in Parkinson's disease. The relationship between MAO B inhibition and the therapeutic action of deprenyl has been questioned, however, because (1) deprenyl possesses a number of additional properties which may contribute to its mechanism of action, and (2) the absence of MAO B within dopaminergic terminals does not support a major role of this enzyme in dopamine metabolism. The purpose of this study was to test the hypothesis that, since MAO A activity is expressed in dopaminergic terminals, chronic administration of deprenyl may also affect this enzyme. The action of deprenyl as a selective MAO B inhibitor was compared in C57BL/6 mice after a single injection and chronic treatment (three times per week for three consecutive weeks). Results of the study indicate that doses of deprenyl (1, 5 and 10 mg/kg) which cause selective inhibition of MAO B after a single injection also decrease MAO A activity when administered chronically. A slight but statistically significant increase in dopamine concentrations and a marked decrease in DOPAC levels were also measured in the striatum following chronic deprenyl administration, but not after a single injection of the drug. Thus, in the experimental conditions of this study, deprenyl loses its MAO B selectivity after repeated administration and, by acting as a MAO A inhibitor, affects striatal dopamine metabolism. These results support the view that MAO A plays a more relevant role than MAO B in striatal dopamine metabolism and raise the possibility that the delayed onset of symptomatic benefits after deprenyl treatment may be due, at least in part, to MAO A inhibition.

490.12

INTRACRANIAL MICRODIALYSIS OF SALICYLIC ACID TO DETECT HYDROXYL RADICAL GENERATION IN THE RAT: EFFECT OF MONOAMINE OXIDASE INHIBITOR. T. Obata* and Y. Yamanaka. Department of Pharmacology, Oita Medical University, Hasama-machi, Oita 879-55, Japan

We examined the effect of pargyline, a monoamine oxidase inhibitor, on the generation of hydroxyl free radicals (·OH) using striatal microdialysis. The probe was washed with simple Ringer's solution for at least 30 min prior to stereotaxic implantaion in the striatum and then the infusion was continued (1 $\mu l \cdot min^{-1}$) for at least 60 min before switching to the experimental drug solution using liquid switch. The hydroxyl free radicals react with salicylate and generate 2,3- and 2,5-dihydroxybenzoic acid (DHBA) which can be measured electrochemically in picomole quantity by HPLC. When pargyline (100 $nmole \cdot \mu l \cdot min^{-1}$) was infused in rat brain, the level of 3,4-dihydroxyphenylacetic acid (DOPAC) gradually decreased in a time-dependent manner. In addition, a marked elevation of DHBA was observed. The present results indicate that accumulation of dopamine (DA) in the extracellular fluid elicited by pargyline can be auto-oxidized and in turn leads (possibly by an indirect mechanism) to the formation of cytotoxic ·OH free radicals.

490.13

DEGENERATION OF DOPAMINERGIC NEURONS IN THE SUBSTANTIA NIGRA FOLLOWING HALOPERIDOL TREATMENT IN THE RAT.

S.M. Savedia, R.S. Bobba, E.T. Kiriakopoulos, S. Garside, P. Rosebush, M. F. Mazurek*. Neuropsychiatry Research Programme, McMaster University Medical Centre. Hamilton, Ontario. L8N 3Z5.

Chronic administration of the D2 dopamine receptor antagonist haloperidol (HAL) is known to cause extrapyramidal side effects that may persist after the medication is withdrawn. In this study, we examined the effect of HAL on dopaminergic neurons of the substantia nigra (SN). Over 8 weeks, rats received daily intraperitoneal injections of either saline, low dose HAL (1mg/kg/day) or high dose HAL (10mg/kg/day). Two weeks after the final injection, the rats were sacrificed and the brains were immunostained for tyrosine hydroxylase (TH). There was a highly significant dose-dependent depletion of TH positive neurons in the SN of HAL treated rats (with a 32% reduction in the low HAL group and a 46% reduction in the high HAL animals). Also, there was a highly significant dose-dependent decrease in the density of TH neurons (19% reduction in low HAL, 31% in high HAL). These results indicate that HAL may be toxic to the dopaminergic neurons of the SN.

490.14

SYMPTOMATIC EFFECTS OF N-METHYL-D-ASPARTATE (NMDA) RECEPTOR ANTAGONISM IN PARKINSON'S DISEASE. P.J. Blanchet*, L. Verhagen-Metman, M.M. Mouradian and T.N. Chase. Experimental Therapeutics Branch, NINDS, Bethesda, MD 20892-1406.

Nigrostriatal dopaminergic cell loss in Parkinson's disease (PD) and oral levodopa (LD) replacement therapy alter the balance of excitatory amino acid transmission in the basal ganglia. Previous studies have shown that NMDA antagonists can potentiate the effects of LD in reserpinized rats (Klockgether and Turski, Ann Neurol 1990;28:539), 6-OHDA-lesioned rats (Engber et al., Neuro-Report 1994;5:2586) and MPTP-exposed monkeys (Wüllner et al., Neuro-pharmacology 1992;31:713; Greenamyre et al., 1994;35:655). We tested the hypothesis that NMDA antagonists might reduce motor response complications from LD with dextrorphan hydrochloride (DX), a dextrorotatory morphinan (Hoffmann-La Roche Inc., Nutley, NJ), in a double-blind, placebo-controlled, dose-escalation design.

Two PD patients, chronically treated with LD and experiencing motor response complications and dyskinesias, first received incrementing i.v. infusions of DX as monotherapy for 6 hrs. Given alone, DX displayed no antiparkinsonian benefit and a deterioration in parkinsonian disability was documented in one patient in the higher dose range used. In combination with an optimal i.v. LD bolus dose, a well-tolerated DX infusion produced an apparent reduction in the intensity of LD-induced, peak-dose dyskinesia in both patients, but did not antagonize the LD antiparkinsonian response. Dose-limiting adverse effects occurred in both patients (dysarthria, inattention, dizziness, ataxia). These preliminary observations indirectly suggest that antiparkinsonian and dyskinetic effects of LD could be mediated by different mechanisms and that NMDA antagonists could be useful adjuncts for the treatment of LD-induced dyskinesias in PD. [Supported by a fellowship grant from The Parkinson Foundation of Canada to PJB]

490.15

NEUROPROTECTIVE EFFECTS OF RILUZOLE ON A MODEL OF PARKINSON'S DISEASE IN THE RAT. P. Barnéoud, M. Mazadier, J.-M. Miquet, S. Parmentier, P. Dubédat, A. Doble* and A. Boireau. Rhône-Poulenc Rorer S.A., CRVA, Biology Department, 13 quai Jules Guesde, F-94403, Vitry-sur-Seine Cedex, France

The aim of the present study was to analyze whether riluzole, a compound that interacts with the voltage-dependent sodium channel and impairs glutamatergic transmission, would exhibit a neuroprotective activity in a model of Parkinson's disease in the rat. Impaired skilled forelimb use, circling behavior, and altered dopaminergic metabolism of the mesotelencephalic system were evaluated in unilaterally 6-hydroxy-dopamine-lesioned rats. Riluzole (2x5 mg/kg, i.p.) reduced (50%) both the contralateral rotations induced by apomorphine and the ipsilateral ones elicited by amphetamine. Moreover, the decreased dopaminergic metabolism seen after 6-hydroxy-dopamine injection was slightly attenuated in the riluzole-treated animals, both at the striatal and nigral levels. These biochemical and behavioral results demonstrate the ability of riluzole partially to protect the degeneration of the nigrostriatal dopaminergic neurons induced by the toxin 6-hydroxy-dopamine. Perhaps, the most telling evidence of the protection afforded by riluzole was that this compound improved the skilled paw use, a complex sensorimotor behavior which is not easily ameliorated by palliative therapies such as dopaminergic grafts. Noteworthily, riluzole protected the rats against the learning impairment of paw reaching induced by the unilateral 6-hydroxy-dopamine lesion. These results extend previous data showing that riluzole counteracts the toxicity induced by MPTP and MPP+ in rodent dopaminergic neurons. The use of riluzole may be considered of potential interest for the neuroprotective therapy of Parkinson's disease in humans.

490.16

INTERACTIONS OF HU-211, A NOVEL NMDA ANTAGONIST WITH THE DOPAMINERGIC SYSTEM. S. Striem, A. Bar-Joseph, Y. Berckovitch, V. Nadler * and A. Biegon. Pharmos Corp., Kiryat Weizmann, Rehovot, 76326, Israel

HU-211 is a novel NMDA antagonist, devoid of the severe side effects characteristic of many other NMDA antagonists. In the present study, we examined its interaction with the dopaminergic system using in vitro and in vivo systems and comparing its activity to MK-801. Binding studies on forebrain membranes demonstrated that HU-211 (100μM) inhibited the binding of SCH 23390, a D_1 antagonist by 51.8±6.3%. HU-211 also enhanced the conversion of [^{3}H] adenine to cyclic AMP (51.8±29.7%). This increase was not reduced by SCH 23390. A synergistic effect (314.7±14.3%) was evident with a D_1 agonist. This effect is specific to the dopaminergic system. In vivo we examined the ability of HU-211 to reduce catalepsy induced by dopamine antagonists. BALB/c mice were treated with either HU-211(10mg/kg), or MK-801(1mg/kg), immediately after administration of either SCH 23390(10mg/kg), the D_2 antagonist clebopride(20mg/kg) or the mixed D_1 and D_2 antagonist haloperidol(1mg/kg). Catalepsy was then measured every hour for 6 hours. HU-211 significantly ($p< 0.0001$) reduced the total catalepsy time induced by all dopamine antagonists in the present study. Catalepsy times were: SCH 23390; 46±8 for vehicle and 11±4 sec for HU-211, clebopride; 58±8 and 22±7 sec respectively and haloperidol; 82±8 and 29±7 sec. respectively. HU-211 reduced the dopamine antagonists-induced catalepsy in a similar manner to MK-801, but with no behavioural side effects. In conclusion, the present work describes the ability of HU-211 to inhibit the binding of D_1 receptor antagonist, to reduce catalepsy induced by D_1 and D_2 antagonists and to specifically cause an increase in dopamine receptor mediated CAMP production. This may have therapeutic implications in diseases involving disorders of the dopaminergic system, such as Parkinson disease.

490.17

ANTIPARKINSONIAN EFFECTS OF THE NOVEL NMDA ANTAGONIST CP-101,606 Kathy Steece-Collier*, Rodrigo Pazmino & J Timothy Greenamyre Chicago Medical School & University of Rochester, Rochester, NY 14642.

After dopaminergic denervation of striatum, glutamate pathways from subthalamic nucleus to the basal ganglia output nuclei become overactive, and play a major role in the pathophysiology of Parkinson's disease. Therefore, pharmacological manipulation of the glutamatergic system may exert antiparkinsonian effects. In the current study, we examined the ability of CP-101,606 (CP), a selective antagonist of the NR2B subunit of the NMDA receptor, to produce antiparkinsonian effects in MPTP-treated rhesus monkeys with a longstanding, stable parkinsonian syndrome. One hour after injection of CP (1.0 mg/kg; s.c.), the parkinsonian clinical score decreased by 20% to a mean of 7.91 ± 0.54, compared to 9.84 ± 0.55 after vehicle injection ($p<0.01$); the effect was still significant at 2 hours ($p<0.02$). Levodopa methylester (LD) alone (10 mg/kg) had a moderate antiparkinsonian effect at 1 hour, decreasing the score by 23% ($p<0.05$). The combination of CP (0.05 mg/kg) plus LD had a profound antiparkinsonian effect (5.54 ± 0.43) that was better than either vehicle (-44%; $p<0.0005$) or LD alone (-27%; $p<0.05$). At two hours, the effect of CP plus LD (5.86 ± 0.62) was still better than either vehicle (-47%; $p<0.0001$) or levodopa methylester (-33%; $p<0.002$). No side effects were seen. We conclude that CP alone has antiparkinsonian effects and that it enhances the therapeutic effects of LD. *(Supported by Pfizer Inc., a Mallinckrodt Scholar Award, USPHS grant NS33779 and the National Parkinson Foundation Center of Excellence at the University of Rochester.)*

DEGENERATIVE DISEASE: PARKINSON'S—MPTP MODELS

491.1

ACUTE EFFECT ON THE BASAL GANGLIA OF INTRACAROTID ADMININSTARTION OF 1-METHYL-4 PHENYL-1,2,3,6-TETRAHYDRO-PYRIDINE (MPTP) TO RHESUS MONKEYS L.S. Forno*#, D. Nagy, J.Conway, M. Emborg, W. McLaughlin and K.S. Bankiewicz. Somatix Therapy Corporation, Alameda CA 94501 and #VA Medical Center, Palo Alto, CA 94304

Symptoms of Parkinson's disease are primarily related to the degeneration of the nigrostriatal dopaminergic system. MPTP administration to human and non-human primates produces selective dopaminergic neuron lesion in the nigrostriatal system and causes severe parkinsonian motor deficits. In this study we examined the acute cellular toxicity of high dose of MPTP.

Two rhesus monkeys were severely affected and died 3 days after unilateral intracarotid injection of 4 mg of MPTP-HCL. 40μm whole mount frozen sections were stained with hematoxylin-eosin and with antibodies to tyrosine hydroxylase (TH) and to glial acidic fibrillary protein (GFAP). There was grossly visible swelling of the neostriatum on the side of injection that correlates well with acute oedema seen on MRI. Reaction to the TH antibody was very weak in the putamen on the affected side, contrasting with the strong reaction on the uninjected which served as control. The caudate nucleus on the side of injection had only a mild reduction of staining for the TH antibody. There was no cell depletion in the substantia nigra seen on either side in these monkeys. Staining for GFAP was reduced, but mainly in the anterior putamen. The TH antibody reaction was mildely reduced in the substantia nigra. A puzzling finding was recent focal infarcts, one in each monkey, but located in the globus pallidus internus in one, and in the internal capsule and substantia nigra in the other. These lesions were interpreted as embolic infarcts, perhaps without direct relationship to MPTP or MPP+. The findings suggest that the acute effect of intracarotid administration of MPTP is complex and represents more then a simple degeneration of the dopaminergic terminals.

491.2

THE DEVELOPMENT OF A NEW SQUIRREL MONKEY MODEL OF PARKINSON'S DISEASE. S. L. Pinkston, L. De Lanney, M. Emborg*#, D. Nagy, J. W. Langston, I. Irwin, D. Kutzscher, F. Wu# and K.S. Bankiewicz. The Parkinson's Institute, Sunnyvale, CA 94089 and #Somatix Therapy Corporation, Alameda, CA 94501.

The goal of this study was to develop a stable model of Parkinson's disease (PD) in squirrel monkeys. Unilateral intracarotid artery (ICA) infusion plus systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was carried out in 19 squirrel monkeys. Since MPTP toxicity is achieved by first pass ICA administration, the dose of MPTP-HCL was given per brain rather than per body weight: normal saline (n.s.) (n=1), 0.5 (n=2), 0.6 (n=3), 0.7 (n=12) and 1.0 (n=1) mg. 3 ICA infusion rates were tested: 1) 1.0 ml/min in 15 ml n.s., 2) 0.5 ml/min in 10 ml n.s., 3) 0.25 ml/min in 5 ml n.s. MRI brain scans showed lateral ventricle enlargement and hemorrhagic lesions in ipsilateral striata using rates 1 and 2 but no lesions using rate 3. The dose of 0.7 mg produced the most consistent signs of hemiparkinsonism (HPD) with some bilateral effects (e.g., bradykinesia). Additional systemic (s.c.) MPTP at 1-1.5 mg/kg produced progressive parkinsonian signs. L-dopa and apomorphine administration increased total body activity and contralateral turning dose-dependently. Histological, biochemical, and in vivo microdialysis examination of brains showed dose-dependent depletion of the dopaminergic system (DS). TH immunostaining revealed dramatic depletion of cells in the ipsilateral substantia nigra and fibers in the striatum with a mediolateral gradient of lesion contralaterally. Dopamine and dopa-decarboxylase measured in brain punches from striata was reduced 98% ipsilaterally and 50-80% contralaterally. Behavioral recovery was much less likely to occur using this technique than after systemic MPTP administration. The model appears to achieve a more stable level of DA depletion in the DS ipsilateral to the MPTP lesion characteristic of acute parkinsonism, while the contralateral partially lesioned side correlates with advanced parkinsonism. Therefore, this approach appears to be a more effective way to produce stable parkinsonism compared to systemic treatment alone. Because it also represents two models in one (partial lesion on one side and complete lesion on the other) this model should have many uses including the study of neurografting directed to DA replacement therapy on the ipsilateral side and/or trophic approach on the contralateral side.

491.3

REST TREMOR IN RHESUS MONKEYS WITH MPTP-INDUCED PARKINSONISM. M.E. Emborg, J.W. Tetrud*#, W.W. McLaughlin and K.S. Bankiewicz. Somatix Therapy Corp., Alameda, CA 94501 and #The Parkinson's Institute, Sunnyvale, CA 94089.

Rest tremor is a characteristic feature of Parkinson's disease (PD) and can also be present in humans and monkeys with MPTP-induced parkinsonism. We quantified rest tremor in 3 rhesus monkeys who received an unilateral intracarotid artery (ICA) infusion of 2.5 mg MPTP-HCL followed by 2-4 doses i.v. of 0.3 mg/kg MPTP. The animals were trained to remain restrained in a primate chair for periods of 120 min. An angular rate sensor (26g) from a tremor monitor (Motus I, Motus Bioengineering, Benica, CA) was taped on the dorsum of each monkey's hand contralateral to the side of the ICA administration with the opposite arm restrained. The sampling time for each recording was 12 sec from which a frequency power spectrum was produced. Recordings began 45 min prior to and ended 45 min following administration of oral 25/250 mg carbidopa/levodopa (CD/LD). The mean peak of tremor frequency prior to CD/LD in the 3 animals was 7.9 hz (SE:0.2) and following CD/LD, it was maintained (15min:8.1,SE:0.2; 30min:8.1,SE:0.3; 45min:7.8,SE:0.5), but there was a 75% reduction in tremor amplitude. We conclude that: (i) the mean rest tremor frequency in rhesus monkeys (7 to 8.5) is higher than in humans with PD (4 to 6 hz), (ii) as in PD, tremor frequency in the monkey is maintained within a rather narrow band width, (iii) as in PD, the amplitude of rest tremor in the monkey is reduced by CD/LD, and (iv) the Motus I tremor monitor is a useful tool for objective characterization of tremor in the monkey and should provide objective assessment of tremor changes in experimental implantation of dopaminergic tissue, pallidotomy and thalamotomy.

491.4

LONG-TERM COGNITIVE IMPAIRMENT IN MPTP-TREATED MONKEYS. J. Fernandez-Ruiz*, D. Doudet and T. Aigner. Lab. of *Neuropsychology, NIMH, NIH Bethesda, MD 20892-4415.*

Administration of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine) is known to induce a long-lasting Parkinson-like motor syndrome in monkeys. In addition, it has been shown that MPTP monkeys manifest cognitive deficits on tasks known to assess the fronto-striatal system; there are, however, no data regarding long-term cognitive effects. In this study, we examined the cognitive abilities of monkeys seven years after MPTP administration. Four MPTP-treated and four age-matched control male rhesus monkeys (*macaca mulatta*) performed a delayed response spatial task under two different experimental conditions in a Wisconsin General Test Apparatus. In the first experiment each monkey started at 0-sec delay and had to achieve a criteria of 90% correct choices, after which 10 sec were added to the delay for the next 30-trial session. The experiment was concluded if the monkey could not reach the 90% criteria within 400 trials. For the second experiment, delays from 0 to 60 sec (in intervals of 10) were repeated four times each in a random manner during the daily session. The results of the first experiment showed a significant difference ($p<0.01$) between the maximum delay attained by the control group (37.5 sec) and that by the MPTP group (22.5 sec). In the second experiment, both groups performed equally well at 0-sec delay; with longer delays, the MPTP monkeys' perfomance declined leading to a statistically significant interaction between delay and drug ($p< 0.05$). These results suggest that the MPTP-treated monkeys have a delay-sensitive spatial memory impairment that is apparent seven years after treatment. In this respect, the MPTP-treated primate displays spatial deficits similar to those observed in Parkinson's patients and thus may offer a useful model for evaluating novel therapeutic approaches to this disease.

491.5

STRIATAL SOMATOSENSORY ACTIVATION IN PRIMATES WITH OR WITHOUT PARKINSONIAN SIGNS. J.S. Schneider, L.L. Brown*, D.S. Rothblat, D.M. Smith, M.G. Smith and T.I. Lidsky. Depts. of Anat. and Neurobiol. and Neurology, MCP and Hahnemann Univ., Phila., PA 19102; Depts. of Neurol. and Neurosci., Albert Einstein Coll. of Med., Bronx, NY 10461; Inst. for Basic Res., Staten Island, NY 10314.

Electrophysiological studies have suggested that parkinson-producing lesions alter the sensory responsiveness and response characteristics of striatal and pallidal neurons. Such a disruption of information processing may underlie some of the signs and symptoms of parkinsonism. To further examine sensory information processing in the primate striatum, and the influence of dopamine (DA) on the processing of this information, we performed [^{14}C]-2-deoxyglucose autoradiographic mapping studies in M. fascicularis monkeys during somatosensory stimulation of the forearm. Two monkeys were intact, 2 were MPTP-treated and parkinsonian, and 1 was MPTP-treated and functionally recovered from parkinsonism. Forearm somatosensory stimuli caused 1) peak metabolic activation patterns consistent with known corticostriate projection patterns in many putamen regions in an intact animal, 2) a slightly greater area of activation in the recovered animal than in the intact animal, and 3) a remarkably widespread and metabolically greater activation pattern throughout the caudate and putamen in the parkinsonian monkey. Unlike other metabolic mapping studies of unstimulated MPTP-treated primates, the stimulated parkinsonian animal showed increased local cerebral glucose utilization in caudate and putamen that consisted of special, expanded patterns in comparison to a dopamine-intact animal and a DA depleted but asymptomatic animal. These results further emphasize and characterize the abnormal processing of somatosensory information in parkinsonian monkeys, and further emphasizes the DAergic regulation of the specificity and plasticity of afferent information processing in the striatum. Supported by MH 46531, NS 21356, and the F.M. Kirby Fdn.

491.6

QUANTITATIVE AUTORADIOGRAPHIC STUDY OF GM1 GANGLIOSIDE EFFECTS ON GABA$_A$ RECEPTORS IN AN EXPERIMENTAL MODEL OF PARKINSONISM IN MONKEYS. A. Pope-Coleman* and J.S. Schneider. Depts. of Anat. and Neurobiol. and Neurology, MCP and Hahnemann University, Philadelphia,PA.19102

GM1 ganglioside has been proposed as a possible therapeutic agent for the treatment of Parkinson's disease. Previous experiments have shown that GM1 treatment to monkeys can result in small but significant increases in striatal dopamine (DA) levels, increases in striatal DA transporter density, and a reversal of DA D1 receptor density upregulation. In view of the importance of dysfunction of GABAergic striatal projection neurons to the functional consequences of nigrostriatal DA denervation, the present study examined the effects of GM1 treatment on MPTP-induced changes in GABA-mediated neurotransmission. GABA$_A$ receptor density in striatum, pallidum, and substantia nigra was evaluated with [^{3}H]-flunitrazepam (FLZ) binding to the benzodiazepine site of the GABA$_A$/benzodiazepine receptor complex. In parkinsonian animals that received MPTP+saline treatments, there was a rostral-caudal gradient of decreased striatal [^{3}H]-FLZ binding. The greatest decrease in [^{3}H]-FLZ binding was observed in the substantia nigra (SN) and in the internal and external segments of the globus pallidus. Six weeks of GM1 treatment subsequent to the induction of parkinsonism by MPTP, resulted in a partial restoration of [^{3}H]-FLZ binding in all regions sampled. In the striatum, GM1-induced increase of [^{3}H]-FLZ binding was greatest at the caudal level. In the pallidum, GM1-induced increases in [^{3}H]-FLZ binding were greater in the internal segment than the external segment. Restoration of SN [^{3}H]-FLZ binding was of lesser magnitude than that observed in the pallidum. The present results suggest that GM1-induced functional recovery is accompanied by changes in the DAergic system that seem to normalize neurotransmission in striatal output circuits. Supported by the F.M. Kirby Foundation and Fidia Pharmaceutical Corp.

491.7

DIFFERENCES IN STRIATAL DOPAMINE INNERVATION AND PREPROENKEPHALIN mRNA LEVELS IN MONKEYS WITH DIFFERENT DEGREES OF PARKINSONISM. L. DiStefano*, C. Chang, A. Pope-Coleman and J.S. Schneider. Depts. of Anat. and Neurobiol. and Neurology, MCP and Hahnemann University, Philadelphia, PA 19102.

The nigrostriatal dopamine (DA) system in non-human primates appears to consist of several subsystems that display different degrees of vulnerability to MPTP and exert various effects upon peptide expression in different functional striatal territories. Most studies concerned with examining neurochemical changes associated with parkinsonism have concentrated on monkeys made acutely parkinsonian by MPTP and have provided no information concerning the relative contributions of different neurochemical systems in the striatum to cognitive or motor deficits of parkinsonism. In the present study, the DAergic innervation of the striatum (visualized by [^{3}H]-mazindol autoradiography) and preproenkephalin (ENK) gene expression (visualized by in situ hybridization histochemistry) were examined in normal monkeys, MPTP-exposed parkinsonian monkeys (PD), and chronic low dose MPTP-exposed monkeys (CLD) with cognitive but no motor deficits. In PD monkeys, loss of striatal DA innervation was greatest in sensorimotor territories, and, in all striatal territories, exceeded the loss of DA innervation observed in CLD MPTP monkeys. In CLD MPTP monkeys, loss of striatal DA innervation was similar across functional striatal territories, although the loss was generally greater in dorsal vs. ventral regions. In PD monkeys, ENK mRNA expression increased dramatically, particularly in dorsal lateral putamen and caudate. Much smaller increases in ENK gene expression were observed in these areas in CLD MPTP monkeys. These data suggest that progression of parkinsonism from primarily a cognitive disorder to primarily a motor disorder is accompanied by increased regional loss of striatal DA innervation and regional increases in ENK gene expression, particularly in sensorimotor territories. Supported by grant MH-46531.

491.8

FACTORS AFFECTING STRIATAL DOPAMINE RELEASE IN NORMAL, SYMPTOMATIC AND RECOVERED MPTP-TREATED CATS. AN IN VIVO MICRODIALYSIS STUDY. Angeles Fernandez and J.S. Schneider*. Depts. of Anat. and Neurobiol. and Neurology, MCP and Hahnemann University, Philadelphia, PA.19102

MPTP administration to cats causes an initial parkinsonian condition that spontaneously recovers. These animals have more extensive striatal dopamine (DA) terminal loss dorsolaterally then ventromedially. Although extracellular fluid (ECF) levels of DA are significantly increased in recovered animals, the source of this DA and factors influencing its release remain in question. The present studies were conducted to further examine possible DAergic mechanisms contributing to functional recovery in MPTP-treated cats. Amphetamine (AMPH, 10μM), NMDA (1 mM), and KCl (60 mM) were locally infused into dorsolateral caudate (DL CD) or ventromedial caudate (VM CD) via microdialysis probes in normal, symptomatic, and recovered MPTP-treated cats, and effects on ECF DA and metabolite levels were examined. In normal animals, ECF DA levels increased significantly and similarly in response to all test compounds, with AMPH-induced release significantly outlasting the time course of NMDA or KCl-induced release. In symptomatic animals, AMPH, NMDA, and KCl-induced release was undetectable in both DL and VM CD. In animals functionally recovered from parkinsonism, DA release was observed in response to all compounds, but peak releases were significantly less than in normal animals. Peak AMPH-induced DA release greatly exceeded peak NMDA-induced DA release in both striatal regions sampled. The latency to release and clearance time for released DA was prolonged in recovered animals compared to normal animals. These results suggest that AMPH and NMDA may increase ECF DA levels through different mechanisms and that these mechanisms for DA release may be differentially affected during functional recovery from an extensive DA-depleting lesion. Supported by NIH grant NS 23980.

491.9

ALTERATIONS IN EXPRESSION OF DOPAMINE AND EXCITATORY AMINO ACID (EAA) RECEPTORS IN THE STRIATUM OF CATS SYMPTOMATIC FOR AND RECOVERED FROM MPTP-INDUCED PARKINSONISM. David S. Rothblat* and J.S. Schneider Depts. of Anat. and Neurobiol. and Neurology, MCP and Hahnemann University, Philadelphia, PA 19102.

Interactions between the two major sources of afferent input to the striatum, the dopaminergic input from the ventral mesencephalon and the glutamatergic input from the cerebral cortex and thalamus are not completely understood. Loss of striatal dopamine (DA) can have significant effects on behavior as well as the regulation of dopamine receptors. The present study was performed to examine the extent to which striatal DA loss affects expression of striatal D1 and D2 DA receptors and the levels of EAA receptors in the striatum of animals symptomatic for and spontaneously recovered from MPTP-induced parkinsonism. Striatal D1 receptor gene expression was upregulated in parkinsonian cats and further upregulated in recovered cats. Striatal D2 receptor gene expression did not differ from normal in either experimental condition. [^{3}H]-AMPA binding was decreased in the dorsal striatum and and slightly less so in the ventral striatum in parkinsonian cats. There was no difference in striatal [^{3}H]-MK-801 binding (to NMDA receptors) between normal and parkinsonian cats. In cats recovered from parkinsonism, [^{3}H]-AMPA binding in the dorsal striatum partially recovered, while [^{3}H]-AMPA binding in the ventral striatum was similar to that observed in symptomatic animals. [^{3}H]-MK-801 binding in recovered cats did not differ from symptomatic or normal animals. These results suggest that changes in D1 receptor expression may be important for functional recovery and that striatal AMPA receptors, particularly in the dorsal or 'sensorimotor' striatum, may be more responsive to changes in DAergic tone and may better correlate with the functional status of the animal than NMDA receptors. Supported by NIH grant NS 23980.

491.10

CHANGE IN DOPAMINE TRANSPORTER (DAT) SITES AND mRNA LEVELS IN C57BL/6 MICE AFTER MPTP TREATMENT. A.Stadlin*, P.Chan[1], H.L.Choi and L.A.Snider. Dept of Anatomy, Chinese Univ. Hong Kong, Shatin, N.T Hong Kong and [1]The Parkinson's Inst., Sunnyvale, CA 94089.

It has been shown that MPTP treatment results in the loss of dopaminergic neurons in the substantia nigra (SN) and a decrease in dopamine levels in the striatum of C57BL/6 mice. A significant recovery had been reported to occur after MPTP treatment in mice. The present study aims to determine the effects of MPTP on DAT and its relation to the recovery. 6-8 weeks old C57BL/6 mice were treated with a single injection of either MPTP (40mg/kg, s.c.) or saline and sacrificed at 1hr, 4hr, 1, 3 and 7 days after treatment. The striatal DAT binding sites were measured by Scatchard analysis using [^{3}H]WIN35,428 as a ligand. The DAT mRNA levels were determined in the SN by isolating poly(A)$^+$ total mRNA from the same animals used for DAT binding study and hybridized with a 496 bp DAT probe. Results showed that there was a very rapid increase in the no. of DAT during the first 4 hours after MPTP treatment. This was followed by a gradual decrease reaching a maximum of 54% decrease of DAT sites by day 3. At day 7, the no. of DAT sites had significantly recovered to only 26% less as compared to the control. The K_d for DAT was 1 fold higher in the MPTP-treated mice (control K_d = 19.19nM; MPTP-treated K_d = 42.19nM). Northern blot analysis revealed that there was a maximum decrease of 80% in the level of mRNA in day 3 MPTP-treated mice. However, the mRNA level had returned to normal value by day 7 when a marked decrease in striatal DA content was found. It can be concluded that MPTP treatment resulted in a maximum loss of DAT sites and mRNA levels at day 3 after MPTP exposure. However, a compensatory mechanism exists whereby the remaining neurons increase in DAT binding affinity and upregulate mRNA, which may contribute to the significant post-treatment recovery.

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491.11

SECOND MESSENGER MODULATION OF L-AROMATIC AMINO ACID DECARBOXYLASE AND TYROSINE HYDROXYLASE IN NORMAL AND MPTP -LESIONED MOUSE STRIATUM. E.A. Young*, N.H. Neff and M.Hadjiconstantinou Departments of Pharmacology and Psychiatry and The Neuroscience Program, Ohio State University, Columbus OH 43210

Rapid and transient regulation of tyrosine hydroxylase (TH) activity by phosphorylation has been extensively studied, however, much less is known about regulation of L-aromatic amino acid decarboxylase (AAAD) activity. We have previously demonstrated that AAAD activity is transiently increased by activation of cyclic AMP and protein kinase C (PKC) dependent pathways in mouse striatum and midbrain (J. Neurochem. (1993) 60:2331-33 and (1994) 63:694-97). Under these same conditions of intracerebroventricular administration of forskolin or phorbol myristic acetate (PMA), TH activity increases transiently, presumably through phosphorylation. MPTP lesions have been shown to reduce both AAAD and TH activities, but 2 weeks after the lesion, Northern blots reveal at least a 2-fold increase in both TH and AAAD mRNA levels. The lesion also alters regulation of TH and AAAD by cyclic AMP and PKC dependent pathways. Both TH and AAAD are capable of further transient activation by forskolin. The increases are of greater magnitude and TH activity remains elevated longer. MPTP lesions abolish the response of AAAD to PMA, and delays and abbreviates the response of TH. This work ws supported in part by grants NS-34571 andMH-10629.

491.12

MPP+ AND AN ENDOGENOUSLY FORMED ANALOG, N-METHYLATED β-CARBOLINIUM, INHIBIT IN VIVO ACTIVITIES OF STRIATAL TYROSINE HYDOROXYLASE AND MONOAMINE OXIDASE. K. Matsubara*, T. Idzu, Y. Kobayashi, D. Nakahara[2], W. Maruyama[1], K. Kimura and M. Naoi[2]. Section of Neuroscience & Sense Organs, Shimane Med. Univ., Izumo 693, [1]Dept. of Psychology, Hamamatsu Univ. Sch. of Med., and [2]Dept. of Biosciences, NIT, Nagoya 466, Japan.

Intracerebral microdialysis in freely moving rats was used to compare the neurotoxic effects of 2,9-dimethylnorharmanium (2,9-Me2NH+) with those of MPP+ in the striatum. Perfusion with either 2,9-Me2NH+ or MPP+ evoked a marked increase of dopamine (DA) and decreases of its acidic metabolites in the dialysate, even at low doses (≤ 0.1 mM). The effects of both compounds (≤ 0.1 mM) on 5-HT and 5-HIAA efflux were negligible. However, the 5-HT system was affected by perfusion with 1 mM of both compounds. As an index of the in situ activity of tyrosine hydroxylase (TH), the rat striatum was also perfused with NSD-1015, and extracellular DOPA levels were measured. MPP+ irreversibly inhibited in situ TH activities almost completely, even at a dose as low as 0.05 mM. On the contrary, 1 mM 2,9-Me2NH+ reduced TH activities to 10% of the basal level only during its perfusion. Although the modes of TH inhibition were different, these results suggested that both 2,9-Me2NH+ and MPP+ were selectively taken up into the dopaminergic nerve terminal, where these cations eventually inhibited DA synthesis by TH and metabolism by monoamine oxidase.

491.13

(+)MK-801 DOES NOT PROTECT NIGRAL DOPAMINE NEURONS AGAINST MPTP-INDUCED LONG-TERM TOXICITY IN C57BL/6 MICE. J.W. Langston, A.M. Janson[1], D.A. Di Monte, W. Cao* and P. Chan. The Parkinson's Inst., Sunnyvale, CA 94089; [1]Dept of Neurosci., Karolinska Inst., Stockholm, Sweden.

Several studies have suggested that excitatory amino acids and activation of NMDA receptors may be involved in dopaminergic neurotoxicity caused by the neurotoxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), although other investigations reported contradictory results. The present study was designed to evaluate the effects of (+)MK-801, an NMDA receptor antagonist, on MPTP neurotoxicity in the mouse substantia nigra. Six to 8 week old C57BL/6 mice were treated with a single dose of MPTP (40 mg/kg, s.c.). (+)MK-801 (1 mg/kg, i.p.) was given to animals 30 min prior to the MPTP treatment and additional two injections were administered at 4 hr intervals following the first one. Levels of dopamine as well as the total number of dopamine neurons using an unbiased stereological cell counting method were determined at different time points after the exposure. In animals sacrificed one week after MPTP, (+)MK-801 significantly prevented MPTP-induced decrease in levels of dopamine in the ventral mesencephalon (VME), and DOPAC and HVA in both VME and striatum. In contrast, there were no differences between the (+)MK-801/MPTP and the MPTP-alone groups at three weeks. Although the total number of tyrosine hydroxylase positive neurons was slightly but not statistically significantly higher in (+)MK-801 pretreated animals as compared to the MPTP-alone group at one week, their values were not different at three weeks. Furthermore, (+)MK-801 did not affect the formation of MPP$^+$, but it significantly delayed MPP$^+$ elimination from the VME. These data suggest that (+)MK-801 does not have a long-term protection against MPTP-induced neurotoxicity in the mouse substantia nigra, although the possibility that excitatory amino acids are involved in MPTP toxicity cannot be ruled out.

491.14

EVALUATION OF METALLOTHIONEIN INDUCERS AFTER MPTP. P. Rojas, C. Ríos and R. Barroso*. Dept. of Neurochemistry, Instituto Nacional de Neurología y Neurocirugía, México city, 14269.

MPTP induces selective damage in brain dopaminergic neurons and produces parkinsonian syndrome in human. In the present study the possible antiparkinsonian activity of -metallothionein (MT) inducers was assessed in the MPTP model. Adult male swiss albino mice were injected with cadmium (Cd, 1 mg/Kg, i.p.) or dexamethasone (Dex, 5 mg/Kg, i.p.) and 5 hrs. later with MPTP (30 mg/Kg, i.p.) daily for 3 or 5 days. 7 days after the last MPTP injection all animals were sacrificed by decapitation, their -brains were removed rapidly, and their striata were dissected out. Striatal dopamine -- concentrations were obtained by HPLC - electrochemical analysis. We found significant increases in striatal dopamine content as follows: MPTP (3 days) + Cd (38%); MPTP (3 days) + Dex, (28%): MPTP (5 days) + Cd, (48%) and MPTP (5 days) + Dex, (43%) versus animals -treated with MPTP alone. Results indicate that MT induction can protect against MPTP -induce neurotoxicity.

491.15

IN VITRO AND IN VIVO PREVENTION OF MPTP INDUCED NEUROTOXICITY BY A SYNTHETIC SUPEROXIDE SCAVENGER, EUK-134 C. Fonck*, B. Malfroy* and M. Baudry., Neurobiology Program, University of Southern California, Los Angeles, CA 90089-2520 and * Eukarion Inc., Bedford, MA 01230.

Selective damage to neurons in the nigrostriatal pathway by 1-methyl 1-4-phenyl-1,2,3,6 - tetrahydropyridine (MPTP) has been proposed as a model to study Parkinson's disease. It has been suggested that MPTP's deleterious effects result from free radical production due to oxidation of MPTP to MPP^+ in the brain. The present study tested the ability of EUK-134, a synthetic free radical scavenger, to prevent striatal tissue damage produced by MPTP. In vitro experiments consisted in incubating mice striatal slices with MPTP (2 mM) and evaluating the extent of damage to dopaminergic terminals by measuring ^{3}H-dopamine uptake. Incubation with MPTP resulted in a large decrease in ^{3}H-dopamine uptake (> 80%) which was markedly reduced by a synthetic catalytic scavenger of free radicals, the salen-manganese complex EUK-134 (50 uM). Adult mice were injected twice subcutaneously with MPTP (40 mg/kg) and the extent of damage was assessed 7 days later by measuring ^{3}H-mazindol binding to striatal membranes. This regimen resulted in a 70% decrease in ^{3}H-mazindol binding. Chronic treatment with EUK-134 dissolved in drinking water starting two days before MPTP injection and lasting for the duration of the survival period produced a significant inhibition of the MPTP-induced decrease in ^{3}H-mazindol binding. These results confirm the involvement of free radicals in MPTP-mediated toxicity and show the potential useful therapeutic applications of salen-manganese complexes.

This work was supported by a grant from Eukarion Inc.

491.16

SEVERE REDUCTION OF DOPAMINE (DA) FORMATION AFTER CARBIDOPA/L-DOPA (CD/LD) ADMINISTRATION IN MPTP-TREATED MONKEYS. W.W.McLaughlin*, M.E.Emborg, F.F.Wu, I.Irwin# and K.S.Bankiewicz, Somatix Therapy Corp., Alameda, CA 94501,#The Parkinson's Institute, Sunnyvale, CA 94089.

Unilateral intracarotid (ICA), plus systemic administration of MPTP reduces nigrostriatal DA levels in the ipsilateral (98%) and contralateral (50-80%) side, while inducing severe signs of Parkinson's disease (PD) that are stable over time. In order to examine the behavioral and biochemical effects of p.o. CD/LD, 5 rhesus monkeys were treated with ICA (2.5-4.0 mg) followed by 2-4 i.v. doses(0.3 mg/kg) of MPTP and studied for 8-20 months after ICA infusion. Four monkeys were given a single dose of CD/LD (10/100-35/350 mg) and 1 monkey was kept on chronic treatment of 25/250 mg. Clinical responses were recorded, animals were sacrificed 40 min after the last dose of CD/LD and their brains were removed. Tissue punches were obtained bilaterally from dorsal and ventral caudate nucleus (CN) and putamen (PUT), nucleus accumbens (NA) and temporal (TC) and occipital (OC) cortices, and were processed for LD, DA, homovanillic acid (HVA) and dopamine decarboxylase (DDC). Tyrosine hydroxylase immunostaining (TH-IR) was performed in adjacent blocks. Monkeys showed poor behavioral response to CD/LD while chronic animal (more severely affected) only responded to apomorphine administration (0.1 mg/kg, im). LD levels in acute monkeys were 4-12 ng/mg and in chronic one were between 25-60 ng/mg, in all the regions for both sides. DA levels on the ipsilateral side for acute, and on both sides for chronic animal, were below 3 ng/mg in all regions with the exception of NA where DA levels were significantly higher. DDC activity was depleted following the pattern of DA levels. The elevated levels of LD, low levels of DA and decreased DDC activity in CN/PUT indicate reduced decarboxylation of LD to DA. It further indicates that most DDC activity is present in nigrostriatal DA fibers and is related to high DA in NA where a partial lesion was observed on TH-IR. These findings may help to explain the low efficacy of LD treatment in advanced PD patients and validate this model for dopamine-replacement therapy using neurografting and gene therapy.

491.17

INHIBITORS OF NO SYNTHASE (NOS) AND POLY (ADP-RIBOSE) SYNTHASE (PARS) DO NOT BLOCK TOXIC ACTIONS OF ß-CARBOLINIUM CATIONS OR MPP^+ IN MESENCEPHALIC CULTURES. M.A. Collins*, L. Slobodnik and E.J. Neafsey#. Dept. of Biochem. and Dept. of *Anatomy, Loyola Univ. Medical School, Maywood IL 60153.

Human brain contains N-methylated ß-carbolinium ($MßC^+$) cations that structurally resemble the nigrostriatal toxin, MPP^+. $MßC^+$ formation via enzymic N-methylation of environmental ß-carbolines (ßCs) such as norharman (Nh) may occur in Parkinson's disease. Like MPP^+, nigral injections of $MßC^+$s damage nigrostriatal cells in rats, but $MßC^+$s are less selective and potent in terms of dopamine (DA) neurons (**Brain Res.** 675: 279, 1995). Neurotoxic potencies toward DA and GABA neurons have been examined in G-15 fetal rat mesencephalic cells (6-d cultures). Exposure 2 d to MPP^+, 2,9-dimethyl-Nh (2,9-$dMNh^+$), 2-methyl-harmaline (2-MHl^+) or 2-methyl-harmine (2-MH^+) was followed with DA & GABA uptake and tyrosine HOase (TH) immunochemistry (uM IC_{50}'s below).

Cation	IC_{50}(DA uptake loss)	IC_{50}(GABA uptake loss)
MPP^+	0.4	~100
2,9-$dMNh^+$	8.6	12.3
2-MHl^+	9.4	15.2
2-MH^+	37.1	100

Toxicities of MPP^+, 2,9-$dMNh^+$ and 2-MHl^+ were unaffected by nitroarginine methyl ester (30-100uM) or 7-nitroindazole (3-100uM)--inhibitors of NOS--or 1,5-dihydroxyisoquinoline (3-30uM), a potent inhibitor of PARS, indicating that NOS (NO production) and PARS do not mediate the mechanisms of MPP^+ or the $MßC^+$ analogs. TH assays indicate that DA uptake blocker GBR12909 inhibits most of MPP^+'s toxicity, but less than 50% of the toxicity of 2,9-$dMNh^+$ and none of 2-MHl^+. Other active uptake routes and and passive uptake may function for $MßC^+$s, but these could be circumvented in vivo by ßC N-methylation within DA neurons. Supported by NIH.

TUESDAY PM SYMPOSIA

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SYMPOSIUM. SENSORY CIRCUMVENTRICULAR ORGANS: BODY-BRAIN COUPLING AND MECHANISMS OF AFFERENT SIGNALING. A.K. Johnson, Univ. of Iowa (Chairperson); W.F. Ganong, Univ. of California, San Francisco; R.R. Miselis, Univ. of Pennsylvania; R. Gerstberger, MPI-W.G. Kerckhoff Institut; P.E. Sawchenko, The Salk Institute.

Circumventricular organs (CVOs) are small midline structures bordering the ventricles of the brain. They have common morphological features that distinguish them from the rest of the nervous system. In particular, they lack a blood-brain barrier. Early work on the 7 or 8 structures considered as CVOs in mammals focused on neurosecretory capacities. However, a neuroendocrine role for some CVOs has been difficult to establish. This is especially true for the subfornical organ, organum vasculosum of the lamina terminalis, and the area postrema. For these 3 structures, more recent results indicate that they function in a sensory mode to detect blood-borne factors. Detection of circulating signals by sensory CVOs has important functional implications for many physiological processes that include those involved in maintaining body fluid, cardiovascular and energy homeostasis and in protecting against infectious and toxic agents. The speakers in this symposium will review recent studies on the neurobiology and physiology of sensory CVOs. Particular attention will be paid to new methods for studying cellular mechanisms of CVOs signal transduction in sensory CVOs and the neural connectivity of CVOs with other brain structures.

493

SYMPOSIUM. GDNF: A NEW NEUROTROPHIC FACTOR WITH MULTIPLE ROLES. L. Olson, Karolinska Institute (Chairperson); F. Collins, Amgen Inc.; B.J. Hoffer, Univ. of Colorado Health Sciences Center; C.E. Henderson, INSERM.

GNDF is a TGFβ member with potent trophic effects on dopamine and motoneurons. The symposium will summarize current knowledge about the distribution and roles of GDNF and discuss how the new data may relate to the etiology and/or therapy of neurodegenerative diseases such as Parkinson's disease and ALS.

Dr. Frank Collins, who together with his team discovered GDNF, will present the molecular biology of GDNF and its position in the TGFβ family. He also has data on the effects of GDNF on both dopamine neurons and motoneurons which will complement the focused presentations of these two aspects given by Speakers 3 and 4.

Dr. Lars Olson will present an overview of the expression of GDNF in the organism, mainly as a result of in situ hybridization studies. Several new surprising aspects have been uncovered, suggesting that GDNF plays important roles not only in the developing nigrostriatal dopamine system and for the motoneurons, but also e.g. in cerebellum, thalamus, and spinal cord as well as in the GI-tract and in the kidney.

Dr. Barry Hoffer will present several lines of converging evidence suggesting that GDNF has potent trophic activity on dopaminergic (DA) ventral mesencephalic neurons in vivo. In nonlesioned rodents, intranigral injection of GDNF produces an increase in locomotor behavior and an upregulation of DA levels and TH immunoreactivity. In both the 6-OHDA rat model and the MPTP mouse model of DA neurodegeneration, GDNF exerts protective and restorative roles. Finally, GNDF augments DA neuron survival and fiber outgrowth in transplant models.

Dr. Chris Henderson will present the evidence that GDNF plays a biological role in regulating motoneuron survival during the cell death period in both chicken and rat embryos. In particular, the cellular origin of GDNF as compared to that of the neurotrophins BDNF and NT-3 will serve as the basis for a hypothesis to explain the multiplicity of factors that support motoneuron survival. The in vivo effects of GDNF on motoneurons are also striking: the relevance of experimental models to potential therapeutic strategies in motoneuron diseases will be discussed.

497.1

NATURAL ANTISENSE TRANSCRIPTION IS INVOLVED IN PROENKEPHALIN REGULATION. G.Weisinger[*,1,2], O.Zinder[1], J.D.DeCristofaro[2] & E.F.LaGamma[2], Faculties of Medicine, Technion, Haifa, Israel[1] and SUNY, Stony Brook, N.Y.[2].

The level of cholinergic induction of striatal preproenkephalin (ppEnk) RNA is 10-15 fold greater when measured at the level of RNA initiation compared with steady state mRNA levels. This was verified by experiments probing the same northern blots with different oligomeric fragments that hybridize to ppEnk exons 1 and 3. Hence, a novel transcriptional or posttranscriptional event occurred between the end of ppEnk exon 1 and the beginning of exon 3. To address this issue run-on transcription assays were initiated using single-stranded DNA (M13) probes derived from different regions of the rat ppEnk gene. The resulting data would indicate the relative rate of RNA transcription along the gene. Our data not only demonstated the presence of the expected ppEnk sense RNA transcripts in four tissues (Striatum, Cerebellum, Olfactory Bulb & Adrenal Medulla), but also the presense of natural antisense RNA ppEnk transcripts showing a definite tissue specificity. Additionally, our data is also consistent with the presence of a new species of somatic ppEnk sense RNA, initiated from within the first intron of the gene. This transcript seems to be important following whole animal cholinergic agonist treatments. A mechanistic understanding ppEnk regulation will require an understanding of these new findings.

497.2

THE BRN-4 POU DOMAIN MEDIATES PROTEIN-PROTEIN INTERACTIONS BETWEEN BRN-4 AND OTHER CLASS III POU FACTORS. K.F. Malik* and W.S. Young, III. Laboratory of Cell Biology, NIMH, NIH, Bethesda, MD 20892.

We previously demonstrated DNA-independent protein-protein interactions among class III POU factors (Malik et al., SFN Abstract, 1994). To determine Brn-4 domains important for these interactions, truncated Brn-4 constructs bacterially expressed as GST fusion proteins were used as both targets and radiolabeled probes in Farwestern blot assays. The Brn-4 POU domain (a.a.s 192-361) was able to interact with full length Brn-4 and itself but did not interact with GST, the Brn-4 N-terminal transactivating domain (N-Term; a.a.s 2-188), the Brn-4 POUspecific domain (POUs; a.a.s 192-275) or the Brn-4 POUhomeodomain (POUhd; a.a.s 261-361). To corroborate these findings a modified two-hybrid assay was used. Brn-4 constructs (same a.a.s as above) were expressed as fusions with GAL-4 DNA binding or VP-16 transactivating domains in CV-1 cells cotransfected with a reporter vector that contained the GAL-4 upstream activating sequence 5' to a viral E1b promoter driving luciferase expression. In these assays Brn-4 POU domain interacts with Brn-4, itself and POUhd but not N-Term or POUs. Together these findings indicate that: 1) the POU domain is critical for class III POU protein-protein interactions; 2) the POUhd may importantly contribute to these interactions; and 3) GST may interfere with POUhd binding in the Farwestern blot assay.

GST/Brn-4 was also used as a probe in Farwestern blot assays to screen nuclear extracts of several cell lines including HeLa, CV-1, and a Brn-4 expressing neuronal precursor cell line for factors that interact with Brn-4. A ~120 KDa Brn-4 interacting factor was found in all three cell lines. This Brn-4 interacting factor (BIF120) is highly enriched in the flow-through fraction of HeLa nuclear extracts passed over a phosphocellulose column. Experiments designed to determine the specificity of Brn-4 interactions with nondenatured BIF120 and the identity of BIF120 are currently underway.

497.3

CLONING OF PUTATIVE PROOPIOMELANOCORTIN STEM-LOOP RNA -BINDING PROTEINS USING A NORTHWESTERN SCREEN. C.M. Spencer*, S.D. Flagg and J. Eberwine, Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Regulation of proopiomelanocortin (POMC) gene expression is an important component of the body's response to stress. In previous studies, we have shown that a stable (-45 kcal) stem-loop structure in the protein coding region of rat or mouse POMC mRNA plays a role in the regulation of POMC translation in rabbit reticulocyte lysates. UV-crosslinking and RNA gel mobility shift assays have indicated that four protein-RNA complexes from mouse corticotroph AtT-20 cytoplasmic extracts are formed in a heparin-resistant and sequence-specific manner with radiolabeled riboprobes containing the POMC stem-loop sequence. At least two of the complexes are formed in a tissue-specific manner.

We are currently attempting to clone POMC stem-loop RNA-binding proteins from AtT-20 cells using a Northwestern screening technique. A lambda-ZAP cDNA expression library (300,000 clones) was screened using a radiolabeled 63-nt riboprobe that corresponds to the stem-loop structure of POMC. Seventy-two clones remained positive after two rounds of screening/plaque-purification and were partially sequenced. After sequences were compared to available databanks, ten novel clones were selected based upon sequence similarity to known DNA- and RNA-binding proteins. These clones were expressed in bacteria and tested further in UV-crosslinking and RNA gel shift assays. One clone, which has tested positive in preliminary assays, contains sequences that are highly similar to mouse adenine nucleotide translocase. This is interesting because a known stem-loop RNA-binding protein, the iron-responsive element binding protein, contains a consensus sequence for binding adenine nucleotides. A shorter sequence in this clone contains significant sequence similarity to FMR-1, another RNA-binding protein. This clone, as well as others, will be further analyzed for POMC stem-loop RNA-binding activity, appropriate tissue expression and the ability to regulate POMC mRNA translation in rabbit reticulocytes.

497.4

GENETIC, PHYSICAL AND TRANSCRIPT MAPPING OF THE MOUSE *VIBRATOR* LOCUS: A JUVENILE TREMOR MUTANT WITH DEGENERATION OF SPINAL, BRAINSTEM AND DORSAL ROOT NEURONS. B. A. Hamilton*, J. L. Nemhauser, R. T. Bronson[1], and E. S. Lander. Whitehead Institute for Biomedical Research, Cambridge, MA 02142 and [1]Tufts University School of Medicine, Boston, MA and The Jackson Laboratory, Bar Harbor, ME.

Vibrator homozygotes exhibit a progressive action tremor beginning soon after they acquire coordinated locomotion. Vacuolated and faint-staining neurons are seen in the spinal cord, brainstem, and dorsal root ganglion after progression of the disease. Affected animals do not survive beyond postnatal day 40 on most inbred genetic backgrounds, although affected animals derived from outmatings to one strain have survived beyond one year, suggesting the presence of suppressing alleles of at least one modifier locus in this strain.

We have genetically mapped *vibrator* to within a 0.5 cM interval on chromosome 11. This interval is spanned by a single yeast artificial chromosome. A contiguous set of bacteriophage P1 clones from this interval has been used to isolate brain, spinal, and neonate-derived cDNA fragments by direct selection. Preliminary sequence analysis of these clones has identified four previously known genes from GenBank, with one to six independent clones for each, as well as several novel transcribed sequences. Efforts to identify which of these genes is defective in *vibrator* are underway.

497.5

CHARACTERIZATION OF THY-1 ENHANCER ELEMENTS IN TRANSGENIC MICE. K.A. Kelley*, A. Sonshine, and A. Ho. Fishberg Res. Ctr. for Neurobiology, The Mount Sinai School of Medicine, New York, NY 10029.

Previous studies of the mouse Thy-1 gene have suggested that the thymic enhancer is located within the third intron while the CNS enhancer is most likely in the first intron. We have further characterized the Thy-1 regulatory elements by deleting flanking sequences to leave only 388 bp 5' to the transcription start site and 154 bp 3' to the polyadenylation signal. The resulting 5.6 kb gene fragment was used to produce three transgenic mouse lines which all expressed the transgene at high levels in brain and thymus, indicating that very little flanking sequence is required for appropriate expression. The 374 bp third intron was then removed from this minimal gene fragment, and the resulting 5.3 kb gene was used to produce four transgenic mouse lines. While none of the lines expressed this transgene in the thymus, three of the four lines expressed very high levels of transgene message in the brain, confirming that the thymic enhancer is indeed in the third intron. A reciprocal construct (3.1 kb) from which the 2.5 kb first intron was removed was used to produce four transgenic lines. Surprisingly, this construct was not expressed in either the brain or thymus of any of the four lines, even though the known thymic enhancer within the third intron was retained in this gene fragment. Finally, thymic expression was also lost from a Thy-1/lacZ constuct in which the lacZ coding region was inserted directly into the second exon (Soc. Neuro. Abst., 19: 1260, 1993). These data suggest that the spacing of the tissue-specific enhancers relative to the promoter may be crucial for appropriate expression, and that the previous putative identification of a brain enhancer within the first intron by deletion studies may not be accurate.

497.6

THE ROLE OF A COMPLEX INTRON ELEMENT IN THE REGULATION OF HUMAN CT/CGRP ALTERNATIVE SPLICING. H. Lou[1], Y. Yung[1], Y. Zhang*[2], S.M. Berget[3] and R.F. Gagel[1] Section of Endocrinology, University of Texas M.D. Anderson Cancer Center[1] Division of Neuroscience[2] and Department of Biochemistry[3], Baylor College of Medicine, Houston, TX 77030.

Cell-specific regulation of alternative RNA processing of the calcitonin/CGRP (CT/CGRP) gene is poorly understood. The key regulatory event in this alternative splice is inclusion or exclusion of exon 4, which codes for the peptide CT, from the final mRNA. Inclusion results in an exon order of 1, 2, 3, and 4 with utilization of the polyA site following exon 4. Exclusion results in an exon order of 1, 2, 3, 5 and 6 with utilization of the polyA site following exon 6. Although several regulatory sequences have been identified, no clear model exists for control of this processing choice. As a part of a continuing effort to understand this processing choice, we identified a highly conserved (among human, mouse and rat) intron element located 200 nt downstream of the exon 4 polyA hexanucleotide by deletion analysis of a minigene construct containing exon 4 and flanking intronic sequence. This intron element is required for recognition and polyadenylation of exon 4. The intron element contains two 5' splice site sequences, the second flanked by two pyrimidine-rich sequences. Point mutations in these four motifs affected the inclusion of exon 4 differently. Disrupting the first 5' splice site increased the inclusion of exon 4 while disrupting the downstream 5' splice site resulted in skipping of the exon. Mutations in the two pyrimidine motifs decreased, but did not abolish, the inclusion of exon 4.

To determine if this intron element regulates polyadenylation of exon 4, we performed in vitro cleavage/polyadenylation assay in HeLa nuclear extract using the second half of CT exon 4 (250 nt) and first half of CT intron 4 (250 nt) as substrate. Point mutation of the upstream 5' splice site within the intron element increased polyadenylation slightly, whereas 2 different point mutations in the downstream 5' splice site reduced polyadenylation by 75%. Mutations in one of the pyrimidine motifs also decreased the cleavage activity more than 66%. Oligonucleotide-mediated-depletion of U1 snRNA from the HeLa nuclear extract abolished cleavage of the CT exon 4, but had no effect on a substrate derived from SV40. This result suggests a specific role for U1 snRNP in polyadenylation of CT exon 4. The possible roles of this intron element on the regulated exclusion of CT exon 4 in neuron cells are also being evaluated. We conclude that polyadenylation of CT exon 4 requires a novel and complex 5' splice containing intron element and provides a potential site for regulation of this splice choice.

497.7

HTLV-1 TAX ACTIVATES THE cAMP-REGULATED ENHANCERS IN THE HTLV-1 LTR AND CELLULAR GENES BY DIFFERENT MECHANISMS. R.P.S. Kwok*, M.E. Laurance, J.R. Lundblad, P.S. Goldman, H.M. Shih, and R.H. Goodman. Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

Because tumor viruses typically co-opt host cell pathways for regulating viral replication, viral gene products can provide reagents for dissecting the mechanisms underlying cellular signaling processes. The human T cell lymphotrophic virus (HTLV)-1 LTR contains an enhancer that is regulated synergistically by the cellular transcription factor CREB and the viral co-factor, Tax. A pathway involving CREB and Tax also underlies the ability of HTLV-1 to activate cellular genes that lead to transformation. Although Tax activates gene expression if fused to a heterologous DNA binding domain, the native protein does not contact DNA directly. Consequently, Tax is thought to function by increasing the dimerization and binding of CREB to DNA. Our observation that Tax converts the inhibitory protein CREMα into a transcriptional activator is not consistent with this hypothesis, however. Using fluorescence anisotropy measurements, we showed that Tax increases CREB and CREMα binding to the HTLV-1 LTR, but not to the prototypical cellular CRE sequence. Furthermore, Tax increases the binding of CREB and CREMα to the cellular co-activator CBP, but only in the context of the HTLV-1 CRE. Activation of cellular genes probably results from the ability of Tax to bind to CBP directly, which we showed by using both *in vitro* and *in vivo* binding assays. Thus, Tax activates viral and cellular enhancers through transcriptional pathways that involve CREB and the co-activator CBP. The virus can activate this system in the absence of protein kinase A (PKA)-stimulation, while the cellular CRE requires the simultaneous activation of PKA. The HTLV-1 Tax system provides a useful paradigm for examining how viral transforming proteins interact with second messenger signaling pathways.

497.8

UPSTREAM SEQUENCES DETERMINE CELL-SPECIFIC EXPRESSION OF THE PNMT GENE. M.J. Evinger* and Y-S.E.Lee, Depts. of Pediatrics and Neurobiology & Behavior, SUNY at Stony Brook, Stony Brook, NY 11794

Cell-specific expression of genes for the catecholamine synthesizing enzymes is an important determinant of neurotransmitter phenotype. Although analogous sequences have been identified in the tyrosine hydroxylase and DBH genes, this report now characterizes those sequences responsible for cell-specific expression of the epinephrine-synthesizing enzyme phenylethanolamine N-methyltransferase (PNMT).

Expression of constructs containing nested deletions of the rat PNMT promoter has been compared following transient transfection into continuous cell lines. Luciferase reporter gene activity for each PNMT promoter construct in the cell lines is expressed relative to expression of that construct in primary bovine adrenal chromaffin cells, the system closest to expression *in vivo*.

We have identified sequences which restrict expression of the PNMT gene to cells of adrenergic and of neuronal lineage. Sequential deletion of these sequences enhances reporter gene expression 2 to 5-fold, depending upon the neural vs. non-neural origin of the cells. PNMT promoter expression in neuroblastoma lines is greater than in PC12 or TE671 cells or in non-neuronal C6 glioma or L6 fibroblast lines. The most distal element appears to be unique to the PNMT gene; its influence restricts expression to adrenal medullary cells. Two additional sequences share similarity with neural specific elements described in other genes. Removal of these portions of the promoter increases expression in non-neural cell lines, e.g. C6 and L6 cells.

The array of multiple adrenergic and neural specific sequences in the PNMT gene effectively restricts peripheral expression of PNMT to differentiated chromaffin cells. Cell-specific expression of the PNMT gene thereby constitutes the critical transcriptional event governing determination of the adrenergic phenotype. Supported by NIH GM46588 (MJE).

497.9

PROMOTER ELEMENTS AND SECOND MESSENGER PATHWAYS INVOLVED IN TRANSCRIPTIONAL ACTIVATION OF TYROSINE HYDROXYLASE BY IONOMYCIN. B. Nankova*, B. Hiremagalur and E.L. Sabban. Dept. Biochem. & Mol. Biology, New York Medical College, Valhalla, NY 10595.

Membrane depolarization, or agents which increase intracellular calcium, elicit transcriptional activation of tyrosine hydroxylase (TH). In this study we analyze the factors involved in the regulation of the TH promoter by a calcium ionophore. PC12 cells were transiently transfected with plasmids containing wild type or mutated 5' flanking sequences of the rat TH gene, fused to bacterial chloramphenicol acetyl transferase (CAT). Point mutations introduced into the consensus cAMP-regulatory element (CRE) abolished the induction of CAT by ionomycin indicating that it is essential for mediating the calcium response. An intact and functional AP1 site did not confer calcium inducibility when the CRE/CaRE sequence was mutated. The extent and kinetics of the increase in intracellular calcium as well as the induction of CAT activity under the control of TH promoter by ionomycin were similar in PC12 cells and in the A123.7, protein kinase A (PKA) deficient cell line. In both cell lines addition of ionomycin rapidly increased the phosphorylation of transcription factor CREB at Ser 133. These results suggest that the activation of TH transcription by ionomycin does not require PKA. However, KN62 an inhibitor of Ca^{2+}/calmodulin dependent (CaM) kinases prevented the induction indicating possible involvement of CAM kinases in the calcium response.

497.10

INTRACELLULAR CALCIUM AS A MEDIATOR OF INCREASED TYROSINE HYDROXYLASE GENE EXPRESSION IN PC12 CELLS. A. Menezes*, R. Zeman, B. Hiremagalur and E.L. Sabban. New York Medical College, Valhalla, NY 10595.

Elevation in $[Ca^{2+}]i$ that occur in response to a variety of stimuli have been implicated in regulating cellular processes such as neurotransmitter secretion, neurite outgrowth and gene expression. The importance of the rise in $[Ca^{2+}]i$ for regulation of tyrosine hydroxylase (TH) gene expression for various treatments which elevate $[Ca^{2+}]i$ by different mechanisms was examined in PC12 cells. Spacial and temporal changes in $[Ca^{2+}]i$ were monitored by calcium imaging and confocal microscopy. Upon continuous depolarization with 50 mM KCl, $[Ca^{2+}]i$ was raised from 50-60 nM to about 120 nM for several hours. TH mRNA levels were elevated 3-4 fold, maximally after 2-12 hrs. Release of calcium from intracellular stores by bradykinin or thapsigargin, raised $[Ca^{2+}]i$ to about 250 nM, and also induced TH transcription with similar kinetics, even in low extracellular calcium. In contrast, nicotine treatment, which raised $[Ca^{2+}]i$ to similar levels and subcellular distribution, required over 12 hrs exposure to induce TH. All these agents utilize the Ca/CRE region of the TH promoter to increase gene expression. Treatments with agents which block release of calcium from intra- and extra- cellular pools were used to determine the source of intracellular calcium required to elevate TH transcription.

Influx of calcium from intracellular or extracellular pools was found sufficient to initiate induction of TH gene expression. However, the results with nicotine indicate that the changes in gene expression are not simply due to a read-out of the rise in calcium. (Supported by NIH & STRC)

497.11

CELL LINE-SPECIFIC TRANSCRIPTIONAL AND POSTTRANSCRIPTIONAL REGULATION OF THE RAT CKB GENE BY ITS INTRONS. E. V. Kuzhikandathil*, S. E. Ilyin, J. Zhao and G. R. Molloy. Department of Biology, University of Delaware, Newark, DE 19716.

The brain creatine kinase gene (CKB) is expressed in a range of tissues with expression being highest in glial cells of the brain (J. Neurochem. 59:1925-1932[1992]). The expression of the rat CKB gene is regulated by both distal and proximal promoter sequences in some cell types. However, in cell lines such as rat C6 glioma, rat RT4 B8 neuroblastoma, human U87 MG glioblastoma and human U251 glioma, our CKB gene promoter-deletion experiments have failed to identify any significant upstream promoter regulatory sequence. In this report, we show that certain introns of the rat CKB gene can regulate its expression at the transcriptional level in HeLa cells and at the posttranscriptional level in U87 and U251 glial cells. We demonstrate that an enhancer-like sequence in intron 1 of rat CKB has a negative regulatory effect in all cell lines tested. In addition, we also show that introns 3, 4, 5, and 6 has a positive regulatory effect on the transcription of the rat CKB gene in HeLa cells. In contrast to HeLa cells, in U87 and U251 glial cells, these introns affect the expression of the rat CKB gene at the posttranscriptional level by generating an altered form of rat CKB mRNA. This altered rat CKB mRNA retains only intron 7 and 145 ntds of the 5' end of exon 8 of the rat CKB gene. The generation of this glial-specific altered CKB RNA and its possible physiological role is under investigation. These results further demonstrate the complex regulation of the rat CKB gene and the mechanisms by which introns can regulate gene expression.

498.1

CHARACTERIZATION OF PICROTOXIN BLOCKADE OF GLUTAMATE-GATED CHLORIDE CHANNELS REVEALS STRUCTURAL AND FUNCTIONAL SIMILARITIES TO $GABA_A$ AND GLYCINE RECEPTORS.

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The predicted protein sequences of the *Caenorhabditis elegans* glutamate-gated chloride channel subunits (GluClα and GluClβ) contain the conserved motifs present in $GABA_A$ and glycine receptors. To establish a functional link between the GluCl family and other ligand-gated chloride channels we studied the properties of picrotoxin (PTX) blockade. *In vitro* RNA synthesized from pGluClα and pGluClβ and was either coinjected (α&β) or injected individually (α or β) into *Xenopus* oocytes. Recordings were made under two electrode voltage clamp. Oocytes injected with α&β formed heteromeric channels which responded to glutamate and ivermectin (IVM), a widely used antiparasitic agent. These currents were weakly inhibited by PTX (EC_{50}=50 μM). Homomeric α and β channels were selectively activated by IVM and glutamate, respectively. The EC_{50} for PTX block of homomeric α channels was 50 μM, and current from homomeric β channels was potently inhibited by PTX with an EC_{50} of 50 nM. Opening of homomeric β channels with glutamate was required for PTX block and unblock. Resistance to PTX has been reported for a Ser to Ala mutation in the M2 domain of a *Drosophila* GABA receptor (ffrench-Constant, R.H., et al, 1993, Nature v363, p449). To test this position for the GluCl family we substituted the Ala of GluClβ with a Thr (as found in GluClα) and channels became resistant to PTX (20 % block at 1mM). In conclusion, resistance of the heteromeric α&β channel is conferred by a single subunit (α), blockade and recovery from PTX block requires channel opening, and a single point mutation in M2 confers PTX resistance. These properties have been described for PTX blockade of glycine and $GABA_A$ receptors and demonstrate a functional as well as structural similarity of the GluCl family to these receptors.

498.2

LOCALIZATION OF A GEPHYRIN BINDING SITE ON GLYCINE RECEPTOR ß SUBUNIT

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The tubulin-binding protein gephyrin copurifies with the inhibitory glycine receptor (GlyR) and is essential for its postsynaptic localization. Gephyrin contributes to the formation of GlyR clusters in the postsynaptic membrane of spinal neurons, as antisense oligonucleotides interfering with gephyrin synthesis drastically reduce clustering. Here, we analyzed the interaction between the GlyR and different recombinant gephyrin isoforms (P1-P3) and identified a gephyrin binding site within a 49 amino acid sequence in the cytoplasmic loop between the third and fourth transmembrane segments of the β subunit. GlyR α subunits and γ-aminobutyric $acid_A$ receptor proteins failed to bind recombinant gephyrin. However, insertion of a central 18 residue segment of the GlyR β 49mer into a γ-aminobutyric $acid_A$ receptor β1 subunit construct conferred gephyrin binding. This data defines a minimal gephyrin binding motif and suggests that β subunit expression is essential for formation of a postsynaptic GlyR matrix.

498.3

STRUCTURAL DETERMINANTS OF ATP-GATED ION CHANNELS

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P_{2X} receptors cloned from rat vas deferens (Valera *et al.* 1994 Nature, **371**, 516) and PC12 cells (Brake *et al.* 1994 Nature, **371**, 519) differ in their pharmacological profiles and kinetics. PC12 P_{2X} receptors are distinguished from the rapidly desensitizing smooth muscle form by their insensitivity to the agonists αβmeATP and ADP, and their lack of desensitization during a 10 s application of agonist. To probe the functional domains important for ligand-binding and channel gating, chimeric receptors were constructed and expressed in *Xenopus* oocytes. A major determinant of agonist selectivity was found to be the region between the two transmembrane domains, which is thought to form a large extracellular loop. The PC12 receptor became desensitizing with the introduction of vas deferens receptor segments corresponding to the transmembrane domains along with neighboring residues. The results indicate that regions controlling P_{2X} ion channel gating are independent of the ligand-binding site, and that rapid desensitization can not be accounted for by any single domain.

498.4

CLONING AND EXPRESSION OF A FAMILY OF ATP-GATED ION CHANNELS

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P_{2X} receptors cloned from the rat vas deferens and PC12 cells form cation-selective channels gated by extracellular ATP when expressed in heterologous cells (S. Valera et al. Nature 371, 516, 1994; A.J. Brake et al. Nature 371, 519, 1994). We have isolated further members of the P_{2X} receptor family by a PCR-based approach, and expressed them by transfection into HEK293 cells. One was isolated from a rat superior cervical ganglion cell cDNA library; however, its RNA is found predominantly in epithelial tissues. It is generally similar in its functional properties to the PC12 form of the receptor (insensitive to α,βmeATP, nondesensitizing). Another cDNA was cloned from a rat dorsal root ganglion library, and in situ hybridisation showed RNA also in trigeminal ganglia and retina. In HEK293 cells this clone has properties intermediate between those of the bladder and PC12 forms; it is sensitive to α,βmeATP but shows less desensitisation than the bladder receptor. All receptors are 40 - 55% identical at the amino acid level, indicating that P_{2X} receptors belong to an extended gene family whose products quite distinct electrophysiological properties and tissue distribution.

498.5

SELECTIVITY OF ANTAGONISTS AT SUBTYPES OF CLONED P_{2X} RECEPTOR EXPRESSED IN MAMMALIAN CELLS.

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The effects of the antagonists suramin, pyridoxal 5-phosphate (P5P) and pyridoxalphosphate-6-axophenyl-2',4'-disulphonic acid (PPADS) were studied on the responses to ATP of HEK293 cells expressing four molecular species of P_{2X} receptors (cloned from human bladder, rat PC12 cells, rat superior cervical ganglion (scg) and rat dorsal root ganglion (drg). Suramin acted as an antagonist at bladder, PC12 and drg receptors; it was apparently competitive only up low dose-ratios (<10); it did not antagonise responses of this scg clone. P5P and PPADS acted as essentially irreversible antagonists at the bladder and PC12 receptors (50% inhibition by 3 - 10 μM), had no effect (up to 100 μM) at the scg receptor, and caused rapidly reversible antagonism (50% inhibition by 10 μM) at the drg receptor. The differences in primary sequence that underlie these differences in antagonist sensitivity are being determined by expression of chimeric and point-mutated receptors. The results indicate that not all P_{2X} receptors are sensitive to blockade by the usual antagonists suramin, P5P and PPADS.

498.6

CLONING AND FUNCTIONAL PROPERTIES OF A CENTRAL P2x ATP RECEPTOR.

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Using a degenerate PCR strategy for cloning novel members of the P2x ATP-gated channel family, we report the primary structure of a new subtype expressed in the rat brain. This clone (P2x3) encodes a 388 aa protein which has the characteristic features of known ATP-gated channels according to their transmembrane topology, post-translational modifications and ion selectivity. The predicted sequence of this central receptor subunit has 47% identity with the rat smooth muscle subtype and 37% with the PC12 subtype. Functional homomeric P2x3 channels expressed in *Xenopus* oocytes present a distinct pharmacological profile (ATP> 2methylthioATP > αβmethyleneATP) and are sensitive to blockade by P2 antagonists. Their potentiation by Zn ions may be of particular functional relevance since P2x3 is localized by *in situ* hybridization in CA3 hippocampal pyramidal cells heavily afferented by Zn-containing terminals of mossy fibers. This central subtype is also expressed at high levels in cerebellum, limbic system and motor nuclei of brainstem. These data support the hypothesis that fast purinergic transmission mediated by extracellular ATP is widespread in the mammalian CNS. (Supported by MRC and NSERC)

498.7

ROLE OF THE METABOTROPIC GLUTAMATE RECEPTOR mGluR5 IN THE HIPPOCAMPUS REVEALED WITH ANTISENSE DEOXYNUCLEOTIDES. J.M. Wojtowicz*, F. Dorri, D. Hampson and A. Baskys. Department of Physiology, University of Toronto, Toronto, ONT, M5S 1A8.

Several metabotropic glutamate receptor subtypes are expressed in the hippocampus but their functions are poorly understood due to the lack of specific ligands and blockers. We have used the antisense deoxynucleotide (oligo) technology to study the effects of mGluR5 on neuronal excitability and synaptic transmission in CA1 area of the rat hippocampus. The antisense oligos to mGluR5 were stereotaxically injected into the CA1 area of the hippocampus of 30 day old male Wistar rats. After repeated injections *in vivo* for three days, standard hippocampal slices were prepared for electrophysiological and Western blott analysis. In uninjected controls two effects of a metabotropic agonist ACPD (50 μM) were observed: a reduction in the evoked field EPSP and an increase of the population action potential. The antisense-treated animals showed a significantly reduced effect of ACPD on the action potential (ANOVA, $P<.05$, $n_c=20$, $n_{AS}=39$). The reduction was sequence and target specific since a mismatched oligo had no effect, and the response to ACPD of the field EPSP in the antisense-treated group was unchanged. Also, the magnitude of long-term potentiation in the affected slices was unchanged. Reduced expression of mGluR5 was confirmed by immunoblotting with a specific antibody. Supported by MRC of Canada.

EXCITATORY AMINO ACIDS: PHARMACOLOGY—NMDA/AMPA RECEPTORS

499.1

METABOTROPIC GLUTAMATE RECEPTOR ACTIVATION MEDIATES NITRIC OXIDE (NO) PRODUCTION IN THE HIPPOCAMPUS *IN VIVO*. A. Bhardwaj*, F.J. Northington, L.J. Martin, D.F. Hanley, R.J. Traystman R.C.Koehler.
Johns Hopkins University School of Medicine, Baltimore, MD 21287.

Utilizing the technique of microdialysis we have demonstrated that NMDA and AMPA receptor stimulation and blockade modulates NO production *in vivo*. Because of the known role for G-protein coupled glutamate receptors in regulating intracellular calcium levels, we tested the hypothesis that metabotropic glutamate receptors modulate NO production *in vivo*. Under controlled conditions of normoxia, normocarbia and normothermia bilateral microdialysis probes were placed in the hippocampus of adult male Sprague-Dawley rats and perfused for 5 hours with perfusates containing 3μM [^{14}C]-L-arginine. Effluent [^{14}C]-L-citrulline was used as a marker of NO production. At 5 hours of perfusion, [^{14}C]-L-citrulline in the effluent (femtomoles/minute $\pm$ S.E.M.) was greater on the side perfused with selective metabotropic receptor agonist, 1 mM trans-(±)-1-amino-(1S,3R)-cyclopentanedicarboxylic acid (ACPD) (149 $\pm$ 35) than on the control side perfused with artificial CSF (81 $\pm$ 16) (N=5). ACPD enhanced [^{14}C]-L-citrulline recovery was attenuated by the addition of 1 mM metabotropic receptor antagonist (±)-alpha-methyl-4-carboxyphenylglycine (MCPG) (143 $\pm$ 10) as compared to ACPD alone (280 $\pm$ 21) (N=5). ACPD enhanced [^{14}C]-L-citrulline recovery was attenuated by 70% with the addition of 1 mM L-nitroarginine. ACPD + 1 mM dantrolene, an inhibitor of intracellular calcium release attenuated the citrulline recovery (93 $\pm$ 18) as compared to ACPD alone (179 $\pm$ 18)(N=6). These data indicate that the metabotropic glutamate receptor activation can mediate the production of NO *in vivo* by modulating intracellular calcium release.

Supported by the National Stroke Association.

499.2

Stable cell lines expressing recombinant human N-methyl-D-aspartate receptor subtypes.
P. J. Whiting*, S. Grimwood, B. Le Bourdellès, W. Cockett, J. Myers, P. Hutson and C. I. Ragan, P. Laughton and T. Priestley. Neuroscience Research Centre, Merck Sharp and Dohme Research Laboratories, Harlow, Essex, U.K.

To enable detailed pharmacological and functional analysis of human N-methyl-D-aspartate (NMDA) type glutamate receptor subtypes we have established permanently transfected cell lines expressing recombinant human NR1a/NR2A and NR1a/NR2B heteromeric receptors. In order to overcome the cytotoxicity associated with expression of recombinant NMDA receptors in transfected cells, the subunit cDNAs were engineered so as to be under control of a dexamethasone inducible promoter. Radioligand binding studies allowed pharmacological analysis of the glycine, glutamate and channel (MK801) sites of recombinant receptors. Similarly, electrophysiological approaches allowed analysis of the functional pharmacology of these recombinant human receptor subtypes, and comparson with the properties of native rat NMDA receptors expressed by cultured neurons.

499.3

PHARMACOLOGY OF STABLY EXPRESSED RECOMBINANT HUMAN N-METHYL-D-ASPARTATE RECEPTORS USING A $^{45}Ca^{2+}$-INFLUX ASSAY. S. Grimwood, E. Gilbert, J. Myers, B. Le Bourdellès, W. Cockett, N. Rupniak*, C.I. Ragan, P.J. Whiting and P.H. Hutson. Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR.

$^{45}Ca^{2+}$-influx experiments were performed on monolayers of cells stably expressing human NR1a/NR2A and NR1a/NR2B receptors under the control of a dexamethasone inducible promoter. Using this assay we have characterised in detail the pharmacology of the different modulatory sites of the NMDA receptor complex with a view to identifying sub-type selective compounds. $^{45}Ca^{2+}$-influx was maximal in the presence of 100 µM L-glutamate and 100 µM glycine, addition of 100 µM MK-801 determined background levels. No significant difference was observed between affinity values obtained with the two cell lines for the ion-channel ligands MK-801, phencyclidine and ketamine, whilst the polyamine-site antagonist ifenprodil was 600-fold more potent at NR1a/NR2B receptors (see Table).

	NR1a/NR2A	NR1a/NR2B
MK-801	0.191 (0.125, 0.291)	0.125 (0.098, 0.159)
Phencyclidine	2.14 (1.68, 2.74)	1.07 (0.652, 1.75)
Ketamine	10.1 (4.22, 24.0)	4.88 (3.35, 7.11)
Ifenprodil	298, 329*	0.491 (0.395, 0.612)

Data are K_e values (geometric mean (-SEM, +SEM)) (µM) ($n\geq3$, *n=2).

These results show that $^{45}Ca^{2+}$-influx can be used to characterise the pharmacology of stably expressed NMDA receptors, and that the sub-type selective compound ifenprodil will distinguish between recombinant human NR1a/NR2A and NR1a/NR2B receptors.

499.4

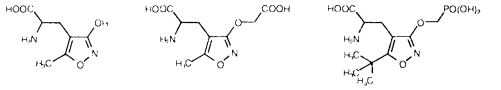
AMPA INDUCES THE ACTIVATION OF MAP KINASE IN RAT CORTICAL NEURONS BY A CA^{2+}-DEPENDENT PROTEIN KINASE CASCADE. Y. Wang, M. Black, P. Morley, K. Chaundy* and J.P. Durkin. Cellular Neurobiology Group, Institute for Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6.

AMPA (α-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid) receptors play important roles in plasticity, neurotransmission and neurotoxicity. Despite the importance of the AMPA receptor to normal and aberrant neuronal behavior, the signalling pathways coupled to the AMPA-mediated influx of Na^+ and Ca^{2+} ions are only poorly understood. In this study we demonstrate that AMPA receptor stimulation in primary cortical neurons triggers a protein kinase cascade leading to the activation of MAP kinase (MAPK). 100µM AMPA caused a rapid 3-fold increase in MAPK activity within 3 min of stimulation and which slowly returned to basal levels over the ensuing 60 min. By contrast, KCl-induced depolarization caused a rapid but highly transient activation of MAPK which returned to basal levels within 10 min of stimulation. The AMPA-induced activation of MAPK was dependent on the presence of Ca^{2+}, but not Na^+, in the culture medium. While the L-type Ca^{2+}-channel blocker, nifedipine, effectively blocked the activation of MAPK by KCl-mediated depolarization, it had no effect on AMPA-induced MAPK activation. AMPA also stimulated *ras* activity in cortical neurons, as measured by an increase in the amount of GTP-bound *ras* relative to the inactive GDP-bound form of *ras*. Using an anti-*ras* antibody, it was determined that the coprecipitation of *ras* with *raf* kinase and the MAP kinase/ERK-activating kinase (MEK-1) increased within 3 min of AMPA stimulation. As was the case with MAPK activation, the association of *ras*, *raf* and MEK-1 was dependent on extracellular Ca^{2+} but not Na^+. A gel mobility shift assay was used to determine that the phosphorylation and activation of MEK-1 occurred in an AMPA-concentration dependent manner. Collectively, these results suggest that the stimulation of the AMPA receptor triggers a Ca^{2+}- dependent kinase cascade in cultured cortical neurons in which *ras* activation leads to the activation of *raf* kinase, MEK-1 and MAPK.

499.5

SYNTHESIS AND PHARMACOLOGY OF HIGHLY SELECTIVE PHOSPHONO AMINO ACID AMPA RECEPTOR ANTAGONISTS. U. Madsen*, B. Ebert and P. Krogsgaard-Larsen. Department of Medicinal Chemistry, Royal Danish School of Pharmacy, DK-2100 Copenhagen, Denmark.

Excitatory amino acid (EAA) receptors are subjects of extensive exploration as potential targets for drug intervention in different neurodegenerative disorders. Ligands for the *N*-methyl-D-aspartic acid (NMDA) and 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) subtypes of EAA receptors are of interest in this regard as pharmacological tools and potential drugs. The availability of a broad spectrum of phosphono amino acids showing potent and highly selective competitive antagonism of NMDA receptor function has played a crucial role in the characterization of this subtype of EAA receptors. AMPA has previously been converted into the AMPA antagonist (*RS*)-2-amino-3-[3-(carboxymethoxy)-5-methyl-4-isoxazolyl]propionic acid (AMOA). In continuation of this line of research, structural hybrids containing a distal phosphono group and an isoxazole nucleus have been synthesized and tested pharmacologically. The pharmacology of the compounds has been investigated in electrophysiological and receptor binding assays using rat brain tissue. The most potent compound, (*RS*)-2-amino-3-[3-(phosphonomethoxy)-5-*tert*-butyl-4-isoxazolyl)propionic acid (ATPO), has a potency as AMPA antagonist (IC_{50} = 27 μM) approximately 20 times greater than that of AMOA and shows higher selectivity for the AMPA receptors compared to the parent compound AMOA. In context with this series of AMPA antagonists, a parallel series of AMPA agonists was synthesized.



AMPA AMOA ATPO

499.6

PHARMACOLOGICAL INTERACTIONS OF THE AMPA ANTAGONIST NBQX WITH CNS DEPRESSANTS. G.H. Jones*, A. Huth, E. Ottow and L. Turski. Research Laboratories of Schering AG, Müllerstr. 178, D-13342 Berlin, Germany.

AMPA receptor antagonists have been shown to have marked neuroprotective effects in variety of animal models of cerebral ischemia and trauma which indicates that this compound class may have clinical potential for the treatment of stroke or head trauma. AMPA receptor antagonists reduce excitatory synaptic neurotransmission in the CNS which leads at higher doses to sedation and loss of motor coordination. It is therefore important to investigate the potential interaction of these compounds with other commonly used CNS depressants. We examined the interactions between the competitive AMPA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX, i.v.) and several CNS depressants, including ethanol, diazepam, morphine, hexobarbital and urethane on measures of motor coordination and toxicity in NMRI mice (20-25 g). Of particular interest was the finding that ethanol (3.125 g/kg, p.o.) potentiated the loss of righting reflex and impairment of rotarod performance, but did not increase the toxic effects, produced by NBQX. Diazepam (80 mg/kg, i.v.) also greatly increased the loss of righting reflex produced by NBQX. In contrast, the presence of hexobarbital (100 mg/kg, i.v.) greatly increased the toxic effects of NBQX, but there was no marked interaction between these two compounds on loss of righting reflex. In conclusion, these results indicate the specific nature of interactions of NBQX with other CNS depressants which may be of clinical importance.

499.7

GLUTAMATE ANTAGONISTS PREVENT CONVULSIONS INDUCED BY INSECTICIDES. W.A. Turski* , P. Blaszczak, E. Urbanska and L. Turski. Institute of Agricultural Medicine, PL-20950 Lublin and Research Laboratories of Schering AG, 13342 Berlin

Human exposure to insecticides may occur in agriculture, during occupational exposure, their improper use in medicine, or by intake of contaminated foods. The symptoms of toxicity in humans in non-fatal cases are nausea, mental confusion, myoclonic jerks and severe convulsions. Current therapy of insecticide-poisoning is symptomatic and consists of removal of the poison, control and support of respiratory and cardiovascular systems, hemodynamic stabilization, and anticonvulsant treatment. Clinical experience suggests that antiepileptic drugs may not be sufficient to control insecticide-induced convulsions which may immensely affect clinical outcome. Therefore we examined whether glutamate antagonists (E)-4-(3-phosphoroprop-2-enyl)piperazine-2-carboxylic acid (D-CPPene, 20 mg/kg i.p.), dizocilpine (MK-801, 0.4 mg/kg i.p.), felbamate (400 mg/kg i.p.) and 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzø(F)quinoxaline (NBQX, 100 mg/kg i.p.) influence seizures induced by chlorinated hydrocarbon insecticides γ-lindan and dieldrin. γ-Lindan induced clonic seizures in Swiss mice, 20-25 g, with an ED50 of 27 mg/kg and tonic seizures with an ED50 of 47 mg/kg. Clonic seizures induced by γ-lindan were prevented by the non-NMDA antagonist NBQX but not by the NMDA antagonists D-CPPene, MK-801 and felbamate. γ-Lindan-induced tonic seizures were blocked by either type of glutamate antagonists. Dieldrin induced clonic seizures in mice with an ED50 of 21 mg/kg and tonic seizures with an ED50 of 29 mg/kg. Clonic seizures induced by dieldrin were prevented by both non-NMDA and NMDA antagonists. Dieldrin-induced tonic seizures were also sensitive to the treatment with glutamate antagonists. Such observations suggest that glutamate may be involved in the evolution of insecticide-induced convulsions, and that antagonism at glutamate receptors may offer a novel therapeutic approach for seizure prevention in insecticide poisoning

499.8

CYCLOTHIAZIDE INTERACTIONS WITH AMPA AND KAINATE RECEPTOR ANTAGONISTS. K.A.Yamada*, Depts of Neurology and Pediatrics, Washington Univ.Sch. of Med., St. Louis, MO 63110.

We studied interactions between cyclothiazide, GYKI 52466 (GYKI), and NBQX using native AMPA/Kainate receptors and whole-cell voltage-clamp recording. 30 μM GYKI blocked Kainate-activated whole-cell currents in hippocampal neurons (16±8.3% of control) and the onset of the block was best fit by a double exponential function (τ_1=94±18, τ_2=388±228 ms). The estimate for GYKI's IC_{50} against hippocampal Kainate currents was 15 μM. 100μM cyclothiazide potentiated the Kainate current 2.6±1.0 fold and reduced the GYKI block (62±12% of control) Moreover, the time course of onset of the block was slower (τ_1=164±39, τ_2=861±455 ms; $P \leq 0.001$). NBQX blocked Kainate currents to 6.2±4.4% of control, and unlike GYKI the kinetics of block was more complex than a two exponential function. However, similar to GYKI, 100μM cyclothiazide reduced NBQX block to 42±16% of control and the time course of NBQX block was also slower. Kainate activated a slowly desensitizing current in DRG neurons which was unaffected by cyclothiazide. GYKI blocked DRG Kainate currents, but with lower affinity than for hippocampal AMPA receptors. Cyclothiazide did not affect GYKI's antagonism of DRG Kainate currents, against which GYKI's IC_{50} was between 120 and 400μM, several fold greater than for hippocampal neurons. These results suggest that the site of action of cyclothiazide is an important allosteric modulatory site on the AMPA receptor, affecting the actions of both competitive and non-competitive antagonists, and that receptor subunit composition influences the affinity of the non-competitive AMPA/Kainate antagonist GYKI. Because AMPA receptors mediate excitatory responses at central synapses and contribute to excitotoxic neuronal injury during hypoxia-ischemia, these findings may have both functional and therapeutic implications. Supported by NIH NS 01443.

499.9

NEURONAL ACTIVITIES OF IBOGAINE AND NORIBOGAINE AT THE NMDA RECEPTOR COMPLEX: LIGAND BINDING AND ELECTROPHYSIOLOGICAL STUDIES. I. Pablo*, I.K. Staley, A.M. Holohean, J.C. Hackman, R.A. Davidoff, and D.C. Mash. Dept. of Neurology, Univ. of Miami Sch. of Med., Miami, FL, 33136.

Ibogaine is purported to be a naturally occurring anti-addiction agent based on preclinical studies and on anecdotal reports from addict self-help groups. Popik et al., (1994) have suggested that the anti-addictive properties of ibogaine are due to an interaction with NMDA receptor-coupled cation channels. The non-competitive NMDA receptor antagonist, MK-801, has been reported to block sensitization to the behavioral activating effects of cocaine and amphetamine. We report here a comparison of the activities of ibogaine and its O-demethylated metabolite (noribogaine) at the NMDA receptor complex. The potency of both drugs at [^{3}H]MK-801 binding sites were compared to their electrophysiological actions on isolated frog spinal cord, using differential sucrose gap recording of the IXth ventral root. Ligand binding profiles demonstrate that ibogaine was six-fold more potent than noribogaine in competition assays with [^{3}H]MK-801. Ibogaine (100 μM) and noribogaine (1 mM) blocked (85 to 90% of control values) the ability of NMDA (100 μM, 5 sec.) to depolarize frog motoneurons in a non-competitive and use-dependent manner. The lower affinity of noribogaine may indicate that the dwell time for the metabolite in the ion channel is too brief to be additive to the activity of ibogaine. The molecular target(s) mediating the putative anti-addictive, psychotropic and neurotoxic properties of ibogaine are not completely understood. If ibogaine's interaction with the NMDA receptor-coupled cation channels contribute to its psychotropic and/or neurotoxic actions, the metabolite may have fewer adverse effects than the parent drug. Supported by NS 17577 and VA Medical Ctr (MRIS 1769 & 3369).

499.10

MODULATION OF AMPA OR $GABA_A$ RECEPTORS ANTAGONIZES ALPRAZOLAM-INDUCED IMPAIRMENT OF LEARNING IN MONKEYS. J. Auta, D.M. Thompson, J.M. Moerschbaecher[1], K. Varner*[1], A. Guidotti, and E. Costa, N.S. Kline Institute for Psychiatry Research, Center for Neuropharmacology, Orangeburg, NY and Dept. Psychiatry, NYU, NY. [1] Dept. Pharmacology, LSU Med. Center, New Orleans, LA.

A multiple schedule of repeated acquisition and performance of response chains was used to assess the effects of IDRA 21 (JPET 272: 300, 1995) and aniracetam (two allosteric modulators of AMPA receptor-induced desensitization) on alprazolam-induced impairment of acquisition in monkeys. Acquisition of the response chain was defined by a decrease in errors as the session progressed. Responding in each component was maintained by food presentation under a fixed-ratio schedule. Alprazolam (0.3 or 1 μmol/kg p.o.), a full positive allosteric modulator of GABA action, when administered alone produced dose-related decreases in response rate in each component. Alprazolam also produced a dose-related increase in percent errors in acquisition while having a small effect on accuracy in performance. Imidazenil, a partial positive allosteric modulator of GABA action (JPET 266: 1018, 1993), had little or no effect when given alone (0.003 to 3 μmol/kg p.o) 60 min presession but dose dependently antagonized and at 0.03 μmol/kg completely blocked the disruptive effects of alprazolam when given 60 min before alprazolam. IDRA 21 (13 and 24 μmol/kg p.o) and aniracetam (137 μmol/kg) administered alone had no effect on either rate of responding or accuracy in either component. However, 60 min pretreatment with IDRA 21 (13 μmol/kg p.o) or aniracetam (137 μmol/kg p.o) produced a dose-related attenuation of the effects of alprazolam in both components. These data suggest that in primates alprazolam impairment of learning may depend upon events coordinated by AMPA and $GABA_A$ receptors (Supported by Grant NS 28130-04).

499.11

EVIDENCE FOR REDOX MODULATION AND *S*-NITROSYLATION OF THE RAT BRAIN Na^+/Ca^{2+} EXCHANGER. R.A. Colvin*, J. Schummers, N. Davis, and L. D. Hatem. Program in Neurobiology, Department of Biological Sciences, Ohio University, College of Osteopathic Medicine, Athens, OH 45701

When plasma membrane vesicles derived from whole rat brain were incubated with 1 mM DTT or ascorbic acid at 37°C for 1 hr., a maximal 35% decrease in Na^+/Ca^{2+} exchange activity was seen. Coincubation with 10 mM H_2O_2 prevented the effect of DTT. Sodium nitroprusside (SNP) (0.1 mM) which in solution has the capability to generate NO and ferrocyanide, had no effect by itself, but when added in combination with DTT caused an additional 50% inhibition of the activity seen with DTT alone. The SNP effect was dose dependent with a half maximal effect seen at 2 μM. Ferrocyanide did not cause inhibition when added in combination with DTT. The inhibitory effect of SNP could not be prevented by coincubation with Vitamin E (100 μg/ml). The initial velocity of Na^+/Ca^{2+} exchange was determined at various concentrations of Ca^{2+} and Vmax and Km determined. DTT inhibition resulted from a decrease in Vmax with no effect on the Km for Ca^{2+}. There was no additional effect of coincubation of DTT with SNP on either the Km or Vmax. There was no effect of DTT or DTT/SNP on membrane permeability to Ca^{2+}. Analysis of unidirectional fluxes for Ca^{2+} during a Na^+/Ca^{2+} exchange reaction showed that both DTT and DTT/SNP reduced Ca^{2+} influx. Ca^{2+} efflux was reduced only after preincubation with both DTT and SNP. Western analysis with an antibody specific for the Na^+/Ca^{2+} exchanger, showed a single band with a molecular weight of 116 kDa in the presence of NEM. Incubation with DTT resulted in the release of a 19 kDa protein fragment. In summary, the brain Na^+/Ca^{2+} exchanger appears to contain reactive thiol groups that modulate its activity and structure. The reduced exchanger may be inhibited by *s*-nitrosylation after exposure to NO. The Na^+/Ca^{2+} exchanger may be proteolytically cleaved *in situ*, and a peptide fragment released when disulfide bridges are reduced.

499.12

PEROXYNITRITE DOWN REGULATES NMDA RECEPTOR ACTIVITY IN RAT CORTICAL NEURONS. Stuart A. Lipton*, Won-Ki Kim, Posina V. Rayudu, Mark E. Mullins, and Jonathan S. Stamler. Depts. of Neurology and Chemistry, Harvard Medical School and Harvard Univ., Boston, MA 02115; Dept. of Medicine, Duke Univ. Medical Center, Durham, NC 27710.

Peroxynitrite ($ONOO^-$), formed by reaction of nitric oxide with superoxide anion, is a potent oxidant and neurotoxin (Lipton, Choi et al., Nature 1993). The role of peroxynitrite as a potential second messenger or regulatory agent, however, remains controversial. Peroxynitrite has been reported to oxidize sulfhydryl groups (Radi, Beckman et al. JBC 1991), and NMDA-evoked responses can be modulated by sulfhydryl redox agents (Aizenman, Lipton et al., Neuron 1989; Sullivan et al., Neuron 1994). Therefore, the present study was undertaken to determine if peroxynitrite, at very low concentrations, could modulate the activity of the NMDA receptor. Peroxynitrite, synthesized by stopped flow reaction kinetics, was rapidly applied to rat cortical neurons in culture while monitoring their NMDA-evoked calcium responses with digital imaging of fura-2 or whole-cell recording with patch electrodes. Peroxynitrite, at micromolar concentrations or less ($t_{1/2} \approx 1$ s), decreased NMDA responses. The effect was reversed by subsequent exposure to the reducing agent dithiothreitol. Peroxynitrite did not further decrease NMDA responses of neurons previously treated with another sulfhydryl oxidizing agent 5,5'-dithio-bis-(2-nitrobenzoic acid). Moreover, peroxynitrite had no effect on NMDA receptors irreversibly alkylated with N-ethylmaleimide. These data indicate that peroxynitrite can reversibly downregulate NMDA receptor activity by oxidizing critical sulfhydryl groups comprising the redox modulatory sites of the NMDA receptor.

499.13

PRESYNAPTIC SYNTHESIS OF THE NMDA-ASSOCIATED GLYCINE RECEPTOR AGONIST, D-SERINE. P.L. Wood, D.B. Goodnough, J.E. Hawkinson and R.P. Shank*. Dept. of Pharmacology, CoCensys, Inc., 213 Technology Dr., Irvine, CA 92718

While glycine has been assumed to be the sole endogenous co-agonist at NMDA-associated glycine receptors, recent descriptions of endogenous D-serine levels in the brain force us to reassess this assumption. Indeed in more rostral brain regions, the levels of D-serine are greater than those of glycine. In the current studies, we assessed the levels and metabolic sources of D-serine in neocortical synaptosomal preparations. Previous studies have demonstrated that CNS serine and glycine are synthesized *de novo* via the phosphorylated pathway, originating with phosphoglycerate. The rate-limiting step in the synthesis of serine is the hydrolysis of phosphoserine by phosphoserine phosphatase (EC 3.1.3.3). In our synaptosomal preparations we have demonstrated the uptake of L-phosphoserine and its hydrolysis to both L-serine and D-serine. These data indicate that racemization can occur during or subsequent to the hydrolysis of L-phosphoserine. Another potential route for the generation of D-serine involves the glycine cleavage system (EC 2.1.2.10). In this case, experiments involving precursor loading with excess glycine or [^{13}C,^{15}N]glycine also demonstrated conversion to both L-serine and D-serine. In conclusion, these studies demonstrate that there may be alternate pathways for the synthesis of D-serine by neocortical synaptosomes.

499.14

THE NON-COMPETITIVE NMDA ANTAGONIST ACTIVITY OF THE OPIOD AGONIST KETOBEMIDONE IS POTENTIATED BY THE SPASMOLYTIC AGENT (*RS*)-3-DIMETHYLAMINO-1,1-DIPHENYLBUT-1-ENE (A29). B. Ebert*[a] and S. Andersen[b]. [a]PharmaBiotec Research Center, Department of Medicinal Chemistry, The Royal Danish School of Pharmacy, DK-2100 Copenhagen, Denmark.[b] Department of Anaestesiology, Hvidovre University Hospital, DK-2650 Hvidovre, Denmark.

The opioid ketobemidone has previously been shown to be a non-competitive N-methyl-D-aspartate (NMDA) antagonist[1]. Ketobemidone is in Scandinavia available in a formulation (Ketogan®) which contains ketobemidone and a spasmolytic agent (*RS*)-3-dimethylamino-1,1-diphenylbut-1-ene, hydrochloride (A29) in a one to five ratio. Lately, however, ketobemidone has been introduced in a sustained release formulation without the spasmolytic (Ketodur®). Clinical observations indicate that Ketogan® is more potent than Ketodur® on a mg to mg basis suggesting that A29 might potentiate the effects of ketobemidone. Using the rat cortical wedge preparation and receptor binding studies, we investigated whether the potentiation of ketobemidone by A29 was caused by an interaction with the NMDA receptor, other glutamate receptors, the γ aminobutyric acid ($GABA_A$) receptor or the GABA uptake system. A29 inhibited synaptosomal GABA uptake (IC_{50} value 180 ± 26 μM) and [^{3}H]MK-801 binding (K_i value 16 ± 4.5 μM), but was inactive in assays measuring affinities for other glutamate receptors and the $GABA_A$ receptor complex. In the rat cortical wedge preparation A29 was a non-competitive NMDA antagonist. The inhibitory effects of 20 μM ketobemidone and 100 μM A29 on responses to 10 μM NMDA were additive. NMDA activity was completely abolished by 1 mM A29. These results suggest that the potentiating effect of A29 may be a consequence of a combined blockade of the NMDA receptor and the GABA uptake system.

Ketogan®, thus, seems to exert its analgesic actions *via* interaction with three different neurotransmitter systems; namely the opiod system, the NMDA receptor and the GABA uptake system.

1. Ebert et al. *Neurosci Lett* (1995) **187**, 165-168

499.15

SELECTIVE, HIGH POTENCY INHIBITION OF CLONED KAINATE RECEPTORS BY SYM 2081. K.A. Jones,* Z-Q. Gu, J.R. Howe,[1] K.A. Yamada,[2] and A.M. Costa. Symphony Pharmaceuticals Inc., Malvern, PA; [1]Dept. Pharmacology, Yale University Sch. Med., New Haven, CT; [2]Depts. Neurology and Pediatrics, Washington University Sch. Med., St. Louis, MO.

Among the four diastereoisomers, (2S,4R)-4-methylglutamate (SYM 2081) displays 10^3 fold higher affinity for kainate (KA) compared to AMPA or NMDA receptors in brain membranes. Here we report the functional activity of SYM 2081 at cloned GluR6 receptors expressed in HEK293 cells. Whole-cell currents elicited by KA (100 μM) in voltage clamped cells expressing GluR6 were reversibly abolished by preapplications of 300 nM SYM 2081. The dose response relationship for 1 minute preapplications of SYM 2081 yielded an IC_{50} of 10 nM. In contrast, no inhibition of AMPA currents elicited by KA in primary cultures of rat neocortex was observed at doses up to 10 μM. Rapid applications of μM concentrations of SYM 2081 to HEK293 cells expressing GluR6 receptors resulted in rapidly desensitizing KA-like currents. The KA-like behavior of SYM 2081 at high doses (>30 μM) was confirmed in cortical neurons that responded to the drug with non-desensitizing currents. We tested the possibility that receptor desensitization was responsible for the inhibitory effects of SYM 2081 by treating cells with concanavalin A (Con A). Whereas untreated cells produced no detectable current in response to rapid applications of 30 nM SYM 2081, Con A-treated cells responded with a slowly developing, non-desensitizing current. In addition, Con A prevented the inhibition of KA currents by SYM 2081. SYM 2081 thus appears to act as an inhibitor of KA channel function by inducing receptor desensitization at doses well below that which cause macroscopic channel activation. The unique properties of this compound make it ideal for studying the physiological roles of the KA class of glutamate receptors.

499.16

GLUTAMATE-MODULATED VERSUS SPONTANEOUS Ca^{2+} OSCILLATIONS IN HIPPOCAMPAL GLIA. P.P. McCaslin* and G. Hammond. Div. Res., Ochsner Med. Found., New Orleans, LA 70121.

Cytoplasmic Ca^{2+} oscillations occur in hippocampal glia as well as other cell types. Oscillations are induced by ligands which activate a large variety of G-protein-linked receptors including ATP, glutamate and 5-HT, and do not require the movement of extracellular Ca^{2+}. We report that oscillations in glia are modulated by G-protein-linked receptors, but light is an important component of the oscillations. In non-perfused buffer, spontaneous oscillations occur in >99% of cultures of hippocampal glia in the absence of exogenous receptor ligands. At a light intensity of 50 cd/m^2, oscillations begin within 20 min and last about 90 min; after oscillations have stopped, increases and decreases in $[Ca^{2+}]_i$ (but not Ca^{2+} oscillations) are nevertheless induced by agents such as ionomycin and cytidine, respectively. If 20 μM glutamate is added to non-perfused buffer and cells are then exposed to light, regular oscillations begin almost immediately and continue for about 60 min; when a new, non-light-exposed region (but glutamate-exposed for 60 min) on the same slide is exposed to light, oscillations begin within 5 min and continue for 60 min with a similar oscillation pattern to the previous cells. Similar results occur a third and fourth time even though cells were exposed to glutamate for 120-180 min before light exposure. Moreover, glutamate-modulated oscillations display a different pattern from spontaneous oscillations which is best illustrated by the return maps of the time series. Interestingly, if cells are perfused for one hour under 50 cd/m^2 light, they do not oscillate (as opposed to the above non-perfused cells); furthermore, they do not oscillate when exposed to glutamate. However if glutamate is added after 5-15 minute of light exposure in perfused cells, oscillations do occur. Collectively, these results suggest that light induces the release of a mediator which is necessary for cytoplasmic Ca^{2+} oscillations in glia. Glutamate apparently modulates oscillations in conjunction with this mediator.

500.1

THRESHOLD PROPERTIES OF OPERANT CONDITIONING IN *LYMNAEA*. K. Lukowiak*, P.M. Hermann and A.G.M. Bulloch. Neuroscience Research Group, University of Calgary, Calgary, AB, Canada, T2N 4N1.

Lung ventilation in the pond snail *Lymnaea stagnalis* occurs at the water surface by opening and closing the pneumostome. In a previous study it was shown that *Lymnaea's* respiratory behavior can be operantly conditioned. The operant conditioning procedure consisted of stimulating the pneumostome area with a sharp wooden stick each time the animal attempted to open its pneumostome. In successive training sessions of an hour per day, the animals learned to avoid the stimulus by changing their behavior in frequency and performance. The present study was designed to investigate threshold properties of the previously described learning procedure. Different groups of animals were, therefore, subjected to different regimes of reinforcement. Animals which had two operant conditioning training sessions of 1/2 hour per day showed the same behavioral changes compared with animals which were trained for one period of an hour per day. However, one training session of 1/2 hour per day did not induce overt behavioral changes. It seems, therefore, that animals need a total of one hour of conditioning training period per day to exhibit associative learning behavior. At the moment our experiments are focused on the question of whether exposure to a subliminal training scheme (i.e. one session of 1/2 hour per day), or to stimuli that are not contingent with pneumostome opening (i.e. random stimulated), affect the ability to exhibit associative learning in the future (i.e. whether this desensitizes the animal). Our data suggest that animals which have received the subliminal training scheme differ from naive animals in their ability to be operantly conditioned. Supported by MRC (Canada).

500.2

BEHAVIORAL AND NEURAL CHARACTERISTICS OF CONDITIONED INHIBITION IN Hermissenda. *Gabrielle Britton and Joseph Farley*, Program in Neural Science, Indiana University, Bloomington, IN 47405*

Most studies of Pavlovian conditioning in model systems have focussed upon the learning produced by conditioned excitation procedures, viz. pairings of CS and US. For Hermissenda (H.c.), pairings of light and rotation result in suppression of phototactic behavior. A second major category of associative learning, which has been conspicuously neglected in model systems research, is that of conditioned inhibition, i.e. the learning which results when a CS signals the absence of a US. We report that H.c. learn to approach a light that has been explicitly unpaired with rotation, i.e. their phototactic behavior is enhanced when light signals the absence of rotation. The behavior of H.c. in a light gradient was time-sampled over a ten min period in an underwater open-field. Two measures of phototactic behavior were computed: 1) the average distance from the center of the light gradient, 2) the change in the animal's orientation (with respect to light) from one sample period to the next. On retention days following 3 consecutive training sessions of explicitly-unpaired presentations of light and rotation, animals moved closer to (and were more likely to be oriented towards) the center of the light gradient. Animals exposed to equivalent numbers of random presentations of light and rotation, or no training at all, failed to show these changes. Intracellular recordings from synaptically-intact Type B photoreceptors indicated that explicitly-unpaired training produced a selective reduction in the amplitude of the light-evoked generator potential, as well as a decrease in action potential frequency. Our results demonstrate the occurrence of conditioned inhibition in an invertebrate model system in which cellular mechanisms of learning and memory can be studied, and further suggest that some of the cellular changes underlying conditioned excitation and inhibition are localized to the same cells (Type B photoreceptors) but are apparently encoded in opposite fashion (increases and decreases in excitability, respectively).

500.3

CONTEXTUAL BLOCKING IS A CENTRALLY MEDIATED PHENOMENON IN THE MARINE INVERTEBRATE, *HERMISSENDA*

R.F. Rogers*, I.A. Muzzio, A.C. Talk and L.D. Matzel
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Contextual blocking of a CS-US association was investigated behaviorally and neurophysiologically in the marine mollusc *Hermissenda*. As indexed by the development of a conditioned foot contraction to a light CS, it was found in two experiments that US (rotation) preexposure within a diffuse chemosensory context (shell fish extract) blocked subsequent learning about a CS (light) paired with the US in that context, but not in a second, neutral context. In a subpopulation of these animals, the membrane resistance across the B photoreceptor soma (a cellular correlate of the light-rotation association) was investigated electrophysiologically. Despite their differential responding at the behavioral level, an equivalent increase in membrane resistance was found in blocked and paired control animals relative to unpaired controls. A third experiment investigated light-induced multi-unit activity in a pedal nerve known to convey motor information to the foot and thought to participate in the behavioral expression of the CS-US association. Consistent with their behavioral expression of the CS-US association, light-induced nerve activity in paired controls was suppressed relative to the unpaired animals. In contrast, no difference was found in light-induced nerve activity between behaviorally blocked animals and their unpaired controls, despite the associative increase in B cell excitability.

One hypothesis to account for these results is that the development of a context-US association in the preexposure phase results in plastic modifications within a network of centrally-located interneurons known to receive information from all three sensory systems. The result of this plasticity is a biasing of this central network toward contextual stimuli, such that the behavioral expression of the light-rotation association is blocked.

500.4

INCREMENTAL TRANSLOCATION OF PROTEIN KINASE C UNDERLIES THE ACQUISITION CURVE DURING *IN VITRO* ASSOCIATIVE CONDITIONING

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Department of Psychology, Rutgers University, New Brunswick, NJ 08903

An ubiquitous characteristic of learning is the improvement in performance observed over successive acquisition trials. This acquisition curve is presumably based in biophysical and biochemical alterations within the nervous system. In the isolated nervous system of the marine mollusc *Hermissenda*, an *in vitro* conditioning preparation was employed that is analogous to associative conditioning in the intact animal. Pairings of light (CS) with mechanical stimulation of the vestibular hair cells (US) resulted in an incremental change in the excitability of the photoreceptors. This increase became asymptotic within 3-5 training trials. A single pairing of light and rotation produced a small increase in excitability that dissipated over time. The incremental step function observed as a result of successive training was not obtained in the presence of NPC-15437, a selective inhibitor of protein kinase C (PKC), or if the stimuli presentation occurred in an unpaired fashion.

Colorimetric analysis of PKC in the cytosol and membrane components extracted from groups of isolated eyes indicated that while total PKC activity remains constant under different training conditions (0, 1, 3, or 5 pairings of light and rotation), successive training trials produce an incremental translocation of PKC from the cytosolic to membrane fractions, that like the increase in excitability, asymptotes within 5 trials. Artificial translocation of the kinase by phorbol ester produced a redistribution of PKC analogous to asymptotic training. Unpaired training produced no such translocation, nor did paired training in the presence of NPC-15437. These results suggest that redistribution of PKC may play an important role in the acquisition of new learning.

500.5

GENE TRANSFER OF OCTOPAMINE RECEPTOR THAT IS LINKED TO THE ADENYLYL CYCLASE INTO THE SENSORY NEURONS OF *APLYSIA*. B-K. Kaang*[1], X.-C. Li, Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, NY, NY 10032; [1]IMBG, Seoul Natl. Univ., Seoul, Korea.

Several processes are thought to contribute to the short-term synaptic facilitation produced by 5-HT, including membrane depolarization, an increase in the duration of the action potential, enhanced membrane of excitability and enhanced neurotransmitter release. To further analyze the role of a second messenger cAMP in these processes, we used the ectopic gene transfer into the sensory neurons of the novel octopamine receptor that is coupled to adenylyl cyclase (Li et al., 1994, *Soc. Neurosci. Abstr.* 20:1160). Since the pleural sensory clusters do not seem to express the octopamine receptor endogenously, we overexpressed this receptor gene in the sensory neurons by the microinjection method using the green fluorescent protein (GFP) as a marker for gene expression. We then activated the expressed receptor by the application of octopamine thereby resulting in the production of cAMP in the sensory neurons. In control experiments, the expression of GFP itself did not seem to affect the physiology of cells including resting potential, input resistance, and the response to 5-HT. The treatment of octopamine produced the spike broadening (37.3 ± 5.0%, N = 14) as well as the increase in the membrane excitability (number of spikes = 11.8 ± 0.5; N = 22) in the sensory cells that expressed the *Aplysia* octopamine receptor, all of which were observed by the treatment of 5-HT to the control cells expressing the GFP protein alone (45.6 ± 10.3%, N = 6). The occlusion experiments showed that cAMP produced by octopamine alone was responsible for at least 73.9% of maximum spike broadening achieved by both octopamine and 5-HT together. Supported by HHMI and SNU Daewoo.

500.6

EGG MASSES ARE A SIGN STIMULUS FOR RELEASE OF BAG CELL PEPTIDES AND REPRODUCTIVE BEHAVIOR IN *APLYSIA*.

Valerie L. Begnoche, Earl Mayeri*, and Samuel K. Moore. Dept. of Physiology, University of California, San Francisco, CA 94143-0444 USA.

Many behaviors associated with reproduction are instinctive, or genetically determined, and involve a fixed action pattern that is triggered by a highly specific stimulus in the environment, the sign stimulus. The sequence of neural events between the sign stimulus and expression of the fixed action pattern is in many cases poorly understood. One idea is that the sign stimulus leads to release of endogenous peptide neurotransmitters. To test this idea we observed spontaneous reproductive behavior in groups of *Aplysia* in a recirculating sea water aquarium. We found that contact by the mouth with an existing egg mass is a sign stimulus for release of egg laying behavior and two other fixed action patterns in the same individual, mating as a female during egg laying, and mating as a male after egg laying.

In animals where electrical activity from the CNS was recorded, contact with the egg mass initiated repetitive spike activity in bag cell neurons, which are part of a peptidergic neural system and are known from previous work to modulate neuronal activity in the CNS for up to several hours and to initiate spontaneous egg laying in isolated individuals. Thus, the egg mass is a sign stimulus that initiates the release of neuropeptides and this serves as an innate releasing mechanism for control of the fixed action patterns.

(Supported by NIH grants NS16490 and NS16033.)

500.7

VISUALLY GUIDED NAVIGATION IN THE GIANT TROPICAL ANT, *PARAPONERA CLAVATA*. A.P. Baader*, Zoologisches Institut der Universität, Winterthurer Str. 190, CH-8057 Zuerich, Switzerland

Scouts of the ponerine ant, *Paraponera clavata*, forage many meters away from the nest. They travel over the foliage of the forest ground as well as up in the trees, thus occupying an immense three-dimensional space in rain forests of Central America. Pheromone trails, although known to be used during foraging, homing, and recruitment, provide only limited information for long-distance explorations in complex terrain. This study investigated the use of visual cues by *P. clavata* scouts during foraging and homing, with the goal of characterizing the coding properties of visual pathways within the central nervous system.

Behavioral field experiments revealed that information from both the top and the sides of the visual fields was essential for successful homing of foragers, even when initially laid chemical trails were still present during the tests. The ants were capable of finding their way back to the nest even after nearby landmarks and pheromone cues had been eliminated, presumably by using distant visual cues and/or canopy information. There was no indication of vector-based orientation. Scouts which were spotted during their extended foraging excursions and transferred to a different place homed successfully, although their visual surroundings at the release site were different from their original site. This indicates that the foragers utilized more complex visual strategies than can be explained by the most common navigation theories.

Intracellular recordings were performed on tethered ants walking on a freely movable treadmill while stimulated with various visual signals. The electrophysiological investigation aims at characterizing descending neuronal visual pathways in behaving animals. (The behavioral part of the project was carried out with Esther Denzler at the Biological Station of the Organization for Tropical Studies (OTS) in La Selva, Costa Rica. Partly funded by the Swiss National Science Foundation).

500.8

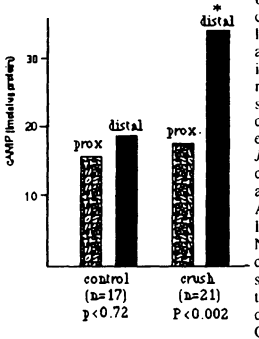
GENETIC MODULATION OF *SHAKER* CHANNELS CHANGES THE SENSITIVITY OF THE CNS OF *DROSOPHILA* TO GENERAL ANESTHETICS. M. Lin* and H. A. Nash, Laboratory of Molecular Biology, NIMH, NIH, Bethesda, MD 20892

This laboratory has reported that some channel mutations influence the sensitivity of *Drosophila* to volatile general anesthetics in behavioral assays. In our present study we use electrophysiological recording from flight and jump muscles to monitor the response of a particular neural pathway when different stimulating voltages are applied across the eyes to the brain. We find that the more distal part of the pathway (from giant fiber through one or two neurons to muscle), represented by a short latency response (<3 msec) to high voltage, is very insensitive to halothane. However, synaptic transmission in the fly brain upstream of the giant fiber, represented by a long latency response (4-6 msec) to lower voltage, is inhibited by clinical doses of halothane. When *Sh* and *eag* mutants are assayed, there is a good correlation between the published effect of the mutations on the peak amplitude of the A-type current in larval muscle and their effect on halothane sensitivity of the long latency response. Genetic tests, such as outcrossing and deficiency mapping, indicate that the phenotype of these strains is due to the corresponding mutation in the potassium channel genes. These results indicate that the A-type K^+ channel in CNS is a target for general anesthetics or at least influences the target.

500.9

cAMP INCREASES AT THE INJURY SITE IN APLYSIA NERVES R.T. Ambron*, D. Ambron, X-P. Zhang, M. Povelones, Anatomy and Cell Biology, Columbia University, New York, NY 10032

Crush injury to a peripheral nerve causes the transport of protein sp97 to the cell body. Sp97 is one of several axonal proteins that have a nuclear localization signal, but it alone changes direction in response to crush. Sp97 accumulates at a ligation located proximal to the crush site. When axoplasm extruded from the ligation site is injected into the soma of non-injured neurons, it induces many of the changes seen when the axons of these cells are crushed. Thus, sp97 has the properties expected of an injury signal (Ambron et al. *J. Neurosci.* 1995). What causes sp97 to change direction? Sp97 is phosphorylated at the crush site. Since PKA is present in Aplysia nerves, we asked whether cAMP levels are elevated in the axon after injury. Nerves p6-p9 of anesthesized animals were crushed and frozen 1 min later. The cm segment of nerve proximal to the crush and the next proximal cm segment were collected separately and assayed for cAMP. Control nerves were not crushed. As seen (fig.), cAMP levels were signifcantly higher (Wilcoxon matched pairs test) at the crush site (distal) than in either control segment or in the proximal segment of the crushed nerve. Axoplasm extruded from the crush site had roughly half of the total cAMP in the segment. PKA activated by cAMP could phosphorylate sp97 and the catalytic subunit would be available for transport to the nucleus to phosphorylate the transcription factor CREB.



500.10

AXOTOMY CAUSES LONG-TERM HYPEREXCITABILITY OF SINGLE *APLYSIA* SENSORY NEURONS IN CELL CULTURE. A. Salim and D. L. Glanzman*, Dept. of Physiological Science, UCLA, Los Angeles, CA 90024.

Walters and Ambron (1995) have suggested that the cellular signals which mediate long-term memory may converge with those which mediate the effects of axonal injury. This suggestion is based on investigations of the effects of nerve crush on the excitability of sensory neurons in *Aplysia* pleural ganglia (Walters et al., 1991; Gunstream et al., 1995). When tested hours-to-days after nerve crush, axotomized sensory neurons are significantly more excitable than control sensory neurons. Similar hyperexcitability of *Aplysia* sensory neurons has been observed 24 hr after repeated applications of 5-HT (Dale et al., 1987), a transmitter which mediates behavioral sensitization in *Aplysia*. A question raised by the nerve crush experiments is whether the injury-induced changes in the properties of the sensory neurons are a direct consequence of trauma suffered by the sensory axons. The changes might be due, at least in part, to modulatory factors released from non-sensory neurons whose axons also run in the crushed nerves, or to nonneuronal substances produced by tissue damage.

To determine whether the cellular changes in *Aplysia* sensory neurons produced by nerve crush are due to intrinsic or extrinsic signals, we examined the long-term effects of axotomy on the excitability of individual pleural sensory neurons in dissociated cell culture. All of the neurons had been in cell culture for 2 days at the start of the experiments. Sensory neurons were impaled with intracellular microelectrodes and injected with 2-sec pulses of varying levels of depolarizing current. We recorded the number of action potentials generated by each sensory neuron in response to the current pulses. Following the current injections, some of the sensory neurons were axotomized. Approximately 24 hr later, the sensory neurons were reimpaled and their excitability retested. The axotomized sensory neurons exhibited a significant increase in their excitability, whereas the control sensory neurons did not. These in vitro results establish that the cellular changes in sensory neurons observed after nerve crush are, in part, an intrinsic effect of damage of the sensory axons.

500.11

MODULATION OF A Na^+/K^+ ELECTROGENIC PUMP AS NEW MOLECULAR MECHANISM UNDERLYING NON-ASSOCIATIVE LEARNING PROCESSES IN INVERTEBRATES M. Brunelli,*, R. Scuri, R. Mozzachiodi, M. L. Zaccardi and M. Lazzerini, Dept. of Physiology and Biochemistry "G. Moruzzi", University of Pisa, Italy.

Electrophysiological experiments performed onto T sensory neuron of the leech *Hirudo medicinalis*, have demonstrated that the after-hyperpolarization (AHP) which follows the electrical activation of this cell may be modulated during non-associative learning processes. The AHP is mainly due to the activity of a Na^+/K^+ electrogenic pump and partly to a gK/Ca. Serotonin, acting through cAMP, reduces the AHP amplitude by inhibiting this Na^+/K^+ pump (J. Physiol. 462: 229-242, 1993). More recently we have demonstrated that low rate intracellular stimulation (L.R.I.S.) of T cell produces after 15 stimuli a lasting increase of AHP amplitude. This effect is still present when the gK/Ca is blocked by 0,1 mM $CdCl_2$. 5 µM NDGA or 25 µM nifedipine application blocks the AHP potentiation as well as the inhibition of the pump by serotonin. On the contrary, it is still possible to obtain AHP increase after 5 µM indometacine application. Our data demonstrate that the increase in AHP amplitude after L.R.I.S. is due to a potentiation of the Na^+/K^+ pump, is triggered by voltage-dependent L-calcium channels and is mediated by lipoxygenase metabolites of arachidonic acid. The increase in AHP amplitude may provide an interesting cellular mechanism underlying the habituation: it might block the traffic of action potentials along the sensory fibers, thus decreasing the synaptic efficacy of T neuron. By utilizing the experimental model of swim induction we have demonstrated that the leeches subjected to repetitive tactile or electrical stimulation exhibit habituation; this reduction of the response to tactile stimulation disappears when either 50 µM NDGA or 1 mM nifedipine is injected into the animals.

501.1

THE IMMEDIATE EARLY GENE CYCLOOXYGENASE-2 IS EXPRESSED IN CA1 NEURONS DESTINED FOR APOPTOTIC DEATH FOLLOWING GLOBAL ISCHEMIA. M. Nakayama*, J. Chen, T. Lowry, T. Asakura, S.H. Graham. Dept. Neurology, Univ. of Pittsburgh, PA 15261 and Dept. Neurosurgery, Univ. of Kagoshima, Kagoshima, Japan

Cyclooxygenase (COX) may play an important role in normal brain functions as well as in the pathogenesis of neurodegenerative diseases. COX-2, the inducible form of COX, is an immediate early gene that is expressed in brain neurons in response to increased synaptic activity.

We studied the temporal profile of COX-2 expression in brains following global ischemia. Anesthetized rats were subjected to 15 min of 4 vessel occlusion and 2h, 4h, 8h, 24h, 48h, or 72h of reperfusion. Transcriptional expression of COX-2 was studied by *in situ* hybridization using a 35S-labeled oligonucleotide probe, and protein expression was studied by immunocytochemistry using a monoclonal antibody against COX-2. Results were compared with TUNEL staining at 24h and cresyl violet staining at 72h after ischemia respectively. Forebrain ischemia induced COX-2 expression in a similar pattern at both the mRNA and protein levels. COX-2 was transiently expressed in neurons throughout CA1-4, dentate, caudate putamen and superficial cortex at 2-8h. However, COX-2 was persistently expressed in majority of CA1 neurons at 24-48h where positive TUNEL staining indicative of apoptosis were also found. In contrast, the stress protein HSP72 was not expressed in CA1 at these times. COX-2 expression was completely lost at 72h when there was near complete death of CA1 neurons.

COX-2 could play an important role in the expression of excitotoxicity since Ca++ activates phospholipases which provide arachidonic acid substrate for COX and each molecule of AA metabolized by COX produces an oxygen free radical. The persistent expression of COX-2 preceding DNA damage and cell death in CA1 following global ischemia are consistent with the hypothesis that COX-2 activity may contribute to oxidative stress and subsequent apoptotic cell death.

501.2

UPREGULATION OF BAX PROTEIN LEVELS IN NEURONS FOLLOWING GLOBAL CEREBRAL ISCHEMIA. S. Krajewski, J.K. Mai#, M. Krajewska, M. Sikorska@, M.J. Mossaakowski+, S.M. Park*, and J.C. Reed. La Jolla Cancer Res. Fnd., La Jolla, CA 92037, #Univ. of Duesseldorf, @National Res. Council of Canada, Ottawa and +Med. Res. Center, Warsaw.

The patterns of expression of the *bcl-2*, *bax*, and *bcl-X* genes were examined immunohistochemically in neurons of the adult rat brain before and after 10 min. of global ischemia induced by transient cardiac arrest. High levels of the cell death promoting protein Bax and concomitant low levels of the apoptosis-blocking protein Bcl-2 were found in some population of neurons that are particularly sensitive to cell death after ischemic incidence, such as CA1 sector of the hippocampus and Purkinje cells of the cerebellum. Moreover, within 0.5 to 3 hrs after cardiac arrest, immunostaining for Bax was markedly increased in neurons with morphological features of degeneration in many regions of the brain. Use of a two-color staining method for simultaneous analysis of Bax protein and in situ detection of DNA-strand breaks revealed high levels of Bax immunoreactivity in many neurons undergoing apoptosis. Post-ischemic elevations in Bax protein levels in the hippocampus, cerebral cortex and cerebellum were also demonstrated by immunoblotting. At early times after transient ischemia, regulation of Bcl-2 and Bcl-x protein levels varied among neuronal subpopulation, but from 3 hrs on, those neurons with morphological evidence of degeneration uniformly contained reduced levels of Bcl-2 and particularly Bcl-X immunoreactivity. The findings suggest that differential expression of some members of the Bcl-2 gene family may play an important role in determining the relative sensitivity of neuronal subpopulations to ischemia and that post-ischemic alterations in the expression of the *bax*, *bcl-2*, and *bcl-X* may contribute to the delayed neuronal cell death that occurs during reperfusion phase after a transient ischemic episode.

501.3

TARGET DISRUPTION OF CuZn-SUPEROXIDE DISMUTASE GENE IN MICE CAUSES EXACERBATION OF CEREBRAL INFARCTION AND NEUROLOGICAL DEFICITS AFTER FOCAL CEREBRAL ISCHEMIA AND REPERFUSION. Kondo T*,[1] Reaume A,[2] Huang T-T,[3] Carlson E,[3] Chen S,[1] Scott R,[2] Epstein C J,[3] Chan P H.[1] (Department of Neurosurgery and Neurology[1], Pediatrics[3], University of California, San Francisco, CA94143 and Cephalon, Inc[2], 145 Brandywine Parkway, West Chester, PA19380.)

Oxygen radicals are be involved in the pathogenesis of infarction, edema and neurological deficits following cerebral ischemia and reperfusion. We have previously demonstrated that these brain injuries are significantly reduced in transgenic mice overexpressing human CuZn-superoxide dismutase (CuZn-SOD). In order to confirm further the beneficial role of CuZn-SOD in neuronal protection against ischemic insult, we employed two independent lines of heterozygous knockout mutant mice that were generated by targeted disruption or deletion of the mouse CuZn-SOD (Sod-1) gene. Heterozygous male Sod-1 knockout mutant mice and wild-type littermates (34±4 g) were identified by the activity of Sod-1 in red blood cells (0.73 vs. 1.60 unit / mg protein). These mice were subjected to middle cerebral artery occlusion (MCAO) and reperfusion by intraluminal suture blockade, plus ipsilateral common carotid artery occlusion. At 24 h after 60 min MCAO, the mice (n=9) were evaluated for neurological deficits prior to decapitation. To evaluate infarct volume, one group of fresh brain sections (2 mm) were stained with 2,3,5-triphenyltetrazolium chloride (n=3). In another group, the frozen brain sections (20 μm) were stained with cresyl violet (n=6). The mutant mice showed significantly greater neurological deficits (17.9 vs. 11.1, $p<0.05$) and larger infarct volume (88.5±10.3 mm^3 vs. 41.6±6.1 mm^3, $p<0.05$) than the wild-type mice. There were no differences between the mutant and the wild-type animals in cerebral blood flow (rCBF), systemic blood pressure and arterial blood-gas analysis. These results indicate that reduction of Sod-1 activity exacerbates the tissue damage after ischemic reperfusion injury without affecting the physiological status, including rCBF. It is suggested that without an adequate level of Sod-1, the overaccumulation of superoxide anions is deleterious to the brain after ischemia and reperfusion. (Supported by NS-14543, NS-25372, AG-08938.)

501.4

CEREBRAL INFARCTION IS EXACERBATED IN MITOCHONDRIAL MANGANESE SUPEROXIDE DISMUTASE (*Sod-2*) KNOCKOUT MUTANT MICE AFTER FOCAL CEREBRAL ISCHEMIA AND REPERFUSION • Mikawa, S,[1] Li Y,[1] Huang T-T,[3] Carlson E,[3] Chen S,[1] Kondo T,[1] Murakami K,[1] Epstein CJ,[3] Chan PH*.[1,2] Departments of Neurosurgery,[1] Neurology,[2] and Pediatrics,[3] University of California, San Francisco, CA 94143

Oxygen radicals have been shown to be involved in the development of cerebral infarction following cerebral ischemia and reperfusion in various experimental animal models. We have previously demonstrated that infarct volume, blood brain barrier breakdown, vasogenic edema and neurological deficits are significantly reduced in transgenic mice overexpressing CuZn-superoxide dismutase (*Sod-1*). Since mitochondria are known to be the site for oxygen radical formation during reperfusion, we sought to elucidate the role of manganese superoxide dismutase (Mn-SOD), a mitochondrial-specific antioxidant enzyme, in ischemic infarction. Targeted disruption of manganese superoxide dismutase gene (*Sod-2*) in mice by homologous recombination has been successfully achieved in our laboratory. The *Sod-2* knockout animal (*Sod-2*^{mlucsf}) contains the mutant gene as demonstrated by PCR and RT-PCR and by the enzymatic assays. The heterozygous mice were subjected to 30 minutes focal cerebral ischemia using intraluminal suture to block the middle cerebral artery and followed by 24 hr. reperfusion. There were no significant differences between the mutant and wild-type littermates in physiological parameters ($_pH$, PCO_2, PO_2 and MABP) before, during and after the onset of ischemia. However, infarct volume was significantly increased in mutant as compared to wild-type animals. Furthermore, mutant animals exhibited a greater severity in neurological deficit scores than those of the wild-type mice. These data suggest that deficiency in mitochondrial defense against oxidative stress in mutant mice results in exacerbation of ischemic infarction. Supported by NS 14543, NS 25372, and AG 08938.

501.5

AN ANTISENSE OLIGONUCLEOTIDE TO TNF-α mRNA INCREASES THE SIZE OF CEREBRAL INFARCTS IN THE RAT. Q. Zhai, N. Futrell*, F. Chen, M.J. Paredes. Cerebrovascular Laboratory, Medical College of Ohio, Toledo, OH 43614

Both TNF-α mRNA and protein are upregulated following brain injuries of various types. Our laboratory has documented TNF-α mRNA within cerebral infarcts, from 2 -12 hours (peak 6 hours) following middle cerebral artery occlusion (MCAO) in the rat. Astrocytes and neurons are positive for TNF-α by double-labeled immunoperoxidase. To study the role of TNF-α in tissue damage following cerebral infarction, we used an antisense oligonucleotide to rat TNF-α mRNA. Sixteen male Fisher rats, 8-9 weeks old, were used. Intraventricular injection of 40μl (5 nmol) of antisense oligo was given to 8 animals, with controls given 40 μl (5 nmol) of sense oligo (n=4) or 40μl of normal saline (n=4). MCAO was produced 16 hours later, using a craniotomy and cauterization of the MCA. Animals were sacrificed 24 hours later. Coronal microtome sections were cut at 400μm intervals and stained with H&E. Infarct volume was measured by computerized image analysis. Mean infarct volumes were 22.06 ± 17.10 mm^3 for normal saline controls and 34.07 ± 14.50 mm^3 for sense controls. Three of 8 antisense treated animals died at 2-3 hours following injection, before MCAO was performed. Five surviving animals had MCAO with a mean infarct volume of 118.00 ± 13.61 mm^3 ($p<0.05$, ANOVA and Newman-Keuls test). The early death of some antisense animals suggests a physiological role of TNF-α in brain homeostasis. Factors potentially contributing to larger infarct size in antisense animals include: 1) a rebound up regulation of TNF-α, which may function as a neurotoxic factor, may have occurred as the quantity of antisense oligo decreased during the study period (40 hours from injection to sacrifice), or 2) a neuroprotective effect of TNF-α is possible within cerebral infarcts, as TNF-α can induce nerve growth factor and calbindin-D_{28k}. Alternate injection times and doses are being studied. The effects of the antisense oligo on mRNA expression and on protein synthesis of TNF-α are under investigation using RT-PCR and Western Blot, to clarify the overall role of TNF-α in cerebral infarction in the rat.

501.6

Ischemia Increases Cyclin-dependent Kinase 5 Activity in Gerbil Brain. S.L. Green*, and P.R. Vulliet. Dept. Molecular Biosciences, UC-Davis School of Vet. Med., Davis, CA 95616

Cyclin-dependent kinase 5 (CDK5) is the catalytic subunit of a recently characterized neuronal cdc2-like protein kinase. It may regulate the cytoskeleton rather than cell cycle control. We have examined the effects of transient global ischemia and reperfusion on CDK5 activity in gerbil brain. Activity increased by 2 to 5-fold in the cytosolic and particulate fractions in the hippocampus and neocortex after 5, 15, and 30 min reperfusion. There was a marked decline in activity after 2 hrs, followed by a return to control levels after 2 days. On western blot analysis, CDK5 immunoreactive bands increased in intensity at the time points in which an increase in activity was observed. There was no change in immunostaining of the hippocampus and neocortex early after ischemia. Loss of staining occurred after 2 days. This data suggests that CDK5 is altered following brain ischemia and may play a role in neuronal response to injury.

501.7

ASTROCYTE GLUTAMATE RELEASE UNDER "ISCHEMIC" CONDITIONS
R.A. Swanson*, and M.C. Longuemare. Dept. of Neurology, VAMC and University of California, San Francisco, CA 94121

Extracellular glutamate accumulation during cerebral ischemia results from both synaptic and non-synaptic glutamate release. Some studies, using animal models of stroke, suggest non-synaptic release of glutamate to be the major source, while other studies have found non-synaptic release to minimal. We propose that these disparities may stem from differences in the metabolic disturbances caused by different ischemic models (e.g. complete vs. incomplete ischemia). This was tested using primary rat astrocyte cultures devoid of neurons. The cultures were exposed to metabolic stresses that occur during ischemia: glucose deprivation, oxygen deprivation, acidosis, and elevated potassium concentrations. Glutamate efflux was assessed by HPLC determinations of the culture media and by efflux of pre-loaded ^{3}H D-aspartate (D-ASP). Potential routes of non-synaptic release were assessed by pre-loading the cells with specific inhibitors of glutamate transport pathways. Hypoxia caused modest (20-40%) reductions in ATP and did not cause glutamate release. $\uparrow K^+_o$ up to 60mM also failed to provoke glutamate release. Hypoxia plus glucose deprivation reduced ATP to below 25% of controls and caused a 10-fold increase in glutamate release. Release under these conditions was markedly reduced by pre-loading the cells with compounds that specifically block Na^+-dependent glutamate transport (TBHA or PDC). This reduction was more complete for D-ASP efflux than for glutamate efflux. These findings suggest (1) that residual glucose delivery, as occurs during incomplete ischemia, may prevent non-synaptic glutamate release; (2) that glutamate release from astrocytes during energy failure occurs primarily via reversal of Na^+-dependent uptake, and (3) that D-ASP transport does not always mimic glutamate transport.

501.8

MECHANISMS OF ACIDOSIS-INDUCED ASTROCYTE DEATH.
B.A. Stein*, K. Farrell, and R.A. Swanson. Dept. of Neurology, VAMC and University of California, San Francisco, 94121.

Rat cortical astrocyte cultures were employed to study the factors causing astrocyte death under conditions simulating incomplete cerebral ischemia. These conditions are characterized in part by acidosis accompanied by hypoxia and variably increased K^+_o. Astrocyte cultures were subjected to these conditions alone or in combination for periods of 2-6 hours and survival was assessed 24 hours later by measuring LDH activity in cell lysates. Astrocytes under normoxic conditions were resistant to acidosis injury over the range of pH 7.2 to pH 6.2. Hypoxia, produced either by O_2 deprivation, azide, or antimycin A, similarly caused no cell death with exposures of up to 8 hours. Astrocyte sensitivity to acidosis was, however, markedly accentuated by hypoxia, with 4 hours at pH 6.2 killing 60-80% of the cells. Evaluation with the pH-sensitive fluorescent probe BCECF showed that hypoxia impaired intracellular pH regulation during acidosis, with pH_i in the presence of azide falling to values 0.5 to 1.0 pH unit below those reached in the absence of azide. The changes in pH_i were paralleled by reductions in glycolytic rate and ATP content. ^{3}H 2-deoxyglucose accumulation showed glycolytic rate during hypoxia at pH 6.2 plus to be only 28 ± 8% of controls. ATP content fell to 63 ± 8 % of control after 30 minutes of hypoxia at pH 7.2 and to 28.1 ± 9% at pH 6.2. The ATP depletion preceded irreversible cell injury by several hours, suggesting a causative role for energy failure. Both glycolytic rate and cell survival were increased in media containing 40mM K^+. These findings suggest that the cause of astrocyte death under conditions of incomplete ischemia is energy failure resulting from intracellular acidosis impairing glycolytic production of ATP.

501.9

EFFECT OF HYPOXIA ON ISCHEMIC AND NON-ISCHEMIC BRAIN.
R.N. Auer* and O. Miyamoto†, *Department of Pathology, University of Calgary, Calgary, AB T2N 4N1, Canada and †Departments of Basic Sports Medicine and Anatomy, Kagawa Medical School, Kagawa, Japan.

The aim of these experiments was to study the potential of hypoxic hypoxia (*i.e.* hypoxia produced by lowering inspired oxygen content) in causing brain necrosis, with or without ischemia. Male Wistar rats were ventilated and in the first (hypoxia only, n=13) group, arterial pO_2 was decreased to 25 mm Hg by lowering the oxygen content in the inspired gas mixture. When mean arterial blood pressure (MABP) spontaneously fell to 30 mm Hg, the oxygen content was adjusted to keep MABP at 30 mm Hg for 15 min. In a second (ischemia only, n=13) group, MABP was decreased to 30 mm Hg and kept there for 15 min by withdrawal or reinfusion of blood after ligation of right common carotid artery (CCA). In a third (hypoxia plus ischemia, n=9) group, after ligation of the right CCA, MABP and pO_2 were lowered to 30 and 25 mm Hg respectively. To further study the contribution of hypoxia and hypotension, additional groups (n=57) varied pO_2 and MABP systematically in animals with unilateral carotid occlusion. Pathological examination was performed one week after the experiments.

Neuronal necrosis was absent in the hypoxia only group. Caudate and hippocampal necrosis was seen in all ischemia plus hypoxia groups. Subsequent groups showed that this depended on both MABP and pO_2. At a fixed MABP of 30 mm Hg, hypoxia led to brain necrosis in 33%, 66%, 60% and 100% of animals at pO_2 of >100, 45, 35 and 25 mm Hg respectively. At a fixed pO_2 of 25 mm Hg, 20%, 60%, 88%, 78% and 100% of animals showed cerebral necrosis at MABP's of 70, 60, 50, 40, and 30 mm Hg respectively. These results suggest that hypoxia augments brain damage when cerebral blood flow is impaired, but in the absence of arterial occlusion is incapable of causing brain damage by itself, even with lowering of the BP to 30 mm Hg in the rat.

501.10

INDUCTION OF VASCULAR ENDOTHELIAL GROWTH FACTOR PROTEIN EXPRESSION BY TRANSIENT CEREBRAL ISCHEMIA IN RAT BRAIN.
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Vascular endothelial growth factor (VEGF) expression in retina, heart and glial tumor can be upregulated by hypoxia or ischemia. To investigate possible role of VEGF in cerebral ischemia, immunohistochemical study was used to detect the induction of VEGF protein expression in the brain by a transient cerebral middle artery occlusion. In the present study, we observed that VEGF-like positive immunostain cells were widely expressed in rat brain. Highest density of VEGF-like positive immunostain cell in the brain was maimly located in superoptic and lateral dorsal septal nucleolus, caudate putamen and medium density, in thalamus, hypothalamus, molecular layer of cerebral cortex and pyramidal layer of hippocampus. The number of VEGF-like positive immunostain cell was dramatically and time-dependently increased in pyramidal layer of cerebral cortex and molecular layer of hippocampus after cerebral ischemia.

The results suggest that VEGF may play a role in the neuronal function and participate in the pathphysiological process after brain ischemia.

501.11

CHANGES IN PHOSPHORYLATION OF TAU INDUCED BY ISCHEMIA AND REPERFUSION.
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The microtubule-associated protein tau plays an important role in the dynamics of microtubule assembly necessary for axonal growth and function. In this study the effect of ischemia and reperfusion on the expression and phosphorylation of tau was examined. Using a reversible model of spinal cord ischemia in rabbits, tau was found to be dephosphorylated in response to ischemia with a time course that closely matched the production of permanent paraplegia. Dephosphorylation of tau was observed only in the caudal lumbar spinal cord that is defined as ischemic by blood flow measurements. Similarly Ca^{2+}/calmodulin-dependent kinase II activity was reduced only in the caudal lumbar region. Thus dephosphorylation of tau and loss of Ca^{2+}/calmodulin-dependent kinase II activity are early markers of ischemia. Tau, however, was rapidly rephosphorylated during reperfusion, at site(s) that cause a reduction in its electrophoretic mobility, regardless of the neurological outcome. Changes in tau phosphorylation were monitored using both the phosphate-sensitive antibody Tau-1 and the phosphate-insensitive antibodies T14 and 5E2. The observed dephosphorylation of tau during ischemia and its rephosphorylation during reperfusion were confirmed using a rat model of global cerebral ischemia. The activities of kinases that may phosphorylate tau during reperfusion is currently being investigated. Alterations in tau phosphorylation or degradation may affect microtubule stability, possibly contributing to disruption of axonal transport but also facilitating neurite regeneration.

501.12

REDOX EFFECTS ON NO-MEDIATED POSTEXCITOTOXIC INJURY IN HIPPOCAMPAL SLICES.
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Transient exposure of hippocampal slices to high concentrations of excitatory amino acids (EAA) or to hypoxia is followed by a lasting increase in the rate of EAA release and of opening of NMDA receptor-gated ionic channels (NMDArc). These changes may play a role in the amplification phase of excitotoxic or hypoxic injury. We studied their mechanism by measuring EAA release by reverse phase HPLC and NMDArc opening by ^{3}H-MK801 binding, 30 min. after a transient(5 min) exposure to 10 mM glutamate+glycine.

Postexcitotoxic perfusion with 100μM nitroarginine(NA) inhibited both EAA release and NMDArc opening. This was reversed by 1 mM L-arginine but not by D-arginine, suggesting that it required NO synthesis. It was reduced by the free radical scavengers U74389G and SOD+catalase, but unaffected by 8br-cGMP, methylene blue or by ADP ribosylation inhibitors. Sodium nitroprusside (500 μM) exposure mimicked the effects on NMDArc opening; this was increased by the reducing agent cysteine (1mM) and reversed by SOD+catalase, suggesting mediation by NO· and/or peroxynitrite.

These results suggest that free radicals such as NO play an important role in this model of postexcitotoxic hippocampal injury.
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502.1

EYE MOVEMENT DISORDERS AFTER PREFRONTAL CORTEX LESIONS IN HUMANS. C. Pierrot-Deseilligny*, R.M. Müri, S. Rivaud and B. Gaymard. INSERM 289, Hôpital de la Salpêtrière, 47 bd de l'Hôpital, 75651 Paris Cedex 13, France.

Eye movements were recorded electro-oculographically in 3 patients with a small ischemic lesion affecting the left dorsolateral prefrontal cortex (PFC), i.e. area 46 of Brodmann, and 10 control subjects. The frontal eye field was spared. Reflexive visually guided saccades (gap and overlap tasks), antisaccades, several paradigms of predictive saccades, memory-guided saccades, double-step saccades, sequences of memory-guided saccades (using 4 successively presented visual targets, which remained illuminated) and smooth pursuit were studied. In patients, latency was slightly shorter in the gap task (with more express saccades) and slightly longer in the overlap task, compared to controls. The difference between latencies in the overlap and gap tasks was greater in patients (contralaterally to the lesion), compared to controls. Saccades accuracy was normal in both tasks. The percentage of errors in the antisaccade task was significantly increased, bilaterally. For memory-guided saccades, latency (bilaterally) and the percentage of error in amplitude (contralaterally) were significantly increased. The chronological order of saccades in memory-guided saccade sequences and accuracy of double-step saccades (i.e. the first and second saccades) were normal. Smooth pursuit was also normal. These results suggest, for the first time in the same patients, that the PFC is involved in disengagement from fixation, inhibition of unwanted reflexive visually guided saccades, triggering of predictive saccades, and spatial working memory controlling saccades. In contrast, the PFC does not appear to be involved in temporal working memory controlling memory-guided saccade sequences, in double-step saccade control and smooth pursuit. Therefore, the PFC, which controls different types of cognitive mechanisms preparing saccades, could play a major role in human ocular motor behavior.

502.2

SPATIAL WORKING MEMORY AND OCULOMOTOR CONTROL IN THE ELDERLY

I.A. Sweeney*, R.A. Berman University of Pittsburgh School of Medicine, Pittsburgh PA 15213

The evaluation of saccadic eye movements provides a promising strategy for studying changes in motor and cognitive processes over the age span. In this study, we examined eye movement activity in groups of 32 young (18-34 yrs) and 21 elderly (65-80 yrs) healthy subjects. The accuracy of visually-guided saccades of the elderly subjects was comparable to that of younger subjects. However, peak eye velocity was reduced in the elderly subjects, particularly for larger eye movements, indicating a decline in basic motor processes that maintain the main sequence of saccades.

Antisaccade and oculomotor delayed response tasks were administered to assess the capacity for voluntary response suppression and spatial working memory. Initiation of saccades in these tasks, as in the visually-guided saccade task, was delayed in the older subjects. Elderly subjects made more antisaccade errors, indicating some loss in the ability to inhibit responses to compelling visual stimuli. The delayed saccade initiation and the reduced ability to intentionally suppress saccadic eye movements suggest an age-related decline in the functional integrity of frontostriatal circuitry. In contrast, the accuracy of memory-guided saccades of elderly subjects was preserved with delays up to 8 seconds. The maintained accuracy of visually- and memory-guided saccades indicates that elderly subjects can make accurate saccades to both sensory cues and remembered spatial locations. Therefore, the widely distributed dorsal cortical circuitry which holds spatial information *on-line* for delayed oculomotor responses may be relatively unaffected by normal aging processes.

502.3

EFFECTS OF FEATURE EXPECTANCY ON TARGET SELECTION IN MACAQUE FRONTAL EYE FIELD. N.P. Bichot*, K.G. Thompson & J.D. Schall. Department of Psychology, Vanderbilt University, Nashville, TN 37240

We have found that the initial visual responses of frontal eye field (FEF) neurons do not discriminate the target from distractors in the receptive field during visual search for a salient stimulus (e.g., red target among green distractors or green target among red distractors) (Schall & Hanes, 1993). In subsequent work with a monkey trained only to make a saccade to a red target among green distractors, we found that the initial visual response of a significant proportion of subcortical visuomotor neurons discriminated the red target from a green distractor in the receptive field (Schall & Thompson, 1994 *Soc Neuro Abstr*).

We now report findings from FEF of that monkey. Neurons with visual activity were recorded in the FEF of the monkey performing the search for a red target. When presented a search array with a green target among red distractors, the monkey never fixated the green target but instead directed gaze to the red distractors. A oneway ANOVA of the initial visual response of FEF cells during search trials indicated significant variation in a higher fraction of cells compared to the previous data. The timecourse of the selection decision was estimated by comparing spike density functions, constructed by convolving spike trains with a growth-decay pulse (τ_{growth} = 1 ms, τ_{decay} = 20 ms), across trials when the target or distractors were in the response field. For the cells showing significant variation of the initial visual response, the visual response latency averaged 95 ms, and the decision latency averaged 15 ms after the visual response began. For the cells showing no variation of initial visual response to the search display, the visual response latency averaged 65 ms, and the decision latency averaged 60 ms. These data indicate that neurons with later visual response latencies may be subject to more extraretinal modulation. (Supported by F32-EY06495, McDonnell-Pew and R01-EY08890)

502.4

TIME-COURSE OF TARGET SELECTION IN MACAQUE FRONTAL EYE FIELD DURING VISUAL SEARCH. K.G. Thompson*, D.P. Hanes & J.D. Schall. Department of Psychology, Vanderbilt University, Nashville, TN 37240

We have shown that during visual search the initial visual responses of frontal eye field (FEF) cells do not discriminate the target from distractors. After this initial indecision, the activity evolves to signal the location of the target (Schall & Hanes *Nature* **366**:467, 1993; Thompson, Hanes, Tu & Schall *Soc. Neurosci. Abstr.* **19**:27, 1993). The purpose of this study was to determine accurately the visual activation latency of FEF neurons and to determine when neural activity in FEF shows evidence of the decision whether the stimulus in the receptive field is the target of the impending saccade.

The visual activation latency, determined by a Poisson spike train analysis (Hanes, Thompson & Schall *Exp. Brain Res.* **103**:85, 1995), averaged 70 ms after target presentation (range: 40 - 130 ms). On average, visual response latencies did not differ either between search and detection trials or between search trials when the target or distractors were in the cells' receptive field. To estimate the evolution of the target location decision, spike density functions, constructed by convolving spike trains with a growth-decay pulse (τ_{growth} = 1 ms, τ_{decay} = 20 ms), were compared across trials when the target or distractors were in the response field. The two spike density functions diverged on average 110 ms after search array presentation, corresponding to 50-80 ms before saccade initiation. The decision time was statistically more related to the time of stimulus presentation than to the time of saccade initiation. For some cells, the decision time tended to be later in trials with longer saccade latencies, suggesting that saccade initiation was postponed by the target decision delay. (Supported by F32-EY06495, T32-EY07135, McDonnell-Pew and R01-EY08890)

502.5

DISCHARGE OF SUPERIOR COLLICULUS FIXATION CELLS DURING GAZE SHIFTS IN THE HEAD-FREE CAT. D. Guitton* and H. Jiang. Montreal Neurological Institute and McGill University, Montreal, Quebec, Canada, H3A 2B4.

The tonic discharge of fixation cells (FCs) in the rostral superior colliculus (SC) may suppress gaze shifts initiated by the motor map. FCs tend to pause during gaze shifts; we studied how pauses are initiated and terminated. Cats faced an opaque barrier of variable width, and generated horizontal gaze shifts in response to a hidden food target that suddenly appeared on one side. Ambient light could be turned off 100 ms after target appearance. In some gaze shifts, head motion (hence gaze motion) was mechanically stopped for brief periods. RESULTS; Ipsilateral gaze shifts: FCs pause in light and dark. Activity is gradually reactivated as gaze approaches final desired position -with a burst at gaze end- irrespective of whether the overall displacement is made in a single step or a staircase series of multiple-steps. In the latter movements a few FCs discharge weakly during intersaccadic plateaus. Activity peaks at final position and then decreases to a lower level. FCs maintain their pause in light and dark during perturbed gaze shifts and are reactivated at gaze end, as in normal movements: i.e. head brakes increase the duration of both gaze shifts and pauses. Gaze rapidly moves to target after brake release. Contralateral gaze shifts: FCs that burst before small (e.g. 2°) contralateral gaze shifts may reduce their frequency or briefly pause during large gaze shifts. Activity increases and peaks just before final position is reached. FCs recorded more rostrally do not burst before small contra-movements. In large contra-moves they pause; more like ipsi-moves. Results support the hypothesis that FCs are reactivated by feedback when gaze motor error approaches zero.

502.6

VISUO-MOTOR TRANSFORMATION DEFICITS AFTER INACTIVATION OF THE CAUDAL FASTIGIAL NUCLEUS IN THE HEAD-FREE CAT. D. Pélisson* and L. Goffart. Vision et Motricité, INSERM U94, 16 av Doyen Lépine, 69500 Bron, France.

Unilateral inactivation of the caudal Fastigial Nucleus (cFN) in the cat strongly alters the accuracy of visually-triggered gaze shifts: ipsiversive movements are hypermetric whereas contraversive movements are hypometric (Goffart and Pélisson, J. Neurophysiol., 1994). Whereas the systematic overshoot of ipsiversive movements likely reflects a spatial bias in planning the impending movement, the significance of hypometric contraversive gaze shifts was not clear and was further investigated.

First, it will be shown that gaze main sequence relationships and eye-head coordination stay unchanged. Second, we will present the effects of perturbations, resulting from transient electrical stimulation of the Superior Colliculus (SC), on orienting gaze shifts towards visual targets. Collicular stimulation was a train of pulses (0.2 ms, 500 Hz) applied during either the latency or the execution of the gaze shift. During the control session, gaze landed ultimately very close to the target, irrespective of whether the stimulation was applied during the latency or the movement period. In contrast, after muscimol injection in the cFN, contraversive gaze shifts were hypometric but ended at locations that depended on the stimulation period. When the SC was stimulated during the on-going movement, gaze landed at approximately the same spatial location as when no perturbation occurred. When applied during the reaction time, the stimulation led to a larger gaze inaccuracy; in fact, the position reached by these compensatory responses corresponded to the endpoint of unperturbed gaze shifts starting from the same position.

These data suggest that gaze hypometria induced by cFN inactivation does not result from deficits in processes that control the on-going movement but rather result from a disturbance in the processes that specify, during movement preparation, a desired gaze displacement command that will drive the movement generators.

502.7

SACCADIC ADAPTATION DEFICITS AFTER MUSCIMOL INACTIVATION OF THE CAUDAL FASTIGIAL NUCLEUS IN MACAQUE.

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Saccade-related discharge of neurons in the caudal fastigial nucleus (CFN) of the monkey cerebellum helps assure saccade speed, accuracy and consistency. We show here that CFN activity also is necessary for adapting saccade gain to compensate for neuronal damage that makes saccades the wrong size. To simulate such damage by behavioral means, we jump the target backward as the monkey makes a targeting saccade. The initially overshooting saccade gradually decreases in size until after about 1000 trials, the saccadic gain (saccade amplitude/initial target amplitude) has decreased by an amount proportional to the backward step.

After we inactivate one CFN with the $GABA_A$ agonist muscimol, the monkey no longer can adapt its saccadic gain. For example, in one monkey an injection made ipsilateral saccades too big (avg. gain=1.87) and contralateral saccades too small (avg.gain=0.63). After 2200 saccades, these gains had changed little (ipsi gain=2.07, contra gain=0.73).

CFN inactivation blocks adaptation, not just expression of the gain change. If, after the above experiment, the monkey spends 20 hrs in the dark, the muscimol dissipates without the monkey making visually guided saccades to affect whatever adaptation that had occurred. Saccade gain retruns to normal (~1.0) after the drug dissipates so there is no evidence that the gain had adaptated. We know that if gain had adapted covertly under the influence of muscimol the change would be evident after 20hrs in the dark because an uninjected animal retains gain adaptation after 20 hrs in the dark.

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502.8

THE SUPERIOR COLLICULUS OF THE MACAQUE PRODUCES INDEPENDENT FUNCTIONAL CHANNELS FOR HEAD AND EYE MOVEMENTS

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Numerous studies have suggested that the superior colliculus provides a gaze signal for the combined movements of the head and eyes. Such a model suggests that an individual neuron has identifiable bursts for the head and eyes or that a single burst produces tightly coupled movements of the head and eyes. To test this hypothesis of a single gaze signal, we have electrically stimulated the superior colliculi of two monkeys, free to make head and eye movements. We made many penetrations normal to the collicular surface extending from the dorsal aspect through the deepest laminae and measured the threshold current for evoking eye movements and head movements. These two values varied as the penetration descended but the head and eye thresholds did not vary together, as might be expected if a single gaze signal were organized here. Furthermore, the individual head thresholds and eye thresholds did not have a systematic relationship. Also, at individual sites we stimulated using currents more than twice threshold for the head or eye movement. At one type of site we observed that latencies, amplitudes, and peak velocities for eye movements were very regular whereas the same stimuli evoked head movements of high variability. At other sites the latencies, amplitudes, and peak velocities of the head movements were relatively constant while those properties of the eye movements were quite variable. We conclude from these data that the colliculus of the monkey does not produce a single gaze signal but has separate channels for accessing head and eye premotor systems.

502.9

SHIFTS OF ATTENTION CHANGE THE SACCADES EVOKED BY STIMULATION OF THE MACAQUE SUPERIOR COLLICULUS

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There has been considerable interest in the relationship of attentional shifts and visually guided saccadic eye movements. Some have argued that they are independent whereas others propose that attentional shifts accompany all visually guided saccades. The aim of the present experiments was to test the premotor theory of attention which claims the same mechanisms are involved in overt and covert orienting. The study was conducted in two monkeys trained to make saccadic eye movements and in two attentional tasks. Central and peripheral cues were used to direct the monkeys' attention to the location at which a visual target was most likely to appear (80% valid cues). The target onset was delayed for random times between 0 and 500 msec after the onset of the cue (cue-target-onset-asynchrony, CTOA).

We electrically stimulated the superior colliculus during the CTOA at twice threshold with trains of 40 msec. Stimulation during fixation always evoked fixed vector saccades. When the identical stimulation was applied before the monkey initiated a visually guided saccade, there was a shift of the evoked saccade towards the direction of the target. Similar effects were induced during the peripherally cued attention task as well as the centrally cued attention task. The stimulation evoked saccades deviated further from the fixed vector saccades as the stimulation was applied later within the CTOA. These time courses were similar to the changes in saccadic reaction times of the visually guided saccades in the two cuing tasks. These observations help to explain the effectiveness of cues in various psychological attentional experiments. The data also suggest that the superior colliculus mediates components of attentional shifts.

502.10

EVIDENCE FOR A CARTESIAN MODEL FOR THE GENERATION OF OBLIQUE SACCADES IN HUMANS

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We measured horizontal, vertical, and oblique eye movements of three sisters with Niemann-Pick type C disease that caused a selective defect of vertical saccades, which were slow and hypometric. Horizontal saccades, smooth pursuit and vestibular eye movements in both planes were similar to control subjects. The defect was investigated further by examining the dynamic properties of oblique movements from primary to tertiary eye positions. The saccadic trajectory was strongly curved. The initial saccadic movement was mainly horizontal and most of the vertical component was achieved after the eye had completed the horizontal part; during this time the eye oscillated horizontally with a frequency of 10 to 17 Hz and an amplitude of 0.2 to 1.5 deg until the vertical component ended. In contrast, the onset of smooth pursuit with oblique step-ramp stimuli was characterized by a nearly-oblique movement; in some cases, the vertical component had a larger velocity than the horizontal - presumably representing an attempt to compensate for the saccadic defect. Using the model of Zee et al. (J Neurophysiol, 1992), for saccade generation we were able to closely simulate the characteristics of the patients' vertical saccades by reducing the asymptotic value of the burst response curve. Combining normal horizontal and "lesioned" vertical versions of the model in simple Cartesian addition yielded trajectories similar to the patients' oblique saccades. We conclude that Niemann-Pick type C disease selectively affects vertical burst neurons which lie in the riMLF and that oblique saccades are normally generated by independent horizontal and vertical burst cell populations.

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502.11

DIRECTIONAL EFFECTS OF EXTRAOCULAR MUSCLE AFFERENT SIGNALS ON SINGLE UNIT VISUAL RESPONSES IN THE OPTIC TECTUM OF THE PIGEON.

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The role of afferent signals from the extraocular muscles (EOM) in both the oculomotor and visual systems remains unclear. However, having recently shown that EOM afferent signals are involved in both the vestibulo-ocular and vestibulo-collic reflexes, and therefore in the control of gaze, these present experiments were designed to investigate interactions between EOM afferent signals and visual signals in the superficial layers of the avian optic tectum (OT).

Adult pigeons were decerebrated under ether anaesthesia and paralysed with gallamine. Extracellular single unit visual responses were recorded from the superficial layers of the left OT with glass-coated tungsten microelectrodes. Visual stimuli were projected to the right eye. EOM afferent signals were induced by moving the left eye passively (PEM) by means of a suction contact lens. In initial experiments, in which the visual stimulus was always a horizontal bar moving vertically up through the visual receptive field, the visual responses of 33 of 56 single units (59%) were significantly modified (usually inhibited) by PEM. PEM in the horizontal plane produced the greatest modification of the visual responses of 21 (64%) of these 33 units. In subsequent experiments, the visual directional preference of a unit was first established, and then this direction of visual stimulus movement was combined with different directions of PEM. Of 27 units which responded best to a visual stimulus consisting of a horizontal bar moving vertically down, 23 (85%) preferred PEM with a horizontal component; only 4 preferred vertical PEM.

These results confirm that an EOM afferent signal reaches the superficial layers of the optic tectum in the pigeon and suggest that there is a relationship between the visual directional preference of a unit, and the direction of PEM which has the largest effect.

502.12

MOTOR ADAPTATION OF 2-D AND 3-D ASPECTS OF EYE-HEAD COORDINATION IN MONKEYS.

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To un-couple the normally stereotyped coordination between eye and head, 3 *Macaca fascicularis* were trained to search "head only" for visual targets while wearing opaque goggles with a 10° aperture. A similar task employed acutely in humans produced modest kinematic effects (Tweed et al. *Soc. Neurosci. Abst.* 1993). Our animals took 1, 4, and 7 weeks respectively of daily training sessions to learn the task for all movement directions. In the first two, dual search coils were implanted for 3-D recordings of eye and head position. For "head only" gaze shifts, eye-in-head quick phases were reduced in size, truncated early by slow phases that kept eye-in-head gaze near the aperture. The normal "head-mainly-horizontal, eye-mainly-vertical" strategy was changed to the "same-direction" strategy required to return the eye to the aperture. However, the behaviour was more complex than simply scaling the horizontal and vertical components of eye vs. head. When the location of the aperture was suddenly shifted, animals could not perform the task without re-training: surprisingly, they continued to drive the eye towards the location of the old (now covered) aperture. It thus appeared that a neural mapping from desired gaze direction onto desired eye-in-head position had been modified. Finally, whereas 3-D eye-in-space position was normally constrained to a curved 2-D surface, eye-in-space position now conformed to a plane, like Listing's plane for eye-in-head. Thus the neural operators for Listing's law and the analogous eye-in-space law may utilize a similar mechanism capable of parametric adjustments to suit the task.

503.1

***in situ* RT-PCR DETECTION OF INFLUENZA VIRAL RNA**

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We have previously assessed the sensitivity, specificity, and reproducibility of *in situ* RT-PCR amplification with labeled-probe hybridization (*in situ* RT-PCR/LPH) to detect and localize viral RNA in archival paraffin-embedded CNS tissue sections from several neurological diseases of known viral origin (i.e. measles virus, poliovirus, HTLV-I). Some of these tissues were embedded over 25 years ago, and frozen specimens are not available.

The etiologic agent of encephalitis lethargica (von Economo's encephalitis) has never been identified. Although its temporal proximity to the 1918 influenza pandemic has suggested that this virus may be implicated, attempts to demonstrate influenza virus as the cause have been inconclusive. Since no frozen tissue is available, we used *in situ* RT-PCR/LPH to determine if influenza viral RNA could be detected in archival paraffin-embedded brain tissue from seven patients who died with postencephalitic parkinsonism as a sequela of encephalitis lethargica. We also studied mice experimentally infected with the neurotropic NWS strain of influenza A. Highly conserved influenza A genomic sequences were detected in the brains of these mice by *in situ* RT-PCR/LPH. We were unable to detect influenza RNA in simultaneously examined postencephalitic brains. Whether influenza RNA may have been present earlier in the disease course is not known. Alternatively, a different agent may have caused this pandemic.

503.2

DOPAMINE TRANSPORTER IMAGING IN PARKINSON'S DISEASE AND PARKINSON PLUS SYNDROMES.

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[123I]β-CIT (2β-carboxymethoxy-3β(4-iodophenyl)tropane) labels monoamine transporters located on the terminals of dopaminergic projections from the substantia nigra to the striatum providing a marker for neurons which degenerate in Parkinson's disease (PD) and related neurodegenerative disorders. [123I]β-CIT SPECT imaging was performed in 42 idiopathic PD patients, 17 Parkinson's plus patients and 27 healthy subjects 18-24 hour post tracer administration. Data was analyzed relative to two outcome measures: ratio of specific:non-displaceable striatal activity and striatal uptake expressed as percent of injected dose. Disease severity was assessed by Hoehn and Yahr Staging, UPDRS scales and timed motor events following 12 hours off drug. In the PD patients striatal [123I]β-CIT uptake was dramatically reduced in an asymmetric and region specific manner. The reduction in age corrected transporter loss in PD patients was 70% in the putamen and 53% in the caudate contralateral to initial symptoms. In Parkinson's plus patients including MSA (SND and Shy-Dragers syndrome), and PSP [123I]β-CIT uptake was similarly, but more symmetrically reduced. The reduction in age corrected transporter loss in patients with Parkinson's plus syndromes ranged from 60-95% in putamen and from 40-90% in caudate. The loss of [123I]β-CIT uptake reflected the severity of the patient's motor deficit. These data suggest that imaging of the dopamine transporter may be a useful in vivo marker for idiopathic PD and Parkinsonian syndromes.

503.3

GDNF UPREGULATES NIGRAL DOPAMINE NEURONS AND IMPROVES MOTOR FUNCTIONS IN HEMIPARKINSONIAN RHESUS MONKEYS

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Glial cell line-derived neurotrophic factor (GDNF), a novel member of the TGF-β superfamily, exerts potent effects on CNS dopamine neurons in vitro and in vivo. In the present study, the effects of either vehicle or human rGDNF-injected into the CNS of 19 adult female hemiparkinsonian rhesus monkeys were evaluated from standardized video taped tests by blinded raters using a primate parkinsonism rating scale. All animals were at least 3 months post MPTP treatment and consistently expressed moderate parkinsonian features. Baseline parkinsonian features were evaluated for at least four weeks prior to trophic factor treatment. Using sterile MRI-guided stereotaxic procedures, three routes of administration were evaluated-intranigral (150 μg GDNF), intrastriatal (450 μg GDNF) and intracerebroventricular (ICV, 100 μg and 450 μg GDNF). Animals were behaviorally evaluated for 3-19 weeks, then euthanized with their brain tissue recovered for neurochemical and immunohistochemical analysis.

No significant behavioral changes were measured in the seven vehicle injected animals during the period following treatment. However, the results from GDNF injections into the same sites were quite different. Improved motor functions lasting for at least four weeks were seen following all three routes of administration. ICV administrations repeated every four weeks maintained the functional improvements. Dopamine neurons in the lesioned nigra showed evidence of increased stimulation as revealed by immunohistochemical staining and increased levels of dopamine and its metabolites.

503.4

INCREASED VIABILITY OF GENETICALLY MODIFIED PRIMARY RAT FIBROBLASTS PREPARED FOR IMPLANTATION IN THE BRAIN IN BUFFER CONTAINING N-ACETYL-L-CYSTEINE AND SERUM

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Optimizing *in vivo* cell viability after grafting is important for gene therapy protocols in which one desires to maximize the expression of gene product from a limited number of genetically modified cells. Experiments involving primary Fischer rat Dermal Fibroblasts (FDFs) prepared for implantation in the brain demonstrated that the choice of media used for the preparation of the cells is critical for their survival *in vitro*. The viability of both tyrosine-hydroxylase (TH) transduced and non-transduced cells after preparation in our standard buffer, PBS, was compared with that in a variety of buffers containing factors known to enhance cell growth in culture. The cells were trypsinized, washed and resuspended in a given buffer, passed through an injection cannula, and then assessed by propidium iodide (PI) exclusion via Fluorescence Activated Cell Sorting (FACS) over a four hour time course. Preparation of the cells in PBS yielded inconsistant results, ranging from a mean of better than 90% viability at the time of passage through the cannula, to less than 60% at four hours. PBS containing 10% heat-inactivated rat serum and 1 mM N-acetyl-L-cysteine (NAC), which regulates the redox state of the cell by potentiating intracellular glutathione levels, had the most significant protective effect. A robust 95% survival was observed in this media for the duration of a given experiment. Neither 10% serum nor 1 mM NAC was as effective, although both were significantly more protective than PBS alone. In addition, a high correlation between cell survival and intracellular glutathione levels was demonstrated. *In vivo* experiments with primary FDFs are in progress, in order to determine whether or not these modifications in the buffer composition will lead to the increased survival of implanted cells.

503.5

INTRASTRIATAL IMPLANTATION OF BDNF-PRODUCING FIBROBLASTS PREVENTS NEURODEGENERATION IN A RAT MODEL OF PARKINSON'S DISEASE (PD).

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We recently developed a rat model of partial lesion of the nigrostriatal pathway using intrastriatal injection of 6-OHDA. BDNF has neuroprotective properties on DA neurons *in vitro*. However, BDNF is difficult to deliver into the CNS and therefore its *in vivo* effects are not well defined. Herein, we tested the protective effect of grafts of fibroblasts genetically modified to produce BDNF (BDNF[+]) in our partial lesion model of PD. Control groups consisted of: (i) animals which received no grafts prior to the injection of 6-OHDA, (ii) animals which received BDNF[+] grafts only, and (iii) animals which received grafts of non-transduced fibroblasts prior to the injection of 6-OHDA. Lesions were assessed by [3H]mazindol binding quantitative autoradiography and cell count on Nissl staining. Compared to lesioned only animals, rats with BDNF[+] grafts were protected against 6-OHDA-induced neurodegeneration, while grafts of unmodified fibroblasts failed to protect against 6-OHDA. In animals that received only BDNF[+] grafts and no subsequent lesion, there was no difference in binding or cell count compared to the controlateral nonoperated side. This suggests that the neuroprotective effect of BDNF against 6-OHDA, which was evaluated quantitative autoradiography and cell counting, is not a reflection of a trophic effect of BDNF on remaining neurons. This study demonstrates that, in this model of partial lesion of the nigrostriatal pathway, grafts of fibroblasts genetically modified to produce BDNF protect against 6-OHDA. These results open new possible neuroprotective therapeutic avenues aiming at slowing down neurodegeneration in early stages of PD, using neurotrophic factors such as BDNF. (Supported by PDF & UPF, USA, and FNRS,Belgium, as well as NIH - Fogarty, Fulbright and NATO fellowships).

503.6

COEXPRESSION OF HUMAN GTP CYCLOHYDROLASE I (GTPCHI) AND HUMAN TYROSINE HYDROXYLASE CDNAS TO POTENTIATE L-DOPA PRODUCTION BY FIBROBLASTS: APPLICATIONS TO PARKINSON'S DISEASE GENE THERAPY.

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We and others have been developing protocols for treating Parkinson's Disease using cells that have been genetically modified to express recombinant human tyrosine hydroxylase (hTH) with the aim of achieving local L-DOPA delivery in the CNS after grafting. However, efficient L-DOPA delivery using these protocols involving most cell types may be limited by the absence of "activating" levels of tetrahydrobiopterin (BH_4), an essential cofactor for TH. To increase the efficiency of L-DOPA production, we are coexpressing cDNAs encoding the human GTPCHI, the rate-limiting enzyme for de-novo BH_4 synthesis, with the cDNA encoding hTH. Co-expression of the rat GTPCHI with TH cDNAs in rat fibroblasts was first reported by C. Bencsics *et al* (Soc. Neurosci. Abst., 20:176, 1994), who showed that L-DOPA production by these cells *in vitro* could be achieved in the absence of added BH_4. We have cloned the full-length human GTPCHI cDNA using PCR of human liver cDNA. Our cDNA sequence corresponds to the active type I form described by Guttlich *et al*. The GTPCHI cDNA has been placed in a retroviral vector, MFG-s-GTPCHI, or in a bicistronic vector, MFG-s-hTH2-ires-GTPCHI, and retrovirus has been packaged using the ψ-CRIP line. Results on the ability of these systems to enhance L-DOPA production *in vitro* and *in vivo* after grafting will be presented.

503.7

APOPTOTIC-LIKE CHANGES IN HUMAN SUBSTANTIA NIGRA M. M. Tompkins*, W. D. Hill. Department of Cellular Biology and Anatomy, Medical College of Georgia, Augusta, Georgia, 30912.

The substantia nigra (SN) undergoes cell death during aging and in neuropathological diseases such as Parkinson's disease (PD) and diffuse Lewy body disease (DLBD). The morphology of this cell death process has not been previously described in detail. We have found apoptotic-like changes in cells undergoing degeneration in the substantia nigra of control and diseased patients. Midbrains of 28 individuals (neurologically normal, 3 cases; Alzheimer's disease (AD), 7 cases; concomitant AD/PD, 4 cases; DLBD, 9 cases; and PD, 5 cases) were probed for DNA fragmentation with a TUNEL assay using an FITC label (Apotag kit, Oncor, Gaithersburg, MD), and counterstained for nucleic acid with propidium iodide. Nigral nuclei with an apparently normal morphology were seen stained with propidium iodide in the presence and absence of FITC labeling. Abnormal FITC(+) nuclei were divided into subgroups based on appearance of the chromatin and the neuromelanin (NM) pigment. The subgroups included condensed chromatin with normal-appearing NM, condensed chromatin with clumped NM resembling apoptotic bodies, and condensed chromatin with strands of DNA radiating away from the main body. Some morphologies resemble early-stage apoptosis, while others appear to be apoptotic cell fragments awaiting or undergoing phagocytosis. The percentage of apoptotic-like neurons was found to be lower in controls and AD cases as compared to AD/PD and DLBD cases, while the PD cases ranged from low to high percentage. Our observations suggest that in PD, AD/PD and DLBD neurons are dying by apoptosis. In this case, therapies aimed at interfering with the apoptotic mechanism may be useful in rescuing neurons.

503.8

APOPTOSIS-LIKE NEURONAL CELL DEATH *IN VIVO* IN THE 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) MOUSE MODEL OF PARKINSON'S DISEASE (PD). A.M. Janson*. Dept of Neuroscience, Karolinska Institutet, Doktorsringen 17, S-171 77 Stockholm, Sweden.

Apoptosis may represent an important mechanism of cell death in some experimental models of neurodegeneration as well as in human neurodegenerative disorders. Although the MPTP mouse model of PD has been studied extensively over the last decade, the mechanism by which the neuron ultimately dies remains unknown.

A single dose of MPTP (40 mg/kg, sc) was given to 10 weeks' old male C57 Bl/6 mice. The nuclear alterations were studied in midbrain sections and analyzed with stereological unbiased quantitative techniques at various time-points after the lesion. Two different in situ labeling techniques analysing DNA fragments were compared. The first one uses DNA polymerase I, which detects the 3'-OH-ends of single-stranded DNA breaks. The second technique employs terminal deoxynucleotidyltransferase labeling the 3'-OH-ends of both single- and double-stranded DNA breaks.

The results for the first time indicate a role of apoptotic-like cell death mechanisms in the MPTP mouse model *in vivo*, possibly also related to the pathogenetic mechanism in PD.

503.9

BIPHASIC PATTERN OF MPTP NEUROTOXICITY IN THE NIGROSTRIATAL SYSTEM IN MICE. P. Chan, D.A. Di Monte, A. Stadlin[1] and J.W. Langston*. The Parkinson's Inst., Sunnyvale, CA 94089, [1]Dept of Anat., Chinese Univ. of Hong Kong, Hong Kong.

The cascade of events leading to MPTP neurotoxicity *in vivo*, particularly during the acute phase of toxicity, is still not fully understood. The purpose of the present study was to evaluate the temporal and possibly causal relationship between pharmacological and toxic events leading to neuronal degeneration after MPTP exposure. C57BL/6 mice were treated with a single injection of MPTP (40 mg/kg, s.c.) and killed at time points ranging from 30 min to 7 days. MPTP/MPP$^+$ pharmacokinetics and changes in levels of DA, ATP, GSH/GSSG, Glut/Asp, mRNA levels and binding sites of dopamine transporter (DAT) were determined in the nigrostriatal system. MPTP was fully converted to MPP$^+$ within 1 hr, which was selectively accumulated (peak concentration: 1.5 hr) in and then eliminated from striatum (within 16 hr). MPTP-induced changes occured in a biphasic pattern based on the presence (first phase) and absence (second phase) of MPP$^+$. The first phase was characterized by a rapid decrease in striatal levels of ATP which was followed by a marked decrease in Glut/Asp and DA, but without loss of the DAT binding sites. These changes returned to normal (ATP and Glut/Asp) or were recovered (DA) within 24 hr. However, a second and long-lasting decline of DA began at day 2; it was accompanied by a significant decrease in the number of DAT binding sites and mRNA levels (maximum decrease: 3 days). No changes in GSH and GSSG content were found at any time point studied. These data suggest that MPTP/MPP$^+$ causes an initial reversible changes followed by long-term neurotoxic effects. Although the precise relationship between these two phases remains unclear, it is likely that transient events in the first phase set in motion a process which may ultimately lead to neurotoxic damage. The MPTP model may provide a valuable tool to study factors which may initiate the neurodegenerative processes.

503.10

TRK-B EXPRESSION IN THE SUBSTANTIA NIGRA PARS COMPACTA (SNC) AND PARKINSONISM IN THE RAT. S.P. Jaw*, D.D. Su, S. Wellman and D.D. Truong. Dept. of Neurology, Univ. of California Irvine, Irvine, CA 92717.

Roles of brain-derived neurotrophic factor (BDNF), neurotrophin-4/5 (NT-4/5), and their common high-affinity receptor, Trk-B, in the development of Parkinsonism were investigated in both young (2-month-old) and aged (30-month-old) male Sprague Dawley rats. As determined by reverse transcriptase followed by polymerase chain reaction (RT-PCR), significant reductions of BDNF and NT-4/5 gene expressions, were found in the SNC of old rats. The levels of Trk-B were also lower in the SNC of old rats as measured by immunoblot assays. Furthermore, following 6-hydroxydopamine (6-OHDA) lesion in the SNC, old rats developed apomorphine-induced rotational behaviors faster (5 days post lesion) than young ones (14 days post lesion). These results indicate that lower expressions of BDNF, NT-4/5, and their receptor, Trk-B may contribute to the temporal difference in the development of Parkinsonism in rats.

503.11

ACUTE TREATMENT OF MICE WITH THE GLUTATHIONE SYNTHESIS INHIBITOR BUTHIONINE SULFOXIMIDE CAUSES DAMAGE TO THE NIGRO-STRIATAL SYSTEM REMINISCENT OF PARKINSON'S DISEASE. J. K. Andersen*, J. Q. Mo, D. G. Hom, T. H. McNeill. Division of Neurogerontology, Ethel Percy Andrus Gerontology Center, University of Southern California, Los Angeles, California 90089-0191.

In this study, we have analyzed the acute effects of treatment with the compound buthionine sulfoximine (BSO), a synthesis inhibitor of the antioxidant glutathione, on the nervous system of C57Bl male mice. Following multiple injections of BSO, levels of GSH were found to be dramatically reduced in the substantia nigra (SN). The reduction in levels of GSH correlated with increases in brain levels of lipid peroxidation and oxidative stress. BSO treatment was found to have detrimental effects on the nigro-striatal system. These effects included decreased dopamine content per cell, increased lipofuscin accumulation, and increased numbers of dystrophic axons in dopaminergic fibers of the mesolimbic and nigrostriatal systems, as well as decreases in striatal dopamine (DA) and its metabolites, HVA and DOPAC. The detrimental effects of BSO treatment on these cell populations were not generalized to other catecholaminergic neurons. Results from our study suggest that BSO administration has effects on the nigro-striatal nervous system which are morphologically similar in appearance to those changes which occur with advancing age and following treatment with MPTP.

503.12

BEHAVIORAL AND NEUROCHEMICAL DYSFUNCTION IN THE CIRCLING RAT. A NOVEL GENETIC ANIMAL MODEL OF PARKINSONISM? W. Löscher[1]*, A. Richter[1], G. Nikkhah[2], C. Rosenthal[2], U. Ebert[1], and H.-J. Hedrich[3] Department of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine, [2]Neurosurgical Clinic, Nordstadt Hospital, and [3]Institute for Laboratory Animal Science, Medical School, D-30559 Hannover, Germany.

One of the crucial breakthroughs in research on parkinsonism was the observation of circling behavior in rodents after unilateral intranigral injection of 6-hydroxydopamine (6-OHDA). This Ungerstedt model still is one of the basic animal models of Parkinson's disease. We report here the first mutant rat strain with abnormal circling behavior and several other features reminiscent of the Ungerstedt Parkinson model. The neurological disorder in the novel mutant rat strain is determined monogenetically by a recessive autosomal gene termed circling (*ci*). Mutant rats of both sexes exhibit an intense asymmetric circling in an open-field or rotometer, which is enhanced by amphetamine. Neurochemical determinations show that mutants of both sexes have significantly lower dopamine and dopamine metabolites in the striatum ipsilateral to the preferred direction of rotation. Furthermore, in a forelimb reaching test for assessing the skilled motor capacities of rats, *ci* rats show a marked deficit on the side contralateral to the preferred direction of turning, which is analogous to motor deficits previously described for rats subjected to unilateral 6-OHDA lesions. The new mutant rat strain thus exhibits remarkable similarities to the Ungerstedt model and may serve to study the endogenous processes, particularly the genetic components, that might eventually lead to progressive neurological disorders such as idiopathic parkinsonism.

504.1

ORGANIZATION OF EXTRASTRIATE VISUAL AREAS IN HUMAN OCCIPITAL CORTEX INFERRED FROM CALLOSAL CONNECTIONS. D.C. Van Essen*[1], S. Clarke[2], H.A. Drury[1], N. Hadjikhani[2], T. Coogan[1], R. Kraftsik[2]. [1]Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, USA; [2]Institute of Physiology, University of Lausanne, Lausanne, Switzerland.

Callosal connections provide valuable markers for the borders of topographically organized visual areas, because they terminate preferentially along representations of the vertical meridian. We report here on the pattern of degenerating callosal fibers in the left hemisphere of a human brain that had a right occipital infarct. A computerized three-dimensional reconstruction of the entire occipital lobe was generated from a series of contours through cortical layer 4. Custom software was used for smoothing and flattening the cortical surface, thereby allowing ready visualization of buried cortex. Our results substantially extend the findings reported last year on a more restricted portion of a different hemisphere (Van Essen et al., Soc. Neurosci. Abstr. 20:428, 1994) as well as the original study of Clarke and Miklossy (J. Comp. Neurol., 1990).

Extrastriate visual cortex contains a pattern of callosal-free regions surrounded by callosal-recipient rings that has many similarities to the pattern known for the macaque monkey. We confirmed the presence of callosal-free regions adjoining V1 dorsally (presumed dorsal V2 and area V3) and ventrally (presumed ventral V2 and area VP). These callosal-free regions are separated by callosal-recipient regions at the representation of the fovea (occipital pole) and the far periphery (anterior calcarine sulcus). In ventral cortex, there is a more lateral callosal-free region in the inferior temporal gyrus, that is likely to include the ventral (upper-field) portion of area V4. In dorsal cortex, there are at least two additional callosal-free regions, including a dorso-medial one that may include area V3A and a dorso-lateral one that may include the dorsal (lower-field) portion of V4. These boundaries provide a useful anatomical framework for comparisons with activity foci revealed by PET and fMRI.

504.2

CORTICAL MAPS AND MODULES AS A SOLUTION TO THE PROBLEM OF VOLUME MINIMIZATION. E. Todorov[1], K. Zipser[1] and D. Zipser*[2] BCS, MIT, E25-634, Cambridge, MA 02139[1]; Cognitive Science Dept., UCSD, La Jolla, CA 92093-0515[2]

The connections among cells, rather than the cell locations, determine the functionality of the brain. Assuming development could achieve the same connectivity with different cell locations, we may argue that cortical evolution will favor the configurations that result in minimal volume (i.e., minimal axonal or dendridic length). If this form of volume minimization is a dominant principle underlying cortical structure, it should be possible to explain the existence of both cortical visual maps and modules without reference to any specific developmental mechanisms. Here we design simulations in which connections among model cortical neurons and subcortical inputs are prespecified, and the positions of neurons in a model cortical sheet are varied (by starting at a random configuration and using simulated annealing) until a minimum of the connection length is found. In simulations in which cortical cells receive input from elongated patches of retinal (LGN) cells of different weight from each eye, ocular dominance columns, orientation columns, and retinotopic mapping resembling those in area V1 are obtained as a result of minimizing total connection length. In simulations in which cortical neurons are connected in a hierarchical manner so that "higher level" cells have larger receptive fields than their afferent cells, all cells are found in register in a single retinotoptic map. If lateral connections among cells of each hierarchical level are added, separate retinotopic maps do arise, but only if the ratio of lateral to forward connections exceeds a threshold, which we show both through simulation and analytically to be approximately 20. Thus, functionally distinct groups of cells can be mixed, clustered into modules, or separated into areas, depending on the interplay between hierarchical and lateral connections.

504.3

OPTIMAL HIERARCHIES FOR THE PRIMATE VISUAL SYSTEM DERIVED WITH A NOVEL NETWORK PROCESSOR. C.C. Hilgetag, M.A. O'Neill and M.P. Young*, Neural Systems Group, Univ. Newcastle, Ridley Bldg., Newcastle upon Tyne NE1 7RU, UK.

Connections between visual cortical areas in primates can be classified as 'ascending', 'descending' or 'lateral' according to the pattern of their origins and terminations within cortical layers. This classification yields 318 pairwise hierarchical constraints that describe relations between 30 visual areas. The available anatomical constraints are incomplete, some of the constraints are unreliable, ambiguous, and inconsistent with others, and the number of possible orderings that might fit the constraints is very large ($\sim 10^{37}$). Nonetheless, the data are widely assumed to contain important information about the organization of the visual system, and hierarchies have been derived by ordering the visual areas by hand while trying to satisfy the constraints as well as possible.

We have automated this procedure and analysed the data with stochastic optimization. This computational approach employed an annealing algorithm which was integrated with an algorithm for re-arranging the wiring of complex networks. The program searched for optimal hierarchical orderings of the visual system. Starting from an arbitrary structure, it proceeded by cumulative modification and cost evaluation of candidate solutions, where the cost related to the number of constraint violations.

The processor found optimal hierarchical orderings that possessed only a very low number of constraint violations, suggesting that the visual system is a surprisingly strict hierarchy. There were, however, at least 55000 different optimal orderings of the visual cortical areas. The presently-known hierarchical constraints were therefore not sufficient to constrain a unique hierarchy, and no single hierarchy can properly describe the organization of the system. Widely-reported hierarchical orderings of the primate visual system that were derived by hand have higher cost than the optimal solutions found by the processor. We also used the network processor for computer experiments to determine the causes of the few remaining violations in the optimal solutions. We found that solutions with an even lower cost could be derived under the assumption that one of the visual areas, FST, consists of two distinct sub-areas.

504.4

SYNAPTIC INTEGRATION OF GENICULOCORTICAL AND INTRACORTICAL FEEDBACK INPUT IN RAT PRIMARY VISUAL CORTEX. A.Burkhalter, J.M.Nerbonne* and Z.Shao. Dept. Anatomy and Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Intracortical feedback connections that link higher to lower cortical areas are likely conduits for top-down influences by which attention modulates processing of visual features. How these higher order processes are synaptically integrated with afferent information supplied by the geniculocortical system is poorly understood. Feedback pathways constitute anatomically distinct microcircuits whose inputs to inhibitory interneurons are five times weaker than in forward circuits (Johnson and Burkhalter, 1992; 1994). This represents an imbalance of inputs to excitatory and inhibitory neurons which results in PSPs that are mainly excitatory (Shao et al., 1995). In contrast forward circuits show more balanced inputs to excitatory and inhibitory neurons and, similar to the geniculocortical system (LGN), activation evokes a sequence of monosynaptic EPSPs followed by disynaptic IPSPs (Douglas and Martin, 1991; Shao et al., 1995).

We have used intracellular recording in slices from rat visual cortex to study how feedback inputs from secondary visual cortex and LGN inputs interact in layer 2/3 pyramidal neurons of area 17. Electrical stimulation was employed for simultaneous activation of feedback inputs and LGN afferents. When stimulated separately, feedback pathways showed large EPSPs and no undershooting IPSPs. Stimulation of LGN inputs alone typically revealed EPSPs followed by IPSPs. For coincident activation, stimulus strengths in both pathways were adjusted to evoke EPSPs but no undershooting IPSPs. Surprisingly, with co-activation in every cell, the response was dominated by inhibition: EPSPs were smaller than the sum of responses to either pathway alone, whereas IPSPs were extremely large, indicating strong non-linear inhibition.

The results suggest that depending on the strength of coincident inputs feedback pathways can act to enhance or suppress striate cortical responses.

504.5

DISTINCT SYNAPTIC MECHANISMS IN FORWARD AND FEEDBACK CIRCUITS WITHIN RAT VISUAL CORTEX. Z.Shao, G.W.Harding* and A.Burkhalter. Dept. Anatomy and Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

The multiple visual areas of mammalian visual cortex are reciprocally linked by forward and feedback connections. The function of forward pathways is to convey information from lower to higher cortical areas, whereas feedback pathways provide 'top-down' influences from higher to lower areas. Each of these functions is generated by anatomically distinct circuits in which 90% of forward inputs go to pyramidal cells and 10% of inputs are sent to GABAergic interneurons (Johnson and Burkhalter, 1994). In contrast 98% of feedback inputs go to pyramidal cells and only 2% to GABAergic neurons (Johnson and Burkhalter, 1992). Although this difference is significant, the absolute magnitude is small and the question arises whether different synaptic properties are generated.

Using slices of rat visual cortex we have performed intracellular recordings in secondary (area LM) and primary visual cortex after stimulation of forward and feedback connections, respectively. In addition these responses were compared to those evoked after stimulation of white matter and horizontal connections within area 17. In every case activation of forward inputs evoked monosynaptic EPSPs followed by undershooting disynaptic IPSPs and closely resembled responses after stimulation of geniculocortical and local horizontal connections. In contrast only 22% of feedback inputs showed undershooting IPSPs.

These results suggest that the synaptic mechanisms in forward and feedback circuits are distinct and that modest differences in the input to inhibitory circuitry can dramatically alter the function of cortical circuits.

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504.6

OPTICAL IMAGING OF ORIENTATION TUNING MAPS IN THE FOCALLY LESIONED CAT VISUAL CORTEX. U.T. Eysel*, Z.F. Kisvárday, M. Rausch Department of Neurophysiology, Faculty of Medicine, Ruhr-Universität Bochum, D-44780 Bochum, F.R. Germany.

Photocoagulation of the cat visual cortex produces well defined heat lesions of 1-2 mm diameter and about 1500 µm depth. Surrounding the lesion we have observed a zone of neuronal dysfunction (Eysel & Schmidt-Kastner, Neurosci Lett. 131:45, 1991). The functional disturbances of single cells included hyperactivity, reduced intracortical inhibition and weakening of orientation tuning. To further understand the spatial distribution of lesion-induced dysfunction we have applied optical recording of intrinsic signals (Grinvald et al., Nature 324:361, 1986) during stimulation with drifting gratings of variable direction and orientation (adult cats, pentobarbital anaesthesia, artificial respiration). Important vital parameters (blood pressure, body temperature, end-expiratory CO_2, EEG) were monitored. The visual cortex (areae 17/18) was bilaterally exposed and a chamber filled with silicone oil attached. Several normal maps were recorded to ascertain the reproducibility. The heat lesion was produced through the intact chamber. After lesioning the cortex was remapped every 2 h for a period of 12-36 h. Postmortally the size of the lesion was measured in Nissl stained sections. Immunohistochemistry was used to estimate the extent of edema and neuronal damage.

Optical imaging of stimulus evoked intrinsic signals proved to be very sensitive for the detection of lesion effects with high spatial resolution. The maps were analysed for possible changes of the spatial distribution of orientation domains and centers. In addition, the strength and sharpness of orientation tuning was quantitatively evaluated. The functionally disturbed region (with a completely silent core and surrounding changes in the orientation map and in strength and specificity of tuning) surpassed the histologically verified border of the lesion by 0.5-1 mm. This spatial extent is closely related to the lateral range of effective intracortical inhibition and the size of the hypercolumns.

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504.7

FUNCTIONAL IMAGING AND CORTICAL CONNECTIONS OF V2 THIN AND INTERSTRIPE COMPARTMENTS. Y. Xiao and D.J. Felleman*. Department of Neurobiology and Anatomy, Univ. Texas Medical School, Houston, Texas 77030.

Previous evidence suggests that areas V4 and perhaps V3A of macaques contain distinctive modules that receive segregated input from V2 thin or intersripes. However, the number, size and organization of these modules is not known. In the current study, V2 thin- and interstripes were visualized, *in vivo*, according to their differential activations to isoluminant chromatic and luminance gratings of different temporal and spatial frequencies. Iontophoretic injections of anterograde and retrograde tracers (Pha-L, BDA, ^{3}H-proline, FITC and TRITC-dextrans, WGA-HRP, BB, NY) were aimed at different stripes or different parts of the same stripe. Clustered intrinsic connections in V2 were most dense within 4 mm of the injection site, but were observed up to 9 mm away. Injections restricted to either V2 thin stripes or interstripes produced clustered terminal labeling in areas V3, V3A, and V4. Individual axons often branched in the white matter and traveled obliquely in gray matter before terminating in several areas. Labeled axons gave off terminal branches usually in layer 4 but occasionally extending to layer 1. Terminal fields in V4 were often restricted to 1-4 narrow foci ~250-500 μm in width, but the total projection field often extended dorso-ventrally for 4-5 mm. Retrogradely labeled cells also formed patches which overlapped, but were not identical to the terminal patches. Terminal projections from thin- and interstripe injections appear to segregate in a complex, interdigitating pattern in V4. Injections at thin-interstripe borders labeled terminal fields in V4 that were wider than those originating from single stripe injections. Supported by RO1-EY08372 and Texas ARP 011618-025 to DJF, and P30-EY10608.

504.8

EVIDENCE FOR THE PRESENCE OF THE DORSOMEDIAL VISUAL AREA (DM) IN FIVE PRIMATE SPECIES. Pamela D. Beck* and Jon H. Kaas Department of Psychology, Vanderbilt University, Nashville, TN 37204.

The dorsomedial visual area (DM) in owl monkeys can be identified by its complete representation of the visual hemifield, dense myelination and high cytochrome oxidase reactivity, and distinct pattern of connections with other visual areas. Our goal in the present study was to determine if DM is an area common to other primates. We injected 1-4 tracers (wheatgerm agglutinin conjugated to horseradish perxoidase, fluororuby, diamidino yellow, fast blue, and fluorescein) into cortex in the expected location of DM, just rostral to dorsomedial V2, in one macaque monkey (*Macaca nemestrina*), two squirrel monkeys (*Saimiri sciureus*), two owl monkeys (*Aotus trivirgatus*), and one galago (*Galago crassicaudatus*). We examined the injection sites and resulting label, cytochrome oxidase staining, and myelination in tangential sections from unfolded, flattened cortex. In all these primates, a DM-like area could be identified by more dense myelination and cytochrome oxidase reactivity. Injections in this region labeled neurons in areas V1, V2, and MT. The labeled neurons in V1 were largely co-extensive with CO blobs. Other connections were with posterior parietal cortex, cortex caudal to MT, and cortex on the ventral surface of the occipital lobe. These similarities in cortical architecture and connections suggest that DM exists in most or all primates.
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504.9

RETINOTOPIC ORGANIZATION OF MULTIPLE VISUAL AREAS IN HUMANS REVEALED BY FUNCTIONAL MRI. M.I. Sereno*, A.M. Dale, R.B.H. Tootell, J.B. Reppas, K.K. Kwong, J.W. Belliveau, and B.R. Rosen. Cognitive Science Department 0515, University of California San Diego, La Jolla, CA 92093, University of Oslo, Oslo, Norway, and MGH NMR Center, 149 13th St., Charlestown, MA 02129.

We previously showed how four techniques (multislice fMRI, stimulus phase-encoding and Fourier analysis, cortical surface reconstruction, and visual field sign calculations) could be combined to reconstruct the internal retinotopic organization of visual areas V1, V2, VP, V3, and V4 in humans, and to accurately trace out the borders between these areas in the human brain (Sereno et al., 1995, *Science*).

This analysis was performed for a larger pool of subjects. We also used thin ray and ring stimuli in an attempt to recover retinotopy from areas with larger receptive fields. The resulting isoeccentricity, isopolar angle, and visual field sign maps were viewed on the partially unfolded cortical surface of each subject. We found a large amount of variability in the absolute size, relative size, and location relative to gyral landmarks of both striate cortex as well as extrastriate cortical areas.

Cortical magnification factor curves for striate and extrastriate cortical areas were also determined, which showed that human visual areas have a greater emphasis on the center of gaze than their counterparts in monkeys. An increase in the foveal density of human midget ganglion cells with respect to macaque monkeys could explain our result. This may also help to explain the increase in the size of the region between foveal V1 and MT in humans compared to monkey (relative to the size of V1).

One subject with early, unilateral, localized retinal damage (with foveal sparing) viewed the mapping stimuli with the damaged eye, and then with both eyes. V1 in this subject showed a localized region of reduced activation with damaged-eye-only stimulation that whose location was predicted in large part by the visual field defect measured for that eye.

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504.10

SPATIOTEMPORAL IMAGING OF COHERENT MOTION SELECTIVE AREAS IN HUMAN CORTEX. AM Dale1*, SP Ahlfors2, HJ Aronen3, JW Belliveau4, M Huotilainen5, RJ Ilmoniemi6, WA Kennedy4, A Korvenoja3, AK Liu4, JB Reppas4, BR Rosen4, MI Sereno7, GV Simpson2, C-G Standertskjöld-Nordenstam3, J Virtanen1, RBH Tootell4. 1University of Oslo, Norway, 2Albert Einstein College of Medicine, Bronx, NY, 3Radiology, Helsinki Univ. Central Hospital, Finland, 4Massachusetts General Hospital, NMR Center, Charlestown, MA, 5Cognitive Brain Research Unit, Univ. of Helsinki, Finland, 6BioMag Laboratory, Helsinki Univ. Central Hospital, Finland, 7University of California-San Diego, CA

The purpose of the study was to demonstrate and characterize human visual areas sensitive to coherent vs. incoherent motion. Six subjects were presented with two classes of random dot rotation/dilation flow field stimuli. In one condition (coherent motion) each randomly placed dot moved as part of the same flow field, while in the other condition (incoherent motion) each dot moved independently, but with the same local statistics as the coherent case. The same subjects were measured using fMRI and simultaneous EEG/MEG (32/122 channels). The results from each measure converged to reveal activity in areas previously shown with fMRI to be retinotopically organized (Sereno et al., Science 1995) as well as in other areas sensitive to motion (Tootell et al., J. Neuro. 1995). In addition, at least two spatially disparate areas were found to exhibit coherent motion selectivity as early as 130ms post stimulus onset. These foci may be human homologues of visual areas identified in nonhuman primates, including MSTd and VIP.

504.11

SPATIOTEMPORAL IMAGING OF PATTERN VERSUS MOTION SELECTIVE AREAS IN HUMAN VISUAL CORTEX S.P. Ahlfors1*, HJ Aronen2, JW Belliveau3, A Dale4, M Huotilainen5, RJ Ilmoniemi6, WA Kennedy3, A Korvenoja2, AK Liu3, R Näätänen5, BR Rosen3, C-G Standertskjöld-Nordenstam2, RBH Tootell3, J Virtanen5, GV Simpson1
1Albert Einstein College of Medicine, Bronx, NY, 2Radiology, Helsinki Univ. Central Hospital, Finland, 3Massachusetts General Hospital, NMR Center, Charlestown, MA, 4University of Oslo, Norway, 5Cognitive Brain Research Unit, Univ. of Helsinki, Finland, 6BioMag Laboratory, Helsinki Univ. Central Hospital, Finland

The purpose of the study was to isolate motion selective human visual cortical areas and to examine the dynamics of their activity. Five subjects were presented with two types of stimuli which consisted of onset-offset of small wedge-shaped patterns in the upper and lower left visual quadrants. One type had moving contrast-modulated radial bands within the wedge, the other had stationary bands. The same subjects were measured with fMRI and simultaneous EEG and MEG (32 and 122 channels). The combined measures revealed pattern onset responses in retinotopically organized visual cortical areas, whereas, motion specific responses were less sensitive to retinal location. Subtraction of responses to stationary stimuli from responses to moving stimuli revealed activity in two cortical regions, one corresponding to fMRI defined MT/V5, the other located in the temporal parietal junction. Initial motion-specific responses in these areas peaked at approximately 140-180 ms post-stimulus onset and again at 260-300 ms.

504.12

DOUBLE DISSOCIATION OF MOTION AND FORM PROCESSING IN VISUOPARIETAL AND VISUOTEMPORAL CORTEX OF THE BEHAVING CAT. S. G. Lomber*, B.R. Payne, P. Cornwell and K.D. Long. Laboratory for Visual Perception and Cognition, Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA 02118.

The purpose of this study was to elucidate functional differences between visuoparietal cortex lining the middle suprasylvian (MS) sulcus and visuotemporal cortex lying ventrally on the posterior suprasylvian (vPS) gyrus. Freely behaving cats were trained to perform a battery of four motion and/or form processing tasks. Following midline sections of the optic chiasm and visual fibers of the corpus callosum, cooling probes were permanently implanted into the MS sulcus of one hemisphere and over vPS cortex of the other hemisphere to permit reversible deactivation of each cortical site. Deactivation of MS, but not vPS, cortex resulted in a profound deficit in the discrimination of direction of motion. In contrast, deactivation of vPS, but not MS, cortex severely impaired retention of object discriminations. Two final tasks both involved two-dimensional static patterns with an overlying grid-mask. While the mask was in motion, deactivation of either MS or vPS cortex impaired discrimination of the underlying patterns. However, when the mask was static, like the underlying pattern, discrimination was only impaired by deactivation of vPS cortex. These double dissociations show that visuoparietal cortex contributes greatly to visual motion processing, while visuotemporal cortex contributes significantly to form recognition. These functional dissociations have many features in common with the visuoparietal and visuotemporal processing streams identified in monkey and humans. Supported by NS32137.

505.1

PRESYNAPTIC INHIBITION OF PROPRIOCEPTIVE SENSORY NEURONS DURING VOLUNTARY MOVEMENTS OF THE LOCUST. M. Burrows* and B. Hedwig. Dept. of Zoology, Univ. of Cambridge, Cambridge CB2 3EJ, U.K.

The central terminals of sensory neurons from a proprioceptor at the femoro-tibial joint of a leg receive inhibitory depolarising synaptic inputs that are mediated by interneurons releasing GABA. During imposed movements of this joint the presynaptic inputs in a particular sensory neuron are greatest for movements that evoke its best spike response. To determine when presynaptic inputs occur during voluntary movements we recorded from the terminals of these sensory neurons during kicking. Locusts use a distinctive motor pattern to extend the tibia of a hind leg rapidly and the force for the movement is generated by an almost isometric co-contraction of the extensor and flexor tibiae muscles followed by a sudden release of the stored energy when the flexor motor neurons are inhibited. During this motor pattern, the spikes of the sensory neurons are superimposed on a depolarising synaptic potential generated near their output terminals that is linked to the time in the motor pattern when the sensory neurons spike. Flexion-sensitive neurons spike and their terminals are depolarised when the tibia is initially flexed. The depolarisation of the terminals precedes the spikes and persists during the co-contraction phase. Extension-sensitive neurons spike and their terminals are depolarised only when the tibia is rapidly extended, and they may repolarise during co-contraction. Some of these synaptic potentials occur before movements have started, suggesting that they may result from central elements of the motor pattern, whereas others are clearly consequent upon joint movements. The effect and timing of the presynaptic inhibition of the terminals during this voluntary movement should be to reduce the effectiveness of the sensory neurons in transmitting signals to their postsynaptic neurons. This could therefore be part of a mechanism that allows voluntary movements to proceed in the presence of self-generated sensory feedback which might impede that movement. Supported by NIH grant NS16058.

505.2

HOW ARE LIMBS ON DIFFERENT SEGMENTS OF THE BODY COORDINATED? A TEST OF THE "EXCITABILITY GRADIENT" HYPOTHESIS IN THE SWIMMERET SYSTEM. B.Mulloney* and W.M.Hall. Neurobiology, Physiology and Behavior, Univ. Calif. Davis, Davis CA 95616.

The swimmerets of crayfish are paired abdominal limbs that normally move rhythmically in a sequence of alternating power-stroke and return-stroke movements that thrust the animal forward. The phases of these movements in different segments lag progressively from posterior to anterior segments. To explain this metachronal progression and the observation that swimmeret beating normally begins with the most posterior pair, Ikeda and Wiersma (1964) proposed that posterior segments were intrinsically more excitable that anterior ones, and so reached threshold in each cycle before the anterior segments did. We tested this idea by comparing the periods of swimmeret motor patterns produced by isolated segments of the central nervous system of crayfish, *Pacifastacus leniusculus*, that were excited by carbachol.

When pairs of abdominal ganglia (A2-A3 and A4-A5) were exposed to the same concentrations of carbachol, we observed no significant differences in their periods. This lack of difference persisted in different concentrations of carbachol, and in different experiments that used TTX, or sucrose, or cutting to isolate pairs of ganglia.

We conclude that excitability of the modular swimmeret pattern-generating circuits do not differ systematically in different abdominal segments, and that another mechanism must account for the normal metachronal coordination that is characteristic of the swimmerets.

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505.3

SPATIAL CODING OF A MECHANICAL STIMULUS BY THE LEECH LOCAL BEND NETWORK: MAPPING STIMULUS LOCATION TO BEHAVIORAL OUTPUT. J.E. Lewis* and W.B. Kristan Jr., Biology Department., University of California, San Diego, 9500 Gilman Dr., La Jolla, CA, 92093-0357.

Local bending in the leech, *Hirudo medicinalis*, is a withdrawal reflex elicited by a mechanical stimulus to the body wall. Bending away from a stimulus results from the contraction of longitudinal muscles in the vicinity of the stimulus and relaxation of muscles opposite to the stimulus. Two classes of mechanosensory neurons mediate the reflex: low threshold T cells and higher threshold P cells. Previous work has described a subset of the neurons underlying the reflex, namely the interneurons and motor neurons responding to individual and paired electrical stimulation of P cells. We extend this work by using a more realistic mechanical stimulus that recruits both T cells and P cells. Video motion analysis of intact preparations and muscle tension measurements in semi-intact preparations reveal that the behavioral output varies smoothly with stimulus location around the circumference of the body wall. These results are consistent with a model involving the continuous coding of stimulus location by the local bend network. We are continuing this work by describing, as a function of stimulus location, the responses of the T cells, P cells, and motor neurons. A detailed characterization in this way will provide a framework for using the local bend network to investigate principles of sensory to motor transfer of spatial information.

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505.4

A VISUALLY-SENSITIVE HALTERE CONTROL MUSCLE OF THE BLOWFLY, *CALLIPHORA*. M. H. Dickinson*. Dept. of Organismal Biology & Anatomy, Univ. of Chicago, Chicago, IL 60637.

The metathoracic wings of all flies have been transformed during evolution into a pair of complex mechanosensory organs called halteres. The drumstick-shaped halteres beat anti-phase to the wings during flight, driven by their own asynchronous power muscles. The oscillations of each haltere are encoded by several hundred campaniform sensilla arranged in five fields at the base of the structure. Perturbations in the motion of the haltere caused by rotations of the animal during flight are thought produce compensatory alterations in wing kinematics via feedback from the campaniform afferents onto thoracic motorneurons.

The haltere afferents are thought to detect externally induced changes in haltere beating. Like the wing, however, each haltere possesses a discrete array of small synchronous control or 'steering' muscles, which could provide a mechanisms for internally induced changes in haltere kinematics. Extracellular recordings from one of these muscles reveals strong descending visual input. This input shows a marked directionality, such that the haltere muscle is excited by rotation of large field visual stimuli towards the ipsilateral but not the contralateral side. The response elicited by a step change in pattern rotation is phasic-tonic: the muscle first adapts rapidly, but then maintains a low tonic discharge for the duration of the motion stimulus. The firing rate of the muscle is roughly proportional to the angular velocity of the visual stimulus.

These results have an important functional implication for the role of haltere in flight control. Given the strong monosynaptic input between haltere campaniforms and wing steering motorneurons, the haltere muscles could provide an alternative pathway for descending flight control interneurons. Instead of driving the wing steering muscles directly, descending visual input could change haltere beating which in turn could modify the output of wing muscles *via* feedback from haltere afferents.

505.5

DEVELOPMENT OF A MECHANOSENSORY PATHWAY IN LOCUSTS. H.J. Pflueger*, S. Meuser, Freie Univ. Berlin, Inst. Neurobiologie, Koenigin-Luise-Str. 28-30, D-14195 Berlin, Germany.

A mechanosensory pathway has been identified in adult locusts that serves flight steering behaviour. It is comprised of filiform hairs on the locust frontal parts, a mediating projection interneuron in the ventral cord, and motor neurons to pleuroaxillary flight muscles. This pathway is found in all larval instars, although locust larvae do not possess functional wings. The receptive field of the interneuron comprises filiform hair receptors on the locust frontal part. In newly hatched first instars 30 filiform hairs are present, and in each larval instar new hairs are added, until in adults a full compliment of 300 hairs is reached. The central nervous projections of these filiform hair receptors exhibit a structural dynamic during larval development, i.e. ipsilateral axon collaterals degenerate whereas contralateral ones are strengthened. Correspondingly, synapses to the ipsilateral interneuron are lost, those to the contralateral interneuron strengthened. This reorganisation is dependent on the neuronal activity of the receptors.

The pleuroaxillary target muscles are also reorganised: Each larval muscle consists of three discrete bundles whereas each adult muscle has only two discrete bundles. Correspondingly, larval muscles are innervated by three large motor neurones, adult muscles by only two motor neurons. This reorganisation correlates with the adult moult, and there is evidence that this reorganisation depends on a low juvenile hormone titre. This finding is supported by chemically induced precocious adults which possess only two muscle bundles and two large motor neurons.

505.6

REORGANIZATION OF PROPRIOCEPTIVE INPUT DURING MATURATION OF THE LOCUST FLIGHT SYSTEM J.R. Gray*, C.E. Gee & R.M. Robertson, Dept. Biology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Current theories suggest that the flight circuitry of the locust is formed during embryogenesis and requires only appropriate activation at the imaginal moult. An alternative hypothesis is that adult maturation represents a time that is required to reorganize the circuits to produce effective flight. We tested the second hypothesis by examination of normal adult maturation of the flight system and maturation following removal of wing proprioceptors. During normal maturation an increase in the wingbeat frequency, from 15 -25 Hz, is accompanied by growth of centrally projecting afferents and central flight interneurons. There is also is an increase in the sensitivity of one of the afferents, the wing hinge stretch receptor (fSR), to rhythmic wing movements, demonstrating that proprioceptive input changes during maturation. Moreover, the ability of the fSR to entrain the flight motor pattern is greater in mature adults than in immature adults. Maturation following removal of proprioceptive input also results in reorganization of the flight circuitry. When the wing tegulae are ablated the wingbeat frequency decreases. During subsequent maturation the wingbeat frequency increases, approaching that of intact animals. There is, however, no difference in the central flight rhythm recorded from recovered and intact animals. This implies that changes in remaining proprioceptive input mediate functional recovery. We suggest that there is significant reorganization of the the flight circuitry during adult maturation and most of these changes are manifested within the proprioceptive pathways.

505.7

DYNAMICS OF CRICKET WIND-SENSING HAIRS: QUANTIFYING INPUT TO A MECHANOSENSORY SYSTEM. Leslie C. Osborne and John P. Miller*, University of California at Berkeley, Berkeley, CA 94720

Crickets, like other orthoptera, are able to sense air disturbances caused by objects moving nearby. This sense is mediated by the cercal sensory system. The cricket has about two thousand mechanoreceptive filiform hairs on two antennal cerci projecting from the rear of the animal. Each hair's deflection is transduced into spikes by a single receptor neuron which carries this information to the cricket's CNS. The information about air movement direction, intensity, and frequency available to the cercal sensory system is determined by the mechanical properties of the filiform hairs. The mass, spring stiffness, and damping vary as a function of hair length, such that longer hairs have lower resonant frequencies than shorter hairs. Additionally, the boundary-layer of dampened air movement near the surface of the cercus affects the amplitude and phase of a hair's response to oscillating flow.

Using a high-speed video microscope, we have characterized filiform hair responses to calibrated single frequency and bandpassed noise stimuli generated by speakers in an acoustically isolated laminar flow chamber. Near-field sound stimuli of variable direction, calibrated by a displacement sensitive microphone, were delivered by two speakers. Single hairs and groups of hairs of differing lengths were observed. Hair length was recorded, hair motion digitized, and angular motion over time extracted and analyzed. Tuning curves, power spectra, and the coherence of hair motion with the stimulus were computed. The results are interpreted in light of recent electrophysiological studies of signal encoding by the receptor neurons.

505.8

AIR CURRENT VELOCITY ENCODING BY ENSEMBLES OF FILIFORM MECHANORECEPTORS OF THE CRICKET CERCAL SENSORY SYSTEM. J. C. Roddey and G. A. Jacobs*. 195 LSA, Dept. of Molecular and Cell Biology, Univ. of California at Berkeley, Berkeley, CA 94720.

Techniques of stochastic systems analysis and principles of information theory were applied to analyze stimulus encoding by ensembles of wind-sensitive, filiform receptor neurons in the cricket cercal sensory system. White noise air current stimuli were presented to the cricket, and the spike train responses elicited by individual receptor neurons and pairs of receptors were recorded. The spike trains elicited in single cells by multiple presentations of the same stimulus waveform were combined to represent the simultaneous responses of an ensemble of receptors to a single stimulus presentation. The rate at which information about the stimulus could be encoded in ensemble responses was then estimated with the stimulus reconstruction technique. The information encoding rates of receptor ensembles were significantly higher than those of single neurons. Also, spike trains of pairs of receptors with different frequency response characteristics were simultaneously recorded in response to white noise air currents. Preliminary results confirm the hypothesis that such receptors have disparate phase response characteristics as well.

505.9

SYNAPTIC DEPRESSION IN THE CRICKET CNS CAN BE CHARACTERIZED BY THE DEPLETION HYPOTHESIS. A.A.V. Hill*, P. Jin and R.K. Murphey, Neuroscience and Behavior Program, Biol. Dept.,Univ. of Massachusetts, Amherst, MA 01003.

We have characterized synaptic depression at the synapses between wind-sensitive sensory neurons (SNs) and primary interneurons in cricket cercal sensory system. Identified SNs were stimulated with a train of 60 depolarizing pulses applied through a saline filled pipette placed over the cut end of a wind-sensitive filiform hair. The amplitude of the EPSP, recorded postsynaptically in either interneuron 10-3 or MGI, declined during the train at stimulus frequencies from 5 to 100 Hz. Based on the correspondence between the experimental results and the predictions of the depletion hypothesis this depression is most likely due to the depletion of transmitter (Liley and North, 1953, J Neurophysiol 16:509-527). According to the depletion hypothesis as the frequency of stimulation is increased the time constant of depression (g) should decrease and the amplitude of the steady-state plateau should become lower. This prediction accurately describes the experimental data. For example, as the stimulus frequency was varied (5, 10, 20 Hz), for a synapse between SN 5p and MGI, g varied linearly (2500, 909, 588 ms). Also, the plateau EPSP amplitude was lower with higher stimulus frequencies. Additionally, we found that reducing the extracellular Ca^{++} concentration results in a decrease in the fraction of transmitter (F) released with each stimulus and a change in the dynamics of depression that is consistent with transmitter depletion. When a synapse from SN 2c to 10-3 was stimulated in low Ca^{++} saline F was reduced by 50% and g increased by 150%. Our aim is to understand how synaptic dynamics shape the temporal response properties of the primary interneurons. Computer simulations suggest that differences in synaptic depression (Jin et al., 1995 Soc. Neurosci. Abstr.) and facilitation influence the phasic and tonic response properties of MGI and 10-3 respectively. Supported by NSF grant # IBN 9121063 to R.K.M.

505.10

ELEMENTARY COMPUTATION OF APPROACH IN A VISUAL INTERNEURON. Nicholas Hatsopoulos*, Fabrizio Gabbiani, & Gilles Laurent. CNS Program, 139-74, Caltech, Pasadena, CA 91125.

We investigated the coding properties of the descending contralateral motion detector (DCMD), a wide-field visual interneuron that extends from the brain to the metathoracic ganglion of the locust. We recorded its activity extracellularly while monocularly presenting objects on a computer screen that appeared to approach the animal.

The time of the post-stimulus histogram (PSH) peak correlated extremely well with the collision time of the stimulus despite variations in the object's physical size and approaching velocity. This suggested that DCMD may be warning the animal of impending collision, particularly for large and slow objects.

We developed a simple, mathematical model which matched the response profiles of DCMD quite well for a variety of different object sizes and velocities. Time-to-collision can be computed by taking the ratio of the angular velocity of an edge in the image to its angular distance from the focus of expansion. Our model replaces the denominator with the exponential of the angular extent of the image.

This non linearity was verified by presenting laterally moving objects of different sizes. Our model not only fit the PSH's resulting from objects moving at constant approaching velocities but also predicted the profiles from decelerating objects whose projected edges move at a constant angular velocity.

We also demonstrated that the critical variable in the denominator is the angular extent of the image and not its angular distance from the focus of expansion. We presented four approaching objects, all of the same size, surrounding the focus of expansion. The model fit the PSH's well only when the angular extent of each image was used.

Supported by ONR and NSF-PFF to GL

505.11

SENSORY INHIBITION OF PROLEG MOTOR ACTIVITY IN *Manduca sexta* LARVAE. D.J. Fickbohm, S. Landau, and B.A. Trimmer*, Dept. of Biology, Tufts University, Medford, MA 02155.

Our observations of *Manduca sexta* larvae suggest that control of proleg movement has both excitatory and inhibitory components. For example, proleg retraction normally evoked by stimulation of mechanosensory planta hairs (PHs) is often prevented during grasping. The excitatory factors are mediated, in part, by a monosynaptic reflex arc involving nicotinic and muscarinic mechanisms (*e.g.*, Trimmer 1994 *J. Neurophysiol.* 72). Although inhibition of proleg reflexes is easy to see in freely moving larvae, it is harder to detect neurophysiologically. However, we made the following observations that suggest sensory input can inhibit motoneurons involved in proleg movement: 1) In extracellular recordings from sub-branches of the ventral motor nerve stimulation of lateral proleg hairs or PHs causes a decrease in the activity of motor units controlling proleg movement, 2) Similar mechanosensory stimulation can hyperpolarize and decrease the spike frequency of VPO and PDV motoneurons that control the ventral posterior oblique and the posterior dorsal ventral muscle groups.

To test the possible role of mAChRs in the inhibition of VPO we pressure-applied the muscarinic agonist, oxotremorine-M (1×10^{-5}M), into the ipsilateral neuropil. Rather than causing an inhibition, this depolarized VPO and increased its firing frequency. Therefore, it is likely that the inhibitory effects of mechanosensory hair stimulation on motor activity are mediated by polysynaptic pathways, perhaps employing a non-cholinergic neurotransmitter, such as GABA or 5-HT.

Supported by a Sloan Foundation Fellowship, Whitehall Foundation Grant, and NIH Grant NS 30566 to BAT.

506.1

REGENERATION OF LESIONED NEONATAL MAMMALIAN SPINAL CORD IN CULTURE FOLLOWING APPLICATION OF ANTIBODY AGAINST GROWTH INHIBITORY PROTEIN .

Z. Varga, M. Schwab# and J.G. Nicholls*, Biozentrum, Univ. of Basel, 4056 Basel, Switzerland: #Brain Research Institute, 8029 Zürich, Switzerland

In the spinal cord of the neonatal opossum (*Monodelphis domestica)* in culture, neurites grow profusely and rapidly through a crush or a cut provided that the animal is less than 12 days old. At this critical period, oligodendrocytes, myelin, growth-inhibitory proteins and astrocytes have developed and regeneration stops. In the present experiments the antibody IN-1 was applied to the fluid bathing isolated lesioned spinal cords of animals after the critical period. This antibody blocks growth inhibitory actions of protein NI-35/250 which is associated with myelin. Penetration of antibody IN-1 into the tissue occured rapidly and reliably under these conditions. Out of 33 preparations from animals aged 13-14 days without antibody none showed any outgrowth of fibers labeled by DiI into the crush. By contrast, in 17 out of 31 preparations from animals aged 13-17 days clear regeneration of axons occurred into and beyond the crush. The effect was specific for antibody IN-1; other antibodies failed to promote regeneration after the critical period even though they penetrated the CNS. These results suggest that the myelin associated antigen NI-35/250 plays a part in preventing regeneration as the animal matures. The use of an isolated central nervous system in culture permits assessment of the effects of trophic as well as inhibitory molecules singly or in combination.

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506.2

MECHANISM OF RECOVERY FROM SPINAL CORD INJURY IN NEONATAL *MONODELPHIS DOMESTICA* (S. AMERICAN OPOSSUM)

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There is increasing evidence that injury to the spinal cord in immature birds and mammals is followed by a significant degree of recovery and development of normal function providing injury is sufficiently early in development (eg, chick embryo, Hasan, *et al*, 1992, J. Neuroscience, 13, 492-507; opossum neonate, Saunders, *et al*, 1995, Clin. Exper. Pharm. Physiol. In press.). The extent to which this recovery and development in the opossum is dependent upon regeneration of neurites from damaged axons as opposed to growth of new axons not present at the time of injury is not known. Nor is it clear whether the remarkable degree of locomotor activity demonstrated in adult opossum that receive complete spinal transection in the first week of life, is due mainly to local circuits in the spinal cord, or whether the connections that develop following injury are normal. These problems have been studied in *Monodelphis domestica* whose spinal cords were crushed with fine forceps under anaesthetic and sterile conditions in the first week of life and allowed to develop to adulthood. Evoked potential and retrograde pathway tracing methods are being used to study the connectivity involved in walking and swimming movements in these animals. In order to remove tactile and proprioceptive inputs which may stimulate rhythmic walking in spinal operated animals, they were subjected to a swimming test and movements analysed on video. These experiments show that the animals are able to display rhythmic movements even when swimming, thus indicating that the activity is controlled at a supraspinal level.

Supported by the Australian Research Council

506.3

REGROWTH OF SPINAL MOTORAXONS AFTER SPINAL CORD INJURY; DISTRIBUTION OF PROSTAGLANDIN RECEPTORS AND G-PROTEINS IN THE CNS SCAR TISSUE.

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Spinal motoneurons in the adult cat and rat have an ability to regenerate central nervous system (CNS)-type axons after a lesion in the ventral funiculus of the spinal cord. Regrowing motor axons penetrate the CNS-type of scar tissue and enter adjacent ventral root fascicles. This scar tissue is mainly composed of a loose trabecular web of astrocytic processes and invading leptomeningeal cells, which have high and low affinity receptors for NGF. The reactive astrocytes express NGF and a truncated species of *trk*B. Laminin, tenascin and collagens are upregulated in the extracellular matrix. In addition, there is a persistent defect in the blood-brain barrier function. Thus, blood-borne macrophages and trophic substances may have longterm access to the lesion area. Cytokines released from macrophages have been suggested to play a key role in the early response to CNS trauma, by regulating the synthesis of neurotrophins and matrix molecules in the scar tissue. Prostaglandins (PGs), induced by interleukins or transforming growth factor (TGF) alpha, represent potent mediators in cytokine evoked actions and exert their effects via specific PG receptors, which are functionally linked with G-proteins. Many of the PG receptors have just recently been cloned. In the present study we have studied the distribution of PG receptors and (relevant) alpha subunits of the trimeric G-proteins in CNS scar tissue using in situ hybridization and/or immunohistochemistry. Spinal motoneurons and astrocytes were shown be labelled with markers for the PGF2alpha receptor. 10 days after a lesion, could an upregulation of the alpha i, s/olf and q/11 subunits of trimeric G-proteins be observed in reactive glial cells and/or the blood vessels of the scar tissue. In addition, an upregulation of the receptor for TGFalpha was observed in the cicatrix. These findings are consistent with cytokine evoked effects during the formation of a traumatic CNS scar.

506.4

Growth Factors Increase PNS Regeneration Accuracy.

Roger D. Madison[1,2,3], Victor Wang[1], and Simon J. Archibald*[1]. Division of Neurosurgery[1], Department of Neurobiology[2], Duke University Medical Center, and the Research Service of the VA Medical Center, Durham, NC[3].

Preferential Motor Reinnervation (PMR) refers to the proven ability of regenerating motor axons in rat femoral nerve to preferentially, albeit incompletely, reinnervate motor vs. sensory distal nerve branches. We have now found that the addition of growth factors (aFGF & NGF) to the nerve transection site significantly increases **PMR** in the rat femoral nerve.

The motor and sensory neuronal pools projecting to the quadriceps muscle via the terminal motor branch of the femoral nerve were prelabeled with DiI (J. Neurosci. Meth., 39:123-129,1991) in 27 adult male Sprague Dawley rats. Ten animals served as controls for the double labeling method and seventeen animals received femoral nerve entubulation repair of a 4 mm nerve gap. The lumens of polyethylene nerve guides were flushed and filled with: **A)** N=10, control solution of lactated Ringer's, USP (Abbott Labs.); or **B)** N=7, growth factor solution containing 100 ng/ml of 2.5S NGF and 400 ng/ml acidic-FGF. Following 4 weeks of regeneration, Fluorogold was applied to the motor branch. After 72 hrs the spinal cords and the L3 DRGs were processed to display retrogradely labeled neurons.

The accuracy of motor axon regeneration into the original terminal motor branch was profoundly increased from 51% to 90% ($p<.001$)by the addition of growth factors to the repair site. Sensory axon regeneration into the original terminal motor branch also increased from 59% to 69%, but this trend did not reach statistical significance. We propose that the increase in regeneration accuracy is due to increased axonal branching at the repair site.

Understanding the mechanisms of increased PMR may have significant benefits to clinical nerve repair. NS22404-09 and VA Merit Review (RDM).

506.5

ECTOPIC EXPRESSION OF THE NEURAL CELL ADHESION MOLECULE L1 BY MOUSE ASTROCYTES IMPROVES NEURITE OUTGROWTH.

H. Mohajeri*, U. Bartsch, H. van der Putten1, G. Sansig1, L. Mucke2 and M. Schachner. Neurobiology, Swiss Federal Institute of Technology, 8093 Zürich, Switzerland, 1: CNS Mol. Cell. Biol., Ciba, 4002 Basel, Switzerland, 2: Virology, RISC, 10666 N. Torrey Pines Rd., La Jolla, CA 92037 USA.

Transgenic mice lines were generated to test whether ectopic expression of L1, driven by GFAP regulatory elements, in astrocytes will overcome the inhibitory influences exerted by adult CNS tissue. *In situ* hybridization analysis of optic nerve sections from transgenic mice confirmed expression of L1 by astrocytes. Immunostaining of cultured astrocytes localized L1 protein to the cell surface. L1 protein expression in lesioned optic nerves of transgenic animals was up to 300% higher than in lesioned nerves of non-transgenic controls and up to 30% higher than in unlesioned optic nerves of transgenic animals. Mouse small cerebellar or chick DRG neurons extended up to 400% or 50% longer neurites on cryostat sections of lesioned optic nerves or astrocyte monolayers, respectively, when compared to similar preparations from non-transgenic controls. The potential to promote neurite outgrowth of different transgenic lines was proportional to the levels of L1 expression and was specifically inhibited by anti-mouse L1 antibodies. These observations demonstrate that L1 is able to overcome, at least in part, the non-permissive substrate properties of differentiated CNS glial cells and CNS tissue when expressed ectopically in astrocytes from transgenic mice.

506.6

UP-REGULATION OF RECOGNITION MOLECULES DURING THE SUCCESSFUL REGENERATION OF THE FISH OPTIC PATHWAY.

R.R. Bernhardt*, E. Tongiorgi, M. Schachner. Neurobiology, Swiss Federal Institute of Technology, 8093 Zürich, Switzerland.

Adult fish retinal ganglion cells (RGCs) can regenerate their axons. Here we correlate this capacity with the expression of mRNAs coding for L1.1, L1.2 , zf-ß, and N-CAM (homologs of mammalian cell recognition molecules), as detected by in situ hybridization. The mRNAs are expressed by developing RGCs, consistent with a role during axonogenesis. In adult RGCs, expression of the mRNAs appears reduced, most strikingly in the case of L1.1. RGCs react to an optic nerve lesion by up-regulating L1.1, L1.2, and possibly also N-CAM mRNA levels, zf-ß mRNA levels appear unchanged. The up-regulation seems to be part of a growth program that is re-activated after axotomy and may be an intrinsic determinant of a neuron's regenerative capacity. Presumptive glial cells in the optic pathway also respond to the lesion: L1.1, L1.2 and zf-ß mRNAs are detected in the regenerating but not the control optic pathways. N-CAM mRNA, which is constitutively expressed at low levels, is up-regulated. The mRNAs are expressed by only some of the cells in the pathway. These may be oligodendrocytes, reported to express related molecules in culture (Bastmeyer et al., 1994). The up-regulation of the four mRNAs in the optic pathway could contribute to an environment that allows axonal regeneration.

506.7

BRAIN NUCLEI WITH AXONS PROJECTING TO THE SPINAL CORD IN ZEBRAFISH: REGENERATIVE CAPACITY AND REGULATION OF CELL ADHESION MOLECULES. Thomas Becker*, Catherina G. Becker, Robert R. Bernhardt, Enrico Tongiorgi, Mario F. Wullimann±, Melitta Schachner. Neurobiology, Swiss Federal Institute of Technology, Hönggerberg, 8093 Zürich, Switzerland. +Brain Research Institute, University of Bremen, 28334 Bremen, Germany.

We studied axonal regrowth from brain nuclei projecting to the spinal cord of adult zebrafish after transection of the spinal cord. Axonal projections to the spinal cord in unlesioned animals were established for 20 distinct nuclei in the brain by retrograde labeling using biocytin, horseradish peroxidase, and fluorescent dextrans. Six weeks after spinal cord transection, most of these nuclei showed axonal regrowth. However, the n. ruber, n. tangentialis, n. raphes and the Mauthner cell did not or hardly show axonal regrowth.

To correlate axonal regrowth with expression of L1.1, a homolog of mouse L1, and of the neural cell adhesion molecule (NCAM), both known to promote neurite growth in mammals, the levels of expression of these molecules were investigated by in situ hybridization. L1.1 mRNA was strongly up-regulated in the nuclei with axonal regrowth, when compared to unlesioned animals. Up-regulation was first detectable 3 days after the lesion, remained high for 2 months, and had returned to control levels after 3 months. The nuclei with poor or no axonal regrowth did not show this up-regulation. NCAM mRNA was not up-regulated in any brain nuclei.

Our observation that axonal regrowth correlates with an up-regulation of L1.1 mRNA suggests that up-regulation of this molecule may contribute to regenerative axonal growth.

506.8

SILENCING OF THE GAP-43 GENE IN DIFFERENTIATED ZEBRAFISH RETINAL GANGLION CELLS DEPENDS ON AN INTACT CYTOSKELETON AND THE ACTIVATION OF A SIGNAL TRANSDUCTION PATHWAY INVOLVING PROTEIN PHOSPHATASES. V. M. Zumsteg, P. Bormann and E. Reinhard*. Dept. Pharmacology., Biozentrum of the University of Basel, 4056 Basel, Switzerland.

Neurite outgrowth and axon elongation depend largely on activation of a specific set of growth-associated genes. The single-copy GAP-43 gene is expressed strongly during neuronal development and then decreases drastically as synapses are formed. Axon interruption re-induces GAP-43 expression in nerve cells capable of regeneration. To explore the mechanisms that lead to silencing of the GAP-43 gene in differentiated neurons we studied its regulation in zebrafish retinal ganglion cells (RGCs) using *in situ* hybridization after optic nerve lesions and interference with intracellular structures in intact nerves. GAP-43 is not expressed in adult zebrafish retina. However, 24 hr after a crush of the optic nerve, RGCs start to re-induce GAP-43. Expression is maximal between day 6 and 10 after lesion, declines gradually thereafter and is absent 15 days after lesion. Prevention of functional regeneration by removing a part of the optic nerve results in sustained GAP-43 expression in RGCs up to 50 days after a lesion, suggesting that synapse formation triggers events that lead to silencing of the GAP-43 gene. To identify components of the signalling pathway we applied metal cuffs filled with agarose-embedded drugs to the intact zebrafish optic nerve. Vinblastine and colchicine, drugs that disrupt the axonal cytoskeleton resulted in GAP-43 induction in RGCs without optic nerve lesion. Similarly, inhibition of intracellular phosphatases by okadaic acid and calyculin A reinduced re-expression of the GAP-43 gene; kinase-inhibitors, such as staurosporine, had no effect. These results indicate that GAP-43 expression is actively repressed in differentiated zebrafish RGCs by retrogradely transported factors that may involve activation of phosphatases or inhibition of kinases. Supported by a grant from the SNF # 31-36266.92.

506.9

MECHANISMS OF CONDITIONING LESION ENHANCEMENT OF NERVE REGENERATION K. L. Lankford* & J. D. Kocsis Department of Neurology Yale University Medical School New Haven CT 06510 & Neuroscience and Regeneration Research Center West Haven CT 06516.

In this study, we assessed the mechanisms by which prior nerve injury enhances subsequent regeneration. To examine this issue, we compared the timing of neurite initiation, extent of outgrowth, and branching frequency of cultured adult rat DRG neurons from animals subjected to sciatic nerve injury one week before culture to control neurons and neurons exposed to conditioned media from ligated DRG or Schwann cells or treatments designed to alter intracellular calcium levels. Conditioning lesioned cultures had roughly twice as many process-bearing neurons after 1 day *in vitro* as controls although the average total neurite extension from neurons with neurites did not change. Retrograde labeling of cut neurons showed that precocious neurite initiation only occurred in neurons with previously ligated axons; neurons whose axons exited the sciatic nerve proximal to the site of injury were unaffected. Average interbranch distances also increased in conditioning lesioned cultures and increased with culture time for controls. Elevating intracellular calcium via depolarization or release from intracellular stores likewise increased neurite initiation at day 1, while thapsigargin, which indirectly blocks calcium transients, inhibited neurite initiation and reduced interbranch distances. By contrast, conditioned media from ligated neurons had no effect on any of the parameters measured and conditioned media from Schwann cell cultures increased the average total neurite extension, without affecting neurite initiation or branching frequency. These data suggest that at least one component of conditioning lesion enhancement of nerve regeneration is directly due to nerve injury, rather than changes in the trophic environment, and that calcium signals might be involved in triggering these changes.

Supported in part by the NIH and the VA.

506.10

EVIDENCE FOR GROWTH OF FASCICULUS GRACILIS AXONS THROUGH THE LESION AFTER TRANSECTION OF THE SPINAL CORD IN THE DEVELOPING OPOSSUM, DIDELPHIS VIRGINIANA. G.F. Martin*, J.R. Terman and X.M. Wang, Dept. Cell Biology, Neurobiology and Anatomy, The Ohio State Univ., Coll. of Med., Columbus, Ohio 43210.

We have shown previously that the spinal cord regenerates after transection during early development in the opossum and that brainstem axons grow through the lesion. We have since asked whether comparable growth of fasciculus gracilis axons occurs. In one experiment, the thoracic cord was transected at postnatal day (PD)5 or 12. The pups were maintained for approximately 30 days after which bilateral injections of Fast Blue (FB) were made several segments caudal to the lesion. Three to five days later, they were sacrificed and perfused so that their spinal cord and brainstem could be removed and sectioned for microscopic examination. In the animals subjected to transection at PD5, labeled axons were present in regenerated spinal cord at the lesion site and in the fasciculus gracilis rostral to it. Such axons could be traced to the nucleus gracilis. Labeled axons were not observed rostral to the lesion in the animals transected at PD12. In order to verify that some of the axons which crossed the lesion originated within dorsal root ganglia, we transected the thoracic cord at PD5 in a second group of animals and 7 days later made injections of cholera toxin conjugated to horseradish peroxidase (HRP) into the hindlimb on one side. The intent of the injections was to label dorsal root axons transganglionically. Three days after the injections, the pups were sacrificed and the removed spinal cords and brains were processed for HRP. In all cases, labeled axons could be traced through the lesion site to the nucleus gracilis. The results support our previous contention that the mammalian spinal cord regenerates after transection at early stages of development and show that axons of the fasciculus gracilis, like axons which originate within the brainstem, grow through the lesion. (Supported by NS-25095 and 10165).

506.11

NEUROFILAMENT SPACING, PHOSPHORYLATION AND AXON DIAMETER IN REGENERATING AND UNINJURED LAMPREY AXONS. D. S. Pijak, G. F. Hall, P. J. Tenicki, A. S. Boulos, D. I. Lurie and M. E. Selzer*. Dept. of Neurology and the David Mahoney Inst. of Neurol. Sci., Univ. of Pa. Med. Ctr., Philadelphia, PA 19104-4283.

The relationships among NF phosphorylation, NF spacing and axon diameter were examined in uninjured and spinal cord-transected larval sea lampreys (*P. marinus*). Axon diameters in untransected spinal cords varied from 0.5-50μm. MAbs specific for highly phosphorylated NFs labeled only large axons (>10μm), while mAbs for lightly phosphorylated NFs labeled medium and small axons more darkly than large axons. For most axons in untransected animals, diameter was inversely related to NF packing density, but interfilament distances of the largest axons were only 1.5X those of the smallest axons. In addition, the NFs of the small axons in the dorsal columns were lightly phosphorylated but widely spaced. Regenerating neurites of giant reticulospinal axons (GRAs) have diameters only 5-10% that of their parent axons. While NFs in these slender regenerating neurites had packing densities twice those of their parent axons, they were highly phosphorylated. Following section of these same axons close to the cell body, axon-like neurites regenerated ectopically from dendritic tips. These neurites had NF packing densities 2.5X those of uncut GRAs, but were also highly phosphorylated. Thus NF phosphorylation probably does not control axon diameter directly through electrorepulsive charges that increase NF spacing. Phosphorylation of NFs might influence axon diameter through indirect mechanisms that can be over-ridden under some circumstances, *e.g.*, during regeneration. NIH Grant # NS14837

506.12

SPATIOTEMPORAL ASPECTS OF CRANIAL NEURAL CREST REGENERATION REVEALED BY DiI LABELING AND *SLUG* EXPRESSION. J.W. Sechrist*, M. Angela Nieto, R.T. Zamanian and M. Bronner-Fraser. Dev. Biol. Ctr., UC Irvine, Irvine, CA 92717 and Instituto Cajal, CSIC, Avda. Doctor Arce 37, 28002 Madrid, Spain.

After unilateral ablation of avian cranial neural folds, the remaining neuroepithelial cells regulate to replace the missing neural crest population (Scherson et al., 1993). The effects of unilateral and bilateral ablations have now been compared using DiI, HNK-1 antibody labeling, and gene expression techniques. The results demonstrate that crest regeneration occurs only after apposition of the remaining neural fold and epidermis. We also discovered profound regional differences in the regulative ability of the cranial neural tube; there is a robust regulative response in the caudal midbrain/rostral hindbrain, but much less regulation in the caudal forebrain/rostral midbrain. Embryos with unilateral ablations recovered more completely, suggesting some contribution by the contralateral side. Bilateral ablations yielded significant numbers of neural crest cells, but deficits in neural crest-derived structures (i.e. trigeminal ganglion) were observed 3-4 days following ablation at the 4-7 somite stage. *Slug*, a transcription factor present in premigratory and migratory crest cells (Nieto et al., 1994), was expressed in cells proximal to the ablated region between 5-8 hours after surgery and prior to emergence of neural crest cells, indicating that up-regulation of *Slug* is an early response in crest regeneration. Our results demonstrate that the potential of the cranial neuroepithelium to regulate and replace the neural crest after ablation depends upon the timing, location, and extent of cranial neural fold ablation. Supported by USPHS HD 25138.

506.13

THE REGULATION OF ASTROCYTE GROWTH AND REACTIVITY. E.E. Geisert, Jr.*, L.J. Yang and N. Del Mar. Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis TN, 38163,

Following an injury to the CNS, astrocytes become reactive to form a scar. Many investigators believe that this astroglial scar contributes in part to the lack of axonal regeneration and functional recovery. One approach to defining the role of reactive astrocytes in scar formation is to develop monoclonal antibodies to the surface proteins of astrocytes that will alter astrocytic growth. The present study centers around a monoclonal antibody, AMP1, that depresses the mitotic activity of glial cells, modulates astrocyte-astrocyte adhesion, and affects the morphology of cultured astrocytes. Previous studies demonstrated that the AMP1 antigen is found on the external surface of cultured astrocytes and is involved in adhesive interactions between these cells. Immunohistochemical studies reveal a dramatic increase in AMP1 immunoreactivity following cortical stab wounds, kainic acid lesions of the neostriatum, and focal ischemia. The monoclonal antibody AMP1 recognizes two proteins: a 106 kDa protein with a high degree of homology to non-muscle alpha actinin, and a 26 kDa protein that is related to the target of the antiproliferative antibody, TAPA-1. The characterization of these antigens indicates that the AMP1 antibody recognizes the 26 kDa protein on immunoblots and in tissue; while, the 106 kDa protein can be identified only on immunoblots and not in tissue or cells. Following CNS injury, the increase of immunoreactivity at the site of injury is due solely to the upregulation in the 26 kDa antigen, TAPA. Taken together these data indicate that TAPA plays a prominent role in regulating the astrocyte response to injury.

BETA-AMYLOID: AGGREGATION

507.1

MATHEMATICAL MODEL OF FORMATION AND GROWTH OF SENILE PLAQUES IN ALZHEIMER DISEASE. S. Buldyrev, S. Havlin, E.T. Hedley-Whyte*, H.E. Stanley and B.T.Hyman Center for Polymer Studies and Dept Physics Boston University, Boston, MA 02215 & Neurology Service, Massachusetts General Hospital, Boston, MA 02114

Studying the geometrical properties of senile plaques may lead to insight into the various factors that contribute to amyloid deposition. Senile plaques are accumulations of a small amphipathic protein, Aß, in the cortex of individuals with Alzheimer disease (AD). Recently,the distribution of plaque size was found to be log-normal (1). This finding suggests that the growth mechanism of the plaques is a random multiplicative process. Neuropathological studies distinguish several different types of plaques, e.g., diffuse and compact, but the relationship among these is unknown. Since tracing the development of plaques in vivo is impossible, creating a biologically-motivated growth model is of interest. We developed a mathematical model of Aß amyloid aggregation that accurately models the stable log-normal distribution of plaque sizes and the geometrical morphology of both diffuse and compact plaques. Crucial aspects of this model are that there are both aggregation and dissociation kinetics of Aß interaction with plaques, and that these vary within defined ranges near a critical point.

(1)Hyman BT, West HL, Rebeck GW, Buldyrev SV, Mantegna RN, Ukleja M, and Stanley HE, PNAS 92 , 3586-3590 1995)

507.2

KINETIC ANALYSIS OF Aß AGGREGATION D.B. Teplow*[Δ†], A. Lomakin[Δ†#], D.A. Kirschner[+†], and G.B. Benedek[#]. [Δ]Department of Neurology, Brigham and Women's Hospital; [+]Neurology Research, Children's Hospital; and [†]Department of Neurology (Neuroscience), Harvard Medical School, Boston, MA 02115. [#]Department of Physics, and Center for Material Science and Engineering, Massachusetts Institute of Technology, Cambridge, MA, 02139.

Deposition of fibers of the amyloid ß-protein (Aß) in the cerebral parenchyma and vasculature is a major neuropathologic feature of Alzheimer's disease. Since Aß normally exists in a soluble form in human plasma and CSF, a key etiologic issue is how this soluble molecule is converted into amyloid fibers. Although fibrillogenesis has been studied extensively *in vitro* , the initial steps in this process are poorly understood. We have applied the techniques of quasielastic light scattering spectroscopy (QLS) to this question. We report here the results of kinetic studies of the aggregation of synthetic human Aß(1-40) and various structural analogues thereof. These studies have led to the development of a new theory describing Aß aggregation and fibrillogenesis.

507.3

A biotechnological method provides access to aggregation competent monomeric Alzheimer's 1-42 residue amyloid peptide ($Aß_{1-42}$)

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Deposition of $Aß_{1-42}$ appears to be fundamental for AD pathogenesis. In contrast to $Aß_{1-40}$. $Aß_{1-42}$ is characterized by its extreme tendency to aggregate into fibres or amorphous precipitate. A tailored biotechnological method prevents aggregation of $Aß_{1-42}$ monomers during its production. The method is based on a protein tail fused to the amino terminus of Aß. This tail leads to a high expression in *E.coli* , and a histidine affinity tag facilitates purification. Selective cleavage of the fusion tail is performed with cyanogen bromide by immobilizing the fusion protein on a reversed phase chromatography column. Cleavage then occurs only at the methionine positioned at the designed site but not at the methionine contained in the membrane anchor sequence of Aß. Furthermore, immobilization prevents aggregation of cleaved Aß. Elution from the HPLC column and all succeeding purification steps are optimized to preserve $Aß_{1-42}$ as a monomer. Solutions of monomeric $Aß_{1-42}$ spontaneously aggregate into fibres within hours. This permits the investigation of the transition of monomers into fibres and the correlation of physico-chemical properties with biological activities.

Mutations of $Aß_{1-42}$ at postion 35 influence the aggregation properties. Wild-type $Aß_{1-42}$ with methionine at position 35 has similar properties as Aß with a methionine sulfoxide residue. The fibre formation tendency, however, is reduced when position 35 is occupied by a serine, leucine or glutamic acid residue.

The biological effect has been investigated with rat PC12 cells using the MTT reduction assay as a measure. Inhibition of MTT reduction correlates with the presence of fibres: the EC_{50} for the methionine sulfoxide derivative is ~1nM and for the glutamic acid mutant ~1μM.

507.4

QUANTIFICATION OF SOLUBLE AND AGGREGATED Aß USING A NOVEL METAL-COATED MICROTITER PLATE ELISA. R.D. Moir, A. I. Bush, K. M. Rosenkranz, L. A. Rodes, S. Guénette* and R. E. Tanzi. Genetics and Aging Unit, Massachusetts General Hospital, Building 149, 13th St, Boston, MA 02114.

Aß is the principal component of cerebral amyloid in Alzheimer's disease (AD). Recent studies have identified Aß as a strong copper and zinc binding protein and have shown that physiological concentrations of Zn^{++} rapidly induce amyloid formation *in vitro*. We exploited these interactions to develop an ELISA which uses these metals to capture the Aß metalloprotein. Immobilized Cu^{++} or Zn^{++} ions replace bound antibodies used to capture Aß in conventional sandwich ELISA protocols. Both soluble and aggregated $Aß_{1-40}$ and $Aß_{1-42}$ are captured by the metal-coated microtiter plates. The present limit of sensitivity for Aß is 20 pg/ml (≈5 pM).

We have also developed simple methods for enriching and purifying Aß from biological samples using filtration techniques, anion exchange and metal chelating chromatographic procedures. Using these techniques in combination with immunoblots, we have verified the specificity of the novel metal capture ELISA for CSF (20-35 ng/ml), neuronal cell culture media (0.5-1 ng/ml), brain extracts (0.1-0.5 ng/g) and plasma (0.5-1 ng/ml).

Preliminary findings indicate that, although there is considerable overlap, Aß levels in the CSF of patients diagnosed as having AD are decreased relative to controls. These novel technologies were also used to demonstrate that Zn^{++} induces Aß aggregation in the presence of CSF.

507.5

UNDERSTANDING NUCLEATION-DEPENDENT AGGREGATION OF β-AMYLOID PEPTIDE AND COMBINATORIAL SEARCHES FOR PEPTIDES THAT INTERACT WITH β-AMYLOID. S. R. Sahasrabudhe*, J. E. Sun, S. Goyal, J. Knaeblein, P. Gonzalez-DeWhitt, M. A. Fortes, N. G. Riedel and S. R. Hughes. Neuroscience PGU. Hoechst-Roussel Pharmaceuticals Inc., P O Box 2500,Somerville, NJ 08876.

The kinetics of amyloid fibril formation by β-amyloid peptide (Aβ) are typical of a nucleation-dependent polymerization mechanism. This type of mechanism suggests that the study of the interaction of Aβ with itself can provide some valuable insights into Alzheimer disease amyloidosis. Interaction of Aβ with itself *in vivo* was explored with the yeast interaction trap system. Fusion proteins were made by linking the Aβ fragment to a LexA DNA binding domain (bait) and also to a B42 trans-activation domain (prey). Protein-protein interactions were measured by expression of these fusion proteins in *Saccharomyces cerevisiae* harboring a lacZ (β-galactosidase) and LEU2 (leucine utilization) gene under the control of a LexA-dependent operator. This approach suggests that the Aβ molecule is capable of interacting with itself *in vivo*. LexA protein fused to the Drosophila protein bicoid (LexA-bicoid) failed to interact with B42 fragment fused to Aβ, indicating that the observed Aβ-Aβ interaction was specific. Also no significant interaction was observed in yeast bearing LexA-Aβ bait when the B42 trans-activation domain was fused to Aβ fragment with phenylalanine^{19}-phenylalanine^{20} substituted by threonine-threonine (AβTT). These findings suggest that the yeast system described above can be used to study the interactions of Aβ monomers and to define the peptide sequences that are important in nucleation-dependent aggregation. We have performed saturation mutagenesis in the VFFA region of Aβ in the context of the prey plasmid. This library screen identified several peptides that interact strongly with Aβ.

507.6

Zinc Induces Aggregation of PN2 and βAP in Dog, Monkey and Human Cerebrospinal Fluid A. M. Brown, D. M. Tummolo, J. Hofmann, J. S. Jacobsen*, and J. Sonnenberg-Reines. Department of Central Nervous System Biological Research, Wyeth-Ayerst Research, American Home Products Corporation, Pearl River, NY 10965.

It has been suggested that abnormalities in zinc metabolism may play a role in the pathogenesis of senile dementias. Recently it was reported that addition of zinc to buffered solutions of βAP induced accelerated formation of insoluble aggregate (Science 265, 1464-7, 1994). The studies reported the behavior of βAP at concentrations of ~0.8 - 8 μM βAP at 25 μM Zn^{++}, or 1.6 μM βAP and 1-300 nM Zn^{++}. In contrast, the concentration of βAP in cerebrospinal fluid (CSF) is typically 2-3 nM. Furthermore, the relevance to βAP found in its native milieu, which includes many potential carriers of both βAP and zinc, is unclear.

We have developed a highly sensitive western blotting system that allows detection and quantitation of PN2 and βAP from 50 μl samples of CSF. Using this technique we have examined the effect of added zinc upon the solubility of PN2 and βAP in CSF, determined by sedimentation of aggregates by ultracentrifugation. We find that addition of ≥ 50 μM zinc to CSF induces the rapid formation of sedimentable aggregates that include both PN2 and βAP. The reaction is specific, since most (≥ 90%) of the CSF protein remains soluble under the same conditions. We also observe evidence of spontaneous aggregation of βAP in CSF from different species without any additions. We are analyzing the structure of the amyloid-containing pellet to learn if there is evidence of fibril formation. Investigation of aggregation of βAP in biological fluids will aid in assessing the biological relevance of agents that have been reported to accelerate fibril formation.

507.7

HOMOPHILIC- AND COLLAGEN-BINDING SITES OF APP ARE OVERLAPPING BUT DIFFERENTLY REGULATED. G. Multhaup, D. Beher, L. Hesse, A. Schlicksupp, J. Beer*, C.L. Masters# and K. Beyreuther. ZMBH-Center for Molecular Biology, Im Neuenheimer Feld 282, D-69120 Heidelberg, Germany. #Department of Pathology, The University of Melbourne, Parkville, Victoria 3052, Australia.

The specific binding of the amyloid precursor protein (APP) to extracellular matrix molecules (ECM) suggests that APP regulates cell interactions and has a function as a cell adhesion molecule (CAM) and/or substrate adhesion molecule (SAM). On the molecular level APP has binding sites for collagen, laminin and glycosaminoglycans (GAG) which is a characteristic feature of cell-adhesion molecules. We have examined the interactions between the APP and collagen types I, III, IV and V and identified the corresponding binding sites on APP and collagen type I.

We show that ^{125}I-APP bound most efficiently to collagen type I in a concentration-dependent and specific manner in the native and heat denatured states suggesting an involvement of a contiguous binding site on collagen. This binding site was identified on the cyanogen bromide fragment α1(I) CB6 of collagen type I which also binds heparin. APP did not bind to collagen type I-heparin complexes which suggests that there are overlapping binding sites for heparin and APP on collagen.

Since a peptide encompassing residues 448-465 binds to collagen type I and inhibits APP-collagen type I binding in nanomolar concentrations, this region may comprise the major part of the collagen type I binding site of APP. Moreover, our data also indicate that the collagen binding site represents a homophilic binding site for APP that can be modulated by zinc(II), copper(II) and heparin. Taken together, the data suggest that the regulation of APP binding to collagen type I by heparin occurs through the competitive binding of heparin and APP to collagen, and the collagen binding sequence of APP competes with homophilic binding of APP.

507.8

ZINC INDUCES AGGREGATION OF $Aβ_{1-40/42}$ AT PHYSIOLOGICAL CONCENTRATIONS. A. I. Bush*, R. D. Moir, K. M. Rosenkranz, L. A. Rodes and R. E. Tanzi. Genetics and Aging Unit, Mass. Gen. Hospital, Boston, MA.

$Aβ_{1-42}$ is the main component of plaque amyloid in Alzheimer's disease (AD). We are systematically appraising neurochemical factors which promote Aβ amyloid formation *in vitro*. Among the biochemical lesions of AD is a cerebral and systemic defect of zinc metabolism. We have shown that Aß specifically and saturably binds zinc, when, at physiological concentrations, it increases the peptide's adhesive properties, resistance to proteolysis and rapidly induces amyloid formation (Bush *et al.*, Science, 265, 1464; 1994). The rat homolog of Aß is not sensitive to the effects of zinc, perhaps explaining why this species is not subject to cerebral amyloid deposition.

We have developed two novel technologies to study the differential effects of metal ions upon the physicochemical behaviour of the 1-40 and the 1-42 Aß species. We analysed the solubility of peptides using a filtration assay where the retention of aggregated peptide (>0.2 μ diameter) permits the high-throughput quantification of metal-induced precipitation (Bush *et al., ibid*; Bush *et al.*, Science, in press). Using this technique we determined that at 1.6 μM, the spontaneous aggregation of the 1-42 Aß form exceeds that of the 1-40 form. However, seeding of 1-40 solutions with pre-aggregated 1-42 induces far less aggregation than incubation with Zn(II). Although Fe(II) and Cu(II) are capable of inducing some aggregation in this system, only Zn(II) induces a readily sedimentable, relatively large (10-40 μm), congophilic reaction product.

Using Zn(II) or Cu(II) immobilized onto microtiter plates, we have developed a novel capture ELISA to quantify Aß in biological fluids, and for characterizing the physicochemical interactions of metalloproteins. This capture assay can quantify aggregated Aß, and was used to determine that aggregation of Aß at low nM concentrations can be induced by physiological concentrations of zinc.

507.9

EFFECTS OF CONGO RED ON Aβ PRODUCTION, STABILITY AND AGGREGATION IN CHO CELLS. M.B. Podlisny*, B. Ostaszewski, C.H. Haass, D.J. Selkoe*Harvard Med. Sch., Boston.

Fibrillar deposits of amyloid β-protein (Aβ) characterize AD. Studies with synthetic Aβ peptides show that soluble, monomeric Aβ is relatively inert whereas fibrillar aggregates of Aβ are neurotoxic *in vitro*. These findings, along with genetic and transgenic mouse data, suggest that aggregated Aβ is pathogenetic in AD. Aβ is proteolytically cleaved from its precursor protein (βAPP) and constitutively secreted as a monomeric 4 kDa peptide. Understanding the processes that mediate the conversion of monomeric Aβ to aggregated Aβ is thus important. We have recently shown that βAPP-transfected CHO cell conditioned medium contains small amounts (<10^{-9}M) of SDS-stable oligomers of Aβ, primarily dimers and trimers. Overnight metabolic labeling of these cells in the presence of 10 μM Congo red (CR) leads to a marked increase in 4 kDa Aβ and a modest decrease in oligomeric Aβ in the medium. Experiments on the effect of CR on Aβ production, stability and aggregation were conducted. CR was found to stabilize Aβ only slightly from degradation by endogenous proteases in the medium. CR had no detectable effect on full-length βAPP or soluble APP_s levels. It produced a slight decrease in the amount of Aβ secreted during short-term pulse-chase experiments. CR also caused an increase in 12 and 14 kD C-terminal βAPP fragments, which were subsequently turned over. These rather subtle effects of CR on Aβ production and stability cannot account for the large increase in Aβ monomer found after overnight labeling in CR. Therefore, CR may be inhibiting the aggregation of monomeric Aβ into higher molecular weight species that are currently not detectable by our immunoprecipitation protocol.

507.10

THE HEPARIN-BINDING OF $Aβ_{WT}$ AND $Aβ_{E22Q}$ DEPENDS ON THE AGGREGATION STATE OF Aβ AND IS DIFFERENTIALLY BLOCKED BY CONGO RED. D.J. Watson[1], A.D. Lander[2]* and D.J. Selkoe[1], [1]Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School and [2]Department of Brain and Cognitive Sciences, M.I.T., Boston, MA 02115.

Heparan sulfate proteoglycans (HSPGs) are components of many types of amyloid deposits, including the diffuse and cored plaques of Alzheimer's disease and the vascular amyloid of Hereditary Cerebral Hemorrhage with Amyloidosis, Dutch type. To help to understand the close association of Aβ fibrils with HPSGs in these amyloid deposits, we examined the binding of synthetic $Aβ_{1-40}$ to a model glycosaminoglycan, ^{125}I - low molecular weight (<~6 kD) tyramine-heparin, using affinity coelectrophoresis (ACE) [Lee and Lander, PNAS 88(7), 1991]. Fibrils of Dutch mutant (E22Q) or wildtype $Aβ_{1-40}$ were formed by resuspending lyophilized peptide in a phosphate-buffered salt solution to 100 μM and rocking for 2 days at 25°C, then diluting and sonicating the resultant pellet. Fibrillar wildtype Aβ shifted the mobility of heparin half-maximally at about 1 μM Aβ. Fibrillar mutant $Aβ_{E22Q}$ had a slighlty increased affinity for heparin. In addition, we found that pre-incubation of Aβ fibrils with the amyloid-binding dye Congo Red completely blocked the binding of heparin to wildtype fibrils but not to mutant E22Q fibrils. The importance of fibrillar Aβ structure for heparin binding is emphasized by the fact that freshly resuspended $Aβ_{WT}$ did not shift the mobility of heparin, and by a reverse ACE experiment in which concentrations of Na-heparin up to 30 mg/ml did not shift the mobility of nonfibrillar E22Q or wildtype ^{125}I-$Aβ_{1-40}$. However, freshly resuspended $Aβ_{E22Q}$ in water or in 5 mM NaOH still produced a shift of heparin mobility similar to the shift caused by mutant fibrils. We are further characterizing these results by doing electron microscopy on the Aβ fibrils and by the use of different peptide preparations, aggregation protocols, glycosaminoglycans, and blocking agents.

507.11

EFFECTS OF APOLIPOPROTEIN E AND PERLECAN ON AGGREGATION AND TOXICITY OF β-AMYLOID PROTEIN EXPRESSED IN CULTURED CELLS. K. Fukuchi*[1], T. Ohman[1], A.D.Snow[2], R.C. LeBoeuf[3], C.E. Furlong[3], and J.R. Hassell[4]. [1]Dept. of Comparative Medicine, Univ. of Alabama at Birmingham, Birmingham, AL 35294; Depts. of [2]Pathology and [3]Medicine, Univ. of Washington, Seattle, WA 98195;[4]Dept. of Ophthalmology, Univ. of Pittsburgh, Pittsburgh, PA 15213.

Characteristic pathological changes found in the brains of patients with Alzheimer's disease (AD) are deposits of β-amyloid protein (Aβ) aggregates and loss of selected neurons. Aβ and its precursor protein (βPP) are thought to play a causative role in the pathogenesis of AD. To study amyloidogenicity (aggregation) and neurotoxicity of Aβ, we had previously established cell culture systems (COS and P19 cells) where overexpression of cDNA for a C-terminal region of βPP brings about aggregation of the C-terminal fragments in COS cells and degeneration of neurons in P19 cells (Fukuchi et al., 1992. BBRC 182:165). Since apolipoprotein E (ApoE) and perlecan, a specific heparan sulfate proteoglycan, are intimately associated with Aβ deposits in the brains of patients with AD, we have been using the cell culture systems to investigate the effects of these proteins on amyloidogenesis and β-amyloid-mediated neurotoxicity. When both ApoE3 and the C-terminal fragment of βPP were overexpressed in COS cells, Western blot analyses revealed partial inhibition of Aβ-aggregation. On the other hand, overexpression of perlecan domains I and II, or domain III, with the C-terminal fragment of βPP appeared not to influence Aβ aggregation. We are currently evaluating ApoE4 and other domains of perlecan to study their potential influence on Aβ aggregation. The effects of ApoE and perlecan on Aβ-mediated neurotoxicity are being studied using P19 cell cultures. This work was supported by the National Institute of Health and the Alzheimer's Association, Inc.

507.12

FURTHER STUDIES IMPLICATING THE IMPORTANCE OF PERLECAN (A SPECIFIC HEPARAN SULFATE PROTEOGLYCAN) IN AN ANIMAL MODEL OF FIBRILLAR Aß AMYLOID DEPOSITION IN VIVO: COMPARISON OF Aß (1-42) VERSUS Aß (1-40). A.D. Snow[1], J.A. Cummings[1], C.T. Ngo[1], W. Yang[1], D. Nochlin[1], K. Rimvall[2], M.J. Sheardown[2] and M. Judge*[2]. Dept. of Pathology, Neuropathology Labs Box 356480, University of Washington, Seattle, WA 98195 and Novo Nordisk, 2760 Malov, Denmark.

Previously, we described a new consistent animal model to study the effects of fibrillar beta-amyloid protein (Aß) in brain, by continuous coinfusion of perlecan and Aß into rodent brain (Neuron 12:219-234, 1994). In the present study, we have compared the effects of perlecan infusion into rat hippocampus in the presence of Aß (1-42) versus Aß (1-40). Sprague-Dawley rats (6 per group) were infused for 1 week with Aß (1-42), Aß (1-40), perlecan only, Aß (1-42) + perlecan, Aß (1-40) + perlecan, or vehicle only. 100% of animals (12 of 12) infused with perlecan plus Aß (1-42 or 1-40) demonstrated congophilic and Thioflavin S positive deposits (indicative of amyloid) at the infusion site and penetrating into brain parenchyma. Only 50% (6 of 12) of animals infused with Aß (1-42 or 1-40) showed any indication of amyloid deposition in brain. A significant 4-fold increase ($p<0.01$) in the extent of Aß amyloid deposition (as measured by blind scoring of Congo red stained sections) was observed in animals infused with Aß (1-42 or 1-40) + perlecan , versus Aß (1-42 or 1-40) only. No significant differences in congophilia were observed between Aß (1-42) versus Aß (1-40), in the presence of perlecan. Current analysis includes blind scoring of sections to evaluate potential neurotoxic effects, and evaluation of amyloid effects on glia and induction of Alzheimer's disease-like antigens. This study demonstrates that perlecan enhances Aß amyloid deposition in vivo regardless of the predominant Aß amyloid protein isoform (ie. 1-42 or 1-40) and reconfirms the importance of perlecan in Aß amyloidosis.

CALCIUM CHANNEL STRUCTURE, FUNCTION AND EXPRESSION I

508.1

BIOPHYSICAL AND PHARMACOLOGICAL CHARACTERIZATION OF STABLY EXPRESSED CLASS B AND CLASS E Ca^{2+} CHANNELS IN HEK 293 CELLS. D.M. Rock*[1], S.J. Stoehr[1], J. Offord[2] and A. Palma[3], [1] Neuroscience Therapeutics and [2] Biotechnology, Parke-Davis Research, 2800 Plymouth Rd., Ann Arbor, MI 48105 and [3] Neurex Corporation, Menlo Park, CA 94025.

Molecular biological experiments have shown that several different subtypes of the α_1 subunit of Ca^{2+} channels exist. Co-expression of different α_1 subunits with α_2 and β subunits results in Ca^{2+} channels that have different biophysical and pharmacological properties. In these experiments, we have stably expressed the human α_{1b} (Class B) or α_{1e} (Class E) along with rabbit skeletal muscle α_2 and human neuronal β in HEK293 cells. We determined the biophysical and pharmacological properties of these expressed channels.

Conventional techniques were used to obtain whole-cell voltage-clamp recordings of Ca^{2+} currents, with 2 mM Ca^{2+} as the charge carrier. Both classes of Ca^{2+} channels were high voltage activated, with activation first occurring at a test potential of -20 to -10 mV, with peak current at a test potential of +10 to + 20 mV. Steady state inactivation properties of both classes of channels were also similar, with channels becoming 50% inactivated at a holding potential of around -50 mV. However, the rate of inactivation of these two classes of Ca^{2+} channels was different. While both classes of Ca^{2+} channels showed voltage-dependent inactivation during test depolarizations, Class B channels inactivated more slowly than Class E channels. Similar to what has been previously reported in oocytes injected with the same subunits, Class B channels were sensitive to ω-conopeptides and Class E channels blocked only by the nonselective toxin ω-Aga IIIA.

These biophysical and pharmacological properties of expressed channels will be compared with Ca^{2+} channels expressed in superior cervical ganglion neurons (N-type) and a subtype of Ca^{2+} channels expressed in cerebellar granule cells (R-type).

508.2

N-TYPE CALCIUM CHANNELS ASSOCIATE WITH MULTIPLE BETA SUBUNITS V.E.S. Scott, M. De Waard, C.A. Gurnett, H.Y.Liu, D.R. Witcher*, V.A. Lennon‡ and Campbell, K.P. Howard Hughes Medical Institute, Department of Physiology and Biophysics, University of Iowa, Iowa City, IA 52242; ‡Neuroimmunology Laboratory, Mayo Clinic, Rochester, MN 55905.

The N-type Ca^{2+} channel is central to neurotransmitter release at the presynaptic membrane in mammalian neurons. Purification of the N-type Ca^{2+} channel shows that it contains α_{1B}, $\alpha_2\delta$ and β subunits, together with a novel neuronal specific 95 kDa component. Monoclonal antibodies specific for the α_{1B} subunit and polyclonal antibodies for each representative of four different β subunit genes have been exploited in further analyzing the subunit composition of these channels. Different β subunits were transiently transfected into COS7 cells. The cells were then harvested, subjected to SDS-PAGE and immunoblotted with affinity-purified antibodies to confirm the selectivity of each antibody. The α_{1B} specific monoclonal antibody coupled to Avidgel served to purify ω-conotoxin GVIA receptor solubilized from bovine brain membranes. Protein eluted at pH 2.5 was subjected to SDS-PAGE followed by Western blot analysis with affinity-purified antibodies specific for β_1, β_2, β_3 or β_4. This revealed that the α_{1B} subunit is associated with both β_3 and β_4 subunits in brain. In addition, the affinity of α_{1B} interaction domain (AID_B) for each of the *in vitro* translated ^{35}S-labeled β subunits was determined. The results demonstrate that β_3 and β_4 have similar affinities for the α_{1B} subunit of the N-type Ca^{2+} channels. Our data suggest heterogeneity in the β subunit composition of N-type Ca^{2+} channels.

508.3

AN ESSENTIAL STRUCTURAL DOMAIN THAT DETERMINES THE BIOPHYSICAL PROPERTIES OF THE HUMAN α_{1A} HIGH-VOLTAGE ACTIVATED CALCIUM CHANNEL. M.E. Williams, M. Hans*, P. Sionit, E.C. Johnson and S.B. Ellis. SIBIA, Inc., La Jolla, CA 92037.

The human α_{1A} and α_{1B} type calcium channels have distinct biophysical properties. Transient expression of human $\alpha_{1B\text{-}1}\alpha_{2b}\delta\beta_{1\text{-}3}$ calcium channel subunits in HEK293 cells results in robust voltage-activated barium (Ba^{2+}) currents (2.71±3.02 nA, n=13) which inactivate rapidly (t_{inac}= 200 ms). In contrast, co-expression of the $\alpha_{1A\text{-}2}\alpha_{2b}\delta\beta_{1\text{-}3}$ calcium channel subunits result in relatively small inward Ba^{2+} currents (0.185±0.166 nA, n=74) that inactivate more slowly (t_{inac}= 346 ms). The size of the α_{1A}-type current is independent of the α_{1A} splice variant or β subunit coexpressed. Moreover, α_{1A} calcium channels expressed in Xenopus oocytes gave rise to robust Ba^{2+} currents (>1 μA). To identify structural elements within the $\alpha_{1A\text{-}2}$ subunit responsible for the relatively small and slowly inactivating Ba^{2+} currents expressed in HEK293 cells, we constructed a series of chimeras combining portions of the $\alpha_{1A\text{-}2}$ and $\alpha_{1B\text{-}1}$ subunits and coexpressed them with $\alpha_{2b}\delta$ and $\beta_{1\text{-}3}$ subunits.

Expression of an α_{1A} subunit that contains the IVS3-COOH terminus of α_{1B} resulted in macroscopic Ba^{2+} currents with ~10x larger current magnitude than the wild type α_{1A} subunit. The inactivation kinetics of these Ba^{2+} currents were faster (t_{inac}=108 ms) compared to the wild type α_{1A} or α_{1B} calcium channel. In contrast, expression of an α_{1A} subunit containing only the COOH terminus of α_{1B} resulted in peak current amplitudes of <30 pA. Whole cell recordings of subsequent chimeras revealed that substitution of the α_{1B} IVS5-IVS6 region within the α_{1A} subunit is sufficient to enhance Ba^{2+} current amplitude (>30x) and increase the rate of inactivation. Site specific mutations within the α_{1A} IVS5-IVS6 segment are being used to help identify specific amino acids that affect the biophysical characteristics of human α_{1A} calcium channels. Further analysis is required to determine whether the increase in current magnitude is a result of an increase in the expression of the number of functional channels or, alternatively, reflect changes in the open probability of the channel.

508.4

Ca^{2+}-DEPENDENT INTERACTION OF N-TYPE CALCIUM CHANNELS WITH SYNAPTIC DOCKING/FUSION CORE-COMPLEX. Z.-H. Sheng*, J. Rettig, T. Cook, M. Takahashi#, and W. A. Catterall. Department of Pharmacology, University of Washington, Box 357280, Seattle, WA 98195-7280, USA, #Mitsubishi Kasei Institute of Life Sciences, Machida, Tokyo, Japan.

The specificity of Ca^{2+}-dependent exocytosis requires the tight structural and functional association of calcium channels with synaptic fusion machinery in the presynaptic active zone. To characterize this protein association, we performed binding experiments with recombinant proteins and partially purified, ^{125}I-ω-CTx labeled native N-type calcium channels. We report here that a syntaxin- and SNAP25-binding site is located in the cytoplasmic loop ($L_{II\text{-}III}$) between domains II and III of the N-type calcium channel (α_{1B}). The N-type calcium channels interact with syntaxin-SNAP25 heterodimer or syntaxin-SNAP25-VAMP trimeric complex in a Ca^{2+} dependent manner. These data suggest that the direct interaction of N-type calcium channels with synaptic core-complex is a Ca^{2+}-sensing process and may play a key role in docking and fusion of synaptic vesicles.

508.5

IDENTIFICATION OF A VESICULAR POOL OF CALCIUM CHANNELS IN *APLYSIA* BAG CELL NEURONS. *B.H. White* and L.K. Kaczmarek. Dept. of Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06520.*

Protein Kinase C (PKC) acutely up-regulates one of two calcium conductances in bag cell neurons (BCNs). Previous evidence suggests that PKC may do so by selectively recruiting channels to the membrane surface. To test this hypothesis we have made antibody probes to calcium channels expressed in BCNs by first amplifying calcium channel fragments by RT-PCR and then using the fragments to make fusion proteins for the production of polyclonal antisera.

PCR amplification yielded two distinct fragments, one (BCCa-I) with ~65% sequence identity to the corresponding regions of mammalian α_{1A} and α_{1B} calcium channels, the other (BCCa-II) with ~65% identity to the corresponding region of α_{1D} channels. Antibodies against the two fusion proteins specifically recognize distinct proteins of >200 kD on immunoblots of BCN membranes, consistent with recognition of calcium channel α_1-subunits. Immunofluorescent staining of cultured BCNs with the two antibodies yields strikingly different patterns. αBCCa-II antibodies uniformly stain BCNs, apparently at the level of the plasma membrane as suggested by confocal analysis. In contrast, αBCCa-I antibodies punctately stain the cytoplasm of BCNs, consistent with vesicular staining. Typically, BCN somata and growth cone core regions are stained, with variable staining of neurites. Occasional "hot spots" occur, often in regions of contact. We speculate that BCCa-I is the PKC-sensitive channel of BCNs.

508.6

A CALCIUM CHANNEL DENSITY GRADIENT IN NEUROBLASTOMA CELL GROWTH CONES Fritz Zimprich, Michael Duchen* and Stephen Bolsover. Department of Physiology, University College London, Gower Street, London WC1E 6BT.

Depolarization of N1E-115 neuroblastoma growth cones to voltages that activate L type calcium channels causes calcium to rise in spatially restricted hotspots. In the present study we selected non-branching growth cones in which the leading edge could be unequivocally identified. In these, a hotspot was usually located at the leading, distal edge so that on average the Fluo-3 fluorescence increase was significantly larger at the distal 5μm than in more proximal growth cone regions. Neither dantrolene at 40μM nor ryanodine at 200μM affected depolarization-induced calcium changes at the growth cone, indicating that calcium-induced calcium release from intracellular organelles can not account for the observed gradient.

Calcium channels of the T and L type were observed in cell-attached patches. L channels had a conductance of 24.8±0.8pS (n=12) in 110mM barium, but also displayed subconductances in the range 9 to 16pS. L channel open probability was 0.011±0.003 (n=9) at +10mV and was the same in all growth cone regions. The number of L channels per patch was six times greater in patches from the distal 4μm than in more proximal patches (taken 11 to 22μm from the tip). These results demonstrate directly that the calcium concentration gradient seen in depolarized growth cones has its origin in a gradient of L type calcium channel density.

508.7

FUNCTIONAL EXPRESSION OF A RAT CALCIUM SENSING RECEPTOR AND LOCALIZATION TO NERVE TERMINALS. M. Ruat, A.M. Snowman*, L.D. Hester, M.E. Molliver and S.H. Snyder. Dept. of Neuroscience, Johns Hopkins U. Sch. of Med., Baltimore, MD.

Calcium ions play a key role in a variety of neuronal functions. Recently, we cloned a calcium sensing receptor (CaSR) from a rat brain library displaying seven putative transmembrane domains and significant homology with the metabotropic glutamate receptors.

Immunochemistry performed on rat brain sections using a polyclonal antiserum developed against a peptide designed from the N-terminal domain of rat CaSR, reveals discrete punctate localizations throughout the brain reflecting nerve fibers and terminals. For example, staining surrounding the Purkinje cell bodies reflects terminals of basket cells, while the Purkinje cells themselves are not stained. Pyramidal cell bodies in the hippocampus are not immunoreactive but are surrounded by intensely stained puncta in CA1 to CA3 regions indicating synaptic terminals. Ependymal cells of the third ventricle are not labeled but are surrounded by positive staining indicating that CaSR is associated with a dense plexus of nerve fibers located at the ependymal surface. CaSR in the ependymal zone may respond to changes in ventricular calcium concentration whereas CaSR on nerve terminals may regulate the disposition of neurotransmitter in response to calcium levels in the synaptic space.

A Chinese Hamster Ovary cell line, CHO(CaSR), permanently expressing CaSR proteins was selected by western blot analysis. Preliminary experiments suggest that calcium and magnesium in the millimolar range stimulate second messenger pathways in CHO(CaSR) cells. Experiments are underway to characterize the pharmacology of these transduction systems.

508.8

TRANSMEMBRANE TOPOLOGY AND FUNCTIONAL ORGANIZATION OF THE VOLTAGE SENSITIVE CALCIUM CHANNEL $\alpha_2\delta$ SUBUNIT C.A.Gurnett, M. De Waard and K.P. Campbell*. Howard Hughes Medical Institute and Department of Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, IA 52242.

Voltage dependent Ca^{2+} channels minimally consist of three subunits: α_1, $\alpha_2\delta$, and β. Functional effects of the $\alpha_2\delta$ subunit on current amplitude appear dependent on coexpression of β subunits, suggesting either a direct interaction of the $\alpha_2\delta$ and β subunits or a modification of β subunit regulation of channel activity by $\alpha_2\delta$-α_1 interactions. Determination of the transmembrane topology of $\alpha_2\delta$ would support or refute a possible direct interaction with the entirely cytoplasmic β subunit based on the cytoplasmic orientation of $\alpha_2\delta$ in two different transmembrane models. We have utilized *in vitro* translation of full length and truncated $\alpha_2\delta$ proteins in the presence of canine microsomal membranes to investigate the transmembrane topology of the $\alpha_2\delta$ subunit. Deletion of sequences including the first and second hydrophobic domains have no effect on membrane association, suggesting that the hydrophobic region in δ is the sole transmembrane domain. Moreover, tryptic fragments of native skeletal muscle $\alpha_2\delta$ located between the first and second hydrophobic domains are shown to be glycosylated. These data support a model whereby all but the 5 carboxyl-terminal amino acids are extracellular and the $\alpha_2\delta$ must interact with the α_1 subunit through either the transmembrane domain in δ or extracellular sequences in $\alpha_2\delta$. Coexpression of these truncated $\alpha_2\delta$ proteins in *Xenopus* oocytes are underway to further identify regions of interaction between the $\alpha_2\delta$ and α_1 subunits.

508.9

VOLTAGE-DEPENDENT CALCIUM CHANNEL α_1 AND β SUBUNIT EXPRESSION AND INTERACTION IN PC12 CELLS. H.Y. Liu*, M. De Waard, C.A. Gurnett, and K.P. Campbell. HHMI, Dept. of Physiology and Biophysics, and Program in Neuroscience, University of Iowa, Iowa City, IA 52242

We have quantitatively studied the expression level of various voltage-dependent calcium channels in rat PC12 pheochromocytoma cell line. Ligand binding studies of [^{125}I] ω-conotoxin GVIA (ω-CgTX) and [^{3}H] PN200-110 demonstrate that these cells express about ten fold more N-type than L-type calcium channels. Purification data have demonstrated that both calcium channel types contain an α_1 and a β subunit. We have investigated the expression of various calcium channel α_1 and β subunits at the mRNA level by reverse-transcription polymerase chain reaction (RT-PCR). Sequencing of these RT-PCR products demonstrate that PC12 cells express at least three β genes (β_1, β_2 and β_3) and three α_1 genes (α_{1A}, α_{1B} and α_{1C}). In order to study the association of different β subunits with the α_{1B} subunit of the N-type calcium channels, we have produced β subtype specific antibodies against fusion proteins containing β subtype specific regions. These β antibodies recognize native β_1, β_2, β_3 and β_4 subunits from rat tissues. We also used mAb $VD2_1$ which recognizes the skeletal muscle L-type and brain N-type calcium channel β subunits, and also *in vitro* translated β_{1b}, β_{2a}, β_3 and β_4 gene products. In immunoprecipitation studies, mAb $VD2_1$ and polyclonal antibodies to β_3 and α_{1B} subunits all immunoprecipitate equal amounts of [^{125}I] ω-CgTX labeled receptor from solubilized PC12 cell membranes. Currently research is undergoing to determine how the expression level of the β subunits affects their association with the α_{1B} subunit in PC12 cells.

508.10

CHARACTERIZATION OF HUMAN N-TYPE VOLTAGE-GATED Ca^{2+} CHANNELS THAT BIND LAMBERT-EATON AND MONOCLONAL ANTIBODIES. V.A. Lennon, C.W. Zwizinski, T.J. Kryzer, E. Luoma, G.E. Griesmann, P.E. O'Suilleabhain, K.P. Campbell, E.H. Lambert*. Neuroimmunology Laboratory, Depts. of Immunology & Neurology, Mayo Foundation, Rochester, MN 55905, and Howard Hughes Medical Institute Research Laboratories, Univ. of Iowa, Iowa City, IA 52242.

The Lambert-Eaton myasthenic syndrome (LES) is an IgG-mediated presynaptic disorder of peripheral cholinergic neurotransmission that is highly associated with small cell lung carcinoma (SCLC). The targets of pathogenic autoantibodies are voltage-gated Ca^{2+} channels (VGCC) that control acetylcholine release at neuromuscular and autonomic synapses. By immunizing rats with a fusion protein containing a portion of the α_{1B} sequence we have produced monoclonal antibodies against this subunit. The rats did not develop a defect of neuromuscular transmission. One monoclonal IgG (MAb CC18) selectively precipitates N-type VGCCs (high affinity receptors for ^{125}I-labeled ω-peptide GVIA) from membranes of human cerebral cortex and SCLC, and binds to denatured α_{1B} (~230 kDa) in Western blots. MAb CC18 binds minimally to an α_{1A} fusion protein, and exhibits 0.002% cross-reactivity with P/Q-type VGCC's (high affinity ω-peptide MVIIC receptors) which are the presumptive neuromuscular target of LES IgG. The apparent molecular weight of the ω-peptide GVIA receptor (~900 kDa, by density sedimentation) increases more when complexed with LES IgG than with MAb CC18. This observation is consistent with binding of polyclonal IgG in LES serum to multiple epitopes on neuronal N-type VGCCs. These IgGs may be pertinent to the autonomic dysfunction of LES. *Supported by NCI grant CA-37343.*

509.1

NEWBORN MONKEYS HAVE WELL-SEGREGATED OCULAR DOMINANCE COLUMNS ORGANIZED IN AN ADULT-LIKE MOSAIC PRIOR TO VISUAL EXPERIENCE. J.C. Horton*, D.R. Hocking. Dept. of Ophthalmology, UCSF, San Francisco, CA 94143-0730.

To determine if visual experience is required for the formation of ocular dominance columns, time-mated Rhesus monkeys were delivered prematurely by C-section at E-157. Animals were kept in complete darkness and all procedures were performed with the aid of infrared night-vision goggles to eliminate light exposure. A day after C-section, ^{3}H-proline was injected into the right eye. A week later, at the equivalent of P-0 (165 days of gestation), alternate sections of visual cortex were prepared for autoradiography and cytochrome oxidase (CO). All three babies studied had well-segregated ocular dominance columns in striate cortex of both hemispheres, organized into the characteristic mosaic present in adults. The columns were always crisper in the cortex ipsilateral to the injected eye, suggesting that axon terminals driven by the ipsilateral eye segregate earlier and/or more completely than those driven by the contralateral eye, or that there is greater tracer spillover in the contralateral geniculate body. In the upper layers, a mature pattern of CO patches was visible, aligned with the ocular dominance columns in layer IV. Every other row of CO patches in layers II & III was labelled by ^{3}H-proline. In V2 a regular system of distinct pale, thick, and thin CO stripes was present. These findings indicate that stimulation of the retina by light is not necessary for the development of columnar systems in the visual cortex. Ocular dominance columns, CO patches, and V2 stripes are all well formed in newborns, prior to visual experience. Even the thalamic input to the CO patches in the upper layers is segregated by ocular dominance in newborns. (Supported by NEI EY10217)

509.2

NORMAL OCULAR DOMINANCE PLASTICITY WITHOUT LONG-TERM POTENTIATION IN THE VISUAL CORTEX OF PKA RIβ-DEFICIENT MICE. J.A. Gordon*[1], T.K. Hensch[1], E.P. Brandon[2], G.S. McKnight[2], R.L. Idzerda[2], and M.P. Stryker[1]. [1]Keck Center for Integrative Neuroscience, Univ. of California, San Francisco, CA 94143; and [2]Dept. of Pharmacology, Univ. of Washington Schl. of Medicine, Seattle, WA 98195.

An activity-dependent competition between axons for common targets sculpts connections in the developing primary visual cortex (V1). Homosynaptic Long-Term Potentiation (LTP), studied *in vitro*, is an attractive cellular candidate for the mechanism underlying these changes. To test this hypothesis, visual cortical plasticity was examined both *in vitro* and *in vivo* in mice with a targeted disruption in the gene for protein kinase A regulatory subunit Iβ (PKA RIβ). All experiments were conducted using critical period-aged animals (P21-P32) and performed blind to genotype.

We have previously demonstrated (Hensch et al.(1995), IBRO Abstracts) a dissociation between LTP and monocular deprivation (MD) effects in PKA RIβ knockouts (KOs). Layer IV theta-burst stimulation (TBS) failed to induce LTP in layer II/III field potentials in slices of V1 from KOs (post-TBS LTP=19±5% [WT] and 1±5% [KO]; $p<0.05$). 4 days of MD shifted cortical responses *in vivo* toward the open eye equally well in WTs and KOs ($p>0.8$).

A shift toward the open eye after MD could result merely from a decrease in the efficacy of deprived eye inputs, without any potentiation of open eye inputs. Recovery of responses to the initally deprived eye, however, requires a potentiation mechanism. After MD for an initial 5 days, reverse suture allowed a similar recovery of responses to the initially deprived eye in KOs and WTs, the extent of which increased with increasing time after the reversal.

In these mice, Hebbian potentiation of visual pathways *in vivo* occurred despite the absence of TBS-induced LTP during the critical period. Alternative cellular mechanisms of plasticity must, therefore, underlie the activity-dependent rearrangement of connections during visual cortical development. *Supported by the HFSP, ARCS, and HHMI.*

509.3

NPY mRNA EXPRESSION IN ORGANOTYPIC RAT VISUAL CORTEX CULTURES IS REGULATED BY THALAMIC AFFERENTS. K.Obst and P. Wahle*, Ruhr-Universität, Zoologie and Neurobiologie, 44780 Bochum, Germany.

We have reported, that the number of NPY mRNA expressing neurons in the rat visual cortex declines dramatically between postnatal day (P) 30 and 70, from peak values at P21 of 2-4 % to 1-2 % at P70 (Obst and Wahle, Eur. J. Neurosci., submitted). In contrast, in cortical monocultures prepared at P0 and maintained for up to 90 days *in vitro* (DIV) we do not observe a reduction in NPY mRNA expressing neuronal number. Moreover, the percentage of NPY mRNA expressing neurons in monocultures is 6-8 % up to 90 DIV and thus exceeds by far the percentage observed *in vivo*.

In co-cultures of rat visual cortex and thalamus (including the LGN) prepared at P0 the number of NPY mRNA expressing neurons declines dramatically from peak values of 6-8 % at 30 DIV to 2-3 % at 60 DIV and later on. The reduction is not due to an overall cell death, because thionin counterstained OTCs revealed that the neuronal number / unit area remained fairly constant in mono- and co-cultures from 30 DIV onwards. This suggests that the reduction observed *in vivo* and *in vitro* is dependent on the thalamic innervation, although it occured long after reciprocal connections between the two explants had been established.

In a triple arrangement consisting of one thalamus cortex co-culture and an additional cortex on the same coverslip, surprisingly 6-8 % of all neurons expressed NPY mRNA in both cortical explants. Further, medium from monocultures applied to co-cultures from 7 DIV onwards elicits an upregulation of NPY mRNA expression.

Therefore we suggest, that a noninnervated cortex releases a trophic factor into the medium, which leads to the NPY mRNA expression in a higher percentage of cortical neurons. In contrast *in vivo* or in pure co-cultures the production of this factor is progressively suppressed possibly by functional changes of the thalamic input later in development. Supported by DFG *"Neurovision"*.

509.4

NEUROTROPHINS AND ACTIVITY INTERACT TO MODULATE DENDRITIC GROWTH IN DEVELOPING FERRET VISUAL CORTEX A.K. McAllister*, D.C. Lo, and L.C. Katz Department of Neurobiology, Duke University Medical Center, Durham, NC, 27710.

The growth and rearrangement of neuronal circuits in visual cortex is strongly influenced by levels and patterns of neuronal activity, but little is known about the molecular signals that transduce activity into morphological changes. Neurotrophins are likely candidates for such molecular signals. To elucidate the influence of synaptic activity and its possible interactions with neurotrophins on dendritic growth, glutamate receptor antagonists—CNQX, a non-NMDA receptor antagonist, or APV, an NMDA receptor antagonist—were added alone or with particular neurotrophins to slices of P14 ferret visual cortex for 36 hours; dendrites of pyramidal neurons were visualized using biolistic transfection of a lacZ reporter construct. Glutamate receptor blockade alone enhanced growth and branching of basal dendrites in layers 4 and 6 (for example, total dendritic length (TDL) of layer 4 neurons: 243±20μm CNQX vs. 165±11μm for untreated (UT) cells, n=48 and 50, $p<0.01$). Brain-derived neurotrophic factor (BDNF) or neurotrophin-4 (NT-4) alone caused an even greater enhancement of dendritic growth and branching (TDL: 340±29 BDNF vs. 165±11 UT, n=52 and 50, $p<0.001$). However, glutamate receptor blockade completely prevented the BDNF-induced enhancement of growth (TDL: 156±19 BDNF+CNQX vs. 165±11 UT, n=52 and 50, $p=0.7$). Blockade of NMDA receptors alone similarly prevented NT-4-induced growth (TDL: 213±31 APV+NT-4 vs. 165±11 UT, n=45 and 50, $p=0.2$) while blockade of non-NMDA receptors was considerably less effective (TDL: 297±28 CNQX+NT-4 vs. 165±11 UT, n=45 and 50, $p<0.001$). These results suggest that neurotrophin effects on dendritic growth require glutamate receptor activation, implying that neurons require ongoing synaptic activity to be competent to respond to the growth-promoting effects of neurotrophins. Supported by NIH EYO7960 (L.C.K.) and NIH NS32742, the Klingenstein Fund, the Alfred P. Sloan Foundation (D.C.L.) and by Sigma Xi (A.K.M.). Neurotrophins were generously provided by Regeneron, Inc.

509.5

SPATIAL PATTERNS OF INHIBITION IN DEVELOPING AND ADULT VISUAL CORTEX: IMPLICATIONS FOR CORTICAL PROCESSING. M.B. Dalva*, M. Weliky, and L.C. Katz. Department of Neurobiology, Duke University Medical Center, Durham, NC 27710.

Inhibition plays a central role in modulating response properties of visual cortical neurons, but little is known about the organization of inhibitory connections onto single cells. To investigate the adult organization and development of inhibitory connections in primary visual cortex, we used scanning laser photostimulation (Dalva and Katz, Science **265**: 255 1994) to map the pattern of functional inhibitory synaptic inputs onto single neurons in coronal and tangential brain slices. At postnatal day 20 (P20), soon after layer 2/3 has formed, there are few inhibitory inputs onto pyramidal neurons (n=6 cells). These originate within 250μm of the cell body of the recorded neuron and are organized radially. Subsequently, the number and extent evoked inhibitory postsynaptic potentials (IPSCs) increases substantially, reaching a peak at eye-opening (P33-34, n=6). The density of inputs is highest near the cell body and decreases with distance, with no inputs of >1.5 mm. From eye-opening until adult (>P41), the number of IPSCs originating from sites >250 μm from the cell body declines dramatically (n=10), while the number of IPSCs evoked near to the cell body remains constant. In the adult (n=9), few IPSCs originate from >250μm from the cell body. Using coronal slices, inhibitory inputs to layer 2/3 pyramidal cells originate primarily from the supragranular layers of visual cortex (>80%, n=8). To examine the relationship between orientation selectivity and inhibitory connections, we determined the pattern of orientation tuning in striate cortex with optical imaging *in vivo*, and then recorded from single neurons with known orientation selectivity preference *in vitro* (n=1 animal, n=4 cells). As in other adult neurons, most inhibitory input was evoked from within 250μm of the cell body; the circular pattern of inhibition shows no clear relationship to the pattern of orientation selectivity. Taken together with our previous results, we conclude 1) that inhibitory inputs develop with a time course similar to excitatory connections, and 2) that inhibition arises from activation of all orientations, not just cross orientations. Supported by a McKnight Investigators Award (L.C.K.) and NIH grant EY07690 (L.C.K.).

509.6

DISRUPTION OF HORIZONTAL CONNECTIONS IN DEVELOPING VISUAL CORTEX INDUCES ORIENTATION MAP DISCONTINUITIES. M. Weliky* and L.C. Katz. Department of Neurobiology, Duke University Medical Center, Durham, NC 27710.

Iso-orientation domains in visual cortex form a periodic pattern of patches and bands. A number of computational models suggest that lateral interactions are required to generate such periodic patterns. To assess whether horizontal connections shape the organization of orientation domains during postnatal development, these connections were severed by making shallow cuts through the upper layers of developing visual cortex in young ferrets (age p33-35, n=4). Thin Teflon sheets (2.5 mm long by .7-.9 mm deep) were inserted into the cuts. At these ages, orientation tuning is still immature. Two to three weeks later, at which time orientation tuning has matured to an adult-like level, optical imaging of intrinsic signals was used to map the layout of iso-orientation domains. These maps revealed that iso-orientation domains were not continuous across the cut. Cross-correlation analysis demonstrated that the pattern of domains on either side of the cuts were uncorrelated. In contrast, when cuts were made at an age in which orientation tuning had matured to an adult-like level (>p43), orientation domains were more highly correlated across the cut, with correlations reaching random only relatively distant (~500 μm) from the cut (n=2). To assist in interpreting these results, "cuts" were made in simulations of cortical map development incorporating lateral excitatory and lateral inhibitory interactions. Cuts in the simulations induced patterns of discontinuities similar to those observed in the visual cortex. These results suggest that prior to the onset of robust orientation tuning, and prior to fully developed clustered axon collaterals, intracortical horizontal connections contribute to organizing the layout of orientation domains in visual cortex. Supported by NIH grant EY07690 (L.C.K.).

509.7

Mechanisms for initiation and propagation of intercellular calcium waves in the developing visual cortex. K. Kandler* and L.C. Katz. Department of Neurobiology, Duke University Medical Center, Durham, NC 27710.

During the development of the neocortex, many neurons are transiently coupled through gap junctions to form neuronal assemblies exhibiting coordinated activity in the form of intracellular Ca^{2+} transients (neuronal domains). To determine the identity of the intercellular signals, we investigated possible signaling molecules involved in gap junction communication between neurons of the visual cortex of early postnatal ferrets and rats.

Patch pipettes were used to inject the gap junction permeable second messenger inositol trisphosphate (IP_3) into individual neurons in slices loaded with the calcium indicator fura-2. In a second set of experiment we investigated the effects of the depletion of intracellular Ca^{2+} stores with thapsigargin (10 µM), the blockage of Ca^{2+} channels with Ni^{2+} (2 mM), and the stimulation of metabotropic glutamate receptors by t-ACPD (100 µM) on the occurrence of temperature drop (TD) elicited neuronal domains (Yuste et al, Neuron 14:7, 1995).

Injection of IP_3 into single neurons triggered intercellular Ca^{2+} waves which propagated to neighboring neurons. These waves covered an area of 2011 ± 438 μm^2 (mean ± SEM, n=14 waves), which is similar to the area of TD elicited domains (2791 ± 218 μm^2, n=64 waves). Depletion of IP_3 sensitive stores by thapsigargin dramatically reduced the occurrence of neuronal domains (control: 4.9 ± 1.3 domains/TD n=13 slices, thapsigargin: 0.8 ± 0.25 domains/TD, n=24, $p<0.01$). In contrast, blockage of Ca^{2+} channels by Ni^{2+} had no significant effect (2.8 ± 0.9 domains/TD n=5, $p>0.1$). Activation of metabotropic glutamate receptors by ACPD elicited neuronal domains (area 1942 μm^2 ± 201, n=47) probably by increasing the intracellular IP_3 concentration.

The present data, together with previous results demonstrate that cortical neurons can coordinate their behavior through biochemical signals, such as IP3, before they begin to communicate via classical synaptic transmission. We suggest that this novel form of neuronal communication underlies the propagation of intercellular calcium waves present in the developing visual cortex. In addition, domains may be "triggered" by local release of glutamate that interacts with the metabotropic receptors. Supported by the Alexander von Humboldt Stiftung (KK) and NIH grant NS32396.

509.8

OPTICAL RECORDING OF EARLY CALCIUM RESPONSES TO ATP IN THE FERRET CORTICAL VENTRICULAR ZONE. K. Herrmann* and R.O.L. Wong. Lab. Neurophys., NIMH, Poolesville, MD 20837 and Dept. Anat. and Neurobiol., Washington Univ. Sch. Med., MO 63110.

Transmitter-like molecules are now believed to be involved in early aspects of neuronal development such as cell division, migration and differentiation. These molecules affect cell development often by regulating intracellular calcium levels ($[Ca^{2+}]_i$). Recently, adenosine 5'-triphosphate (ATP) has been included in the list of extracellular ligands that mediate intercellular signaling in many cell types, including neurons. Here, we have examined whether cells in the mammalian cortical ventricular zone (VZ) are sensitive to ATP during the period of neurogenensis and early differentiation. We used optical recording techniques and calcium sensitive dyes to monitor the responses of cells in the VZ to exogenously applied ATP (0.05 mM to 1mM) in acute slices of ferret neocortex between embryonic day E31 (gestation is 41 days) and postnatal day P8. ATP evoked an increase in $[Ca^{2+}]_i$ in many cells in the VZ, both in the embryonic and neonatal neocortex. A larger proportion of cells in the VZ appeared responsive to ATP during the first postnatal week, than during the fetal ages. The magnitude of change in $[Ca^{2+}]_i$ observed in the VZ varied between tens to hundreds of nM. The ATP-evoked $[Ca^{2+}]_i$ rise resulted from an influx of Ca^{2+}, since it was blocked by BAPTA (2 mM) or Ni^{2+} (>3 mM). However, this response was not affected by the sodium channel blocker, tetrodotoxin (2 µM). Subsequent intracellular filling of ATP-responsive cells with lucifer yellow and neurobiotin revealed that they were predominantly progenitor cells, with thin processes that were oriented radially or tangentially. The fact that cells in the cortical ventricular zone are clearly responsive to ATP might indicate a role for this ligand in mitosis and/or early cell differentiation. Supported by a L.P. Markey Award to R.O.L.W.

509.9

DEVELOPMENTAL MECHANISMS UNDERLYING THE FORMATION OF LAYER-SPECIFIC CORTICAL CIRCUITS. V. Castellani and J. Bolz*, INSERM U.371 Cerveau et Vision, Bron, France.

There is compelling evidence that neuronal activity is required for the patterning of connectivity within individual cortical layers. In the visual cortex, for instance, neuronal activity controls the segregation of thalamic afferents into eye-specific patches in layer 4 and the formation of column-specific horizontal axonal projections in layers 2/3 (see Goodman and Shatz, Cell 72, 1993). In contrast, little is known about the mechanisms involved in the elaboration of the laminar specificity of cortical connections. Recent in vitro studies provided evidence for the existence of diffusible and substrate-bound guidance factors during the development of layer-specific afferent and efferent cortical projections (see Bolz et al., TINS 16, 1993). We examined whether molecules confined to specific cortical layers play a role in the construction of the intrinsic cortical circuitry. An important component of this circuitry are stereotyped vertical connections which link distinct cortical layers. For instance, axon collaterals of layer 6 neurons branch in layer 6 and the superficial layers, but there are very few branches in the intervening layer 5. In an in vitro assay, cortical explants from E15 or E16 rats (which contain layer 6 and subplate neurons) extended the same number of axons on cell membranes isolated either from layer 6, layer 5 or the superficial layers of P9-P11 rats. In contrast, when given a choice between membranes from superficial layers or layer 5, and likewise between membranes from layer 6 and layer 5, more than 80% and 70% respectively of the axons grew on membranes from the appropriate layer compared to layer 5. The most dramatic effect was observed on axonal arborizations: on layer 6 membranes, branching was 100%, and on membranes from superficial layers 110% more frequent than on layer 5 membranes. Thus substrate-bound molecules differentially expressed in cortical layers contribute to the laminar specificity of collateral branch formation of cortical neurons.

509.10

DEVELOPMENTAL MECHANISMS UNDERLYING THE SEGREGATION OF AFFERENT AND EFFERENT CORTICAL PROJECTIONS. D. Bagnard[1], F. Mann[1], S.Henke-Fahle*[2], and J. Bolz[1]. [1]INSERM U.371 Cerveau et Vision, Bron, France; [2]Dept. Ophthal., Univ. Tübingen, Germany.

The axonal pathway which links specific thalamic nuclei with distinct cortical areas precisely ordered. During cortical development, ascending thalamic axons travel in the subplate (SP) and in the outer region of the intermediate zone (IZ), whereas descending cortical axons grow in the deep region of the IZ towards their target. We have been studying the mechanisms that regulate the segregation of these reciprocal projections. As reported previously (Henke-Fahle et al., Neurosci. Abs. 1994), staining with different monoclonal antibodies directed against components of the extracellular matrix (ECM) transiently delineated the pathway of fibers running to and from the cortex. In a quantitative outgrowth assay, the antibody mab 10, which is concentrated in the SP and outer IZ, influenced thalamic and cortical axons in a 'push-pull' manner: it inhibited the growth of thalamic axons, but at the same time enhanced the outgrowth of cortical axons. However, this antibody did not interfere with the guidance of thalamic or cortical axons. We then used a co-culture system in combination with time-lapse video microscopy to examine potential interactions between individual cortical and thalamic axons. In cortex-cortex co-cultures prepared from E15 and E16 rats, over 60% of the axons encountering another cortical axon grew along each other. These results are consistent with the proposal that the corticofugal pathway is laid down by early pioneer fibers, and that later descending cortical axons extend along earlier-growing cortical fibers (McConnell et al., Science 245, 1989). In contrast, in cortex-thalamus co-cultures of the same developmental stage, in over 70% of the cases thalamic and cortical axons retracted from each other. Thus ECM components in the developing pathway, in concert with cues restricted to the surface of specific axonal populations, contribute to segregate afferent from efferent cortical projections.

GENESIS OF NEURONS AND GLIA III

510.1

THEORETICAL AND EXPERIMENTAL ANALYSES OF LATERAL INHIBITION AND THE ROLE OF NOTCH AND DELTA IN PATTERNING THE XENOPUS NEURAL PLATE. A. B. Chitnis*, D. Henrique and C. R. Kintner, MNL, The Salk Institute, La Jolla, CA 92037 and ICRF Developmental Biology Unit, Oxford OX1 3PS.

Lateral inhibition is a type of cell-cell interaction where a cell that adopts a particular fate inhibits its neighbors from adopting the same fate. This kind of interaction has been shown to play an important role in restricting the number of neural precursors in the Drosophila nervous system. Due to lateral inhibition only a single cell in each proneural cluster eventually delaminates and differentiates as a neuroblast while the rest adopt an epidermal fate. The process of lateral inhibition is mediated by the neurogenic genes Delta and Notch, which encode an inhibitory signal and receptor, respectively, in this process. In Xenopus the CNS is derived from a part of the ectoderm that segregates to form the neural plate; the rest of the ectoderm adopts an epidermal fate. Cells within specific longitudinal domains in the neural plate acquire the potential to differentiate as neurons. However, only a small subset of these cells eventually differentiate as neurons.

We have theoretically and experimentally explored the role of local interactions in patterning the early Xenopus CNS. Computer simulations show that, in principle, lateral inhibition alone could refine the limits of the neural plate, define longitudinal domains with neurogenic potential within the neural plate and select neuron precursors within these domains. Analysis of the effects of experimentally manipulating the activity of Xenopus Notch1 and Xenopus Delta1, however, suggests that these genes mainly restrict the number of neurons that differentiate within specific longitudinal domains in the neural plate. Supported by NIH.

510.2

THE ROLE OF THE *XENOPUS SHAGGY* KINASE IN NEURAL INDUCTION AND *XOTCH*-MEDIATED LATERAL INHIBITION. E. A. Marcus*, D. A. Wettstein, W. A. Harris and C. R. Kintner, Biology Department, UCSD and the Salk Institute, La Jolla, CA 92093.

In light of the recent demonstration that *Xenopus Notch* mediates lateral inhibition of cell fate in the neuroectoderm of developing *Xenopus* embryos, identification of the intracellular events downstream of *Xotch* activation may provide insights into the molecular basis of primary neurogenesis in vertebrates. One candidate element for the signal transduction cascade triggered by *Xotch* receptor activation comes from genetic analyses in *Drosophila* where it has been shown that a null mutation at the locus encoding the protein kinase *shaggy* mimics the neurogenic phenotype of a *Notch* loss-of-function mutation. Moreover, it has also been shown that the *shaggy* null phenotype is epistatic to a *Notch* gain-of-function phenotype in double mutants, suggesting that activity of *shaggy* is required for *Notch*-mediated signaling. In the present study we have cloned and sequenced the *Xenopus* homolog of *Drosophila shaggy* and are examining its role in neural induction and *Xotch*-mediated lateral inhibition in *Xenopus* embryos.

The *Xenopus shaggy* homolog (*X-shaggy*) was cloned by low stringency screening of a *Xenopus* neurula cDNA library using a probe generated by random priming of a human homolog of *shaggy, GSK3b*. The isolated full-length cDNA was sequenced and used to generate a digoxygenin-labeled riboprobe for *in situ* hybridization. At the neural plate stage, *X-shaggy* mRNA is expressed in three dorsal longitudinal stripes. These stripes overlap with the expression of b-tubulin mRNA which selectively labels primary neurons. In later stage embryos, *X-shaggy* is expressed in the eye, cranial nerves, neural tube, notochord and somites. We are currently misexpressing wild-type *X-shaggy* and dominant negative *X-shaggy* DNA constructs alone and in combination with an activated *Xotch* construct in *Xenopus* embryos to determine whether *X-shaggy* plays a role in *Xotch*-mediated lateral inhibition during primary neurogenesis.

510.3

PITUITARY ADENYLATE CYCLASE ACTIVTING PEPTIDE (PACAP) IS AN AUTOCRINE REGULATOR OF CEREBRAL CORTICOGENESIS N. Lu* and E. DiCicco-Bloom, Dep't of Neuroscience & Cell Biology, UMDNJ/Robert Wood Johnson Medical School, Piscataway, NJ.

The sequence of cerebral cortex development has been characterized extensively *in vivo*: young neurons undergo radial migration and differentiation only after precursor proliferation in the ventricular zone has ceased. While molecules stimulating proliferation have been identified, including IGF-I and bFGF, little is known about factors signaling the transition from proliferation to differentiation. Previously, we found that PACAP promotes this ontogenetic switch, inducing mitotic arrest while enhancing neurite outgrowth and *trk*B. The expression of PACAP ligand and receptor mRNA and protein in embryonic brain raised the possibility of autocrine function of the peptide during ontogeny, for which we now provide evidence.

To examine autocrine function, a virtually pure population of MAP-2 positive precursors, derived from embryonic day 13.5 rat cerebral cortex, was cultured for 24 hr in defined medium. Using antiserum raised against PACAP, 83% of cells expressed immunoreactivity which was blocked by PACAP but not the related peptides VIP, Sec, and PHI. In addition to peptide, 67% of precursors expressed PACAP receptor, consistent with a possible autocrine role.

Since PACAP receptors activate cAMP pathways, we examined effects of peptide on nuclear expression of phosphorylated CREB, a well-characterized transcription factor downstream of cAMP. While 3% of control cells exhibited staining, PACAP treatment increased phosphorylated CREB immunoreactivity to 69%, corroborating receptor staining, and indicating that peptide elicits nuclear signaling in cortical precursors.

To establish an autocrine circuit, in which released PACAP inhibits ongoing mitosis, a specific antagonist,$PACAP_{6-38}$, was employed. The antagonist control blocked PACAP-induced mitogenic stimulation in sympathetic cultures. In cortex cultures, antagonist increased by 57% the proportion of mitotic neuroblasts (Con= 10±0.3; Antag=16±1.1), consistent with interruption of ongoing mitotic inhibition mediated by PACAP. These observations suggest that PACAP is an endogenous cortical factor regulating the transition from precursor proliferation to neuronal differentiation.

510.4

CELL PROLIFERATION OBSERVED WITHIN THE RAT CEREBRAL NEUROEPITHELIUM IN VITRO

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We know that the majority of neurons of the mammalian cerebral cortex are generated from precursor cells within the pseudostratified neuroepithelium but we know very little of the nature of the cell movements and interactions that underlie neurogenesis within this region. To address this problem a number of methodologies are being used to follow the behaviours of cells in the neuroepithelium of the developing rodent cerebral cortex *in vitro*. Organotypic cultures of sheets and slices of cortex continue to display mitotic activity for one or two days after explantation into a defined culture medium. Vital chromatin dyes and lineage tracers may then be applied *in vitro* and the stained cells followed by time lapse confocal microscopy over periods of several hours. The mitotic process at the ventricular surface may be followed in great detail. It has already been reported that the mitotic spindles of the vast majority of cells align parallel to the surface of the tissue and that the spindles of many cells actively rotate within the plane of the tissue through metaphase and but stop at the onset of anaphase (Adams, Soc Neurosci Abstr. **20**:30 (1994)). The statistical alignment of rotating metaphase spindles is strongly biased parallel or antiparallel to the direction of anaphase and cell division. Consistent with the orientation of cell division being determined at the entry into mitosis. The fates of these dividing cells are now being investigated in more detail to related this process to the generation of post mitotic neurons.

510.5

DIFFERENTIAL REQUIREMENT FOR BCL-X DURING THE DEVELOPMENT OF NEURONS AND GLIA. N. Motoyama*, K. A. Roth†, S. Senju, D. Y. Loh. H. H. M. I., and †Department of Pathology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Regulation of programmed cell death (apoptosis) is thought to be important for the development and homeostasis of multicellular organisms. In the developing nervous system, more than 50% of neurons undergo programmed cell death. Bcl-x is one member of the Bcl-2 gene family, which regulates programmed cell death. Bcl-x is widely expressed in the nervous system during development and in the adult. Many differentiating immature neurons of central and peripheral nervous systems in Bcl-x-deficient embryos undergo apoptosis (N. Motoyama et al., Science. **267**, 1506, 1995). Because of embryonic lethality, we could not analyze the role of Bcl-x in late-embryonic development or in the adult nervous system using germline mutant mice.

Here, we generated Bcl-x-deficient somatic mutant chimeras using the double knock-out method. We injected *bcl-x*$^{-/-}$ embryonic stem (ES) or control parental wild-type ES cells into blastocysts from C57BL/6 (B6) mice to generate chimeric animals. By glucose phosphate isomerase allozyme analysis, which allows us to distinguish between ES and B6-derived cells, we showed that the contribution of Bcl-x-deficient ES cells to the brain, spinal cord, sensory ganglia and eye was small compared to other organs such as kidney, heart, and lung. In situ hybridization for an ES cell-specific marker was performed to determine the cell-specific contribution of ES cells to the nervous system. In comparison to control chimeras, *bcl-x*$^{-/-}$ chimeric mice exhibited a marked decrease in the number of ES cell derived neurons in the brain, spinal cord, and sensory ganglia. ES cell derived glial cells were comparable in control and *bcl-x*$^{-/-}$ chimeric mice. These finding suggest that Bcl-x is required for the development and survival of the vast majority of neurons but not for glia.

510.6

LOCAL INDUCTION OF OLIGODENDROCYTE PRECURSORS IN EMBRYONIC CHICK SPINAL CORD BY THE NOTOCHORD. D. M. Orentas* and R. H. Miller. Department of Neurosciences, Case Western Reserve University, Cleveland, Ohio, 44106.

During spinal cord development oligodendrocytes, the myelinating cells of the CNS, arise from precursors initially located in the ventral ventricular zone. In the chick spinal cord at E6 (stage 29) oligodendrocyte precursors, identified by the mAb O4, are neuroepithelial cells located directly dorsal to the floorplate. The dorsal-ventral polarity of the developing spinal cord appears to be established by signals derived from the notochord and such signals may also influence the initial location of oligodendrocyte progenitors. To determine whether the notochord had the capacity to locally induce spinal cord oligodendrocyte precursors, additional notochords were transplanted adjacent to the dorsal neural tube and host embryos allowed to develop to at least E6. O4+ cells developed in the dorsal neural tube adjacent to the transplanted notochord. Heterochronic transplants demonstrated that the notochord was capable of inducing O4+ oligodendrocyte precursors in the dorsal neural tube until stage 12 (E2). Similarly, dorsal neural tubes were competent to respond to notochord induction until the same stage. The localization of SC-1+ floorplate cells in experimental animals suggested that induction of floorplate did not directly correlate with the induction of O4+ oligodendrocyte precursors. To assess the requirement for the notochord during normal development of oligodendrocytes, the endogenous notochord was ablated at different developmental stages. Ablations subsequent to stage 12 did not perturb the pattern of oligodendrocyte precursor development. These data suggest that signals originating from the notochord are capable of inducing oligodendrocyte precursors from cells in the dorsal neural tube. Furthermore, after E2 signals from the notochord are not essential for the normal development of spinal cord oligodendrocytes at E6. The 4 day time lag between the signaling from the notochord and appearance of oligodendrocyte precursors in the spinal cord suggest an indirect mode of signaling. If the notochord was releasing a signal directly inducing O4+ cells, such a signal would be need to be long acting and stable. If however, the notochord is directing ventralization of the neural tube, signals from the notochord may act upon intermediate structures which are then capable of inducing oligodendrocyte progenitors.

510.7

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED mRNA SPECIES IN THE DEVELOPING CENTRAL NERVOUS SYSTEM OF MOUSE Nakayama K.,Tomooka Y.[2], Okada G., Moriizumi T.[3]* Iwai K. and Takahashi M. Dept. Immunobiology, Cancer Res. Inst. Kanazawa Univ., 13-1 Takaramachi, Kanazawa, Ishikawa 920, Japan., 2) Dept. Biological Science and Technology, Science Univ. of Tokyo, 3) Dept. Anatomy, Medical school, Shinsyu Univ.

Vertebrate central nervous system(CNS) derives from a neural tube, in which pluripotent neural precursor cells (NPCs) proliferate and differentiate into various types of neuronal and glial cells. The molecular mechanisms by which NPCs become committed to distinct lineages are not well understood. As a first step to clarify the mechanisms of NPCs differentiation, we screened for genes activated or inactivated during NPC differentiation. We isolated NPCs from mouse neural tube at embryonic day 10 for primary culture. In primary cultures, NPCs differentiate into neural and glial cells by following a time schedule similar to that observed in mouse embryos. Total RNAs were isolated from primary cultures at 2 day intervals, followed by differential display method using the four degenerated dT12MN primers in combination with 26 arbitrary 10mers. In general, each primer set could display more than 100 bands. Visual survey of the mRNA differential display with 104 primer set combinations revealed less than 0.5% bands that appeared to be differentially expressed during NPC differentiation. These bands were cloned for further analysis. Based on time courses of gene expressions , we classified differentially expressed genes under three classes; (1) inactivated genes (decreased expression), (2) activated genes (increased expression), (3) intermediate genes (expressed transiently). These genes should be useful as markers in the molecular dissection of neural development.

510.8

Screening for differentially expressed genes in CNS injury and development using multiplex arbitrarily primed PCR (MAPP)

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Investigating the genetic network program which underlies CNS development and which may become reactivated following CNS injury requires an efficient, unbiased screening technique for the identification of genes which participate in these processes at all levels. We have optimized an arbitrarily-primed PCR technique for this purpose. Since we have found that the principle of arbitrary priming also results in the production of artifacts during high-gain amplification, it is important that primers and reactions conditions are carefully matched. We have eliminated primer concatemer artifacts by using a high-fidelity, low-cycle PCR protocol with modified primers to amplify the first strand PCR products created under low fidelity conditions. This approach requires longer primers than used in the "differential display" protocol, but also takes advantage of the T12VN reverse transcription combined with arbitrary forward priming. We have found that T12VN priming is not confined to the poly-A tail and that forward primers can also operate in the reverse direction because of the inherent reverse transcriptase activity of the Taq polymerase. Nevertheless, we were able to generate diverse and reproducible gene expression spectra that enabled us to identify genes selectively regulated during spinal cord development, PC12 cell differentiation and in response of adult dentate gyrus to kainate-induced seizures. A variety of genes were identified related to redox metabolism and apoptosis, macromolecule synthesis and folding, transcriptional regulation, and neurotransmitter phenotype. Furthermore, we discovered many novel sequences not part of the Genbank database. The expression and regulation of these novel genes was confirmed by PCR using sequence specific primers.

510.9

LOCALIZATION OF mRNA FOR UDP-GALACTOSE: CERAMIDE GALACTOSYLTRANSFERASE IN MOUSE BRAIN DURING DEVELOPMENT. Tetsushi Kagawa, Akio Oba, and Kazuhiro Ikenaka*. Lab. of Neural Information, National Institute for Physiological Sciences, Okazaki, 444, JAPAN.

UDP-galactose: ceramide galactosyltransferase (CGT) is considered to be a key enzyme in the biosynthesis of galactocerebroside, which is absolutely enriched in oligodendrocyte in CNS and is the most frequently used marker of oligodendrocyte. Thus, CGT gene expression should be closely linked to oligodendrocyte development. We applied the in situ hybridization technique to determine the localization of CGT mRNA in mouse CNS during development. Serial paraffin sections were collected and hybridized with CGT cRNA probe labeled with digoxigenin-UTP (DIG-UTP) or with myelin proteolipid protein (PLP) DIG-cRNA probe which can detect both PLP and its isoform DM-20, a putative marker of the progenitor cells of oligodendrocytes. At postnatal day 20 (P20), CGT gene expression was highly restricted to the CNS white matter, which was a quite similar to the pattern of PLP/DM-20 gene expression. We could detect the CGT gene expression in the developing brain and spinal cord as early as embryonic day 17 (E17). The CGT-positive cells were restricted to a longitudinal column of cells in the basal half of ventricular zone around the ventricles quartus. The strongest signals were observed at the most caudal level of medulla oblongata. The PLP/DM-20 gene expression pattern is very similar to that of the CGT gene except in the anterior hypothalamus, where we detected only PLP/DM-20 signals. After birth, CGT-positive cells were distributed in caudal to rostral and ventral to dorsal gradient, for instance we detected the CGT mRNA from P2 in cerebellum.

510.10

CELLULAR BASIS OF METHYLMERCURY POISONING IN MOUSE NEOCORTEX. N.L. Hayes*, M. Philbert, K.R. Reuhl, and R.S. Nowakowski. Dept. of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854 and Dept. of Pharmacology and Toxicology, Rutgers University, Piscataway, NJ 08854.

Methylmercury (MeHg), a widespread environmental neurotoxicant, has a profound effect on cell proliferation in the developing CNS. MeHg (5μg/g) was given intraperitoneally to pregnant CD-1 mice on embryonic day 14 (E14) either 4 hrs prior to double labeling with 2 S-phase markers (iododeoxyuridine (IUdR) followed 2 hrs later by bromodeoxyuridine (BUdR)) or between the two S-phase markers (1 hr after IUdR and 3 hrs before BUdR). Thirty minutes after the BUdR injection embryos were fixed and processed for immunohistochemical visualization of the two markers. Labeled and unlabeled cells in the ventricular zone of the neocortical anlage were counted and labeling indices (LI) calculated. The LI of all nuclei labeled by the second tracer (LI:BUdR) is a measure of the proportion of the total population in S-phase at the time of the BUdR injection, and a measure of the ratio of the S-phase to the total cell cycle, Ts/Tc. The LI of cells labeled by the first tracer only (LI:IUdR) is a measure of the proportion of cells which left S-phase during the interinjection interval. We found a decrease in LI:BUdR in both experimental groups, indicating that, in acute MeHg-treated animals, the Ts/Tc ratio is reduced and the proportion of proliferating cells in S-phase is decreased. A small increase in the LI:IUdR indicated that the effect on the cells exiting S-phase is minimal. The ratio LI:IUdR/LI:BUdR showed that the change in LI:IUdR is insufficient to account for the larger change in LI:BUdR. The most parsimonious explanation of these data is that MeHg reduces the number of cells entering S-phase by lengthening G1 and/or M.

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510.11

CROOKED TAIL (Cd): A MOUSE MODEL OF BRAIN MALDEVELOPMENT & ROSTRAL NEURAL TUBE DEFECTS Y Oofuji, ML Carter, CJ MacNabb, & ME Ross*, Dept of Neurology, Univ of Minnesota, Minneapolis, MN 55455.

Cd is a semidominant mutation in which heterozygote tails are kinked and homozygotes have a high incidence of exencephaly, a rostral neural tube (NT) defect related to anencephaly. We previously cloned a message form of the cell cycle regulatory gene, D2 cyclin (J. Neurosci, 14:6384, '94), which has a highly restricted temporal and anatomic expression pattern in brain during development. On a map of the mouse genome (Jenkins & Copeland, NCI) the MN20/D2 cyclin probe was localized to chromosome 6, near the purported locus for Cd (Lane, '72). To further investigate MN20/D2 cyclin as a potential candidate for the Cd allele, we compared the developmental anatomy of Cd exencephaly with the timing and sites of MN20 expression during normal embryogenesis. On whole mounts and sections of Cd embryos, NT closure was observed to fail at the level of the mesencephalic vesicle by embryonic day (E)10.5. By in situ hybridization, MN20/D2 cyclin mRNA was expressed heavily in the roof of the mesencephalic vesicle and was just detectable in the telencephalon at E10.5, the earliest age yet examined. By E12.5, MN20/D2 cyclin expression is shifted posteriorly to the tectum, and is heavily expressed at the top of the cerebral wall. To define more precisely the chromosome locus for Cd, further linkage analysis was carried out by PCR-based, simple sequence length polymorphism (SSLP) in an intraspecific backcross. Thus far, 30 N2, affected progeny have been genotyped, identifying a linkage group in which the D6Mit300 marker has a LOD score of 9 relative to Cd. We conclude that: (1) Cd exencephaly results from failure of NT closure at the mesencephalic level at ~E10; (2) the temporo-spatial pattern of MN20/D2 cyclin expression correlates well with the site of origin and developmental time-course of Cd exencephaly; (3) SSLP-based linkage analysis supports the MN20/D2 cyclin gene as a candidate for the Cd locus. Thus, abnormal expression of D2 cyclin may be the molecular substrate for the Cd phenotype.

510.12

Developmental expression of novel gene(PK4) mapped on human chromosome 17p13

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Recently, surmountable amounts of genes are being cloned and it has become neccessary to develope new techniques for discovering genes with known chromosomal location and their possible functions.

We made cDNA library from 18 weeks old human fetal brain assuming it expresses many genes involved in neuronal development. To define the location on the chromosome we performed fluorescent *in situ* hybridization on human chromosome with these cDNAs. The bands that showed strong hybridization with our cDNAs were microdissected and amplified by PCR. PK4 was amplified from 17p13 and found to be the new gene by the sequence analysis. To predict the role of this gene, *in situ* hybridization histochemistry was performed on developing and adult rat.

In the nervous system, strong expression of PK4 close to the ventricle was observed in E13 to E16. In the dentate gyrus, PK4 was highly expressed in comparison with other areas of hippocampus in P21. From P7 to P14, PK4 was extensively expressed in the external granular layer.

From these results, we suspect that PK4, located on human chromosome 17p13, may be related with neurogenesis.

510.13

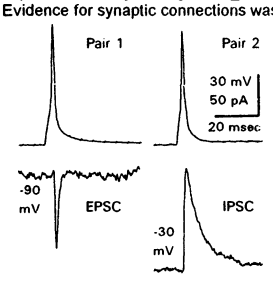
SEX DIFFERENCES IN ADULT NEUROGENESIS IN THE WILD-TRAPPED MEADOW VOLE. L.A.M. Galea* and B. S. McEwen, Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY, USA.

Sex differences favoring males in spatial learning in the adult meadow vole appear to be related to gonadal hormone level and photoperiod. Dentate gyrus and hippocampal size differences are also associated with sex and hormone level in the meadow vole. Adult neurogenesis is seen in mice and rats in the dentate gyrus of the hippocampus. The purpose of this study is to examine the relationship of sex and reproductive status in adult neurogenesis in the dentate gyrus of wild-trapped meadow voles. Wild meadow voles, *Microtus pennsylvanicus*, were captured during a short photoperiod (November, January) in Millbrook, NY. Voles were injected 24 h following capture with ^{3}H-thymidine (5.0μCi/g), a marker of DNA synthesis, and perfused 24 h following injection. Brains were processed for autoradiography and stained for cresyl violet. Significant sex differences favoring females in the number of dividing ($p=.0006$) and degenerating cells ($p=.028$) were shown in both the granule cell layer and the hilus of the dentate gyrus. Voles are also being trapped during the long photoperiod (May, June) in order to compare levels of adult neurogenesis with voles trapped during the short-photoperiod. The meadow vole model may help to elucidate the mechanisms underlying the dynamic nature of cell birth and death in the hippocampus. Research supported by an NSERC-PDF to LAMG and a MH41256 to BSM.

510.14

SYNAPSE FORMATION AND ESTABLISHMENT OF POLARITY BY NEURONS DERIVED FROM P19 EMBRYONIC CARCINOMA CELLS. MFA Finley,* N Kulkarni, and JE Huettner. Department of Cell Biology & Physiology, Washington University, St.Louis, MO 63110.

P19 embryonic carcinoma cells adopt a neuronal phenotype following exposure to retinoic acid. We have used electrophysiology and immunofluorescence to study the development of synaptic connections and neuronal polarity by P19-derived neurons. Paired recordings from P19 cells grown on microislands of rat astrocytes revealed that about 25% of the cells evoked fast excitatory currents (Pair 1) while roughly 5% of the cells elicited rapid inhibitory currents (Pair 2). The remaining cells failed to evoke a detectable postsynaptic response. Nearly all of the epscs were blocked by CNQX; the ipscs were inhibited by bicuculline or in some cases by strychnine. The tau of single-exponential decay averaged 1.4±0.2 msec for epscs and 12±2 msec for ipscs. Evidence for synaptic connections was first recorded 10 days after plating.



Staining with antibodies to GAP-43 and MAP-2 in P19 mass cultures revealed clear segregation into separate axonal and somato-dendritic compartments, respectively. Consistent with our physiological evidence for synapse formation, we observed intense punctate staining with antibodies to the synaptic vesicle proteins SV2 and synaptophysin. Collectively, these data suggest that P19 neurons develop polarity and form functional synapses in a manner similar to normal CNS neurons.

510.15

GABA ELEVATES CYTOPLASMIC CALCIUM IN FRACTIONATED EMBRYONIC RAT CORTICAL CELLS H. Xian, D. Maric, I. Maric and J.L. Barker*, Lab. of Neurophysiology, NINDS, NIH, Bethesda, MD 20892

Modulation of calcium homeostasis by GABA may play an important role in the early developmental processes occurring in the CNS, such as cell proliferation, differentiation and migration. We have found that during CNS development, embryonic cells exhibit different buoyant densities reflecting differences in their ratio of nuclear mass to cytoplasmic volume. Subpopulations of cells can be isolated using gradients in buoyant density during both proliferative and differentiating periods. We have isolated cells according to their buoyant density from the telencephalon-cortex at different embryonic ages to study the developmental appearance of membrane properties. Here we have studied the effects of GABA on cytoplasmic Ca^{2+} (Ca^{2+}_c) in several fractions at later embryonic ages using digital video-microscopy and fura 2. We found that GABA elevated Ca^{2+}_c in subpopulations of cells in the most buoyant fractions. The elevation in Ca^{2+}_c varied from 50 to 200 nM. At E16 - E19, this GABA-induced increase in Ca^{2+}_c depended entirely on extracellular Ca^{2+}, since the response was eliminated in Ca^{2+}-free medium. Moreover, the response appeared to be mediated through activation of $GABA_A$-type receptors since the $GABA_A$ receptor antagonist bicuculline (100 μM) inhibited it completely and reversibly. Pretreatment of cells with thapsigargin (3 μM) or ryanodine (1 - 10 μM) did not prevent the GABA-induced increase in Ca^{2+}_c in most of the cells tested; however, in some cells, the GABA response was eliminated by ryanodine. Most GABA-responsive cells showed an increase in Ca^{2+}_c following exposure to 10 - 40 mM K^+. However, a small percentage did not increase Ca^{2+}_c over this range of [K^+]. These results suggest that GABA-induced increases in Ca^{2+}_c involve Ca^{2+} influx perhaps via voltage-dependent Ca^{2+} channels or receptor- and/or second messenger-operated mechanisms in the plasma membrane, as well as release of intracellular Ca^{2+} from ryanodine-sensitive stores.

510.16

PROLIFERATIVE AND DIFFERENTIATING STAGES OF NEOCORTICAL DEVELOPMENT CAN BE RAPIDLY ACCESSED BY CONTINUOUS GRADIENTS IN BUOYANT DENSITY: II. A FUNCTIONAL CHARACTERIZATION. D. Maric*, I. Maric, and J.L. Barker, LNP, NINDS, NIH, Bethesda, MD

We have used the property of cellular buoyant density to selectively fractionate rat cerebral cortical cells with phenotypes ranging from proliferatively active, tetanus toxin receptor (TnTx-R) lacking to terminally postmitotic, high TnTx-R expressing. Here we test the expression of functional Na^+ channels and GABA, glycine (GLY) and glutamate (GLU) receptors on 20 distinct buoyant density subpopulations of cortical cells isolated on continuous Percoll gradients at embryonic day 19. The cells were double-loaded with voltage-sensitive (oxonol) and Ca^{2+}-sensitive (Fluo-3) fluorescent indicator dyes and their responses to veratridine (VTD), a Na^+ channel agonist, GABA, muscimol (MUSC), a $GABA_A$ receptor agonst, GLY, GLU and kainic acid (KA), an agonist of a subset of GLU receptors, recorded using a dual-laser flow cytometer. The least buoyant and most proliferative cell fraction produced depolarizing responses to VTD, GABA/MUSC and to GLY, but not to GLUT or KA. However, none of these responses, with the exception of a subpopulation of cells depolarizing to VTD, involved a elevation of Ca^{2+} ions. As the cells became more buoyant and lost their proliferative potential, presumably due to their progressive commitment toward differentiation, the percentage of cells that depolarized to all of the above ligands increased, and the rises in intracellular Ca^{2+}, paticularly in response to GLUT and KA became more apparent. At the other end of the spectrum, almost every cell of the most buoyant and virtually completely postmitotic cortical subpopulations produced the most intensive depolarizing responses to all of the above ligands which were accompanied by submicromolar increases in intracellular Ca^{2+}. Most of this Ca^{2+} elevation was presumably achieved via activation of voltage-dependent Ca^{2+} channels which appear to be absent in the least buoyant, most proliferative cells. The results show that buoyant density can be used as a simple property in fractionating distinct cortical subpopulations based on their proliferation/differentiation status and can be readily applied in order to gain access to different stages of neocortical development.

510.17

PROLIFERATIVE AND DIFFERENTIATING STAGES OF NEOCORTICAL DEVELOPMENT CAN BE RAPIDLY ACCESSED BY CONTINUOUS PERCOLL GRADIENTS I. IMMUNOCYTOCHEMICAL CHARACTERIZATION. I. Maric*, D. Maric, and J.L. Barker LNP, NINDS, NIH, Bethesda, MD

We evaluated specific proliferation/differentiation patterns in the embryonic (E) rat neocortex at a single cell level using continuous density gradients of Percoll, immunocytochemistry and flow cytometry. Timed pregnant E19 Sprague-Dawley rats were given a single intraperitoneal injection of BrdU (50 μg/g b.w.) and sacrificed one hour later. The cortical region of the embryonic CNS was dissociated into a single cell suspension and subpopulations of cells further separated based on their natural specific buoyant density using continuous Percoll gradients. Cell proliferation was assessed by anti-BrdU-FITC immunoreaction, propidium iodide staining and two color flow cytometry. Cell differentiation was evaluated using single and double immunostaining with antibodies (Abs) against nestin, tetanus toxin (TnTx) and GFAP. Cortical cells separated reproducibly into multiple specific bands. After immunostaining, flow cytometric analysis revealed that fractionated cells showed a remarkable correlation between their buoyant densities and proliferative and differentiating stages of neocortical development. The most buoyant cells, accumulated in the bands at the top of the gradient, were all $BrdU^-$, $GFAP^-$ and virtually all $TnTx^+$, indicating that most were well-differentiated neurons. Double staining with TnTx and nestin Abs revealed that a small percentage of $TnTx^+$ cells were also $nestin^+$. Going toward the bottom of the gradient, each subpopulation consisted of increasing number of smaller, less buoyant cells that were progressively more $BrdU^+$ and $nestin^+$ and less TnTx single positive. In contrast, the least buoyant cells isolated at the bottom of the gradient were all $TnTx^-$ mostly $nestin^+$ and about 15% of these cells incorporated BrdU during the one hour pulse. Thus, separation of cells according to their specific buoyant density using continuous Percoll gradients offers the opportunity of analysis of cells in different stages of maturation obtained from the same specific region of the developing CNS.

CELL LINEAGE AND DETERMINATION III

511.1

ROLE OF MIDLINE GENES IN THE DEVELOPMENT OF THE GRASSHOPPER CENTRAL NERVOUS SYTEM. K. Menon, T.-Y. Kung, A. Collazo* and K. Zinn. Division of Biology, Caltech, Pasadena, CA 91125.

In insects and in vertebrates a distinct group of cells located in the midline play an important role in guiding commissural axon pathways during development of the central nervous sytem (CNS). The median neuroblast (MNB) lineage in grasshopper gives rise to the majority of these midline cells. A set of 'midline genes' have been identified in *Drosophila* that affect the CNS midline. However, the precise roles of these genes in controlling cell fate is poorly understood. We are using the grasshopper system to address these questions.

We are examining how two midline genes, *single-minded (sim)* and *Toll*, control cell fate decisions in the MNB lineage of the developing CNS. Our progress towards this goal includes isolating and determining expression patterns of the grasshopper homologs of *Drosophila sim* and *Toll* genes. The *sim* clone encodes a protein that is approximately 75% identical to the *Drosophila* sequence within its N-terminus half (including the helix-loop-helix region). The *Toll* clones were isolated by J. Sane's group. We have also performed *in situ* hybridization experiments for both these genes in grasshopper embryos and results indicate that *sim* RNA is present in neuronal and glial progeny of the MNB cluster and in the MP3 progeny. *Toll* 2 RNA is present in the glial cells of the MNB progeny and in the adjacent neuroblasts. Currently we are trying to inhibit expression of *sim* and *Toll* genes with antisense oligonucleotides ithat are microinjected in the MNB nucleus of grasshopper embryos. Analyses of the perturbation experiments will be presented.

511.2

BrdU DETERMINATION OF PROLIFERATION RATES IN MEDIAN NEUROBLAST LINEAGES OF THE GRASSHOPPER CNS. Mark A. Masino and Melody V.S. Siegler*, Dept. Biology, Emory Univ., Atlanta GA 30322.

Incorporation of bromodeoxyuridine (BrdU), which labels actively dividing cells, was used to determine proliferation rates of segmental median neuroblasts (MNBs) of the grasshopper nerve cord. The third thoracic (T3) and first abdominal (A1) neuromeres of the cord are homologous structures that fuse during embryonic development. Thompson and Siegler (J. Neurosci. 13:3309, 1993) showed a difference in the number of neurons that comprise the two groups of MNB progeny, 90 in T3 and 60 in A1. In T3, the MNB arises at 29% embryogenesis and proliferates at a steady rate, adding 10 cells to the group per 5% developmental interval until shortly before the MNB dies at 78%. In A1, the MNB arises at 30% and proliferates 10 cells per 5% interval from 30% to 45%. A plateau occurs from 45% to 60%. After 60% the steady rate of proliferation resumes, until the MNB dies at 73%. In A1, a peak of cell death coincides with the plateau. We questioned whether the cell death was entirely responsible for the plateau in A1 growth or whether slowing of the MNB division rate was also a factor. Embryos (30% to 80% stages) were injected with BrdU and allowed to develop a further 5%. After processing to reveal cells of the MNB groups that incorporated BrdU, counts were made. We found no significant difference between T3 and A1 in the number of progeny labeled per 5% developmental interval. Thus, cell death, rather than a change in the proliferation rate, accounts for the plateau in A1 growth and the smaller population size. Additionally, we found that the MNB groups in A2-A7 contained 25 or fewer neurons total. Significantly fewer progeny were labeled per 5% developmental interval, consistent with an MNB division rate about half that of T3 and A1. Support: NSF IBN-9120863 & NIH KO4NS1484 (NINDS) to MVSS.

511.3

DIFFERENTIATION-DEPENDENT ACTIONS OF VOLTAGE-GATED Ca^{2+} CHANNEL MODULATORS ON VSC 4.1 CELL PROLIFERATION AND APOPTOSIS. R.G. Smith, A.H. Mohamed, S.H. Appel*, L. Colom, D. Mosier, and W.-D. Le. Dept. of Neurology, Baylor College of Medicine, Houston, TX 77030.

Studies on the regulation of neuroblast proliferation and initiation of apoptosis in early nervous system development have suggested a prominent role for altered Ca^{2+} flux through voltage-gated calcium channels (VGCCs). Addition of the dihydropyridine L-type VGCC agonist Bay K-8644 (0.5-10 μM) to undifferentiated motoneuron-neuroblastoma VSC 4.1 hybrid cells produces a Ca^{2+}-dependent increase in cell proliferation rate; both dihydropyridine and non-dihydropyridine L-VGCC antagonists reduce or block VSC 4.1 cell division (ED_{50} for diltiazem, verapamil, and nifedipine = 0.05, 0.5, and 5 μM, respectively), without affecting cell death. In contrast, addition of the peptide N-VGCC antagonist ω-conotoxin GVIA (5-50 nM) increases cell proliferation rate.

Following cell differentiation for one week in 1mM dibutyryl cAMP, Bay K-8644 effects are lost, while nifedipine and ω-conotoxin GVIA instead induce apoptosis, as determined by DNA laddering and blockade with 1 μM aurintricarboxylate or 0.1 nM cycloheximide. The effect of nifedipine on cell loss is slow, is not affected by reducing extracellular Ca^{2+} with EGTA, and is significantly inhibited by prior addition of oxygen radical scavengers. ω-Conotoxin GVIA's effects are more rapidly mediated, are prevented by reduction in extracellular Ca^{2+} with EGTA, and are partially blocked by AMPA-sensitive glutamate receptor inhibitors (GYKI-52466 and CNQX), by NOS synthetase blockers, and by NO scavengers. These data suggest that control of neuron precursor proliferation and apoptosis may be differentially regulated by modulators of different VGCCs, that differentiation-dependent changes in regulation may allow the same VGCC modulators to control different processes at different developmental stages, and that this regulation may involve indirect action of other ion channels. Supported by NIH grants NS33186 and AG08664.

511.4

PROGRAMMED CELL DEATH IN THE FETAL CNS INVOLVES APOPTOTIC MECHANISMS. K.Staley*, A.J. Blaschke and J.J.M Chun. Dept. of Pharmacology, UCSD School of Medicine, La Jolla, CA 92093-0636.

Massive cell death in both proliferative and postmitotic zones of the fetal cerebral cortex has been demonstrated by *in situ* end labeling (ISEL) of fragmented DNA. (see Soc. Neurosci. abstract Blaschke *et al.* 1995). To determine if this early neural cell death involves an apoptotic mechanism, we examined the developing mouse CNS for the presence of a widely accepted hallmark of apoptosis, the endogenous cleavage of genomic DNA into nucleosomal ladders. This characteristic form of DNA degradation could not be detected following the standard methods of agarose gel electrophoresis and either staining with ethidium bromide, or Southern blot hybridisation to genomic DNA. However, the use of a modified ligation-mediated polymerase chain reaction (LMPCR) enabled us to detect nucleosomal-sized DNA fragments in both the developing brain and other mammalian tissues known to undergo PCD. Amplification of nucleosomal ladders was shown to correlate directly with the extent of cell death in an established model of apoptosis, the dexamethasone treated-thymus. Since LMPCR detection of DNA fragments is dependent on the presence of blunt, 5'-phosphorylated double strand breaks, these results suggest that the endonuclease(s) responsible for DNA degradation *in vivo* generates nucleosomal ladders with these distinctive ends. A semi-quantitative analysis of nucleosomal-sized fragments detected by LMPCR in embryonic, postnatal and adult cortex showed ladder formation to correlate with the extent of cortical cell death shown previously using ISEL. This result suggests that PCD in the embryonic CNS indeed involves mechanisms of apoptosis. [supported by the Klingenstein Fund and the UC Tobacco-Related Disease Research Program]

511.5

MAJOR SITES OF CELL DEATH IN THE FETAL CEREBRAL CORTEX ARE THE ZONES OF CELL PROLIFERATION. A.J. Blaschke*, K. Staley, and J.J.M.Chun. Department of Pharmacology, School of Medicine, University of California, San Diego, La Jolla, CA 92093-0636.

During development of the mammalian nervous system programmed cell death (PCD) is believed to participate in the formation of connections between neurons and their appropriate targets. The embryonic period is when most neurons are generated and initial synaptic connections are formed; PCD could thus play an important role during this time. Previous studies have documented cell death in the postnatal cerebral cortex but no thorough analyses have examined this issue in the embryo. In this study we have used *in situ* end labeling (ISEL) of fragmented DNA to show the distribution of dying cells within the murine cerebral cortex during embryonic development.

Tissue sections from Balb/c embryo cortices (E10-E18) were labeled by a modified ISEL technique and compared to adult cortex. Although few labeled cells were seen at E10 or in the adult (less than 1%), from E12-E18 many dying cells (averaging over 50% of total cells observed) were seen throughout the cerebral wall. The quantity of cell death in the cortex is significant, and indicates that PCD has an important role during mammalian neurogenesis. Even more significant, however, is the distribution of dying cells; although many are found in cortical regions where cells are postmitotic, the majority are found in the regions of cell proliferation: the primitive plexiform layer and the ventricular zone. In postmitotic zones cortical neurons are forming synapses, and cell death here can be explained by target-dependent mechanisms of PCD. However, these mechanisms cannot explain cell death in proliferative zones, where cells have not yet made synaptic connections. Our findings suggest that novel regulatory mechanisms may exist in these regions, and that neurons may undergo selection at several stages of development in order to reach maturity. [supported by the Klingenstein Fund, the UC Tobacco-Related Disease Research Program and NIH/NIGMS GM07198]

511.6

EXPRESSION OF A NEW PUTATIVE G-PROTEIN COUPLED RECEPTOR IS ENRICHED IN PROLIFERATIVE ZONES OF THE EMBRYONIC CEREBRAL CORTEX. J. H. Hecht*, J. A. Weiner, and J. J. M. Chun. Dept. of Pharmacology, School of Medicine, University of California, San Diego, La Jolla, CA 92093-0636.

Neurons comprising the cerebral cortex develop from histologically uniform proliferative zones. Molecules mediating cell-cell communication, such as the G-protein coupled receptor (GPR) family, may play an important role in these regions by regulating early cortical neuron development. To isolate novel GPRs expressed in the embryonic cortex, we used degenerate oligonucleotide primers in polymerase chain reaction (PCR) amplification of cDNA derived from novel murine telencephalic cell lines. A PCR product was cloned which had sequence homology to the GPR family. This clone was used as a probe to isolate a full length cDNA, termed *vzg-1*, from a mouse brain cDNA library. *Vzg*-1 shares approximately 35% amino acid identity with the human and rat edg-1 orphan receptors, and the cannabinoid and melanocortin receptors. It contains amino acid residues conserved across the GPR family and has seven predicted membrane spanning domains. Northern blot analysis shows that *vzg-1* is expressed in the embryo as early as embryonic day 13 (E13) and in the cell lines from which it was isolated. *In situ* hybridization patterns in E16 mouse embryos using riboprobes synthesized from different regions of the *vzg-1* clone show primary expression of *vzg-1* in the ventricular zone of the dorsal telencephalic wall along with expression in the marginal zone. Thus, *vzg-1* represents a new putative GPR with enriched expression in the proliferative zones of the embryonic cortex. Its expression pattern suggests that signal transduction pathways mediated by novel GPRs may be important at the earliest stages of cortical neuron development. [supported by the March of Dimes, the NIMH and NIH/NIGMS GM07198]

511.7

NEUROTROPHINS AND bFGF DIFFERENTIALLY AFFECT THE PROLIFERATION AND DIFFERENTIATION OF CORTICAL NONPYRAMIDAL NEURONS. I. S. Pappas, T. Sklaviadis* and J. G. Parnavelas. Dept. of Anatomy, Univ. College London, London WC1E 6BT, U.K.

A host of neurotrophic factors have now been shown to influence both the generation and differentiation of neuronal and glial progenitor cells in the nervous system. Here, we sought to investigate the role of neurotrophins and bFGF in the development of cortical cell types. Primary cultures prepared from rat embryonic cortices (E16) were infected with BAG retrovirus and then treated with neurotrophins [NGF (100 ng/ml), BDNF (10 ng/ml), NT-3 (100 ng/ml)] and bFGF (10 ng/ml) in defined medium for 7 days. The clones formed consisted either of single cells (about 30% of all clones in the control and treated cultures) or clusters of cells. Clusters varied in size according to treatment: 17-32 cells/clone in bFGF-treated cultures or 5-8 cells in untreated and NT-3-treated cultures. Treatment with NGF and BDNF resulted in clusters of a wide spectrum of sizes. These results suggest that growth factors differentially affect the proliferation of cortical progenitor cells. To further define the cortical cell types affected by the various treatments, we focused on the subpopulations of nonpyramidal neurons defined by the presence of the calcium-binding proteins calbindin (CB) and calretinin (CR). Using double-labelling techniques, we observed that clones containing CB+ cells represented 6% of the total clones in control cultures, 14% in cultures treated with bFGF, 3% with NGF, 7% with BDNF and 19% with NT-3. Clones containing CR+ neurons were only found in bFGF-treated cultures. We also established that BDNF, NT-3 and bFGF inreased significally the total number of CB+ neurons; the latter also inceased the total number of CR+ neurons. This trend was confirmed by Western blot analysis. These findings suggest that neurotrophins and bFGF play a role in the proliferation, differentiation and lineage determination of CB- and CR-containing nonpyramidal neurons. Supported by the Medical Research Council.

511.8

PHENOTYPIC DIFFERENCES IN IDENTIFIED, SEGMENTALLY HOMOLOGOUS NEURONS MAY BE CAUSED BY HOMEOBOX GENE *LOX4*. G. W. M. Bothe*, V. Y. Wong and E. R. Macagno. Dept. Biol. Sci., Columbia Univ., New York NY 10027

Processes of regional differentiation in the CNS were studied in the leech, *Hirudo medicinalis*. The anteriormost pair of neurons in each midbody ganglion are segmental homologs that have three different *Lox4* expression patterns. nRMVs never express *Lox4*, RPE motoneurons express *Lox4* transiently during embryogenesis, and pRMVs express *Lox4* continually. We set out to study these neurons asking: Are there phenotypical differences between these neurons, and could those be explained by the *Lox4* expression pattern?

The interaction of RMVs and RPEs with a natural growth substrate, extracellular matrix extract, was tested *in vitro*. nRMVs grew neurites to a radius of 80±40 µm within 1 d. The neurites of pRMVs grew to a radius of 26±11 µm. The RPE, however, grew profusely (radius 150±59 µm) and had more neurite branches.

The interaction between RPE or RMV and potential targets was assayed *in vitro*. RPE axons adhered strongly on the natural target, male organ muscle cells (MOMCs). After 16 h in culture, RPEs and MOMCs could not be separated without tearing the axon apart. A short, thick, nearly unbranched neurite grew on the MOMC in 58% of the cell pairs. RPEs did not adhere strongly to body wall muscle cells and grew little. pRMV did neither adhere nor grow on MOMC.

RPE differ from nRMV and pRMV in the size and shape of their soma action potentials. RPE potentials are relatively large (11.5 ± 3.9 mV), whereas nRMV potentials are small (3.6 ± 0.7 mV). In addition, RPE hyperpolarize strongly after an action potential whereas nRMV and pRMV do not.

To test whether *Lox4* might be involved in establishing the above-mentioned phenotypic differences, *Lox4* mRNA was injected into adult RPEs. Preliminary results indicate that, after 2 d, injected mRNA is translated and that *Lox4*-mRNA, but not ß-gal, mRNA changes the shape of somatic action potentials of RPE neurons.

511.9

CHARACTERIZATION OF SEROTONIN-DEFICIENT MUTANTS IN THE NEMATODE *C. ELEGANS* C. M. Loer* Dept. of Biology, Lafayette College, Easton, PA 18042

We are seeking to identify genes that regulate expression of neurotransmitter phenotype of serotonergic neurons in *C. elegans*. We are using two approaches: 1) isolation and characterization of serotonin-deficient mutants, some of which should identify regulatory genes, and 2) identification of marker genes used by serotonergic neurons, the genes controlled by the regulators of neurotransmitter type.

Among existing serotonin-deficient mutants is *cat-4 (e1141)*, which was first isolated as a dopamine-deficient mutant lacking formaldehyde-induced fluorescence (FIF); it was subsequently found to lack serotonin immunoreactivity as well (Sulston et al., 1975, J. Comp. Neurol. 163: 215; Desai et al., 1988, Nature 336: 638). Although *cat-4* mutant worms were previously described as serotonin hypersensitive, we have found that *cat-4* mutants are also hypersensitive to a variety of other unrelated agents. We first noticed that *cat-4* mutant worms died much more rapidly than wild type worms during routine "cleaning" of worms with bleach. Whereas wild type worms squirm in bleach for several seconds before becoming rigid, *cat-4* mutants stop moving almost instantly (< 1 sec). In addition, *cat-4* mutant carcasses seem to disappear or dissolve during this treatment, unlike those of wild type worms. We have further characterized the hypersensitivity of *cat-4* mutants to a number of agents, including SDS, levamisole and serotonin. Our observations suggest that mutation of the *cat-4* gene affects not only production of neurotransmitters, but also construction of the cuticle.

We are currently screening for additional serotonin-deficient mutants based on behavioral abnormalities, bleach hypersensitivity and serotonin immunoreactivity. We hope to identify additional genes that may be involved in expression and regulation of a serotonergic phenotype.

511.10

EMX1 IS EXPRESSED IN NEURONS DURING LAYERS FORMATION IN THE DEVELOPING TELENCEPHALIC CORTEX. M. Gulisano *[1,2], V. Broccoli [1] and E. Boncinelli [1]. [1]-DIBIT-Istituto Scientifico H San Raffaele, Milan, Italy, 20132 [2]-Istituto di Biologia Generale, Università di Catania, Catania, Italy, 95124.

Two homeobox genes, Emx1 and Emx2, have been cloned as vertebrate homologues of *empty spiracles*, a gene controlling very anterior body regions during early *Drosophila* embryogenesis. Both genes are clearly expressed during early mouse brain formation with specific patterns.

We studied in detail Emx1 and Emx2 expression in the developing cerebral cortex of the mouse between E12.5 and adult hood.

Emx2 expression appears to be confined to proliferating cells in the ventricular zone, with the exclusion of both the transition field and the forming cortical plate. Between E14 and E16 Emx2 shows a weaker expression in anterior regions than posterior regions in the cortex, suggesting a correlation of Emx2 expression with an antero-posterior maturation gradient. Conversely at these developing stages Emx1 is expressed in the ventricular zone, in the transitional field and in the forming cortical plate. Later in development, when cortical layers start to form, Emx1 is expressed in differentiating cells in the cerebral cortex with exclusion of layer 1. In particular, Emx1 seems to be specific for neuronal cells and not for glial cells. In the subplte Emx1 expression is confined to a specific subpopulation of neurons whose function remains to be assessed.

Our data suggest that both Emx1 and Emx2 are involved in cerebral cortex development in the mouse. It is of interest to consider a specific role for Emx2 in proliferating neuronal cells and for Emx1 in neuronal cell identity in the developing cerebral cortex of the mouse.

511.11

NOVEL SEPTAMER-BINDING PROTEINS MARK DIFFERENTIATION EVENTS IN THE GLIAL AND NEURONAL LINEAGE. A.L. Dobi*, M. Palkovits[1], C.G. Palkovits, D.v. Agoston, MCN, LDN, NICHD; LCB, NIMH[1]; NIH, Bethesda, MD 20892.

The differentiation of the multipotent neuroglial precursor requires temporally and spatially coordinated interactions between specific DNA elements and their binding proteins to restrict the expression of genes to a set that characterizes the mature phenotypes of glia or neurons. To identify these elements we used DNA methylation interference footprinting with a 5' fragment (-542; -379) of the rat enkephalin (rENK) gene and identified a truncated octamer motif (TTTGCAT=sept) that specifically binds to nuclear proteins of the embryonic rat brain. Gelshift screening with nuclear proteins derived from seven ontogenetically and phenotypically distinct brain regions between ages of embryonic day 10 (E10) and postnatal 28 (P28) showed the existence of several distinct sept-binding proteins. From E14, when neuronal and glial lineages begin to emerge we identified two very abundant proteins forming low mobility complexes. The appearance of this doublet follows the maturation of distinct brain regions as indicated by their appearance as early as at E12 in the spinal cord and the late peak of expression in the cerebellum. Because only the complex with somewhat higher mobility is present in cultures of primary cortical astrocytes and the developmental appearance of this band in ontogenetically distinct brain regions follows the temporal and spatial pattern of glia proliferation we tentatively identified this complex as glia-specific. These proteins are distinct from known octamer binding proteins because antibodies against known oct-1 or oct-2 proteins do not recognize them.The binding to the sept-motif requires phosphorylation of both proteins as treatment with phosphatases completely diminishes the formation of complexes. The expression pattern of developmental-, neuronal- and glia-specific markers in different brain regions at various developmental ages indicates that the sept-binding proteins may contribute to the early differentiation events of neuronal and glial lineages.

511.12

THE BRAIN-SPECIFIC HELIX-LOOP-HELIX PROTEIN *NEX-1*: A NEURONAL ACTIVATOR OF GAP-43?
A. Bartholomä, M. Schwab, P. Gass, and K.-A. Nave*
Center for Molecular Biology (ZMBH), University of Heidelberg, D-69120 Heidelberg, Germany.

NEX-1 is a novel helix-loop-helix (HLH) protein, cloned from the adult rat brain (*Mech. Development* 48, 217-228, 1994). The rodent NEX-1 gene is expressed only in the CNS, beginning at E14, and is restricted to cells that have left the ventricular zone. Peak expression occurs in the first postnatal week, coinciding with terminal neuronal differentiation and synaptogenesis. In the adult brain, NEX-1 transcripts are most abundant in hippocampus (CA1-4), neocortex, amygdala, and cerebellum. Since HLH proteins are key developmental regulators in other systems, we have begun to address the function of NEX-1 in neuronal differentiation. A candidate target gene for NEX-1 encodes the neuronal growth-associated protein GAP-43. In the CNS, both genes display a very similar temporal and spatial pattern of expression. In undifferentiated P19 cells, the overexpression of NEX-1 and E12 activated the cloned GAP-43 promoter (kindly provided by L.Schrama). Moreover, we observed by indirect immunofluorescence that undifferentiated P19 cells became GAP-43 positive following transfection. Since control cells showed little or no immunoreactivity, this suggests that GAP-43 has been induced *de novo*. To further address the function of NEX-1 in neuronal development, we used homologous recombination to completely delete the gene in mouse embryonic stem cells. Blastocyst injections resulted in the birth of highly chimeric mice. Results of the gene targeting experiment will be discussed. (Supported by grants of BMFT and DFG to K.A.N.)

PROCESS OUTGROWTH, GROWTH CONES, AND SPROUTING IV

512.1

MODIFICATION OF RETINAL AXON BRANCHING PATTERN BY THE EXPRESSION OF CONSTITUTIVELY ACTIVE CaMKII IN XENOPUS TECTAL CELLS. D.J. Zou* and H.T. Cline. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

The plasticity of developing frog retinotectal projection depends on NMDA receptor activity in tectal neurons. The possibility of that the Ca^{2+} influx through the NMDA receptor may act through a CaMKII pathway was studied by expressing the constitutively active CaMKII in tectal cells with the recombinant vaccinia virus and following the dynamic changes of the retinal axon morphology. The morphology of individual DiI labeled retinal axons was first observed *in vivo* with a confocal microscope in the stage 48 albino *Xenopus* tadpoles. Vaccinia virus containing the DNA sequences encoding the truncated CaMKII (amino acid 1-290) and a reporter β-galactosidase (β-gal) (tCaMKII virus) or β-gal alone (β-gal virus) was then injected into the ventricles of the animals. When intensive infection in the CNS occurred as indicated by the expression of β-gal on Day 3, the same axon's morphology was observed again. Quantitative analyses showed that the tCaMKII but not the β-gal axons became less complex. The branchtip number decreased to $66\pm4\%$, while in the control axons it increased to $120\pm7\%$, of the Day 0 values. The branchtip density decreased more than it does in the control animals, $66\pm4\%$ *vs.* $89\pm5\%$ of the initial values. The axon length did not increase ($108\pm9\%$) as much as it does in the controls ($142\pm10\%$). Relatively, there was a selective decrease in the branchtips with high topological branch order (>8) and short segment length (1-10 μm). The maximal length and width of the axon arbor did not change. These results suggest that the activity of CaMKII in the tectal cells could be involved in controlling the branching pattern of retinal axons. We are studying the detailed mechanisms of these tCaMKII effects on the remodelling of retinal axon branching pattern by observing repetitively the morphology of individual axons at shorter intervals.

512.2

DYNAMIC GROWTH PATTERNS OF XENOPUS TECTAL CELLS AND EFFECTS OF OVEREXEPRESSION OF CaMKII Gang-Yi Wu and Holly Cline*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY11724

Relatively little is known about the dynamic growth patterns of dendrites and related cellular and molecular mechanisms. In order to reveal the processes of neuronal growth in vivo, tectal cells near the ventricular layer midway along the rostrocaudal extent of tectum were selectively labeled in Xenopus tadpoles between stages 46 and 49 using iontophoresis of DiI. Individual cells were imaged using time lapse confocal microscope and three-dimension reconstruction techniques. The dynamic growth of rostrally projecting cells is reported here. The development of all rostrally projecting neurons roughly followed the same time course. Data from daily observations showed that the axon was generally initiated before the dendritic elaboration. The axon was tipped with an active growth cone and navigated medially or laterally into the tectothalamic tracts. Dendrites first sprouted after the axon entered the above tracts. Once the axon reached the target area (thalamus), the dendrites dramatically increased in branch numbers and lengths. This period of fast dendritic growth lasted about 1 day and resulted in the formation an initial dendritic pattern. After this period the dendritic arbors continued to grow. No obvious regression process was detected. Observations taken at short-time intervals(5-10min) revealed that the dendritic arbor formation is a dynamic process. Addition and retraction of filopodia-like structure of dendrite growth cone and of primary branches occurred rapidly. To elucidate the functional role of CaMKII in process outgrowth, we have used the Vaccinia virus to deliver the gene for the constitutively active CaMKII (AA 1-290) into tectal neurons in vivo. Overexepression of CaMKII in neurons during the period of rapid dendritic growth significantly reduced the complexity and elongation of tectal cell dendrites after 2-3 days. Transfection in more mature neurons apparently did not result in such a severe reduction in dendritic arbor complexity. Therefore, our results show that the normal growth of neurons is a dynamic process. Furthermore, the data suggest that the activity-regulated enzyme CaMKII is involved in regulation of the dendritic growth of tectal neurons in Xenopus in vivo.

512.3

CHANGES IN INTRACELLULAR CALCIUM MAY BE PREDICTIVE OF MORPHOLOGICAL PLASTICITY IN RETINAL GANGLION CELL AXON ARBORS AND GROWTH CONES. J.A. Edwards* and H.T. Cline. Beckman Neuroscience Center, Cold Spring Harbor Laboratory, CSH, NY 11724.

Two fundamental aspects of neuronal development are axonal navigation and the elaboration of the axon terminal within the target region. Several hypotheses, based upon data from in vitro model systems, attempted to relate changes in intracellular calcium ($[Ca^{2+}]_i$) with morphological changes in either growth cones or complex axon arbors. To date there is no consensus opinion regarding whether changes in $[Ca^{2+}]_i$ are required for neurite elongation and growth cone motility or to what degree these changes affect developmental morphology. Although in vivo recordings of $[Ca^{2+}]_i$ have been correlated with electrical and/or mechanical stimulation of zebrafish spinal cord neurons (Fetcho and O'Malley, 1995), no direct measure of morphological plasticity and $[Ca^{2+}]_i$ has been performed simultaneously in vivo. The present investigation was undertaken to study the in vivo relationship between $[Ca^{2+}]_i$ and morphology in the developing visual system of albino Xenopus laevis tadpoles.

Calcium green-1 dextran (CaGD) was iontophoretically injected into the temporal retina of stage 43/44 tadpoles. CaGD was chosen over other available single excitation/single emission fluorescent probes on the basis of its brighter emission characteristics at lower, basal levels of $[Ca^{2+}]_i$. Confocal laser scanning microscopy was then used to study both changes in $[Ca^{2+}]_i$ and morphology in single retinal ganglion cell complex axon arbors or pathfinding growth cones. Optical image planes were collected at 4 μm intervals using a computer controlled z-motor, and imaging series were collected once every 5 minutes and analyzed using Intervision (Noran Instruments, Inc.) and VolVis (public domain) software. These studies demonstrate, for the first time, that changes in $[Ca^{2+}]_i$ can be correlated with dynamic morphological plasticity in vivo The findings from this work should aid in our understanding of the relationship between changes in $[Ca^{2+}]_i$ and changes in axonal or growth cone morphology within the developing visual system.

512.4

CALCIUM-DEPENDENT SIGNALING MECHANISMS GOVERN DENDRITIC OUTGROWTH AND ARBORIZATION IN DEVELOPING CEREBELLAR PURKINJE NEURONS. *Andrea J. Yool* and Raven Reitstetter.* Dept. of Physiology, University of Arizona College of Medicine, Tucson AZ 85724.

Purkinje neuron development in culture is characterized by the elaboration of a branched dendritic arbor during a time equivalent to postnatal development of the rat cerebellum. This structural development is accompanied by enhanced excitability and the expression of new Ca and K_{Ca} components of the firing pattern. Our data show that the transcription of K_{Ca} channels is developmentally regulated. The delayed K_{Ca} expression correlates with the pattern of K_{Ca} activation *in vitro* and suggests the regulatory mechanisms governing this channel are not substantially affected by the explanting process. We find a dramatic effect of chronic K_{Ca} channel blockade on neuronal development, in support of a key role for these channels in the transmembrane signaling processes that shape dendritic structure. Purkinje neuron morphology is altered by chronic treatment with 1 mM tetraethylammonium, which affects primarily the large conductance K_{Ca} channel. Control neurons show densely branched dendritic arbors; chronic TEA stimulates outgrowth over extended ranges with a generally similar branching pattern. Purkinje neurons are labeled with antibody to calbindin; structures that we have identified as dendrites by morphology are labeled appropriately by antibody to MAP2. Our hypothesis is that the calcium signals that drive outgrowth are in turn regulated by K_{Ca} channels, and that chronic blockade of K_{Ca} channels augments the calcium signals. Surprisingly, despite its importance in modulating Ca^{2+} entry, K_{Ca} has not previously attracted attention as a potentially important regulatory mechanism. Many other studies have shown that transient calcium signals are important in regulating neurite outgrowth in various cell types. In Purkinje neurons, the predominant Ca^{2+} channel is the P-type, sensitive to ω-Aga-IVA toxin. Ongoing studies with ion channel-specific toxins will test for interactions between subtypes of Ca and K_{Ca} conductances during the maturation of the dendritic arbor.

512.5

CHANGES IN $[Ca^{2+}]_i$ PRODUCED BY DEPOLARIZATION OF GROWTH CONES FROM TONIC AND PHASIC MOTONEURONS. K. Arcaro* and G.A. Lnenicka Depart. of Biol. Sci., State Univ. of New York, Albany, N.Y. 12222

In a previous study, we found that prolonged depolarization with 60 mM K^+ had different effects on the growth of regenerating phasic and tonic motor axons in the crayfish (*Procambarus clarkii*): axons of the tonic motoneurons continued to advance while those of the phasic motoneurons were inhibited and eventually degenerated. Experiments with calcium channel blockers indicated that this resulted from the differential effects of calcium influx. To determine whether these results were due to differences in calcium regulation or calcium sensitivity, we measured $[Ca^{2+}]_i$ in neurites and growth cones. Regenerating motor axons were examined using ratio imaging of fura-2 fluorescence. In growth cones from tonic axons, depolarization with 60 mM K^+ produced approximately a 5-fold increase in $[Ca^{2+}]_i$, which is maintained for the duration of the depolarization (up to 4 days). In spite of the elevated $[Ca^{2+}]_i$, the neurites continued to grow at normal or above normal rates. Preliminary experiments on growth cones from phasic axons indicate that similar increases in $[Ca^{2+}]_i$ result in inhibition of growth, suggesting that phasic and tonic axon growth cones have different sensitivities to $[Ca^{2+}]_i$. (Supported by NSF grant IBN-9121757)

512.6

NEURITE OUTGROWTH INDUCED BY LIMBIC SYSTEM-ASSOCIATED MEMBRANE PROTEIN CAN BE REGULATED BY CHANGES IN INTRACELLULAR Ca^{2+}. V.Zhukareva*, N.Chernevskaya, M.Nowycky, and P.Levitt. Dept. Neuroscience and Cell Biology, Robert Wood Johnson Medical School - UMDNJ, Piscataway, NJ 08854 and Dept. Anatomy and Neurobiology, Med. Coll. PA, Philadelphia, PA 19129.

The limbic system-associated membrane protein (LAMP) is an Ig-superfamily member that exhibits homophilic, selective adhesive activity for specific neuronal populations. Monolayers of CHO-K1 cells transfected with full-length *lamp* cDNA (CHO_L cells) show an enhanced ability to promote neurite outgrowth of fetal $LAMP^+$ neurons from perirhinal cortex and hippocampus compared to $LAMP^-$ neurons from olfactory bulb and visual cortex. The mechanism by which this specificity may be regulated has been investigated by examining the role of second messengers in mediating LAMP-induced outgrowth. Treatment of the limbic neurons with L- (20 μm nifedipine) and N-type (1 μm ω-conotoxin) Ca-channel antagonists abolishes LAMP induced neurite outgrowth on the monolyer of CHO_L. Limbic neurons, grown on control CHO cells treated with the same antagonist also demonstrate reduced outgrowth, although to a lesser extent. In preliminary experiments, bath application of soluble LAMP causes an increase of intracellular Ca^{2+}, which begins to decline after 40 min. of incubation. These observations indicate that LAMP may function through the activation of an intracellular signaling system involving changes in intracellular Ca^{2+} levels. Supported by MH45507 (PL) and NS22281 (MN).

512.7

HETEROGENEOUS REGULATION OF NEURITE OUTGROWTH IN EMBRYONIC *XENOPUS* SPINAL NEURONS. Xiaonan Gu* and Nicholas C. Spitzer. Dept. Biology & Center for Molecular Genetics, UCSD, La Jolla, CA.

Neurite extension is regulated by the frequency of transient Ca^{++}_i waves occurring naturally (8-9/h) in growth cones of embryonic *Xenopus* spinal neurons grown in culture. In Ca^{++}-free medium, stimulation of brief Ca^{++} pulses by application of 100 mM K^+/10 mM Ca^{++} mimics endogenous Ca^{++} transients in Ca^{++}-containing medium, and results in neurite outgrowth similar to that of neurons grown in the presence of calcium. From analyses of the neuronal population, both the mean neurite length and the distribution of lengths of individual neurites in stimulated cultures grown in Ca^{++}-free medium match those from cultures grown in Ca^{++}-containing medium. We have investigated the extent of differences in outgrowth of individual neurites, with the same stimulation paradigm.

We measured neurite extension between 12-15 h in culture. Between 13 and 14 h, cultures were either continuously perfused with Ca^{++}-containing medium, or perfused with Ca^{++}-free medium and stimulated at 9/h with high K^+/Ca^{++}. Both treatments inhibited outgrowth in ~60% of neurites (n≥21), by a mean value of 60%. Outgrowth was stimulated in 5% of neurites, and 10% of neurites exhibited no response to both treatments. Different responses to stimulation were not related to initial lengths of neurites. 25% of neurites have already stopped extension by 12 h in culture. During the process of neurite outgrowth, neurites of multipolar cells behaved similarly. As found in population analyses, neurite outgrowth was not inhibited when stimulated at the lower spontaneous frequency of propagated spikes (3/h).

These findings suggest the existence of 3 subtypes of neurons with different neurite extension machinery, since all neurites exhibit spontaneous Ca^{++} transients. Extrapolating observations from 1 h stimulation to 18 h yields an expected mean neurite length consistent with that obtained at 18 h in Ca^{++}-containing medium in previous population analyses. Thus, Ca^{++} transients regulate extension of individual neurites thoughout the 18 h period. Supported by NS15918.

512.8

THE EFFECT OF CALCIUM ON NEURITE OUTGROWTH IS SUBSTRATE-DEPENDENT. Jane Viti and Carl Lagenaur*. Dept. of Neurobiology, Univ. of Pittsburgh, Pittsburgh, PA 15261

Neurite outgrowth and growth cone shape are influenced by cellular interactions with the growth substrate. Many studies have indicated a critical role for calcium in neurite outgrowth and growth cone movement. If neurons use different signaling mechanisms for each substrate then the cells' response to extracellular calcium could differ with different substrates. Mouse cerebellar neurons were plated on four purified substrates; NCAM, L1, P84, and laminin. The cells were then grown in calcium-free media supplemented with varying concentrations of calcium ranging from 0 mM to 8 mM. Cells growing on NCAM had the longest neurites when supplemented with 1-2 mM ca^{++}. With 8 mM ca^{++}, the neurites were slightly shorter but the cells were still healthy. The longest neurites on L1 were observed when 1 or 2mM ca^{++} was added. With 4 mM ca^{++}, cells began to die and neurites were significantly shorter. These toxic effects increased at 8 mM ca^{++}. Cells plated on P84 did not seem to be greatly effected by differing calcium concentrations. Peak growth occured with the addition of 4 mM ca^{++}. At 8 mM ca^{++}, cell viability decreased and neurites were slightly shorter. When cells are plated on laminin, neurite length, number and the amount of fasciculation increased with higher concentrations of calcium, peaking with the addition of 4 mM ca^{++}. At 8 mM ca^{++}, the cells were healthy with long neurites but they were defasciculated. These results identifiy a substrate-dependence of calcium's effects on neurite outgrowth.

512.9

TURNING OF NERVE GROWTH CONES INDUCED BY FOCAL ELECTRIC FIELDS IN Ca^{2+} FREE MEDIUM S. TSUKADA, and J. FUKUDA*. Department of Physiology, National Defense Medical School, Tokorozawa, Saitama, JAPAN.

Transmembrane Ca^{2+} influx and $[Ca^{2+}]_i$ gradient at nerve growth cones has been understood as essential for turning responses of growing tips by electric currents. Here we report an opposite result that the turning responses take place in neurites growing in the absence of Ca^{2+} influx in growth cones. Dorsal root ganglia dissected from newborn rats were placed in a two chamber dish so that their neurites grew in a Ca-free medium. DC current was applied to neurites through a microelectrode placed approximately 50 μm distant from the growth cones. $[Ca^{2+}]_i$ in neurites was measured using Calcium-green 1 under a laser scanning microscope, and Ca^{2+} stores in neurites was evaluated as a transient increase in $[Ca^{2+}]_i$ by applying A23187 (Ca^{2+} ionophore) to neurites bathed in a Ca-free medium. Our findings are: 1) turning responses, similar to those induced in control neurites (grown in normal Ca^{2+} medium), were induced in neurites grown in Ca-free medium. 2) $[Ca^{2+}]_i$, which was 150 nM in control neurites, was reduced to approximately 50 nM in neurites during growth in Ca-free medium. 3) A transient increase in $[Ca^{2+}]_i$, that was induced in growth cones of control neurites during turning responses, was not observed in neurites grown in Ca-free medium. 4) Intracellular Ca^{2+} stores were reduced to approximately 1/3 of control level in the neurites grown in Ca-free medium. We therefore concluded that induction of turning responses were not influenced either by reduction in $[Ca^{2+}]_i$ or by depletion of intracellular Ca^{2+} stores in rat DRG neurites.

512.10

DEPOLARIZATION-INDUCED CHANGES IN THE MORPHOLOGY OF GROWING NEURONS. L.M. Boulanger* and M-m. Poo. Dept. Biol. Sci., Columbia Univ., N.Y., N.Y. 10027

Electrical activity plays a prominent role in the formation and refinement of synaptic connections in the developing nervous system as well as in regulating synaptic efficacy in the adult, but the underlying mechanisms are largely unknown. Previous studies on the effects of electrical activity and neurotransmitters in cultured neurons have shown that depolarization often leads to growth inhibition or retraction of filopodia and neurites. We have examined depolarization-induced changes in the morphology of growing *Xenopus* spinal neurons in culture. Incubation in 45 mM K^+ for 2.5 min caused elevated exocytosis and rapid, long-lasting changes in neuronal morphology. Number of filopodia at the growth cone and along the neurite and apparent plasma membrane surface area were increased by depolarization. These changes were prevented when high K^+ was applied in the absence of external Ca^{2+}. These depolarization-induced changes suggest a rapid, Ca^{2+}-dependent insertion of membrane material, presumably via exocytotic fusion of membrane precursor vesicles from the constitutive pathways. This possibility was further examined by visualizing membrane recycling activity in living neurons using the fluorescent dye FM1-43. Incubation with 10 μM FM1-43 in the presence of external Ca^{2+} and high K^+ caused filopodial sprouting and concomitant staining of recycling vesicles. Stained vesicles were found in clusters along the neurite and their distribution closely paralleled that of new filopodia. This is consistent with the idea that Ca^{2+}-dependent exocytosis triggers local endocytic activity, and a net gain in exocytosis over endocytosis is responsible for local filopodia protrusion and membrane growth. Thus membrane depolarization can cause rapid and sustained increases in extension and complexity of growing neurons, and Ca^{2+}-regulated membrane growth may be closely related to local membrane recycling known to be involved in the retrieval of synaptic vesicles.

512.11

EFFECTS OF INHIBITORS OF NMDA AND PI-LINKED METABOTROPIC GLUTAMATE RECEPTORS ON NEURITE DEVELOPMENT IN CULTURED RAT HIPPOCAMPAL NEURONS. G. Audesirk*, M. Kern, and L. Cabell. Biology Department, Univ. of Colorado at Denver, Denver, CO 80217-3364.

Glutamate activates a variety of both ionotropic and metabotropic receptors. We examined the effects of inhibitors of NMDA receptors and phosphoinositide-linked metabotropic receptors on neurite development in cultured E18 rat hippocampal neurons. Receptors were activated by glutamate present in fetal bovine serum in the culture medium. After two days in culture, we measured survival, neurite initiation, axonal elongation and branching, dendrite number, and dendrite elongation and branching. Neither 200 μM D-AP5, a competitive inhibitor of NMDA receptors, nor 10 μM MK-801, an open-channel blocker, altered any parameter of neurite development. A second competitive inhibitor, (RS)-CPP (100 μM), had no effects on any parameter except axonal elongation. Several parameters of neurite development were decreased by both the active and relatively inactive enantiomers of MK-801 at 50 μM, indicating nonspecific effects at high concentrations. These results indicate that NMDA receptors do not play an important role in the early stages of neurite development in culture. In contrast, competitive inhibitors of PI-linked metabotropic receptors, L-AP3 (100 to 500 μM) and (S)-4-CPG (7.5 to 10 μM), reduced neurite initiation and enhanced dendrite branching. D-AP3, the inactive enantiomer of L-AP3, did not alter initiation or dendrite branching. Therefore, activation of PI-linked metabotropic receptors may modulate neurite initation and dendrite branching.

512.12

POSSIBLE ROLE OF THE N-METHYL-D-ASPARTATE (NMDA) RECEPTOR IN THE REMODELING OF DENDRITIC ARBORS DURING DEVELOPMENT. N.M.J. Blake*[1] and B.J. Claiborne[2]. [1]Program of Neuroscience, Washington Univ., St. Louis, MO 63110. [2]Div. Life Sciences, Univ. Texas at San Antonio, TX 78249.

During late postnatal development, granule neurons in the rat hippocampus lose dendritic segments while their remaining branches continue to elongate; total dendritic length is conserved (Rihn and Claiborne, 1990, Dev. Brain Res. 54:115). Because synaptic activity mediated by the NMDA receptor is thought to affect the maturation of some axons in the central nervous system, we hypothesized that it may also play a role in the remodeling of granule cell dendrites.

To test this hypothesis, 3 rats were sacrificed on postnatal day (PND) 14 to provide baseline data. From PND 14 through 24, 4 littermates were injected with CPP (3-((+)2-carboxypiperazin-4yl-propyl-1) phosphonic acid) every 12 hrs (IP; 10 mg/Kg), and 3 were injected with saline. On PND 25, 400μm-thick, fixed hippocampal slices were prepared. Granule neurons were retrogradely labeled with DiI, and dendrites analyzed in 3-D.

Results indicated that granule neurons (n=16) from 14-day-old pups had 31 ± 1 branches (mean ± SEM) and total dendritic lengths of 2266 ± 76μm. Neurons (n=18) from 25-day-old, saline treated animals exhibited significantly fewer branches (21 ± 1), but similar dendritic lengths (2268 ± 136μm), confirming previous work. In contrast, neurons (n=17) from 25-day-old CPP-treated rats did not exhibit a significant decrease in branch number (28 ± 1), but did show an increase in total dendritic length (2973 ± 136μm). Thus, injections of CPP reduced the branch loss normally seen in granule neurons between PNDs 14 and 24, but did not affect dendritic growth. These data suggest that synaptic activity mediated by the NMDA receptor may play a role in the remodeling of dendritic arbors during late postnatal development. (Supported by NIGMS GM 08194).

512.13

ENDOGENOUS NITRIC OXIDE MODULATES NEURITE GROWTH IN REGENERATING RAT SENSORY NEURONS. D.B. Wayne* and J.H.P. Skene, Department of Neurobiology, Duke University, Durham, N.C. 27710

Application of exogenous nitric oxide (NO) to rat dorsal root ganglion (DRG) neurons in vitro can halt outgrowth of regenerating axons (Hess et al., Nature 366: 562, 1993). In DRG neurons, the enzyme nitric oxide synthase (NOS) is present during development and is upregulated during regeneration (Verge et al., PNAS 89: 11617, 1992). Therefore, we examined the role of endogenous NO in outgrowth and in particular whether NOS, a calmodulin dependent enzyme, may mediate some of the effects of calcium on outgrowth. First we confirmed that in regenerating rat DRG neurons, an increase in intracellular calcium by exposure to calcium ionophore A23187 inhibits neurite extension. We then examined the frequency and rate of DRG axon extension following treatment with the NOS inhibitor N^G-methyl-L-arginine (L-NMMA), under standard culture conditions or in the presence of A23187. Inhibition of NOS enhances outgrowth suggesting that endogenous NO may be an important modulator of axon extension. [Supported by NIH grant NS20178.]

512.14

ROLE OF AFFERENTS IN THE DEVELOPMENT OF DENDRITIC BRANCHING AND SPINES IN CULTURED HIPPOCAMPAL NEURONS. A.H.Kossel*, S.B.Kater. Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523.

Presynaptic afferents likely play a major role in development and plasticity of dendritic morphology in the CNS. The present study asks how afferents can influence the morphology of neurons, and if activity and the activation of different subtypes of glutamate receptors are involved in these changes. E18 rat hippocampal neurons were grown 11-14 days on a net of axons extending from explants of entorhinal cortex, providing a constant, high density of presynaptic afferents. Synapses developed between neurons and the axonal net as determined by immunohistochemistry and EM. After 11 days, neurons on and off the axonal net were clearly different. Neurons in contact with the afferents were 4 times more branched than isolated neurons and also had a high density of spines. To assess the role of afferent activity in branch and spine formation, we grew cultures under chronic blockade of neuronal activity by TTX. Cultures treated with TTX showed a reduction in spine density, as previously reported. However, dendritic branching was not affected. In order to determine if activation of individual subtypes of glutamate receptors is involved in these postsynaptic changes, cells were grown in the presence of different glutamate receptor blockers. However, neither application of the ionotropic glutamate receptor blockers DNQX and APV, nor the metabotropic receptor-blocker MCPG led to a significant reduction in the number of spines or branches. In fact, APV treated cultures showed a transient increase in spine density around day 11. Since blockade of different glutamate receptor subtypes did not affect spine formation, neuronal activity may exert its effects on spine formation via changes in, for instance, release of cotransmitters, neurotrophins or the altered expression of synapse specific molecules. Our findings also show that neuronal afferents clearly increased dendritic branching. In conclusion our results suggest that there are several pathways by which neuronal afferents can influence neuronal structure.

512.15

GAP-43 FACILITATES PEPTIDE HORMONE SECRETION IN MOUSE ANTERIOR PITUITARY AtT-20 CELLS. C. Gamby*‡¶, R. G. Allen†, and L. Baizer‡ . ‡R. S. DOW Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, ¶Dpt of Cell Biology and Anatomy and †Center for Research on Occupational and Environmental Toxicology , Oregon Health Sci. Univ., Portland, Oregon 97209.

The neuronal growth-associated protein (GAP)-43 (neuromodulin, B-50, F1), which is concentrated in the growth cones of elongating axons and in nerve terminals in restricted regions of the adult nervous system, has been implicated in the release of neurotransmitter.

To study the role of GAP-43 in evoked secretion, we transfected mouse anterior pituitary AtT-20 cells with the rat GAP-43 cDNA under control of the Rous Sarcoma Virus (RSV) promoter and derived stably transfected cell lines.

Immunoblot analysis demonstrated that GAP-43 was targeted to the membrane. Depolarization-mediated ß-endorphin secretion was greatly enhanced in the GAP-43-expressing AtT-20 cells without a significant change in Ca^{2+} influx ; in contrast, expression of GAP-43 did not alter CRF-evoked hormone secretion. The transfected cells also displayed a flattened morphology and extended processes when plated on laminin-coated substrates.

We also investigated the interaction of GAP-43 and calmodulin (CaM) in the AtT-20 cells. In the plasma membrane of cells treated with a cross-linking agent, immunoblot analysis with anti-GAP-43 antibodies shows a 70 kDa band corresponding to approximately 30 % of the total GAP-43 immunoreactivity. Immunoprecipitation with anti-GAP-43 antibodies followed by immunoblot analysis with anti-CaM antibodies shows that CaM is associated with GAP-43 in these cells.

These results suggest that AtT-20 cells are a useful model system for further investigations on the precise biological function of GAP-43.

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512.16

NEUROTRANSMITTER RELEASE FROM GROWTH CONES OF CENTRAL CHOLINERGIC NEURONS. H. Tatsumi,+#, S. Tsuji@, H. Soeda+ and Y. Katayama+*. +Department of Autonomic Physiology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan @Département de Cytologie, Institut des Neurosciences, Université Pierre et Marie Curie, Paris, France. #PRESTO JRDC Japan.

Neurons taken from the diagonal band of Broca (DBB) of new born rats (7-9 days after birth) protruded dendrites, axons and growth cones in culture. Neurons acutely isolated from the rat superior cervical ganglion (SCG) were used as a detector of the ACh release, because these cells are endowed with ACh-receptors. SCG neurons were plated on the glass coverslip, on which DBB neurons had been already cultured (2 to 7 days). Using whole-cell patch clamp technique, inward currents were recorded from SCG neurons placed close to the growth cones of DBB neurons electrically stimulated, suggesting that ACh was released from the growth cones. Rapid morphological changes of growth cones were also observed with high resolution video-microscopy at the same time.

AXON GUIDANCE MECHANISMS AND PATHWAYS VI

513.1

EXPRESSION OF A DOMINANT NEGATIVE FGF RECEPTOR IN THE DEVELOPING RETINA. S. McFarlane*, E. Cornel, E. Amaya, and C. E. Holt, Dept. Biology, UC San Diego, La Jolla, CA 92093-0366.

FGF signaling has been implicated in the differentiation of neurons, including retinal ganglion cells (RGCs). For instance, FGFs in culture stimulate neurite extension of RGCs and increase the expression of a differentiation marker, neurofilament protein. Our previous data shows that bFGF is expressed in the developing optic pathway of *Xenopus laevis* and that application of bFGF to the developing retinotectal projection *in vivo* causes retinal axons to grow around and bypass their target, the optic tectum. Our results provide further support for a role for FGF signaling in RGC differentiation. To further test the possibility that FGF signaling is important for the differentiation of RGCs *in vivo* we have blocked FGFR signaling by transfecting developing RGCs with a dominant negative form of the FGF receptor that lacks the intracellular kinase domains. At stage 20, a mixture of DOTAP/ cDNA was injected into the proliferative neuroepithelium of the eye primordium. Embryos were injected with either a dominant negative construct (XFD, Amaya et al., 1991) or control constructs: a non-functional dominant negative (HAVNOT), Green fluorescent protein (GFP), and luciferase constructs. Embryos were allowed to develop for 48 hours until stage 41 when retinal axons have reached their target. Dominant negative and HAVNOT transfected cells were visualized with an antibody against *Xenopus* FGFR, either in cryostat or vibratome sections. GFP transfection was monitored by fluorescence and luciferase with antibody staining.

We observed RGCs expressing high levels of mutant FGFR as judged by intensity of immunostaining. These cells were located in the appropriate retinal lamina and possessed both axons and dendrites suggesting that in the absence of normal FGF signaling many aspects of RGC differentiation occur normally. We are currently investigating whether FGFRs are needed for axonal pathfinding. (Supported by NIH #NS23780, PEW Scholars Award)

513.2

EXPRESSION AND POSSIBLE ROLE OF pp125FAK IN THE DEVELOPING *XENOPUS* RETINAL PROJECTION. T. L. Worley* and C. E. Holt. Dept. of Biology 0366, UC San Diego, La Jolla, CA 92093.

Previously we have reported that the pharmacological inhibition of protein tyrosine kinase (PTK) activity with the drugs herbimycin A and lavendustin A impairs the development of the *Xenopus* retinal projection. In addition, this treatment was found to hinder laminin-dependent retinal neurite outgrowth in culture. One inhibitor-sensitive PTK was identified as pp125FAK, which is known to become stimulated upon integrin-mediated binding in non-neuronal cells. Here we establish the presence and location of pp125FAK with immunohistochemistry in the developing *Xenopus* brain, retina and retinal growth cone. In a Western blot analysis of the same stages of brain and retina, we also show the developmentally regulated expression pattern and phosphorylation levels of pp125FAK. Finally, we observe that cytochalasin D treatment of the developing retinal projection, which in earlier studies was shown to result in aberrant pathfinding and a decrease in filopodial formation, also induces a decrease in tyrosine phosphorylation on pp125FAK. These data suggest a role for pp125FAK kinase activity in the proper establishment of the *Xenopus* retinal projection. This work was supported by NIH#NS23780 and a Pew Scholars Award.

513.3

IN VITRO GUIDANCE OF RETINAL GANGLION CELL AXONS BY RAGS, A 25 KD TECTAL PROTEIN RELATED TO LIGANDS FOR EPH-RECEPTOR TYROSINE KINASES U. Drescher, C. Kremoser, C. Handwerker, J. Löschinger, H. Baier*, M. Noda and F. Bonhoeffer. Max-Planck-Institute for Development Biology, Department of Physical Biology, Spemannstr. 35, 72076 Tübingen, FRG.

Previous in vitro experiments have indicated that a glycosyl-phosphatidylinositol (GPI)-anchored protein may play an important role in guidance of retinal axons during the formation of the topographically ordered retinotectal projection. Within this projection nasal axons of retinal ganglion neurons grow to posterior tectum, temporal retina is connected to anterior tectum. In vitro, in the so-called stripe assay, retinal axons are offered the choise of growing on either anterior or posterior tectal membranes. Temporal axons are repelled by posterior membranes and grow on anterior stripes. The posterior membranes lose their repellent activity after incubation with PI-PLC, an enzyme which cleaves GPI-anchored proteins from the membrane surface. This activity can be detected only between E6 and E12, the time at which ingrowing retinal axons build up a crude scaffold of projections. Therefore, the three criteria assumed for a candidate axon guidance molecule for temporal axons are (i) GPI anchorage, (ii) expression from E6 to E12 and (iii) higher expression in posterior than in anterior tectum. Using 2D gel electrophoresis we identified a 25 kDa protein which fullfills these criteria. Cloning and sequencing of the corresponding cDNA reveals significant homologies to recently identified members of the Eph receptor tyrosine kinase ligand family. By using the stripe assay, we could demonstrate an in vitro guidance activity of this protein, in that retinal axons are repelled from stripes containing membranes derived from p25 cDNA transfected cos cells. These results indicate for the first time a specific function of a member of the Eph receptor/ligand family in processes of axon guidance. Our current investigations focus on the identification of the p25 receptor, an Eph-receptor tyrosine kinase expressed on retinal ganglion cell growth cones.

513.4

AXONAL GROWTH ON A SUBSTRATE GRADIENT OF BASAL LAMINA PROTEINS

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In the early embryonic retina optic axons grow toward the optic disc and form a centripetal axon pattern. To see whether optic axons follow a peripheral-centrally directed substrate gradient in the retina the growth behavior of optic axons was tested on a substrate gradient of basal lamina proteins in vitro. The substrate was an extract from the inner liming membrane from the embryonic retina. The gradients were produced by diffusion of the basal lamina proteins in a drop of PBS on a membrane filter. The slope of the gradients were determined by counting fluorescent beads in the basal lamina extract. Retinal explant stripes were placed perpendicular and parallel to the edges of the gradients and cultured for 30hrs. The growth pattern of the axons, detected by silver staining, showed that optic axons on the border of the gradients became shorter and more fasciculated. However, axons did not turn toward a higher substrate concentration indicating that the gradients were unable to guide optic axons in a particular orientation.

513.5

MATURATION OF AXONAL PATHWAYS WITHIN THE BRAIN AND RETINA OF THE PRENATAL FERRET. J.K. Johnson*, C.L. Winslow, and N.E.J. Berman. Laboratory of Neuroscience, Dept. Anat. & Cell Biol., University of Kansas Medical Center, Kansas City, KS 66160-7400.

The distribution of GAP-43 expression was studied in the ferret (*Mustela putorius furo*) brain and retina in order to examine the ontogeny of prominent axonal pathways.

Animals between the ages of E20 and birth were used in this study. Formaldehyde-fixed brains were embedded in paraffin and serially sectioned. GAP-43 was revealed using standard immunocytochemical techniques. Reacted sections were counterstained with thionin as needed.

Strong GAP-43 immunoreactivity was present by E20, but was limited to punctate staining within the ventricular zone (VZ). No staining was observed within the marginal zone (MZ), nor were any of the central pathways present, including the optic nerve. By E28 the staining within the VZ had ended and axon outgrowth had begun throughout the brain, including the retina. By this time two separate bands of densely-stained fibers within the developing white matter (WM)/intermediate zone (IZ) had formed. Additionally, the MZ and SP had become lightly labeled, with occasional punctate staining present within the CP. Also present were strongly stained optic nerves. Most remaining major intrahemispheric central pathways expressed GAP-43 by this time (e.g., optic nerve, internal capsule). By E34 all major pathways were densely stained, including the newly formed, and densely stained, corpus callosum.

These results indicate that premigratory neurons begin to express GAP-43 while still in the VZ. Prominent central pathways form within a narrow window of time with intrahemispheric pathway development preceding formation of the corpus callosum.

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513.6

CELLULAR LOCALIZATION OF GUIDANCE COMPONENTS IN THE ESTABLISHMENT OF RETINOTECTAL TOPOGRAPHY

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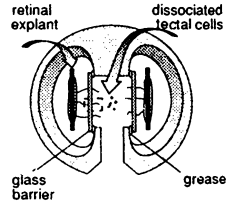
Establishment of retinotectal topography involves guidance activities expressed on the surface of tectal cell membranes. To preserve and present as many cellular guidance components to elongating retinal fibers, we designed a 3-compartment chamber to delimit areas where cultured embryonic retinal and tectal cells encounter one another. Fibers from temporal retinae of the chick (E6-E9) stopped at the edge of posterior (caudal) tectal cells, whether dissociated from chick (E5-E11) or rodent (mouse, E14; rat E17-E18). Substantial invasion by nasal axons occurred in the same culture dish and both temporal and nasal fibers grew well into regions with either chick or rodent anterior (rostral) cells. Thus, dissociated cells yielded results consistent with previous results from tectal membrane fragments: components repulsive for temporal axon extension are expressed in posterior tecta of both chick and rodent.

Cellular localization of guidance cues can indicate sequential processes involved in establishment of topographic projections; therefore, retinal growth cone encounters with individual tectal cells were examined with time-lapse video microscopy. Posterior tectal neurons appear selectively repulsive for temporal retinal growth cones, causing growth cone collapse and retraction. On the contrary, radial glia from all regions of the tectum attenuated retinal axon extension, without inducing sudden retraction. Nasal growth cones turned and tracked along radial glia from their natural targets, potentially indicating a second set of specific guidance cues in this system. This work was performed while RWD held a National Research Council-NICHD/NIH Research Associateship.



513.7

PRENATAL DEVELOPMENT OF RETINOFUGAL PROJECTIONS IN THE RAT: TARGET FINDING OF RETINOGENICULATE AXONS. Y.Kondo*[1,2], M.Takada[1], and N.Mizuno[1]. [1]Dept.Morphological Brain Science, Kyoto Univ., Kyoto 606-01, JAPAN. [2]Dept.Neurosciences, The Cleveland Clinic Foundation, Cleveland, OH 44195.

Employing anterograde axonal tracing with DiI, we investigated the prenatal development of retinogeniculate projections in the rat. The tracer was deposited into the retina at embryonic day(E) 16, 17, 18, 19 or 20. At E16 and E17, retinal axons were directed towards the lateral geniculate body (LG) and superior colliculus (SC), bilaterally with a clear-cut contralateral predominance. Virtually all of the axons passed through the optic tract without their penetration into the LG, although many of them reached as far as the SC by E17. At these ages, a considerable number of axons left the optic tract on the way to the LG and SC to course medially towards the third ventricle. At E18, some of the retinal axons that arrived at the SC turned to come back to the LG, and some of the retinal axons that passed through the optic tract immediately lateral to the LG brought forth axonal branches to enter the LG. These turning and budding axons for innervating the LG increased in number at E19 and E20; at these ages, medially-directed axons towards the third ventricle were no longer detected. The results indicate that retinal axons recognize the SC as the primary target and then the LG as the secondary one.

513.8

PATHWAYS FOLLOWED BY AXONS FROM RETINAE TRANSPLANTED TO CONGENITALLY BLIND MICE. R.D. Lund[1] and M.H. Hankin*[2]. [1]Institute of Ophthalmology, London EC1V 9EL U.K. and [2]Medical College of Ohio, Toledo, OH 43614.

In order to examine the role of pre-existing pathways in providing guidance cues for optic axons, we studied the course followed by axons originating from embryonic retinae implanted in newborn *ocular retardation* (*or*^{J}/*or*^{J}) mice in which the optic nerve fails to develop. Retinae placed on the ventrolateral brainstem (apposed to the site of the optic tract) send axons dorsolaterally to innervate the ipsilateral superior colliculus and several other subcortical visual centers. Not all axons, however, follow the normal direction of the retinofugal projection: some course ventrally to cross at the level of the suprachiasmatic nucleus (or less frequently caudal to the mammillary body) to attain the position of the contralateral optic tract and grow towards visual centers on the contralateral side. Furthermore, retinae implanted in the internal capsule emit axons that follow projection fibers through the striatum to innervate the lateral geniculate and other optic nuclei.

These results show that prior existence of an optic projection is not necessary for axons derived from ectopic retinae to attain visual nuclei. While there seems to be a preference for growth along the course of the optic tract and chiasm (perhaps following molecular cues or pre-existing pathways such as the TPOC), this is not the only route that optic axons can follow in the brain. The fact that axons grow in the "wrong" direction along the course of the optic tract may argue that the orientation of growth is not conferred by the substrates over which the axons grow. Another possibility, however, is that some axons are responding selectively to cues which specify the laterality of projection.

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513.9

METABOLIC LABELING OF GLYCOSAMINOGLYCANS IN THE DEVELOPING TECTUM OF THE SYRIAN HAMSTER.

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It is widely accepted that sulfated proteoglycans (PGs) influence axon development; however, the cellular and molecular mechanisms of this influence remain to be elucidated. In addition, relative contributions of protein core and glycosaminoglycan (GAG) chain(s) to PG function have yet to be clarified. We have examined the distribution of PGs in Syrian hamster tectum during the time retinotectal axons grow in and arborize. Previous immunohistochemical experiments have shown that chondroitin sulfate (CS) and keratan sulfate (KS) GAGs are concentrated primarily at the tectal midline, where retinal axons do not grow. This observation along with results of in vitro studies suggest a role for CSPG(s) and KSPG(s) as barriers to retinal axon growth. However, analysis of CSPG and KSPG protein cores isolated from midline and lateral tectum has suggested that similar cores are present in both areas (D. Hoffman et al., Soc. Neurosci. Abstr. 1994). We have now examined GAG production: coronal slices through developing tectum were incubated for 30 minutes in sulfate-deficient medium, cultured for 5-20 hours in medium containing 250 µCi/ml ^{35}S-labelled sodium sulfate and 50 µM net sulfate, then fixed. To identify the sulfated GAG(s) produced, tissue slices were digested with either chondroitinase ABC, keratanase, heparatinase, or no enzyme. Exposure of undigested slices to film indicated a concentration of radioactivity at the midline, with very little isotope present in lateral tectum. Preliminary results show that in slices treated with chondroitinase ABC, the midline radioactivity concentration decreased substantially. These results suggest that while similar PG cores are present throughout the tectum, midline cells produce more sulfated GAG (particularly CS) than lateral cells.

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513.10

EXPRESSION OF AGGRECAN, A CHONDROITIN SULFATE PROTEOGLYCAN, IN THE DEVELOPING CHICK VISUAL PATHWAY. B.D. McAdams*, M. Domowicz, N. Schwartz and S.C. McLoon. Univ. of Minnesota, Minneapolis, MN 55455 and Univ. of Chicago, Chicago, IL 60637.

The growth of retinal axons through the developing retinofugal pathway involves the interaction of growth cones with a diverse set of cell surface and extracellular matrix (ECM) molecules. One class of ECM molecules, chondroitin sulfate proteoglycans, was previously identified in the path of growing retinal axons. The present study characterized the expression pattern of a chondroitin sulfate proteoglycan, aggrecan, in the developing retinofugal system using the S103L antibody. This antibody recognizes a form of aggrecan expressed in neural tissue which is not susceptible to keratanase digestion and which reacts with antibodies to 6-sulfated and unsulfated chondroitin. Aggrecan immunoreactivity was first detected in the pathway on E6 and was associated with radially-oriented cells of the peripheral retina and optic nerve head. By E7, aggrecan was found in the optic nerve, chiasm, and tract. Initially (E6-7), the aggrecan of the developing tectum was associated with radially oriented cells. In tectum from E7 to E12, aggrecan appeared progressively in a rostral to caudal sequence in the layers of long projecting axons including the retinal axon layer. During the period of retinal axon synaptogenesis, arborization, and refinement (E12-E16), aggrecan also appeared in intermediate tectal layers where these processes occur. The distribution of aggrecan in the developing visual pathway suggests that it may have a role in retinal axon growth and synaptogenesis. To test this possibility, retinal axon growth was examined in a mutant, the nanomelic chick. Nanomelic embryos express a truncated form of aggrecan that is still immunoreactive but is not secreted. Aggrecan immunostaining in the pial endfeet of radial glia as well as in the endoplasmic reticulum of other cells in the optic pathway in nanomelic embryos indicates that these cells normally synthesize aggrecan. Alteration in the timing and pattern of retinal axon growth was not evident in these mutants, which suggests that extracellular aggrecan does not have an essential role in retinal axon extension or guidance; however, the timing and distribution of aggrecan suggests a role for this proteoglycan in other developmental processes.

513.11

GROWTH CONE DYNAMICS OF *IN VIVO* RETINAL PATHFINDING ERRORS INDUCED BY CHONDROITIN SULFATE

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The axons of developing retinal ganglion cells (RGC) navigate along a defined route to reach their target, the optic tectum. Our previous studies have shown that exogenous chondroitin sulfate (CS) has a striking effect on pathfinding: retinal axons disperse widely from their normal trajectory and extend aberrantly in the telencephalon and diencephalon. In this study, we investigated the dynamic behavior of RGC axons and their growth cones in response to the application and withdrawal of CS *in vivo*.

Live time-lapse video experiments of DiI labeled RGC axons were performed on exposed brain preparations to image extending axons during the period when they grow from the base of the optic tract to the tectum (stages 33/34 to 40). Although growth in 10 mg/ml CS was slowed compared to controls, growth cone morphology was not obviously changed as the growth cones exhibited numerous filopodia and active lamellipodia. Pathfinding errors occurred mainly by wide turns in inappropriate directions. These errors could be detected usually 1 to 2 hours after CS application and were more dramatic for newly ingrowing axons than for axons that had already grown a significant distance along the tract.

We have shown previously that if CS is applied for 10 hours and then washed out for 8 hours, optic projections appear normal. We are now using the live time lapse set-up to investige how individual axons behave during recovery to see if misrouted axons can correct their routes or are retracted.

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513.12

ENGRAILED MISEXPRESSION PERTURBS THE TOPOGRAPHIC TARGETING OF RETINAL AXONS IN THE CHICK OPTIC TECTUM.

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The targeting of retinal axons to their topographically correct termination zone (TZ) in the optic tectum is hypothesized to be controlled by guidance molecules distributed in a graded or restricted manner. The expression of these molecules is probably regulated by transcription factors and growth factors. The *engrailed* homeobox transcription factors (*En-1* and *En-2*) are two likely candidates for this function. *En* is the earliest identified molecule to be expressed in a gradient in the chick mesencephalon. In chick/quail chimeras, graded *En* expression correlates with the targeting of retinal axons in the tectum; temporal axons terminate rostrally in low *En* regions and nasal axons terminate caudally in high *En* regions (Itasaki & Nakamura, '92).

To assess more directly the role of *En* in the development of the retinotectal map, we have constructed replication competent virus (RCAS) expressing *En-1* or *En-2*. The virus (10^8-10^9 iu/ml) was injected into the mesencephalic lumenal space of st. 10 to 12 chick embryos. Infection of cells in vitro and in vivo, as determined with an antibody to the gag viral protein, correlated with expression of the exogenous *En* genes, as determined by immunohistochemistry and in situ hybridization. The retinotectal projection was later analyzed after the retinotopic map is normally established by focal injections of DiI in temporal or nasal retina. In virally infected tecta, nasal axons form abnormally large and diffuse TZs, and temporal axons appear to exhibit abnormal behaviors including a failure to end in the correct TZ either due to a slowing of their growth or to retraction. These results suggest that overexpression of *En* disrupts the development of the retinotopic map. (Supported by R01 EY07025 & F32 EY06550)

513.13

EXPRESSION OF PSA-NCAM IS NOT ESSENTIAL FOR THE DEVELOPMENT OF CONGRUENT BINOCULAR MAPS IN *XENOPUS* TECTUM.

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Expression of the polysialylated form of neural cell adhesion molecule (PSA-NCAM) is enhanced during development and has been correlated with expression of plasticity. Studies from this laboratory have demonstrated high levels of PSA-NCAM expression during the critical period for the establishment of binocular maps in the optic tectum of *Xenopus*. As a test of whether PSA is necessary for the alignment of axonal projections from the ipsilateral eye with axonal projections from the contralateral eye, PSA was removed from the tectum by an enzyme, endoneuraminidase (endo N), that specifically cleaves polysialic acid at an α-2,8 linkage. Slices of elvax polymer impregnated with endo N were placed on the surface of the tectum immediately after metamorphosis and left in place for 1-4 months. Removal of PSA in response to endo N treatment was confirmed with western blots and tecta probed with a monoclonal antibody to PSA.

The effects of PSA removal on the morphology of isthmotectal axons were assessed from tectal wholemounts of HRP-labeled isthmotectal axons. We found no qualitative differences in the extent of ingrowth and arborization of isthmotectal axons when comparing endo N-treated tecta with control tecta. In addition, electrophysiological mappings of the binocular visual field revealed no anomalies in the alignment of ipsilateral and contralateral axonal projections in endo N-treated tecta.

These results suggest that although PSA-NCAM expression in the tectum is correlated with periods of plasticity, the PSA moiety is not crucial to the development of congruent binocular maps in otherwise normal brains.

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513.14

RETINAL GANGLION CELL PROJECTIONS ARE MODIFIED BY TRANSPLANTATION OF BDNF PRODUCING CELLS TO THE SUPERIOR COLLICULUS OF DEVELOPING RATS.

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Developing retinal axons must follow a stereotypical course directing them to subcortical visual centres which on arrival they must recognise. Transplantation studies suggest that local substrates close to the surface of the brainstem and diffusible factors emanating from the target region are important. We have already shown that BDNF producing fibroblasts can guide transplanted embryonic retina in the cortex. In order to test for the role of diffusible factors in the developing retino-collicular pathway, we have transplanted BDNF producing fibroblasts to the tectum of neonatal rats which have / have not had an optic axotomy. After 3-4 weeks, the resulting retinal projections were visualised by injecting the retrograde tracer HRP intravitreally and the animals perfused and stained two days later with TMB.

In the developing retino-collicular pathway of the rat, each eye distributes its connections across the whole area of both ipsilateral and contralateral superior colliculus. Most of the ipsilateral projections retract during the first ten postnatal days to produce the normal adult pattern of connections. When one eye is removed at birth this retraction fails to occur and there remains a strong ipsilateral projection. Preliminary results suggest that when BDNF producing fibroblasts are transplanted to the contralateral tectum of an optic axotomised neonatal rat, the ipsilateral projection is reduced. These results suggest that BDNF may play an important role in guiding developing retinal axons.

We would like to thank MRC and Action Research for financial support and F.H.Gage for kindly providing the BDNF cell line.

513.15

EFFECTS OF NEUROTROPHINS ON LAMINA-SELECTIVE BEHAVIOR OF RETINAL GANGLION CELL NEURITES IN CULTURED OPTIC TECTUM.

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In many parts of the central nervous system, ingrowing axons enter, arborize, and synapse in specific laminae. To study such laminar specificity, we designed a co-culture system from chick embryos, in which retinal explants are laid atop tectal slices so that retinal ganglion cell (RGC) neurites have equal, unimpeded access to all tectal laminae. Laminar specificity of RGC outgrowth, branching and arborization is conserved in this system, even when the tectal slice is chemically fixed before RGCs are added (Yamagata and Sanes, Dev. 121:189,1995). These results implicate membrane- or matrix-bound cues as regulators of arbor formation, but studies by others suggest that soluble factors are also involved. To learn how these two classes of factors interact, we asked whether neurotrophins affect RGC outgrowth or arborization on tectal sections. Addition of BDNF to serum-free medium nearly doubled the number of neurites that branched to leave their ingrowth layer (the stratum opticum, SO) and enter the retinorecipient layers (RR). BDNF also increased the number of neurites that formed secondary branches (rudimentary arbors) in the RR. Effects were detectable at 2ng/ml and nearly maximal at 10ng/ml. Stimulation of branching occurred in the absence of significant effects on the total number or maximal length of neurites. Moreover, neither the selectivity of neurite outgrowth on the SO, nor the restriction of branches and arbors to the RR was affected by neurotrophins. NT3 and NT4/5 had similar effects, but NGF was ineffective in this assay. These results favor the idea that soluble and cell surface factors collaborate to shape axonal arbors, with the former predominantly affecting their size and the latter their position. (Supported by NIH).

513.16

AN IN VITRO ASSAY FOR STUDYING INTERACTIONS OF RETINAL AXONS WITH RADIAL GLIA OF THE OPTIC CHIASM

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At the optic chiasm retinal ganglion cell axons from the temporal part of the retina turn into the ipsilateral optic tract, whilst axons arising in the nasal part of the retina cross to the contralateral tract. It is believed that the ipsilaterally projecting axons are prevented from crossing at the chiasm by an inhibitory signal on a sub-population of radial glia in the midline. Our interest is in characterising this differential behaviour of retinal axons with midline glial cells.

We have established an in vitro assay to examine the behaviour of temporo-ventral and dorso-nasal retinal axons co-cultured with ependymal glia isolated from the diencephalon.

Ependymal glia can be isolated from the ventral diencephalon of E17 mouse brains by trypsinisation for one hour followed by mechanical dissociation. These cells are then cultured on a laminin substrate, or on basal lamina isolated from E7 chick retina. After 3 days in culture the glial identity of the these cells has been confirmed by positive staining with α-GFAP and by negative staining with neuron associated antisera (HNK-1; 3A10; 2H3). The antibody SSEA-1 that is a marker for the midline glia of the ventral diencephalon, stains a sub-population of the GFAP positive cultured glial cells, indicating that we have successfully isolated the midline glia. Explants of E13 mouse retina are taken from either the dorso-nasal or temporo-ventral retina and also grown on E7 chick retinal basement membrane. Co-cultures of retina and isolated glia have been successfully established and interactions between retinal axons and these glia studied using time-lapse video microscopy. Our preliminary observations of interactions between retinal axons and midline glial cells will be presented.

This work is supported by grants from the MRC and Wellcome Trust

513.17

AXONAL, GLIAL AND PROTEOGLYCAN INTERRELATIONSHIPS IN THE DEVELOPING OPTIC PATHWAY OF FETAL FERRETS.
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This study has examined the relationship between radial glial fibers, optic axons, and the distribution of chondroitin sulfate proteoglycans in the developing optic pathway. Standard immunohistochemical procedures were used to define the distributions of the cytostructural protein, Vimentin (Vim), and of the glycosaminoglycan, chondroitin sulfate (CS), in fetal ferrets.

On E-24, when the first pioneering optic axons have entered the optic tract, Vim-positive glial fibers extend radially from the ventricular surface to form endfeet at the ventral, pial, surface, where CS is also present. As optic axonal invasion proceeds, Vim-positive radial glial fibers become diverted to course parallel to the pial surface within the dorsal, deeper, parts of the developing pathway (Reese et al., J. Comp. Neurol. **349**, 303); coincident with these glial fibers, the deeper parts of the tract are immunoreactive for CS on E-27 through E-33, when the sub-pial parts are relatively immunonegative. Early removal of one eye on E-25, which produces an almost entirely crossed projection from the opposite retina (Chan and Guillery, J. Neurosci. **13**, 5277), leads to the maintenance of an exclusively radial glial architecture in the ventral diencephalon contralateral to the removed eye on E-30, where the distribution of CS remains elevated at the pial surface.

These results show that the glial architecture of the developing optic pathway is sculpted by the neuronal architecture, and suggest that the CS-bearing proteoglycans within the pathway are produced by the radial glia. This changing glial and molecular architecture may in turn influence the growth of later-arriving optic axons, since these enter the superficial, CS-free, parts of the pathway only, establishing a chronotopic ordering of axonal arrival.

513.18

RETINAL AXON DIVERGENCE: INHIBITORY CUES FROM THE CHIASM MIDLINE DETECTED IN COLLAGEN GEL COCULTURES L.-C. Wang*, R. Rachel, R.C. Marcus, and C.A. Mason. Dept. Path., Cntr. Neurobiol. Behav., Columbia Univ., NY, NY 10032.

During divergence of retinal axons to both sides of the brain, both crossed and uncrossed fibers travel toward the midline, and either cross the midline or turn back to the ipsilateral optic tract, respectively (Godement et al., J. Neurosci. 14: 7024). Recent studies in vitro have demonstrated that cells of the chiasm midline elicit differential outgrowth of crossed and uncrossed fibers, in patterns that mimic avoidance or growth across the midline (Wang et al., submitted; Wang et al., Soc. Neurosci. Abstr. 20: 1081). To continue to investigate how retinal growth cones detect cues for divergence, we analyzed the retinal axon growth from ventral temporal retina (source of uncrossed fibers) and from dorsotemporal, dorso- and ventronasal retina, all sources of crossed fibers. An explant of retina was positioned 200-400 microns away from an explant of the optic chiasm midline, both taken from E15 mouse embryos, and embedded in bovine or rat tail collagen. After coculture for 48 hrs, there was little evidence for either a tropic or anti-tropic effect of the chiasm on retinal neurite outgrowth, using criteria from published studies. However, chiasm explants exerted a general growth-retarding effect on retinal axons manifested as fewer and shorter neurites extending from the side of the explant nearest the chiasm. These patterns were generally not seen when the retinal explant was positioned opposite a piece of control tissue. At present, we cannot distinguish to what extent the neurite growth response depends on contact with the chiasm, and to what extent factors are released and/or are diffusible, all currently under investigation. The growth reducing effects of the chiasmatic midline on retinal axon growth are consistent with our previous studies indicating that the chiasm contains inhibitory factors that contribute to retinal axon divergence. Supported by NS 27615 to CAM.

513.19

THE ADVANCE OF AXONS THROUGH THE OPTIC CHIASM OF FETAL MICE. S.J. Tavendale* and R.W. Guillery. Dept. of Human Anatomy, Oxford University, South Parks Road, Oxford, UK, OX1 3QX.

Previous studies of the retinofugal fibres showed growth cones distributed throughout the optic nerve but accumulated next to the pia as the nerve joins the chiasm and in the tract (Guillery and Walsh, *J. Comp. Neurol.* **265**:203-217; Colello and Guillery, *J. Comp. Neurol.* **317**:357-378). In this region the glial environment also changes from an interfascicular to a radial organisation. Reese *et al.* (*J. Comp. Neurol.* **349**:303-324) showed that within the chiasm itself, some of this order is lost, with growth cones also lying in a deeper position.

Electron micrographs obtained from sections spaced at 20μm intervals, cut parallel to the fibres, and extending from the prechiasmatic nerve to the origin of the optic tract in E16 and E17 mouse embryos, show that as the fibres leave the optic nerve, the growth cones move towards the ventral surface and the finer, more mature fibres, cut longitudinally, accumulate deeper in a more or less continuous field. As the fibres are traced into the chiasm, groups of fine axons and wrists course ventrally and mingle with the growth cones. These form transversely cut groups of 100-200 fibres that cross perpendicular to similar, longitudinally cut groups nearer the midline. The longitudinally cut groups dominate in the dorsal part of the midline, the transverse groups dominate ventrally. Growth cones and wrists are present throughout the dorso-ventral extent of the chiasm, but tend to be more common in ventral regions.

We conclude that growing axons become arranged in a chronotopic order before they enter the chiasm, lose some or all of this order within the chiasm as they negotiate the specialised glia of the midline, and regain a chronotopic order in the tract. The crossing in well defined bundles suggests that axons from the same eye fasciculate preferentially with each other, and that the difference between the adult chiasm of birds and eutherian mammals may be only a minor developmental one.

NEUROTRANSMITTER SYSTEMS AND CHANNELS II

514.1

GABA EXPRESSION IN DEVELOPING SPINAL CORD NEURONS IN RAT PRIMARY CELL CULTURES.
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GABA is expressed in embryonic neurons from retina(Versaux-Botteri et al., -94) and spinal cord (Behar et al., -93). Furthermore, GABA has been shown to be released from growth cones (Taylor et al.,-90) as well as to excite hippocampal neurons in the first postnatal period (Cherubini et al., -90). For these reasons GABA has been proposed to have a trophic role in early neural development. In order to study GABAs role during neural differentiation and synaptogenesis we performed an immunohistochemical study on primary cell cultures in order to establish the basis for further electrophysiological studies of neural and synaptic functions during development.

Cell cultures were prepared from embryonic rat pups (stage E14). Analysis were performed on day 1,3,7,14 and 28 in culture. GABA could be detected already at day 1. The intensity of immunoreactivity (IR) as measured with ELISA increased to reach a peak at day 7. From day 7-14 the immunoreactivity gradually decreased. Immunoreactivity to neurofilament did not decrease. GABA-IR was observed in the cytoplasm at day 1-3. At day 7 the GABA-IR was strongest in the periphery of the cellbodies and in the extending processes. These findings show that GABA is expressed in a similar pattern as in intact spinal cord and that primary cell cultures can be used for studies on GABAs role during development. Experiments are now performed in order to study the effects of GABA on transmittor expression and neuronal excitability.

514.2

GABAERGIC PROPERTIES OF HUMAN NEURONS DERIVED FROM THE TERATOCARCINOMA CELL LINE N-TERA 2 (NT2) S.R.Kleppner*, M.A.Dichter, and V.M.-Y.Lee Inst. of Neurol. Sci., and Depts. of Path. and Neurol., Univ. of Penn. Sch. of Med., Philadelphia PA 19104

NT2 cells are capable of generating large numbers of pure post-mitotic neuronal cells (NT2N) upon treatment with retinoic acid. The NT2N cells have previously been transplanted into nude mouse brain, and can survive for extended periods of time. Preliminary immunocytochemical studies revealed that implanted NT2N cells express the GABA biosynthetic enzyme glutamic acid decarboxylase (GAD) and further immunostaining in vitro showed that NT2N cells express GABA and one isoform of GAD, GAD_{67} but not GAD_{65}. Both GABA and GAD_{67} levels appear to increase with time in culture. Western blot analysis confirmed the GAD immunocytochemistry. In addition, biochemical assays indicate that the NT2N cells show sodium-dependent GABA uptake. NT2N cells immunostain for synaptic densities with synaptophysin, and electron micrographs indicate the presence of synapse-like structures. Functional $GABA_A$ receptors have been electrophysiologically demonstrated on these cells although no functional inhibitory synapses have yet been observed. As there are few GABAergic cell lines available, and NT2 is a human neuronal cell line, these results suggest that the NT2N cells can be useful for studying the development and differential regulation of GABA function and inhibitory synaptogenesis in a human neuronal system. These NT2N cells may also prove useful for transplantation in situations in which GABAergic activity is reduced or where locally increased inhibition is required to dampen excess excitability. Further characterization of the GABAergic properties of these cells by HPLC, GAD activity assays and autoradiography is in progress.

514.3

DISTINCT EXPRESSION PATTERNS OF $GABA_A$ RECEPTOR SUBUNITS IN EMBRYONIC AND EARLY POSTNATAL RAT CNS. F. Lahjouji, W. Ma, A. DA Cunha[1], W. Sieghart[2] and J.L. Barker Lab. Neurophysiology, NINDS; [1]Lab. Cell biology, NIMH, NIH, Bethesda, MD 20892; [2]Dept. Biochemical Psychiatry, University Clinic for Psychiatry, Vienna, Austria

Although there are quite a few reports of transcript expression for $GABA_A$ receptor subunits in the developing CNS, little is known about the pre- and post-natal expression of the subunit peptides. We examined the localization of $GABA_A$ receptor $\alpha1$, $\alpha2$, $\alpha3$, $\beta1$, $\beta3$, $\gamma1$ and $\gamma2$ subunits in the developing cortex, hippocampus, thalamus and hypothalamus using immunocytochemistry with polyclonal antibodies. The $\beta1$ and $\beta3$ subunit immunoreactivity was first detected at E12 in fibers and then at E13 in cell bodies within the mantle zone of the rhombencephalon. At E14, all tested subunits, except $\alpha1$, were detectable in the brain. The $\alpha1$ began to appear in the cortical plate and hypothalamus at E17, and in the hippocampus and thalamus during the first week postnatal. The $\alpha1$ increased and expanded progressively throughout brain. The $\alpha2$, $\alpha3$ and $\beta3$ subunits showed intense immunostaining throughout the four regions studied at E20, but declined dramatically in subcortical regions after the first week postnatal. The $\gamma2$ subunit showed a relatively constant pattern of staining. The $\beta1$ and $\gamma1$ subunits were clearly detected in the cortical ventricular zone at E17 and E20. The results are generally consistent with our findings of subunit transcript expressions and show the early emergence and age- and region-dependent expressions of the $GABA_A$ receptor subunits during embryonic and early postnatal period coincide with neurogenesis and further neuronal differentiation, suggesting that the early detected $GABA_A$ receptor subunit peptides in vivo may play distinctive roles in the development of the CNS

514.4

TRANSIENT APPEARANCE OF ALTERNATIVELY-SPLICED GAD_{67} TRANSCRIPTS IN EMBRYONIC CNS AND THEIR RE-EXPRESSION IN THE TRAUMATIZED ADULT RAT SPINAL CORD

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It is known that there are at least two forms of GAD proteins (GAD_{65} and GAD_{67}) encoded by distinct genes. Recent studies have shown that during embryogenesis the GAD_{67} gene undergoes developmentally-regulated alternative splicing. The translation of the spliced GAD_{67} mRNA (EP10) may result in enzymatically active truncated GAD proteins. However, the functional significance of the truncated GAD proteins is unknown. We examined the cellular expression of GAD family mRNAs in developing and adult rat CNS using *in situ* hybridization. GAD_{65}, GAD_{67} and EP10 mRNAs emerged concurrently at E12 in both the germinal and mantle zones of the ventral parts of the forebrain, rhombencephalon and cervical spinal cord. During the late embryonic/early postnatal period, GAD_{65} and GAD_{67} mRNAs increased in density and cellular distribution throughout the CNS, while EP10 began to disappear progressively in most brain regions and spinal cord. In adult brain, EP10 remained only in the olfactory bulb, dentate gyrus of hippocampus and cerebellum in which granular cell proliferation persists. After compression of the adult male rat spinal cord, EP10 was reinduced within 3 days and persisted up to 5 weeks when rats had recovered from complete paralysis of both hind limbs to ambulatory levels of activity. Transient abundance of EP10 during neurogenesis and its recapitulation in the crushed spinal cord strongly suggest roles for truncated GAD proteins in neuronal generation and regeneration.

514.5

CELLULAR DISTRIBUTION OF TWO GLUTAMIC ACID DECARBOXYLASE ISOFORMS IN EMBRYONIC RAT HIPPOCAMPAL CELL CULTURES.

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In order to estimate the synthesis of γ-aminobutyric acid (GABA) in cultured GABAergic neurons, we used immunocytochemistry to study the development of the two isoforms of glutamic acid decarboxylase (GAD), GAD65 and GAD67, in hippocampal cell cultures during 28 days *in vitro* (28 DIV). The cells with GABAergic phenotype were identified using antibodies recognizing either GAD65, GAD67 or GABA. Two technical approaches were used: the immunoperoxydase and the immunofluorescence methods for single and double-staining experiments, respectively. At 21 DIV, GAD67 and GAD65 antibodies similarly stained the cell bodies and neurites of GABAergic hippocampal neurons. However the density of GAD65-immunoreactive axon terminals (punctate structures) is higher than the density of GAD67-immunoreactive axon terminals. At this stage, double-staining procedures showed a perfect colocalization of GAD65-like immunoreactive (GAD65-LI) neurons with the GAD67-LI neurons. At 7 DIV, GAD65-LI neurons accounted for 39% of the number of GABAergic neurons while GAD67-LI neurons represents 100% of the GABAergic neurons. Between 7 DIV and 28 DIV the amount of GAD65-LI neurons regularly increased to stabilize at approximatly 100% of the number of GABAergic neurons. During the same period of time, the number of GAD67-LI neurons was unchanged. These results show an earlier appearance of GAD67 compared to GAD65 in GABAergic neurons in hippocampal cell cultures. The possibility that different functions could be associated to the two isoforms of GAD as previously hypothesized by Esclapez and al. (J Neurosci., 1994,14:1834) will be discussed in light of functional data. Supported by I.N.S.E.R.M.

514.6

NITRIC OXIDE 'PACES' GIANT GABAERGIC ACTIVITY IN THE DEVELOPING RAT HIPPOCAMPUS THROUGH A TRANSIENTLY EXPRESSED NEURONAL STRUCTURE. F. Strata*, M. Atzori and M. Molnar. Biophys. Lab., Int. Sch. Adv. Studies (SISSA), Via Beirut 2-4, 34013 Trieste, Italy.

The diffusible messenger Nitric Oxide (NO) has been proposed to play a role in the mechanisms underlying synaptic plasticity, development and synchronization of neural activity (Gally et al. PNAS 87, 3547-3551, 1990). We used intracellular and patch-clamp techniques to study a possible role for NO in the origin of giant GABA responses occurring at 0.02-0.2 Hz during development of the rat hippocampus. Experiments were performed on hippocampal slices obtained from neonatal rats (P1-P8) with microelectrodes filled with 3M KCl and patch pipettes filled with 146 mM KCl. Application of the NO precursor L-Arginine (0.2-2 mM) or the NO donor 3-Morpholinosydnonimine hydrochloride (SIN-1, 0.2-2.0 mM) inhibited Giant Depolarizing Potentials (GDPs). In analogy with thalamus (Pape and Mager, Neuron 9, 441-448, 1992) we tested the hypothesis of a hyperpolarization-activated 'pacemaker' current controlled by NO. Voltage-clamp experiments on hilar interneurons patched under visual control revelead the presence of a cationic inward current which was reduced by 39±15% (mean±S.E.M.) in the presence of 0.1-1 mM L-Arginine and by 64±5% by 0.3 mM Cs^+. The same concentration of Cs^+ reversibly inhibited GDPs suggesting a role for such inward current in their generation. Biocytin injected into one hilar neuron spread throughout several hilar interneurons revealing the existence of a neural structure which was absent in the adult. The present data suggest that NO 'paces' the giant GABAergic electrical activity through a transient neuronal meshwork expressing an unusually Cs^+ sensitive inward rectifier current.

514.7

GABA TRANSPORTER (GATs 1-4) mRNA EXPRESSION IN EMBRYONIC AND POSTNATAL MOUSE BRAIN. J.E. Evans*, A. Frostholm and A. Rotter. Dept. of Pharmacology and the Neuroscience Program, The Ohio State University College of Medicine, Columbus, OH 43210.

Four GABA transporter mRNAs (GATs) were examined in developing mouse brain using in situ hybridization with radiolabeled oligonucleotide probes. GATs 1 and 4 were densely distributed throughout the developing central nervous system. They were present over proliferating and migrating cells in subventricular zones at embryonic day 13-14 and throughout prenatal development. During postnatal development, the two signals spread throughout the brain: In adults, the highest GAT 1 signal was present in the hippocampus, olfactory bulbs, and cerebellar cortex, and GAT 4 was distributed throughout the brain stem nuclei, olfactory bulbs, spinal cord, deep cerebellar nuclei and the cerebellar granule cell layer. Unlike GATs 1 and 4, the expression of GATs 2 and 3, was largely restricted to the pia-arachnoid membrane. The GAT 2 mRNA also was expressed in proliferating and migrating cerebellar granule cells. Although GAT 1 also was present over the pia-arachnoid, it was expressed at lower levels and in a more restricted pattern within the membrane. The different developmental profiles of the four transporters may be indicative of their neurochemical roles: Although GATs 1 and 4, may subserve the classical purpose of terminating GABA transmission in neurons and glia, GATs 1, 2 and 3, also may control GABA levels in CNS vasculature. Thus, GATs in the pia-arachnoid may help to regulate the amount of GABA available to developing neurons, such as cerebellar and olfactory granule cells, which migrate along the sub-pial surface during late embryonic and early postnatal development.

514.8

BOTH GAD65 AND GAD67 ARE PRESENT DURING CHICK EMBRYONIC BRAIN DEVELOPMENT. M.-O. Mattsson*, A.-K. Åhman and F. Wågberg. Dept. of Cellular and Developmental Biology, Umeå University, S-901 87 Umeå, Sweden.

We have in the present study investigated some features of GABAergic development, notably some aspects of the GABA-generating enzyme GAD and its expression, in the prosencephalon and telencephalon of the chick embryo. HPLC analysis revealed that the GABA content in the developing telencephalon increased steadily from embryonic day 3 (E3) to E17, which is due to a 20-fold increase in GAD activity. RT-PCR revealed that both GAD65 and GAD67 mRNA is present from E3 onwards. The chick specific probes that we raised indicate one major GAD65 transcript at 5.1 kb and a minor transcript at 3.9 kb on Northern blots. GAD67 mRNA has one principal band at 5.6 kb with a less intense band at 3.9 kb. Both mRNA´s exhibit an increased expression during development, with a peak at E17. Immuno-blot analysis confirm the presence of both GAD65 and GAD67 proteins during the investigated period. Immunoprecipitation studies are in progress to elucidate the contribution of the respective protein to GAD activity. These results will be valuable for understanding the specifik role of the two GAD proteins during brain development.

514.9

MOLECULAR CLONING OF MOUSE GLUTAMIC ACID DECARBOXYLASE AND GABA RELEASE FROM cDNA-TRANSFECTED CELLS. H. Asada*, K. Maruyama, Y. Kawamura, and K. Obata. Lab. of Neurochemistry, Natl. Inst. for Physiol. Sci., Okazaki 444, Japan.

Glutamic acid decarboxylase (GAD) catalyzes the formation of GABA from glutamic acid. There are two isoforms, GAD_{65} and GAD_{67}, which are encoded by two different genes. We screened mouse brain cDNA library with cDNA probes obtained by a reverse polymerase chain reaction to obtain a full length cDNA for mouse GAD_{65}. Sequencing of the resulting clone included an open-reading frame which encoded a 585 amino acid polypeptide that showed 97.8% identity with GAD_{65} from rat brain and 95.2% with that from human brain. The full length mouse GAD_{65} cDNA was expressed transiently in fibroblast (COS cells) and stably in neuroblastoma (Neuro-2a cells) by cDNA transfection. Both of the engineered cells produced significant amount of GABA. The GABA release into the extracellular medium was detected from the engineered COS (347±48 pmol/10^6cells in 30 min.) and Neuro-2a (113±25 pmol/10^6cells in 30 min.) under resting conditions, whereas control cells showed below detectable level. The treatment of high potassium (100mM) stimulation dramatically increased GABA release from Neuro-2a (360±86 pmol/10^6cells in 30 min.) stably expressing GAD_{65} but not from COS (313±78 pmol/10^6 cells in 30 min.). This suggests the presence of active releasing mechanism of GABA produced by exogenously expressed GAD in Neuro-2a cells.

514.10

GABA-ERGIC DEVELOPMENT IN THE EMBRYONIC CHICK FOREBRAIN. P. Lundgren*†, M.-O. Mattsson†, L. Johansson§ and Å. Sellström§.
† Department of Cellular and Developmental Biology, Umeå University, S-901 87 Umeå, Sweden; §Division of Experimental Medicine, National Defence Research Establishment, S-901 82 Umeå, Sweden.

We have investigated the development of the GABAergic phenotype including GABA and GAD in embryonic chick telencephali from embryonic day (E) 6 to E17, by means of *in situ* hybridization and immunohistochemical techniques. The first appearance of both GAD65 and GAD67 mRNA:s was detected in the E8 telencephalon. A weak staining with sheep antisera recocnizing both forms of GAD was seen in a few cells at this stage and some GABA positive cells were also visualized. At E11 a more distinct expression of both mRNA:s were detected concomitant with an increased amount of cells staining for GAD. A bulk of GABA positive cells were observed in the paraventricular zone at this stage. The maximum expression of GAD65 and GAD67 mRNA:s and the GAD protein was reached at E14. At E17 the levels of message and protein are approximately in the same range as at E11. Co-staining with antibodies against GABA and the neuronal marker neuron-specific enolase revealed that all neurons are not GABAergic. Immuno-electron microscopy indicated that as early as E11, cells positive for both GAD and GABA exhibit processes and synapses. During all the investigated stages the message for GAD65 is less abundant than GAD67 mRNA and the antibody staining is colocalized with GAD67 mRNA. This could indicate that GAD67 is responsible for the early GABA production during the chick forebrain development.

514.11

CLOSE RELATIONSHIP OF GABA TRANSPORTERS GAT1 AND GAT4 DURING THE LATE BRAIN EMBRYOGENESIS

E.Jursky, and N. Nelson*. Roche Institute of Molecular Biology, Roche Research Center, Nutley NJ 07110

cDNA clones representing four pharmacologically distinct GABA-transporters (GAT1 to GAT4) were previously identified in mouse brain. Two of these GAT1 and GAT4, were found to be brain specific. We studied GAT1 and GAT4 in the developing rat brain using polyclonal antibodies prepared by recombinant fusion protein strategy. Patterns of the immunoreactivity for both transporters were very similar in the embryonic and early postnatal stages. However, while GAT1 immunoreactivity was detected in distinct patterns in gray matter and growing axons, GAT4 immunoreactivity was found in subset of radial glial cell fascicles frequently oriented perpendicularly to axons expressing GAT1. Our results demonstrate a close relationship of selective GAT4 expressing radial glial elements with GAT1 expressing axons and GAT1 positive areas during the of synaptogenesis. Because the beginning of the expression for both GAT1 and GAT4 correlate with the expression of adult GABA receptor, transporters may play role in the establishment of neuronal and glial components of the GABAergic inhibitory system in the brain .

514.12

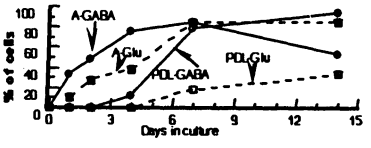
CHARACTERIZATION OF $GABA_A$ RECEPTOR COMPLEX IN GROWTH CONES. H. Fukura[1,2], M. Igarashi[1]*, Y. Komiya[1]. Departments of [1]Molecular and Cellular Neurobiology, and [2]Anesthesiology and Reanimatology, Gunma Univ. Sch. of Med., Maebashi, Gunma 371, Japan.

The nerve growth cone plays a role in neurite elongation, axonal pathfinding, and accurate synaptogenesis. Intercellular calcium concentration in the growth cone is believed important for regulation of these growth cone functions. Recent studies have indicated that activation of $GABA_A$ receptors depolarizes immature neuronal membranes through Ca^{2+} mobilization. To study ontogeny of $GABA_A$ receptor complex in growth cones, we isolated growth cone membranes (GCM) and other membrane fractions from fetal and neonatal rat forebrain, and pharmacologically characterized $GABA_A$ and benzodiazepine binding sites using [^{3}H]-muscimol and [^{3}H]-diazepam. Specific binding sites for [^{3}H]-muscimol were more enriched in GCM compared to those in other membranes. Bmax values of [^{3}H]-muscimol in GCM increased from embryonic day 17 (0.372 pmol/mg protein) through postnatal day 2 (1.44 pmol/mg protein), and then gradually decreased without any significant changes of Kd values. Specific binding sites for [^{3}H]-diazepam were also enriched in GCM, however, no significant changes either in Kd or in Bmax values were observed during early development. We measured $[Ca^{2+}]_i$ of the isolated growth cones by fluorescence spectrometry using Fura2/AM. GABA (100 μM) increased $[Ca^{2+}]_i$, and addition of phenobarbital (100 μM) enhanced this increment from 209% to 336% of the control. Elevation of $[Ca^{2+}]_i$ by GABA plus phenobarbital was blocked by verapamil or picrotoxin. We also measured [Cl], by fluorescence spectrometry using MQAE. GABA(100μM) changed [Cl], value. This change was blocked by picrotoxin ; verapamil showed no effect. Our results suggest that $GABA_A$ receptor complex is enriched in GCM and that activation of $GABA_A$ receptor causes elevation of $[Ca^{2+}]_i$ partly through L-type Ca^{2+} channels and Cl^- channels associated with $GABA_A$ receptor complex, implying that $GABA_A$ receptor in the growth cone plays some role for axonal guidance.

514.13

CORTICAL ASTROCYTES AFFECT THE DIFFERENTIATION OF CULTURED EMBRYONIC RAT SPINAL CORD NEURONS. **Y.-X. Li[1], A.E. Schaffner[1], M.K. Walton[*2], and J.L. Barker[1]. [1]LNP, NINDS, NIH, Bethesda, MD 20892; [2]DCTDA, OTRR, CBER, FDA, Rockville, MD 20852**

Although astrocytes(A) have been suggested to be important during CNS development, their functional roles are still not clear. Here we compared the morphological and electrical properties of spinal cord neurons cultured on poly-D-lysine(PDL) with properties of neurons cultured on A of newborn rats. Neurons were dissociated from embryonic (E) ventral spinal cord at E15 and plated on PDL or A. Differences began to appear after several hours. The processes of neurons cultured on A were thicker and longer than those of neurons cultured on PDL. Patch clamp recordings revealed that co-cultured neurons expressed higher membrane capacitance and lower input resistance at -80mV than neurons on PDL. The amplitudes of voltage-gated Na^+ currents recorded in co-cultured neurons were much greater than those recorded in neurons cultured on PDL. The results from gramicidin perforated-patch recordings showed that GABA-induced responses changed from de- to hyperpolarizing after 4 days in co-culture while neurons on PDL took more than two weeks to complete this process. The initial appearance and the percentage of neurons exhibiting spontaneous GABA and Glutamate(Glu) post synaptic currents (PSCs) differed(see Fig.). A facilitated the appearance of GABA-, then glutamergic transmission.



514.14

ASTROCYTES INFLUENCE NEURONAL DEVELOPMENT IN VITRO. A.E. Schaffner*, Y.-X. Li and J.L. Barker, Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

Astrocytes direct the migration and maturation of neurons in vivo. We compared morphological and electrophysiological properties of spinal cord (SC) and hippocampal (HPC) neurons grown on a monolayer of cortical astrocytes (A) or poly-D-lysine (PL). Within a few days in vitro (div), neurons on A had longer and more numerous processes than cells on PL. Synaptophysin immunoreactivity (SI) was generally confined to neuronal somata on PL at 1 div whereas neurons on A had significant process staining. At 5 div on PL, SI appeared granular and a few neurons had SI in multiple processes; SI was punctate and confined to a single process (presumably the axon) in neurons on A. Neurons grown on A exhibited spontaneous synaptic activity at earlier time points than cells grown on PL. Over a two-week period, a change from exclusively GABA- to mainly glutamate-mediated activity occurred more rapidly and in more cells grown on A versus PL. Interestingly, the number of GABA immunoreactive neurons increases during this time and plateaus at a high level (≥60% for both SC and HPC). The high % of $GABA^+$ cells in the cultures correlates with a similar % of neurons exhibiting GABA-induced spontaneous currents. There was no significant difference between the % of $GABA^+$ SC neurons grown on PL or A after 4 div. There was a significantly higher % of $GABA^+$ HPC neurons on PL relative to those on A after 4 div. We plan to determine if the time course for glutamate immunoreactivity relates to the emergence of spontaneous glutamate activity.

514.15

RAPID ASTROGLIAL UP-REGULATION OF GABA ACTIVATED Cl^- CURRENT IN CULTURED RAT HIPPOCAMPAL NEURONS Q.Y. Liu*, A.E. Schaffner, V. Dunlap and J.L. Barker Lab. of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

Hippocampal neurons from rat embryos at 18 days gestation were cultured in 35 mm dishes coated with poly-D-lysine and with or without a monolayer of confluent cortical astrocytes. The modulatory effects of astrocytes on the development of GABA-activated Cl^- current were studied in neurons cultured for hours-days with whole-cell and outside-out single-channel patch clamp techniques. Drugs were applied to the recorded neuron with a fast-perfusion system. The GABA-evoked current response in these cells involves $GABA_A$ receptors since it reverses polarity at E_{Cl}, has single-channel conductance and kinetics characteristic of $GABA_A$ receptor/Cl^- channels and is completely blocked by bicuculline. Neurons grown on astrocytes have significantly more surface membrane, as indicated by visual inspection and larger whole-cell capacitance values. They exhibit greater GABA-activated Cl^- current than neurons grown on poly-D-lysine, even when normalized using membrane capacitance. GABA-activated Cl^- currents evoked in neurons cultured on poly-D-lysine and astrocytes exhibit similar dose-response curves, block by bicuculline, picrotoxin and Zn^{2+} and enhancement by diazepam. No differences were detected in the properties of Cl^- single-channels activated by GABA. Taken together, we believe that the $GABA_A$ receptor/Cl^- channels in neurons cultured on poly-D-lysine and astrocytes are composed of similar subunits and that there are more functional $GABA_A$ receptors per unit membrane area in neurons cultured on astrocytes than those cultured on poly-D-lysine.

NEUROTROPHIC FACTORS: RECEPTORS AND CELLULAR MECHANISMS III

515.1

EXPRESSION OF TRKB PROTEIN IN NEONATAL STATUS EPILEPTICUS. G.R. Jackson, R. Baldwin, J.L. Saver, and C.G. Wasterlain. Dept. of Neurology, UCLA Sch. of Med. and Sepulveda VA Med. Center, Los Angeles, CA 90024.

Increased hippocampal expression of BDNF and its high affinity receptor, TrkB, has been demonstrated in a number of seizure models. BDNF regulates the survival and differentiation of widespread neuronal populations in the developing CNS, but its physiologic role in mature neurons is less well characterized. Although induction of *trk*B mRNA in hippocampus has been demonstrated by *in situ* hybridization, it is unclear whether this induction is matched by expression of functional receptor protein. Immunoblotting with an affinity-purified, polyclonal antibody directed against a region adjacent to the C-terminal TrkB tyrosine kinase domain identifies a major band of Mr ~150,000. We have used this technique to examine the effects of status epilepticus on TrkB expression in postnatal d4 rat pups. Animals were exposed to flurothyl every 5 min for 2 hr, a paradigm with well-characterized epileptogenic effects (Wasterlain, Neurology 26: 975). By 2 hr following the conclusion of flurothyl-induced status epilepticus, TrkB immunoreactivity was increased significantly in hippocampal extracts but not those from control regions. In the developing hippocampus, seizure-induced perturbation of BDNF-receptor interactions may underlie mossy fiber sprouting, the formation of aberrant excitatory circuits, and the development of temporal lobe epilepsy.

515.2

TRKA IS DOWN-REGULATED DURING DEVELOPMENT IN A SUBSET OF SMALL DRG NEURONS. D.C. Molliver* and W.D. Snider. Dept. Neurology, CSNSI, Washington University, St. Louis, MO 63110.

In the adult rat approximately 45% of dorsal root ganglion (DRG) neurons express TrkA. However, about 80% of DRG neurons die in the *trkA*(-/-) mutant mouse. We recently determined that a subset of small DRG neurons, labeled by the isolectin BSI-B4 and by antibodies to the neurofilament protein α-internexin and the delta isoform of protein kinase C, does not express TrkA in the adult rat, but fails to survive in the *trkA*(-/-) mutant mouse. These results suggest that a population of neurons lacking TrkA in adulthood may express TrkA during development and later down-regulate TrkA. In order to determine whether this is the case, we performed immuno-cytochemistry for TrkA in the mouse hindlimb region at multiple developmental stages. TrkA-immunoreactivity (TrkA-IR) was visible in many DRG neurons at embryonic day 11 (E11), the earliest stage examined. Cell counts taken from stained sections of E13, 15, 17 and postnatal day (PND) 1 animals indicated that approximately 80% of neurons in the DRG contain TrkA up to early postnatal life. However, TrkA-IR was seen in only 63% of DRG neurons in PND7 animals, and in 40% of neurons in adult mice. These results support the hypothesis that most DRG neurons express TrkA during development, but that a subset of these neurons down-regulates TrkA postnatally. Furthermore, BSI labeling was first visible in many small DRG neurons at P7, suggesting that the down-regulation of TrkA expression may be temporally associated with the appearance of a phenotypic marker for this group of neurons. Interestingly, the postnatal down-regulation of TrkA expression appears to coincide closely with the critical period described by Mendell et al. (see TINS 1993. 16:353-59) during which small-diameter DRG neurons show functional plasticity in response to changes in the availability of NGF.

515.3

FUNCTIONAL INTERACTION OF TRKA AND TRKC NEUROTROPHIN RECEPTORS DURING DEVELOPMENT OF POSTNATAL SYMPATHETIC NEURONS. D.J. Belliveau*, J. Kohn, D. Kaplan and F.D. Miller. Centre for Neuronal Survival, Montreal Neurological Institute, Montreal, PQ, H3A 2B4, Canada

Our laboratory has been examining the biological role of high affinity neurotrophin receptors, trkA and trkC, during development of postnatal sympathetic neurons. We have previously shown that sympathetic neurons cultured in the presence of 10 ng/ml NGF for five days respond to the addition of NT-3 with profound neurite outgrowth but when switched to NT-3 alone, do not survive. In addition to Western blotting, we performed immunocytochemistry with a trkC specific antibody directed against the intracellular domain of the receptor, revealing the presence of trkC on NGF dependent sympathetic neurons. To determine the molecular mechanisms whereby activation of trkA leads to survival and growth whereas activation of trkC in the same cells leads to growth but not survival, we are biochemically characterizing the receptors and their downstream targets in sympathetic neurons. Analysis of the autophosphorylation profiles of trkA or trkC revealed that autophosphorylation on both receptors is increased for up to 48 hours in response to constant, long term exposure to the appropriate ligand. IN contrast, over the same time period, no increase in trk receptor levels was detected, either in response to NGF or NT-3. Analysis of whole cell lysates for trk phosphorylation revealed that both NGF and NT-3 led to increased phosphorylation of a number of protein whose sizes corresponded to known trk substrates. At least one of these substrates is Shc as demonstrated by immunoprecipitation. We are currently characterizing the other downstream targets of trkA versus trkC, and determining whether these two tyrosine kinases converge and/or diverge biochemically and whether this correlates with biological responsiveness. This work was supported by MRC of Canada, Network for Centres of Excellence, and Genentech Inc.

515.4

CHARACTERIZATION OF TRKB-MEDIATED SIGNALING PATHWAYS IN RAT CEREBELLAR GRANULE NEURONS - DIFFERENTIAL DISPLAY OF BDNF INDUCED GENES Ute Zirrgiebel*[1], Yoko Ohga[1], Bruce Carter[2], Benedikt Berninger[1], Naoyuki Inagaki[1], Hans Thoenen[1] & Dan Lindholm[1] 1.Dept. Neurochemistry 2.Dept. Neurobiochemistry Max Planck Institute for Psychiatry, Am Klopferspitz 18A D-82152 Martinsried/Munich FRG

TrkB mediates the responses to BDNF and NT-4. TrkB receptors are expressed in developing cerebellar granule neurons. BDNF and NT-4 increased the survival of these neurons and induced auto-phosphorylation of TrkB. This resulted in phosphorylation and binding of phospholipase C γ (PLC-γ) and SHC to the activated TrkB receptors. In keeping with a signaling function of PLC-γ, we observed an increase in phosphatidyl inositol turnover and an elevated $[Ca^{2+}]_i$ level following BDNF treatment. We show PKC activation after BDNF or TPA treatment and the blocking of the survival promoting effects of BDNF and TPA with the PKC inhibitor calphostin C. BDNF activated the ras gene product and led to a long lasting activation of MAP-kinase. These results suggest that two different pathways, the ras and the PLC-γ pathway, are activated by TrkB receptors in primary neurons and that PKC activation is involved in the survival promoting effect of BDNF.

To study which genes are affected a *Differential Display* approach was started to isolate BDNF regulated genes in this cell culture system. We compared BDNF treated neurons with control cells performing a RT-PCR screen. We are currently analysing cloned PCR fragments.

515.5

FUNCTIONAL ANALYSIS OF *TRK* NGF RECEPTOR DOMAINS AFTER TRANSFECTION IN PRIMARY NEURONAL CULTURES. L. Aibel, P. Perez, P. Coll, D. Martin-Zanca and M. V. Chao*. Instituto de Microbiologia Bioquimica, Universidad de Salamanca, Salamana Spain and *Department of Cell Biology and Anatomy, Cornell University Medical College, New York, New York USA 10021

The extracellular domain of *trk* neurotrophin receptors is characterized by several common structural features, including three leucine-rich repeats, two clusters of cysteine-rich domains and two immunoglobulin-like (IgG) motifs. The intracellular domain displays a consensus catalytic tyrosine kinase domain with a short insert and cytoplasmic tail. Previous studies of chimeric cDNAs between *trkA* and *trkB* indicated that the two IgG domains were essential for NGF binding (Perez et al, *MCN*, 1995). To evaluate further the interaction between NGF and the *trkA* receptor, primary cultures of E16 rat hippocampal neurons were transfected with a β-galactosidase reporter gene and expression plasmids containing chimeric *trkA/trkB* cDNAs and mutated *trkA* constructs using calcium phosphate precipitation. The expression of these constructs was monitored by immunocytochemical analysis, which detected a subpopulation of neurons that are β-galactosidase-positive and process bearing. The level of transfection was found to be 1-2% of the total number of cells. Nearly 30% of the cells transfected with the wild type *trkA* cDNA responded to NGF with increased neurite outgrowth after 2 days of treatment. Similar values were observed with *trkA/trkB* chimeric cDNAs which contained both IgG domains of *trkA*. Other transfected constructs which contained only the cysteine-rich domains and the leucine rich repeats of *trkA* in a *trkB* backbone did not respond to NGF. These results indicate that neurotrophin signal transduction can be assessed in transfected primary neuronal cultures.

515.6

PLACODE-DERIVED NEURONS EXPRESS TRKA mRNA BUT DO NOT RESPOND TO NGF. R. Williams*, A. Grapin, R. Rush, N. Le Douarin and T. Ebendal, Department of Developmental Biology, Medical Nobel Insitute, CMB, S-171 77 Stockholm, SWEDEN

Although it is generally considered that only neurons which are derived from the neural crest are dependent on nerve growth factor, since some neurons derived from the placodes respond to nerve growth factor in vitro, we have re-investigated the possibility that some of these neurons are normally dependent on nerve growth factor for survival. In situ hybridization was performed on serial sections taken through all the cranial ganglia of normal birds at various ages of development. Within ganglia which are exclusively derived from the epibranchial placodes, there was a subpopulation of neurons which expressed trkA mRNA. Within the trigeminal ganglion, which is derived from both the neural crest and the ectodermal placodes, we performed extirpations of either the placodes, the neural crest or replaced the neural crest from a chick with a quail neural crest of equivalent developmental stage. From the results of these extirpation/replacement experiments, it is clear that trigeminal neurons derived from both sources are able to express mRNA for all the high-affinity neurotrophin receptors within this ganglion. It also suggests that a subpopulation of neurons derived from the epibranchial placodes are dependent on nerve growth factor during normal embryonic development. To test this idea, purified nerve growth factor was administered to chicks between embryonic days 6 and 10 inclusive. The chicks were sacrificed at E11, the heads sectioned coronally and cell numbers within the geniculate and petrosal ganglia were counted and compared to age-matched controls. There was no significant increase in the number of neurons within either ganglion. The presence of the trkA receptor within neurons which do not respond to NGF suggests a mechanism of heterodimerization with other high-affinity receptors that may modulate the response of the other receptors to their ligands.

515.7

ANTEROGRADE TRANSPORT AND AXO-DENDRITIC TRANSFER OF NEUROTROPHINS IN THE DEVELOPING VISUAL SYSTEM. C.S. von Bartheld[1]*, M.R. Byers[2], R. Williams[3] and M. Bothwell[1]. [1]Dept. of Physiology & Biophysics and [2]Anesthesiology, Univ. of Washington, Seattle, WA 98195 and [3]Karolinska Institute, Stockholm, Sweden.

Afferent trophic signals support neuronal survival. To test if neurotrophins can be transported anterogradely from the cell body, the eyes of E15-16 chick embryos were injected with 10-100 ng radio-iodinated neurotrophins NGF, BDNF or NT-3. Axonal transport by retinal ganglion cells (RGC) to their target, the optic tectum, was quantified 20 hrs after injection by gamma-counting and emulsion autoradiography. NT-3 was transported at a higher level (100 pg) and more consistently than BDNF, NGF, or bFGF. Transport of NT-3 appears to be receptor-mediated as it was competed by excess cold NT-3. Transport is reduced by co-injection of K252a, completely abolished by monensin, but not affected by brefeldin A or tetrodotoxin (TTX). The effect of monensin may indicate that exogenous NT-3 joins the pathway of newly synthesized proteins in the Golgi complex of RGC. More than 95% of exogenous NT-3 arrives in the tectum intact as judged by laser densitometry of SDS gels. NT-3 mRNA is expressed in the retina at E16, and trkC mRNA is expressed by second-order visual neurons in the tectum. High-affinity binding of BDNF and NT-3 indicates that trk B and C protein is located predominantly in dendrites rather than neuronal cell bodies. Autoradiographic analysis of NT-3 distribution in the tectum at the ultrastructural level shows that more than 30% of the transported NT-3 is transferred to second-order visual neurons and is frequently associated with vesicles. Intraocular BDNF and NT-3 can rescue tectal neurons from cell death induced by co-injection of pertussis toxin, but not by TTX. We are now testing if TTX affects the release of NT-3 at the axon terminals.

Supported by NIH grants HD 29177 and NS 30305. The neurotrophins BDNF and NT-3 were kindly provided by Regeneron.

515.8

RETINOIC ACID AND TRKA IN DEVELOPING CHICK SYMPATHETIC NEURONS A. Schröpel, G. Dechant and Y.-A. Barde*. Dept of Neurobiochemistry, MPI for Psychiatry, 82152 Martinsried, Germany

Cultured chick E7 sympathetic neurons survive for four days independently of NGF (Ernsberger et al. 1989). These neurons will eventually all die, even in the presence of NGF. However, the addition of retinoic acid (RA) to the cultures allows the responsiveness to NGF to be induced, as well as the formation of high affinity NGF receptors on these neurons (Rodríguez-Tébar et al. 1991). In the present study, we asked whether retinoic acid affects the expression of the cognate NGF receptor trkA in cultured sympathetic neurons. Sympathetic neurons isolated from E6.5 chicken embryos were cultured in the presence of NGF and/or RA. RNA was isolated from these cultures and analysed for trkA expression. Surprisingly, we found that sympathetic neurons already express trkA at the start of the cultures, in vitro as well as in vivo, and found no up-regulation of trkA mRNA upon addition of RA. To rule out the possibility that the trkA gene is expressed, but the mRNA not translated, we performed cross-linking experiments with iodinated NGF. Following immunoprecipitation using an antiserum directed against the C-terminus of chick trkA, a radioactive band of the size expected for trkA cross-linked with NGF was readily detected, in cultures with and without RA. Surprisingly then, the induction of NGF survival and of high affinity NGF binding cannot be explained by the de novo appearance or up-regulation of trkA, and additional mechanisms are likely to be involved.

515.9

NEUROTROPHIN RECEPTORS ON CHICK SYMPATHETIC NEURONS DURING THE DEVELOPMENTAL SWITCH FROM NEUROTROPHIN-3 TO NERVE GROWTH FACTOR RESPONSIVENESS. G. Dechant*, A. Schröpel and Y.-A. Barde, Dept. of Neurobiochemistry, MPI for Psychiatry, 82152 Martinsried, Germany

Many neuronal populations switch their neurotrophic factor requirements during the time when axons first encounter their target tissues. This change in growth factor dependency might be part of a more general developmental program regulating the transition from proliferative neuroblasts to terminally differentiated neurons. We studied this phenomenon using chick sympathetic neurons as a model system. Between embryonic days 7 and 11 (E7-E11), these neurons switch from neurotrophin-3 (NT-3) to nerve growth factor (NGF) dependency. We have investigated the in vitro survival effects of NT-3 and NGF and asked to which extent the expression of the neurotrophin receptors trkA, trkC and p75 reflects the changes in the biological effects of their ligands. We found that both NGF and NT-3 given at 100 ng/ml concentration support the in vitro survival of sympathetic neurons at all stages, indicating the maintenance of signal transducing receptors for both factors during development. The dose-response curves for NGF and NT-3 were however strongly and inversely regulated. By in situ hybridization we detected transcripts of the trkA gene as early as E4.5 in primary sympathetic chains. At all stages studied, two trkC transcripts were detected, one of them encoding the full-length tyrosine kinase. p75 mRNA was upregulated between E7 and E11. In cross-linking and binding experiments, we found that p75 on sympathetic neurons binds NT-3 with a markedly higher affinity than on recombinant cell lines. The higher binding-affinity was accompanied by an increase in ligand specificity. We suggest that the ratio between the p75 and trk-tyrosine kinases regulates the neuronal responsiveness to neurotrophins during development.

515.10

BSK RECEPTOR TYROSINE KINASE IS INVOLVED IN MULTIPLE ASPECTS OF MOUSE NERVOUS SYSTEM FUNCTION DURING EMBRYONIC DEVELOPMENT. J-H. Zhang* and R. Zhou, Lab. for Cancer Res., College of Pharmacy, Rutgers Univ., Piscataway, NJ 08855.

Growth factor receptors and their ligands are involved in various processes during embryonic development. We have isolated recently a novel growth factor receptor termed Bsk, for brain-specific kinase (independently isolated rat and chicken homologs are named Ehk1 and cek7 respectively). We showed that in adult mouse, Bsk expression was limited only to the brain. Specifically, expression was found mainly in neural structures such as the hippocampus, the piriform cortex, the amygdala, and the frontal cortex, which constitute the limbic system. Analysis of Bsk expression during embryonic development by *in situ* hybridization revealed that Bsk was widely transcribed in the presumptive nervous system, including the forebrain, the midbrain, the hindbrain and the spinal cord. However, Bsk expression in the midbrain, the hindbrain and the spinal cord gradually decreased, while in the forebrain increased over time. By the time of birth, the most prominent expression of Bsk was found only in the limbic system. This high level of expression in the limbic system persisted throughout the postnatal development and remained stable in adult up to 24 months. Furthermore, Bsk expression in the developing nervous system was confined to the postmitotic neurons, since little mRNA was detected in the germinal layer where mitotic neuronal precursors are located. Our analysis of Bsk expression during embryonic development indicated that Bsk may play at least two roles in the development of mouse nervous system. The first role is in the general aspect of neuronal differentiation, since Bsk expression was found in almost all regions of the early nervous system. This function is transient. The second role is in the maintenance and survival of the limbic neurons in late embryonic and postnatal period, as suggested by the long lasting expression of Bsk in the limbic system.

515.11

WITHDRAWN

515.12

DEVELOPMENTAL ALTERATIONS IN THE EXPRESSION OF THE LOW-AFFINITY NEUROTROPHIN RECEPTOR (p75) IN THE TRIGEMINAL BRAINSTEM COMPLEX (TBC) IN RAT: TRANSIENT VIBRISSAE-RELATED PATTERNING IN NUCLEUS INTERPOLARIS.

D.P. Crockett*, Lu Wang and M.D. Egger. Dept. Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ 08854-5635.

p75, expressed by neural crest-derived primary sensory neurons, binds all members of the neurotrophin family. Previously, we reported that in adult rats there is a vibrissae-related pattern of p75 immunoreactivity (IR) in the principal nucleus (PrV) of the TBC. Dense p75 IR was also observed in the superficial laminae of nucleus caudalis (SpC) and in cell bodies of the trigeminal mesencephalic nucleus. Few p75-positive fibers were detectable in nucleus interpolaris (Spl) and oralis (SpO). We now report that, in marked contrast to the adult, during the first week postpartum, dense p75 IR is present throughout the entire TBC. Within PrV, a vibrissae-related pattern of p75 IR emerges at birth (PD0) and remains a permanent feature. Within Spl, during postnatal days 4 - 7, diffuse staining is replaced by a fully formed, vibrissae-related pattern, fading during the third postnatal week. In addition, during the second and third postnatal weeks, p75 IR in SpO and in the deeper laminae of SpC gradually declines. The relative restriction in the distribution of p75 IR in the adult TBC, compared to that in the perinatal period, suggests that p75 IR plays a significant role during periods of active synaptogenesis and pattern formation.

515.13

DEVELOPMENTAL EXPRESSION OF PAN TRK AND P75 RECEPTORS IN THE HUMAN BRAIN.

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The prenatal development of the neurons immunoreactive to the tropomycin-related kinase (trk) and p75 nerve growth factor receptors (p75NGFr) was examined in the brain of developing humans between 13 to 34 week of gestation. In the embryonic week 13, trk-immunoreactive (trk-IR) neurons were observed in the cortical subplate zone, basal forebrain, caudate nucleus, putamen, external segment of globus pallidus, subthalamic nucleus, and substantia nigra. p75NGFr-immunoreactive (p75NGFr-IR) neurons were observed at this time point in the same structures except the caudate and substantia nigra. This pattern remained unchaged at embryonic weeks 15-16 except for the addition of the trk-IR neurons in some thalamic nuclei and in the red nucleus. By embryonic week 18, the expression of trk protein was widely distributed and seen within the cortical subplate zone, entorhinal cortical plate, basal forebrain, caudate nucleus, putamen, external segment of globus pallidus, hippocampus, subthalamic nucleus, most thalamic nuclei, lateral mammillary nucleus, some brainstem nuclei, and dentate nucleus of cerebellum. p75NGFr-IR neurons were found in cortical subplate zone, basal forebrain, putamen, external segment of globus pallidus, hippocampus, subthalamic nucleus, some brainstem nuclei, dentate nucleus and cortex of cerebellum. This staining pattern remained virtually unchanged between embryonic weeks 19-22. From embryonic week 22, the distribution of trk-IR and p75NGFr-IR neurons changed gradually. At this time, neurons in some thalamic and brainstem nuclei became progressively immunonegative for trk. These data further demonstrate an important role of neurotrophins and their receptors in the development of multiple neuronal systems in the human brain. (Supported by NS25655 and a grant from the Alzheimer's Association).

515.14

Characterization of GDNF binding to putative GDNF receptor.

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Glial-derived neurotrophic factor (GDNF) is a distantly related member of the transforming growth factor-beta (TGF-β) superfamily. GDNF has been reported to support the survival and differentiation of a number of diverse neuronal cell populations including peripheral autonomic ganglia and ventral mesencephalic neurons *in vitro*. The distribution of GDNF receptor binding in adult rat brain was analyzed by receptor binding and autoradiography. In rat brain sections, ^{125}I-labeled GDNF bound specifically to the lateral septal nucleus. GDNF binding could be induced in the area of the substantia nigra by transection of the medial forebrain bundle which severs the nigrostriatal projection. Displaceable ^{125}I-GDNF binding was analyzed in crude homogenate and purified membrane samples prepared from adult rat septum and cortex. Only crude homogenate and membrane preparations of septal tissue showed displaceable binding.

515.15

RETROGRADE AXONAL TRANSPORT OF GDNF IN THE ADULT CENTRAL NERVOUS SYSTEM.

A. Tomac, J. Widenfalk, E. Lindqvist, L.F.H. Lin†, T. Kohno†, T. Ebendal‡, B.J. Hoffer§, and L. Olson*. Dept. of Neuroscience, Karolinska Institute, Stockholm, Sweden; †Synergen Inc., Boulder, CO; ‡Dept. of Developmental Neuroscience, Biomedical Center, Uppsala University, Uppsala, Sweden; and §Dept. of Pharmacology, University of Colorado, Denver, CO 80262.

Glial cell line-derived neurotrophic factor (GDNF) is a distant member of the TGFβ family with potent trophic effects on dopamine neurons and α–motoneurons. In situ hybridization studies suggest that GDNF may have several additional roles in the central nervous system during development and possibly in the adult. We have made stereotaxic injections of either [^{125}I]-GDNF or non-labeled GDNF into areas known from in situ hybridization to express GDNF mRNA. To the extent that such expression represents target production of trophic support, the injected GDNF species should be expected to be transported retrogradely. Using autoradiography and GDNF immunohistochemistry to detect labeled and non-labeled GDNF respectively, we found efficient transport from striatum to substantia nigra nerve cell cell bodies. Specificity was demonstrated by the absence of transport of [^{125}I]-GDNF in the presence of a 100-fold higher concentration of non-labeled GDNF. Double labeling using TH and GDNF antibodies demonstrated punctate GDNF labeling of TH-positive dopamine neurons. Injections of GDNF into several other brain areas including thalamus and cerebellum also resulted in retrograde transport. Our results suggest that specific receptor-mediated uptake and transport of GDNF can occur in several different defined areas of the central nervous system, suggesting that GDNF may act as a target-derived trophic factor for several different neuronal populations in addition to nigral dopamine neurons and spinal α-motoneurons.

515.16

RETROGRADE TRANSPORT STUDY OF GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) IN ADULT RAT NERVOUS SYSTEM.

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GDNF, a member of TGFβ superfamily, has profound neurotrophic activity on substantia nigra dopaminergic neurons and spinal and brainstem motor neurons. GDNF mRNA is made by muscle and Schwann cells in the periphery and by type I astrocytes in the CNS. Thus GDNF could be available to and act on other neuronal populations in addition to dopamine and motor neurons. In this study we used retrograde transport followed by autoradiographic techniques to examine potential neuronal populations which can bind, internalize and retrogradely transport GDNF in a receptor-mediated fashion in adult rats. In the CNS, ^{125}I-GDNF was transported by dopaminergic neurons in the substantia nigra and VTA after injection into the striatum or ICV. In addition, we found labeling of neurons in the central linear nucleus of raphe and dorsal nucleus of raphe after both intra-striatal and ICV injection. After injection into the superior colliculus, ^{125}I-GDNF was transported to the contralateral but not ipsilateral retinal ganglion cells. In the periphery, GDNF was transported by spinal motor neurons and DRG neurons after intra sciatic nerve injection. GDNF was not transported by superior cervical ganglia neurons after injected into the anterior eye chamber. The results of this study suggest that GDNF may have neurotrophic activity on neuronal populations which are not previously known to be GDNF responsive.

515.17

CHARACTERIZATION OF GDNF RECEPTORS ON PRIMARY NEURONS AND CELL LINES. M. Trupp* and C. F. Ibáñez. Laboratory of Molecular Neurobiology, MBB, Karolinska Institute, S-17177 Stockholm, Sweden.

Glial cell line-derived neurotrophic factor (GDNF) was initially purified based upon its ability to promote the specific uptake of dopamine by rat embryonic ventral midbrain primary cultures. We have previously shown that recombinant GDNF purified from Sf21 conditioned media promotes the survival of neurons from dissociated embryonic chicken sympathetic neurons. Initially, we have characterized high affinity binding sites on these neurons by displacement of ^{125}I-GDNF with unlabeled ligand. We now apply Scatchard and Hill analyses to saturation binding data to describe cooperative binding of GDNF to these neurons. In addition, a potentiation of GDNF binding was observed after addition of transforming growth factor beta (TGFβ), resulting in a nearly seven-fold increase in the affinity of the receptor complex.

Iodinated GDNF was chemically cross-linked to receptors on neurons and various cell lines and analyzed by SDS-PAGE. Multiple GDNF binding proteins were visualized, including receptors with apparent molecular weights equivalent to the Type I, II and III receptors previously described for other members of the TGFβ superfamily. Analysis of neuronal and non-neuronal cell lines for GDNF binding indicates the existence of multiple specific binding complexes--which appear in distinct ratios providing different binding affinities.

515.18

PURIFICATION, CLONING AND RECEPTOR INTERACTIONS OF AL-1, A LIGAND FOR AN EPH-RELATED RECEPTOR (REK7) INVOLVED IN AXON FASCICULATION. J. W. Winslow*, Ai Shih, P. Moran, J. Valverde, K. D. Beck, F. Hefti, I. W. Caras. Genentech, Inc. S. San Francisco, CA 94080

REK7, also known as EHK-1 and bsk, is an EPH-related tyrosine kinase receptor expressed predominantly in the hippocampus and cortex. Cell culture studies indicate that REK7 may play a role in axon fasciculation. To study the regulation of axonal fasciculation and other neuronal activites by this receptor system, we purified by affinity chromatography a novel REK7 ligand from BT20 human carcinoma cells, and cloned it from a human embryonic brain cDNA library. This ligand, termed AL-1, is a 28 K GPI-linked protein that shares 40-50% amino acid identity with the B61, ELF-1, and EHK-1L family of EPH receptor ligands. AL-1 binds to REK7 and related receptor SEK with affinities of 1 nM and 10 nM, respectively. Membrane attachment of AL-1 appears necessary for receptor activation since endogenous REK7 receptors of cultured rat cortical neurons are efficiently autophosphorylated by transfected cells expressing GPI-linked AL-1, but not by soluble AL-1. Autophosphorylation of cortical cell REK7 can be elicited by a soluble AL-1IgG fusion protein, suggesting that dimeric or oligomeric AL-1 may be necessary for REK7 kinase activation. These results suggest that AL-1 and REK7 may interact primarily as a consequence of cell-cell contact, and support our findings from cortical neuron-astrocyte co-cultures which implicate this ligand/receptor pair in a mechanism of axonal fasciculation.

515.19

REK7, AN EPH-RELATED TYROSINE KINASE RECEPTOR, IS INVOLVED IN AXON BUNDLE FORMATION IN CO-CULTURES OF CORTICAL NEURONS WITH ASTROCYTES. I.W. Caras, J. Valverde, P. Moran, Ai Shih, F. Hefti*, J.W. Winslow, Klaus D. Beck. Genentech, Inc., S. San Francisco, CA 94080

REK7 is an EPH-related tyrosine kinase receptor expressed exclusively in the nervous system, predominantly in hippocampus and cortex. A soluble REK7-IgG fusion protein prevents axon bundling in co-cultures of cortical neurons with astrocytes, a model of late stage nervous system development and differentiation. Immunofluorescent staining of the cultures with an anti-REK7 antibody indicates that REK7 is expressed on the axon fibers of the cortical neurons, consistent with a role in axon fasciculation. We show that a ligand for REK7, AL-1, is also expressed in these co-cultures, predominantly on the surface of the astrocytes. The finding that REK7 is efficiently activated by membrane-bound AL-1 but not by soluble AL-1 suggested that soluble AL-1 might act as an antagonist of axon fasciculation. We verified this by showing that soluble AL-1 blocks axon bundling. Our findings, together with the observation that both molecules are expressed in the brain, suggest a role for REK7 and AL-1 in the formation of neuronal pathways, a feature of nervous system development and regeneration.

515.20

Localization of Al-1, a Novel Membrane-Bound Ligand, in the Mammalian CNS; Comparison with Putative Receptors Rek and TK-7. Mark Armanini*, Paul Moran, Ingrid Caras, Siao Ping Tsai, Arnon Rosenthal, John Winslow, Ai Shih, and Heidi Phillips. Dept. of Neuroscience, Genentech Inc., 460 Point San Bruno Blvd., SSF, CA.

A new family of membrane-bound ligands has been described which bind receptors of the Eph family. **Al-1**, a recently purified ligand of this family, has been demonstrated to have possible activities on cultured mammalian neurons. Based on binding-affinity studies, Eph-receptors **Rek** and **TK-7** have been proposed as putative receptors for **Al-1**.

In-situ hybridization was performed in the rat CNS to compare the expression of **Al-1** with its putative receptors **Rek** and **TK-7** in the adult animal, as well as to compare their developmental patterns in the E15 embryo and P1 rat. In the adult rat, **Al-1** expression was observed primarily in the cortex, while **Rek** and **TK-7** exhibited much wider distribution including the cortex, hippocampus and thalamus. **Al-1** expression was much greater and more extensive earlier in development in the CNS and was observed in numerous non-neuronal embryonic tissues.

In-situ hybridization studies were also performed to localize **Al-1** in regions of human and monkey forebrain. Prominent expression was seen in the cortex with no detectable expression observed in the hippocampus.

These findings suggest a developmental role for **Al-1**, and the adult mammalian cortex as a potential sight of interaction between **Al-1** with its putative receptors.

NEURONAL DEATH VI

516.1

PROGRAMMED CELL DEATH IN THE DROSOPHILA CNS MIDLINE. Lei Zhou, Hassan Hashimi, John R. Nambu* Biology Department, University of Massachusetts at Amherst, Amherst, MA, 01003.

During nervous system development large numbers of cells die via programmed cell death (PCD). In *Drosophila*, embryonic cell death is blocked by chromosomal deletions of the *reaper* gene, which encodes a novel protein expressed in dying cells. We have utilized a *reaper* deficiency strain, Df(3R)H99, to show that PCD is required for normal embryonic neural development. H99 mutants exhibit neural hypertrophy and defects in the projection of the segmental and intersegmental nerves. By using several ß-galactosidase reporter genes expressed in cells of the CNS midline, we further find that prominent PCD occurs in this specific CNS structure. Ectopic 'rescued' midline cells are detected in H99 mutants from stage 12 onward and many come to reside in aberrant locations atop the nerve cord. In wild type embryos, midline cell corpses are phagocytosed by migrating macrophages. A subset of macrophages migrate posteriorly along the CNS midline and are associated with midline pores that extend through the nerve cord. Macrophage precursor cells are still present in H99 mutants and exhibit widespread migration, however they do not adopt differentiated morphologies. The ventral midline may provide cues for the migration and accumulation of macrophages. Both *tinman* and *single-minded* mutants exhibit defects in midline differentiation and macrophage distribution.

516.2

THE *SPACEHEADS* ARE A CLASS OF MUTATIONS AFFECTING NEURAL SURVIVAL IN ZEBRAFISH *DANIO RERIO*. S. Abdelilah*, J. Malicki, S. Neuhauss, Z. Rangini, A. Schier, L. Solnica-Krezel, D.Y.R. Stainier, D. Stemple, F. Zwartkruis and W. Driever. Cardiovascular Research Center, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA. 02129.

We have performed a saturation mutagenesis screen for embryonic lethal mutations in zebrafish *Danio rerio*. Among the more than 700 mutations isolated, we have identified a novel class of 50 zygotic recessive mutations with characteristically altered brain morphology.

At 28 hours of development, most of the *spacehead* mutations have enlarged tectal and fourth brain ventricles. Additionally, the telencephalon appears with an anterior extension, presumably an enlargement of the second brain ventricle.

A characteristic feature of the *spaceheads* is early neural degeneration. At least two of these mutations display a visible degeneration as early as the 7-8 somite stage. In order to asses whether the observed degenerations are tissue-specific, we have employed a detection assay for apoptotic cell death on zebrafish whole-mounts using the terminal deoxynucleotidyl transferase (TdT) system. All mutations tested at 28 hours of development depict increased levels of apoptotic cell death within the entire CNS, indicating that the molecular defect affects primarily the neuroectoderm.

Future analysis of the *spaceheads* might provide a powerful tool for studying the mechanisms that lead to apoptotic cell death within the neural cell lineages.

516.3

APOPTOSIS IN THE DEVELOPING ZEBRAFISH BRAIN. L.K.Cole*, M.E. Murray, and L.S. Ross. Neurobiology Program, Dept. of Biological Sciences, Ohio University, Athens, OH 45701.

Cell death in the nervous system is a normal developmental event which may serve to eliminate neurons failing to establish functional synapses with their targets. The term **apoptosis** is used to refer to cell death which is genetically programmed, and which occurs due to *de novo* synthesis of cytotoxic proteins by either the doomed cell itself or by other cells. Apoptosis may be caused by the lack or removal of a trophic factor when a cell fails to make a synapse with a target cell. We explored the distribution of apoptotic cells in the embryonic zebrafish brain. The fragmented DNA of apoptotic cells was marked using the ApopTag™ kit (Oncor, Gaithersburg, MD). Surprisingly low numbers of apoptotic cells were observed in the developing zebrafish brain. The highest number of apoptotic cells was seen at 24 hours postfertilization, when labeled cells were scattered throughout the extent of the brain. In 3-day old embryos, apoptosis was confined primarily to the telencephalon. By 4 days, apoptotic cells were reduced in the telencephalon and were increased in the otocyst. In embryos beyond 4 days, the few apoptotic cells were confined mainly to the telencephalon, although occasional cells were seen in the ventral hypothalamus. These results indicate that the highest levels of apoptosis occur early in development, during the period when the major axonal tracts are being established (Ross et al., 1992, J. Neurosci. 12:467-482). Supported by NSF IBN-9222896.

516.4

THROMBIN INHIBITS NEURITE OUTGROWTH AND INDUCES CELL DEATH IN CHICK SPINAL MOTONEURON CULTURES. V.L. Proctor*, E.D. Llyod, & L.J. Houenou. Dept. of Neurobiology & Anatomy, Bowman Gray Sch. of Med., Wake Forest Univ., Winston-Salem, NC 27157, USA.

Following injury to the blood-brain barrier, neurons may be exposed to high levels of thrombin, a serine protease. Recently, we have suggested that serine proteases, including thrombin, may be involved in neuronal cell death in vivo (Houenou et al PNAS 92:895,1995). Furthermore, thrombin has been shown to induce apoptosis of cultured neurons and astrocytes (Soc. Neurosci. Abst. 20: 641,1994). However, whether thrombin can directly affect the development and differentiation of motoneurons is still unknown. Here we examined the effects of thrombin and its specific inhibitor, hirudin, on highly enriched cultures of spinal cord motoneurons from 5 day old-chick embryos. The results show that (1-1,000nM) thrombin significantly decreased motoneuron survival in a dose dependent fashion, when cultures were examined 2-3 days following plating. In addition, thrombin decreased the number of neurites/neuron and the number of primary branches occurring on the longest neurite. Co-treatment with hirudin or skeletal muscle extract prevented the deleterious effects of thrombin. The molecular mechanisms underlying the effets of thrombin on motoneurons are currently being examined and will also be discussed. Taken together with the fact that neurons, including motoneurons, express thrombin receptors, these results suggest that thrombin may be an important regulator of neuronal cell survival and differentiation during development and in pathology.

Supported by the MDA and NIH

516.5

DEVELOPMENT OF VIRAL VECTOR-MEDIATED GENE DELIVERY SYSTEMS FOR THE RETINA. D.B. Clarke*, T.N. Jelsma, R. Dunn, R. Slack[1], F. Miller[1], G.M. Bray and A.J. Aguayo. The Montreal General Hospital Research Institute and [1]The Montreal Neurological Institute, McGill University, Montréal.

The intraocular administration of BDNF or NT-4 exerts potent survival effects on injured adult rodent retinal ganglion cells (Mansour-Robaey et al., PNAS 1994; Clarke et al., Soc. Neurosci. Abst., 1993). We are now exploring viral vector technology to express these molecules in retinal neurons and glia.

In vitro, the ability of viral vectors to direct neurotrophin expression in neuronal cells was tested using trkB-expressing PC12 cells (provided by Dr. L. Greene) that differentiate in the presence of BDNF. Cells were infected with an HSV amplicon expressing BDNF and ß-galactosidase. Four days after infection, most cells expressing ß-galactosidase had a morphology characteristic of differentiated PC12 cells. Furthermore, many neighbouring cells also had a differentiated morphology, suggesting that BDNF expressed from the viral vector acted on neighbouring cells.

In vivo observations indicate that both HSV (provided by Dr. X.O. Breakefield) and adenovirus vector systems can be used to direct the expression of a marker gene, ß-galactosidase, to cells of the mouse retina. Delivery of neurotrophin genes to the retina will determine whether this technology can be used effectively to provide trophic support to axotomized retinal neurons.

516.6

DEPLETION OF INTERNAL CALCIUM TRIGGERS APOPTOSIS: COMPARISON WITH PROGRAMMED CELL DEATH IN SYMPATHETIC NEURONS

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We have previously shown that neuronal survival under depolarizing conditions with high K^+ correlates well with sustained levels of cytoplasmic free calcium ($[Ca^{2+}]i$) in rat superior cervical ganglion (SCG) cells and cerebellar granule neurons. This suggests that neurotrophic factors are necessary for the survival of immature neurons which otherwise die due to failure to maintain the basal $[Ca^{2+}]i$. In deed, the basal level of $[Ca^{2+}]i$ in SCG neurons was decreased after NGF withdrawal , and when SCG neurons were deprived of NGF in the presence of the Ca^{2+} chelator, Quin-2 AM (10-50 μM), they were resistant to NGF deprivation (only 16 (+3) % of the neurons died at 40 hrs after NGF withdrawal in the presence of Quin-2 AM, while more than 70% died in its absence). Moreover, when cytoplasmic free Ca^{2+} was depleted by BAPTA AM (≥50 μM) or Quin-2 AM, these neurons underwent apoptosis in the presence of NGF as characterized by cellular shrinkage and chromatin condensation visualized with the bis-benzimide stain, Hoechest 33258. This toxicity occurred in a dose-dependent manner, and was less effective when BAPTA derivatives with higher Kd values were employed. Immunohistochemical study using antibodies against c-*fos* indicated its activation during BAPTA-mediated cell death. It is thus likely that a decrease in $[Ca^{2+}]i$ of SCG cells after NGF withdrawal may be involved in the cell death cascade leading to c-*fos* activation followed by DNA fragmentation and eventually cell death.

516.7

CHARACTERIZATION OF NEURONAL PROPERTIES AFTER ADENOVIRUS-MEDIATED GENE TRANSFER. D.J. Creedon*, R.M. Easton, M. Deshmukh, J.M. Nerbonne, and E.M. Johnson, Jr., Dept. of Pharm., Washington Univ. Med. Sch., St. Louis, MO 63110.

We are interested in using molecular genetic techniques for studying neuronal cell death and the mechanism of NGF signaling. Recombinant adenovirus (Ad) has shown promise as a gene transfer vector for neurons, but there is little information on whether or not Ad vectors affect normal cellular physiology which could confound experimental results. For example, we previously found that HSV amplicon vectors caused an intractable inhibition in neuronal protein synthesis. To assess the effects of Ad vectors, superior cervical ganglion (SCG) neurons were infected with a recombinant Ad vector expressing LacZ. An moi of 40 yielded > 95% x-gal positive cells, but maximal expression was only obtained after 48 hrs. Morphologically, infected neurons were indistinguishable from controls either when they were maintained in NGF or as they underwent apoptosis after NGF deprivation. Five days after infection, protein synthesis assays showed no difference in total protein synthesis between control and infected neurons. Electrical recordings 4 days after infection revealed normal resting membrane potentials, normal Na^+ and K^+ currents in voltage clamped cells; current injection generated action potentials including repetitive spikes during prolonged current application. These results suggest that Ad vectors can provide foreign gene expression in SCG neurons for at least five days without gross cellular perturbations.

516.8

EFFECTS OF INHIBITION OF CYTOSOLIC OR MITOCHONDRIAL PROTEIN SYNTHESIS ON APOPTOSIS INDUCED BY DEAFFERENTATION IN THE DEVELOPING BRAIN.

C. A. Guimarães & R. Linden* - Instituto de Biofisica Carlos Chagas Filho, UFRJ, Rio de Janeiro 21949-900, Brazil

The superior colliculus (SC) of the neonatal rat undergoes programmed cell death (PCD) with apoptotic morphology as part of its normal development. We tested inhibitors of protein synthesis for effects on naturally-occurring and deafferentation-induced PCD in the developing SC. Neonatal rats at postnatal day 2 were injected subcutaneously with either anisomycin (ANI) or chloramphenicol (CMP), respectively a cytosolic and a mitochondrial protein synthesis inhibitor, and the left eye was removed under deep anaesthesia to produce deafferentation of the SC. Unoperated control rats received either injections of inhibitors or were untouched. Three hours after eye removal, the pups were perfused with fixatives under deep anaesthesia. Frozen sections through the brain were stained with neutral red and analyzed by light microscopy. Following deafferentation, there was an increase in the rate of cell death in the SC compared with controls. Treatment with ANI abolished this effect, indicating that the deafferentation-induced apoptosis in the neonatal SC requires cytosolic protein synthesis. Following tratment with CMP the rate of apoptosis was increased both in control and in deafferented rats. This result suggests that CMP has a general effect on PCD. Contrary to previously published results from other laboratories, the effect of inhibiting mitochondrial protein synthesis is not specific to the mechanisms of cell death induced by deafferentation.

(CNPq, FINEP, CEPG-UFRJ, FAPERJ)

516.9

CHANGES IN CYTOPLASMIC FREE CALCIUM DURING APOPTOSIS OF PC12 CELLS FOLLOWING REMOVAL OF SERUM. C.A. Messam* and R.N. Pittman. Dept. of Pharmacology, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Apoptosis is an important process in normal cellular homeostasis and development as well as cellular pathology; however, biochemical pathways involved in apoptosis have not yet been fully elucidated. Increases in intracellular Ca^{2+} are thought to play an important role in excitotoxic death of neurons as well as in apoptosis of nonneuronal cells. In addition, elevated levels of cytoplasmic Ca^{2+} have been implicated in survival of growth factor-dependent neurons; however, little information is available on dynamic changes in cytoplasmic calcium during neural apoptosis induced by growth factor or serum removal. To investigate the role of calcium in apoptosis of undifferentiated PC12 cells following removal of serum, the fluorescent indicator dye, fluo-3, was used to obtain multiple measurements on individual cells during the course of apoptosis. Changes in cytoplasmic free Ca^{2+} were measured 4-10 hours, 24-28 hours, or 48-52 hours after removal of serum from cells. Calcium levels were maintained at fairly constant levels at early times after serum removal; however, during the active motile phase of apoptosis, cytoplasmic free Ca^{2+} increased 3-5 fold and was sustained at these elevated levels until cells died. The initial increase in Ca^{2+} correlated with the onset of the most dynamic phase of membrane blebbing which preceded death by 1-2 hrs. Ongoing experiments are designed to investigate the source of the calcium (intracellular or extracellular), its potential role in killing these cells, and the temporal relationship between increased intracellular free calcium and changes in chromatin morphology.

This work was supported by NIH grant NS32465

516.10

tau PROTEIN IS DEPHOSPHORYLATED BY AN OKADAIC ACID INHIBITABLE PHOSPHATASE DURING NEURAL APOPTOSIS. J.C. Mills and R.N. Pittman.* Grad. Group in Cell Biology and Dept. of Pharmacology, U. of Penn. Sch. of Med., Philadelphia, PA 19104.

Neural apoptosis was studied in an NGF-differentiated subline of PC12 cells. Long-term timelapse videomicroscopy has revealed that apoptosis (after withdrawal of NGF) is characterized by early beading and thinning of neurites followed closely by zeiotic membrane blebbing of cell bodies. The breakdown of neurites suggested that destabilization of microtubules might be critical to the death process and led to the hypothesis that the microtubule-stabilizing protein tau might be modified in apoptotic cells. Since death in this system -- as in most types of apoptosis -- is asynchronous, an immunocytochemical approach was developed to study tau in actively dying/blebbing cells. Apoptotic cells were identified by Hoechst 33342-stained chromatin condensation, and the state of tau in these cells was assessed by indirect fluorescent immunostaining using monoclonal antibodies (tau1, recognizing a dephosphorylated epitope; PHF1, against a phosphorylated epitope; tau49, against all forms of tau). Using this method, a substantial increase in dephosphorylated tau was detected in actively dying cells (i.e. an increase in tau1 immunoreactivity, decrease in PHF1, and little change in tau49). Of cells with apoptotic nuclei, 90±6% had substantially increased tau1 immunoreactivity, while 75±4% of the cells with increased tau1 had apoptotic nuclei; thus, it is probable that, in most cells, tau becomes dephosphorylated slightly before or at the time of chromatin condensation and remains dephosphorylated until complete nuclear fragmentation. Okadaic acid (an inhibitor of protein phosphatases 2A and 1) inhibited tau dephosphorylation significantly within 1.5 hrs at a concentration of 80 nM and within 4.5 hrs at 20 nM, suggesting that PP2A (and/or PP1) is activated in apoptotic cells at the time of morphological changes.

516.11

MODULATION OF CELL CYCLE COMPONENTS DURING NEURAL DIFFERENTIATION AND APOPTOSIS. J.A. Erhardt, A.J. DiBenedetto*, and R.N. Pittman. Dept. of Pharmacology, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104.

PC6-3 is a subline of PC12 cells that becomes irreversibly differentiated over a period of 7-10 days in the presence of NGF (Pittman et al., 1993; J. Neurosci. 13:3669-3680). Following removal of NGF, these cells undergo a transcription-dependent cell death similar to that seen in primary cultures of sympathetic neurons. The primary focus of this study is to investigate changes occurring during apoptosis, as well as to determine specific effects of NGF on cell cycle regulators so that a clearer interpretation of subsequent changes in these proteins during cell death can be obtained. To investigate the relationship between control of the cell cycle and apoptotic cell death, changes in cell cycle components are being characterized using immunoblot analysis. Cell cycle components being investigated include cyclins A, B, D, and E; cyclin dependent kinases (Cdks) 2, 4, 5 and p34 cdc2; retinoblastoma protein (Rb); and Cdk inhibitors, p21 and p27. Changes in these proteins are being characterized at various stages of differentiation (days 3, 5, 7 and 10 of NGF treatment) and during different phases of apoptosis (4, 12, and 24 hrs following removal of NGF). Several components of the cell cycle are modulated during the period when many cells become irreversibly differentiated, and a subset of these proteins change consistently during early stages of cell death following removal of NGF. In particular, significant changes are seen in p21 and cdk2 in populations of dying cells. Potential functional roles of cell cycle regulators in cell death are being investigated by generating lines of PC6-3 cells stably transfected with sense and antisense constructs of cell cycle components under control of an inducible tetracycline transactivator.

516.12

Neuronal cell death of cultured cortical neurons through apoptosis after deprivation of survival support by astroglial cells Yukio Nishizawa*, Makoto Ohgoh, Manami Kimura, and Kouichi Katayama, Eisai Tsukuba Research Laboratories, 5-1-3 Tokodai, Tsukuba, Ibaraki 300-26, Japan

Peripheral neurons dependent on NGF die by apoptosis after NGF deprivation. Since the dependence of central neurons on particular neurotrophic factors has been established only for small population of neurons, a convenient in vitro method for assessing the programmed cell death has not been established. On the basis of the fact that cortical neurons survive in culture for many weeks in the presence of astroglial cells, we have established an in vitro cell death model. The neurons were cultured on a cover slip on top of astroglial confluent culture and were then separated from astroglial cells. The neurons in fresh medium died within four days after separation from glial cells. This type of cell death appeared to be mediated by apoptosis, since the nuclei of dying neurons contained fragmented DNA assessed by the TUNEL method. The cell death was significantly inhibited by neurotrophic factors, NT-3, NT-4, and BDNF, but not by NGF. These results suggest that cortical neurons died after separation from glial cells through apoptosis caused by deprivation of neurotrophic factors provided by the astroglial cells.

516.13

DEVELOPMENTAL PATTERN OF NITRIC OXIDE SYNTHASE EXPRESSION IN *XENOPUS* SPINAL CORD WITH AN EMPHASIS ON MOTONEURON APOPTOSIS. W. L. Muhlach* and E. A. Ruebke. Dept. of Zoology, Southern Illinois University., Carbondale, IL 62901.

Nitric oxide (NO) may play a role in motoneuron development (Neurosci. 57:1,'93; Neurosci. 61:719,'94; Exp. Neurol. 120:153,'93). For example, inhibitors of nitric oxide synthase (NOS) attenuate expression of a marker of motoneuron maturation (CAT-301 antigen), neonatal rat motoneurons transiently express NOS, and adult motoneurons express NOS after ventral root avulsion. Two approaches were taken to address NO's involvement in motoneuron development. First, NADPH diaphorase activity was elucidated in the lumbosacral lateral motor column (LLMC) of *Xenopus laevis* developing tadpoles before, during and shortly after motoneuron apoptosis (stages 48-50, 51-53, 54-55 respectively), prior to metamorphosis (stages 56-59 and 60-63), and in adults. Second, the sciatic nerve of adult *Xenopus* frogs was axotomized near the spinal column. At intervals of 3,7,14,and 21 days post-operation, animals were euthanized, and the LLMC region of the spinal cord was processed for NADPH diaphorase activity. Tissues were fixed in 4% para-formaldehyde and 40um serial x-sections were cut on a cryostat. At no time during their ontogeny , including the period of apoptosis, did the motoneurons express NOS. This implies that NOS is not induced in apoptotic motoneurons as it is in response to motoneuron injury. At stages 56-59 two populations of NOS-positive neurons, interneurons and commissural neurons, appear to synapse with the maturing motoneurons. During stages 60-63 the NOS-positive commissural neurons disappeared while the interneurons remained into adulthood. This suggests that there are NO-dependent aspects of motoneuron afferent development, and that some NO influences on motoneurons remain in the adult. Sciatic nerve axotomy did not produce positive motoneurons. Trophic factor(s) from the Schwann cells, proximal to the lesion, may have suppressed NOS expression.

516.14

SERUM DEPRIVATION-INDUCED APOPTOSIS OF THE MOTOR NEURON-LIKE NSC34 CELL LINE IS ACCOMPANIED BY p53 ACCUMULATION AND CELL CYCLE ARREST. I.T. Shaw*, M Bachetti & N.R. Cashman. Neuroimmunology Unit, Montréal Neurological Institute, McGill University, Montréal, Canada H3A 2B4.

We have shown that serum deprivation-induced apoptosis in the NSC34 hybrid cell line is dependent upon early generation of reactive oxygen species (ROS; Shaw *et al., Soc Neurosci Abs* 1994). We hypothesize that *ROS-dependent DNA damage induces p53-dependent apoptosis* in NSC34 cells, and in human and transgenic mouse motor neurons subjected to oxidative stress by mutation of superoxide dismutase 1 (SOD 1). We now report that: 1) similar to irradiated thymocytes, apoptosing NSC34 cells exhibit accumulation of p53 protein within hours of serum deprivation, suggesting that ROS-induced DNA injury and p53 accumulation are early events in our model of apoptosis. 2) The proportion of G2-M cells in serum-deprived NSC34 cultures which are *spontaneously permeablized* (apoptosing population) is significantly less than the proportion of G2-M cells which are *experimentally permeablized* (whole population), suggesting that cells undergoing apoptosis are arrested at G1 phase, consistent with the cell cycle checkpoint regulatory activity of p53. Our data suggests that apoptosis in motor neuron-like NSC34 cells, and perhaps motor neurons *in vivo*, is dependent upon a cascade characterized by ROS generation, DNA damage and p53 accumulation. By this reasoning, ROS might be regarded as both an effector mechanism in apoptosis by DNA damage, and also as a signaling mechanism by ultimately triggering p53-induced genetically regulated cell death.

516.15

S-ADENOSYL-L-METHIONINE PREVENTS APOPTOTIC CELL DEATH OF CEREBELLAR GRANULE NEURONS. J. Harada, N. Yoshizawa, T. Oda * and K. Matsuda. Neuroscience Research Laboratories, Sankyo Co., Ltd., Hiromachi 1-2-58, Shinagawa-ku, Tokyo 140, Japan.

Differentiated cerebellar granule cells require depolarizing concentrations of extracellular pottasium for survival in culture. Apoptotic cell death of these neurons induced by lowering pottasium concentration in the culture medium, provides a good model of neuronal apoptosis in the central nervous system. Using this model system, we found that S-adenosyl-L-methionine (SAMe), a naturary occurring molecule which has been reported to be neuroprotective *in vivo*, prevented the apoptotic cell death of granule cells. SAMe is known to be involved following three intracellular reactions: transmethylation, glutathione biosynthesis, and polyamine biosynthesis. S-adenosyl-L-homocysteine (SAH), a potent inhibitor of transmethylation reaction mediated by SAMe, did not abolish protective effect of SAMe but was protective by itself. Cysteine and N-acetyl cysteine, potential glutathione precursors, also protected granule cells from cell death with similar extent. In addition, spermine strongly protected granule cells from apoptosis. The effect of spermidine was weaker than that of spermine, and putrescine showed no protective effect. N^1,N^{12}-bis(ethyl)-spermine (BESPM), an inhibitor of SAMe decarboxylase, did not inhibit protective effect of SAMe. BESPM also protected granule cells, suggesting that it mimics the effect of spermine.

These results suggest that SAMe prevents apoptotic neuronal cell death by facilitating the production of glutathione and/or polyamines but not transmethylation reactions.

516.16

INCIDENCE OF APOPTOTIC NUCLEAR PROFILES IN *XENOPUS LAEVIS* CENTRAL NERVOUS SYSTEM IS INCREASED BY EXOGENOUS THYROID HORMONE EXPOSURE. M.S. Beattie*, J.C. Bresnahan, J.N. Masters, M.J. Crowe. Neuroscience Program, Dept. of Cell Biology, Neurobiology & Anatomy, OSU Biotechnology Center, The Ohio State University, Columbus OH 43210

Metamorphosis in *Xenopus laevis* (XL) is induced and sustained by thyroid hormone (TH) which controls morphogenesis, cell death (apoptosis), and structural reorganization in many organ systems, including the CNS. Endogenous TH appears in XL tadpoles at stage (st.) 54, but larval tissues can acquire sensitivity to exogenous TH as early as st. 44. The highest concentration of TH receptor mRNAs in st. 44 tadpoles is present in the brain, spinal cord and gut epithelia. We examined the effect of exogenous TH on the incidence of nuclear fragmentation in the CNS of XL tadpoles. St. 50 and 54-56 tadpoles were exposed for 0, 12, 24, 48 h and 5 days to 100 nM TH. Tadpoles were immersion-fixed, frozen, cryostat-sectioned (20 µm) and incubated in the nuclear stain Hoechst 33342 (10 µg/ml) for 1 h. Sections were examined for nuclei with the condensed chromatin that is a morphological characteristic of apoptosis. No apoptosis was detected in **st. 50 tadpoles** exposed to TH for 0, 12 or 24 h. After 48 h, a few apoptotic nuclei were seen in the brainstem and many were present in gut epithelia. After 5 days, extensive apoptosis was present throughout the spinal cord and brain. **St. 54 tadpoles** demonstrated few apoptotic nuclei in the CNS exposed for 0 or 12 h. A slight increase in the number of apoptotic nuclei was seen in the CNS of 24 h exposed tadpoles and numerous apoptotic profiles were detected in both brain and spinal cord by 48 h. St. 54 tadpoles exposed for longer times became ill and often did not survive past 4 days. Numerous apoptotic nuclei were observed as well as obvious tissue damage within the spinal cord. TH appears to induce precocious apoptosis which is detectable by bisbenzimide staining techniques. (Support: NS10165 & NS07291)

516.17

3,3',5-TRIIODO-DL-THYROXINE (T3)-INDUCED MITOCHONDRIAL PROLIFERATION AUGMENTS GENERATION OF REACTIVE OXYGEN SPECIES (ROS) IN APOPTOSIS OF THE MOTOR NEURON-LIKE NSC34 CELL LINE. N.R. Cashman*, M Bachetti & J.T. Shaw. Neuroimmunology Unit, Montréal Neurological Institute, McGill University, Montréal, Canada H3A 2B4.

Using electron microscopy and flow cytometry with the oxidation-sensitive fluorochrome 5,6 carboxy-2',7'-dichlorofluorescein (DCFH), we have shown that serum deprivation-induced apoptosis in the NSC34 hybrid cell line is accompanied by mitochondrial swelling and an early "burst" of ROS (Shaw *et al., Soc Neurosci Abs* 1992, 1994). To ask if mitochondrial injury is a cause or consequence of observed ROS generation, we now investigate the relationship between NSC34 mitochondrial content and the kinetics of DCFH fluorescence in cells treated with the respirotoxin phenazine methosulfate (PMS), and cells induced to undergo apoptosis by serum deprivation. Mitochondrial proliferation was induced by addition of T3 to culture media (5X and 10X basal concentration). Compared to NSC34 cells cultured in basal media, T3-treated NSC34 displayed a differentiation response characterized by profuse process extension, and had increased mitochondrial content detectable by both citrate synthase activity and Southern blotting. T3-treated cells exhibited: 1) increased duration and area of the time curve of DCFH fluorescence induced by PMS treatment, presumably due to increased mitochondria available for generation of superoxide; 2) increased duration and area of the time curve of DCFH fluorescence in serum-deprived cells, suggesting that mitochondria may be an important source of ROS in this model of apoptosis; and 3) paradoxically *improved* survival of serum-deprived cells. As we have previously found that the rate of cell death in serum-deprived NSC34 is directly dependent upon the duration and amplitude of the early ROS "burst", our data suggest that thyroid hormones may have two opposing activities in neuronal apoptosis: 1) and *apoptosis accelerating effect* secondary to increased cellular mitochondrial content (a potential source for superoxide or cytosolic release of sequestered Ca^{2+}); and 2) an *apoptosis inhibiting effect* perhaps due to gene transcriptional activity of this steroid receptor superfamily, as suggested by induction of NSC34 process extension in serum-fed cells.

516.18

REACTIVE CELL TYPES AROUND STAB WOUNDS IN THE AVIAN BRAIN. A. D. Székely, G. Nagy, M. Kálmán, A. Csillag and G. E. Meredith* 1st Dept. of Anatomy, Semmelweis Univ. Med., H-1450 Budapest, Hungary and Dept. of Anatomy, Royal Coll. of Surgeons, Dublin, Ireland.

Macrophages/microglia and reactive astroglia are the classic participants of the post-lesion demarcation and removal of damaged tissue in the vertebrate CNS. We have previously described a type of reactive glia following lesions in the chicken but not in the rat brain (Székely et al., Anat. Anz. Suppl. 170:689, 1991). These cells displayed spontaneous peroxidase positivity (SPP) with one or two thick or thin processes running to the lesion surface. For further characterization, adult zebra finches, two-day-old chicks and adult rats were anaesthetized, placed in a stereotaxic frame and the right hemisphere was lesioned with a sterile hypodermic needle. The survival times varied between 2 and 14 days, then the animals were perfused with 4 % paraformaldehyde and coronal sections were cut from the region of the lesion. In zebra finches, SPP cells were discernible and they showed NADPH-diaphorase (ND) reactivity. Also macrophages/microglia, reactive for both ND and *Griffonia* lectin staining, appeared close to the lesion. In young chicks, SPP cells were found in the vicinity of the stab wound and they also formed clusters around small vessels in the periphery of the lesion. Some large, vacuolar, DAB positive cells were present in these cell groups, more frequently in young birds, surrounded by lightly stained cells with an arbor of fine processes.

In rats, no similar cellular response was detected following lesions, in particular, we did not observe SPP cells or perivascular reactive cell types, apart from the appearance of macrophages/microglia and the GFAP positive astroglial cordon demarcating the lesion.

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516.19

A COMPARISON OF THE EFFICACY OF THE HERPES SIMPLEX VIRUS VERSUS THE ADENOVIRUS AS VECTORS TO DELIVER FOREIGN GENES INTO PRIMARY SYMPATHETIC NEURONS. Ruth S. Slack, Daniel J. Belliveau, M. Rosenberg, E. Cooper*, R. Dunn and F.D. Miller. Centre for Neuronal Survival, Montreal Neurological Institute, 3801 University, Montreal, Quebec H3A 2B4.

In order to develop a satisfactory strategy to manipulate the genome of terminally differentiated neurons, we are exploring the possibility of using the Herpes Simplex Virus and Adenovirus as potential vectors. Viruses carrying identical expression cassettes, including the CMV promoter driving β-galactosidase were compared. Infectivity studies indicate that Adenovirus is more efficient at infecting primary SCG neurons than Herpes Virus. With Adenovirus at an moi of 10, greater than 80% of sympathetic neurons expressed the reporter gene with no decrease in viability. In contrast infection with Herpes virus at an moi of 10 only 25 to 30 % of cell expressed β-galactosidase. Determinations of long-term survival following viral infection also indicate that sympathetic neurons can tolerate significantly higher titres of Adenovirus in comparison to Herpes vectors. Six days following infection at 100 moi, greater than 70% of cells infected with adenovirus survive, while less than 20% of cells infected with herpes virus remain viable. Determination of cell viability and examination of electrophysiological properties following infection will reveal the utility of these viruses as vehicles for the introduction of foregin genes into primary sympathetic neurons.

516.20

FETAL SPINAL CORD TRANSPLANTS RESCUE AXOTOMIZED RUBROSPINAL NEURONS FROM RETROGRADE CELL DEATH IN ADULT RATS.

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Intraspinal transplants of fetal spinal cord (FSC) may contribute to recovery after spinal cord injury by rescuing axotomized neurons otherwise destined to die. In this study we examined whether FSC transplants can rescue axotomized Red Nucleus(RN) neurons from retrograde cell death in adult rats. RN neurons of adult female Sprague-Dawley rats were labeled by retrograde transport of Fluorogold (FG) injected bilaterally into the spinal cord at C6; 1 week later, the right-sided RN neurons were axotomized by hemisection of the left side of the C3-4 spinal cord, and either a solid piece of embryonic day (E) 14 spinal cord or gelfoam was introduced into the aspirated hemisection cavity. Additional rats underwent C3-4 spinal cord hemisection with tranplantation of FSC or gelfoam but did not receive FG injections. 2 and 4 months after transplantation RN neurons in the caudalmost 500µm of the nucleus were counted and the area of the neurons measured. At both times the number of RN neurons contralateral to the hemisection was reduced by 40% in rats that received gelfoam; mean cell area was also reduced by 40%. RN cell loss was ~20% in rats receiving FSC transplants, but mean cell area was reduced by 40%. FSC transplants rescue about half of the axotomized RN neurons that would otherwise die after spinal cord injury in adults and were less effective than for Clarke's nucleus where rescue is virtually complete (Himes, et al., J. Comp. Neurol. 339; 117-131, 1994). Supported by NS 24707 and VA Research Service.

517.1

CHARACTERIZATION OF THE KITTEN RETINOGENICULATE PATHWAY GROWN IN COCULTURE. R.S. Erzurumlu*, F.-S. Lo & W. Guido. Dept. of Anatomy & Neuroscience Center, LSU Medical Center, New Orleans, LA 70112.

We established a coculture model for the developing cat retinogeniculate pathway in order to study the cellular mechanisms underlying axonal and dendritic remodeling and synapse consolidation. Retinal explants and thalamic slices containing LGN from 1-day old kittens were cocultured in serum free medium. After 3-8 days, some were fixed and the lipophilic tracer DiI was placed in retina or LGN. In others, we conducted intracellular recordings with biocytin filled electrodes to obtain information about the membrane properties and synaptic responses of LGN cells receiving input from ganglion cells. Retinal ganglion cells differentiated, and had exuberant processes, growth cones and dendritic spines. Retinal axons grew into thalamic slices, and formed elaborate arbors within LGN. DiI labeling from LGN showed mostly α, a few γ, but not any β cells, innervated LGN explants. Intracellular recordings indicated that LGN cells responded to small depolarizing current pulses with a train of action potentials. These responses showed rapid frequency accomodation due to the activation of a fast voltage-sensitive (I_C), and a slow voltage-insensitive (I_{AHP}) afterhyperpolarization. When hyperpolarized, many morphologically identified X and Y cells responded with low threshold (LT) Ca^{2+} spikes. Cells with immature morphology showed little or no LT spiking. Many LGN cells were activated synaptically by electrical stimulation of the retinal explant. EPSPs had two overlapping components, an early-fast, DNQX-sensitive (non-NMDA), and a late-slow, AP5-sensitive one (NMDA). With V_m hyperpolarization, EPSPs activated LT spikes. In some cells, increasing stimulus intensity led to multiple EPSP complexes, suggesting they receive input from more than one ganglion cell. These observations indicate that many significant cellular events characteristic of the developing cat retinogeniculate pathway can be captured in vitro. *Support by NIH NS32195 (R.S.E.) and NSF 9396270 (W.G.).*

517.2

MEMBRANE PROPERTIES AND SYNAPTIC RESPONSES OF MATURE, IMMATURE, AND VISUALLY-DEPRIVED LGN CELLS. W. Guido*, and F.-S. Lo. Anatomy & Neuroscience Center, LSU Medical Center New Orleans, LA 70112.

Little is known about the membrane properties of LGN cells during the development and refinement of connections in the cat retinogeniculate pathway. Indeed, the functional state of immature LGN cells and the role played by visual experience has yet to be explored. Using intracellular recordings with biocytin filled electrodes in a thalamic slice preparation, we examined the membrane properties and synaptic responses of LGN cells from normal and monocularly sutured (MS) cats, and 1-day old kittens. Mature X and Y cells had many voltage-dependent properties including a low threshold (LT) Ca^{2+} conductance (I_T) which leads to Ca^{2+} spikes and burst firing, a transient K+ conductance (I_A) which delays the onset of action potential firing, a K^+ conductance (I_C) which contributes to firing frequency, and a mixed cation conductance (I_H) which prevents strong hyperpolarization. EPSPs evoked by stimulation of optic tract fibers also had an NMDA component. Cells from 1-day old kittens lacked I_A and I_H. Cells resembling X and Y types, had overshooting action potentials and a single component afterhyperhpolarization, I_c. LT spikes were common and some showed bursting. Other, with highly immature morphology, had wider action potentials and showed rapid frequency accommodation due to the activation of I_c and the slower I_{AHP}. Highly immature cells lacked or had very small LT spikes and these failed to give rise to bursts. Cells from 1-day old kittens had EPSPs with NMDA and nonNMDA components. In some, increasing stimulus intensity led to multiple EPSP complexes, suggesting they receive input from more than one ganglion cell. Cells recorded from deprived layers of MS cats had properties that were either similar to normal or highly immature cells. Thus prior to eye opening, LGN cells possess many important properties needed to effectively integrate retinal signals. Visual experience also plays a vital role in determining the final modeling of normal morphology and membrane properties of LGN cells. *Support by NSF 9396270 to WG.*

517.3

SENSORY DEAFFERENTATION AFFECTS IMMEDIATE EARLY GENE (IEG) EXPRESSION IN THE VISUAL SYSTEM OF THE ADULT CAT. L. Arckens*[1,2], W. Vanduffel[2], F. Zhang[3], U.T. Eysel[4], J.-J. Vanderhaeghen[3], G.A. Orban[2] and F. Vandesande[1]. [1]Lab. Neuroendocrin. & Immunol. Biotechnol., [2] Lab. Neuro- & Psychofysiol., K.U.Leuven, B-3000 Leuven, Belgium [3] Lab Neuro-pathol, U.L.Bruxelles, B-1070 Bruxelles, Belgium [4]Dept Neurophysiol., Ruhr-Universität Bochum, D-44780 Bochum, Germany

In order to investigate the molecular basis of topographical reorganization occurring in adult cat visual cortex after sensory deafferentation, we have used in situ hybridization to investigate the effect of binocular central retinal lesions on the expression of four IEGs in the dLGN and in twelve visual cortical areas of the adult cat. In the deafferented region of the dLGN the amount of c-jun mRNA increased markedly, but temporarily. In contrast, deafferentation of the central portion of the dLGN resulted in decreased c-fos and junB mRNA levels. These lesion-related effects remained constant for at least one month and the dimensions of the lesioned area matched perfectly the predictions based on the retinotopic map of Sanderson (1971). At the cortical level c-jun expression increased only in layer VI of area 17 subserving central vision and only temporarily. Zif-268, c-fos and junB expression decreased not only in the region of area 17 subserving central vision but also in the corresponding regions of areas 18, 19, 21a, 21b, 7, PMLS, PLLS, VLS and DLS. These results were confirmed in immunocytochemistry using antibodies against c-fos and zif-268. Shortly after the lesion the dimensions of the lesion-affected cortical loci were twice as large in visual degrees as expected from the retinotopic maps of Rosenquist (1985).The area of cortex affected decreased over time following the lesion and the rate of shrinkage differed among areas. Our results show that many cortical areas exhibit an IEG reaction to bilateral retinal lesions. As these reactive cortical regions are larger than the deafferented area, the IEG reaction might be related both to the topographical reorganization and to the deafferentation.

517.4

THE EFFECT OF RETINAL LESIONS ON GAP-43 EXPRESSION IN THE VISUAL SYSTEM OF ADULT CATS. V. Backelandt[1,2]*, U.T. Eysel[3], G.A. Orban[2] and F. Vandesande[1]. [1]Lab. of Neuroendocrinology and Immunological Biotechnology, Zoological Institute, Katholieke Universiteit Leuven, [2]Lab. of Neuro- and Psychophysiology, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium; [3]Lab. of Neurophysiology, Ruhr-Universität Bochum, Germany.

We have studied synaptic plasticity in the adult cat visual system after induction of small binocular central retinal lesions. GAP-43 (growth-associated protein 43) was chosen as a molecular marker for synaptic reorganization. The cats were sacrificed between 1 week and 32 weeks after induction of the retinal lesions. By means of immunohistochemical stainings we observed an increase in GAP-43 expression in the lesion-affected part of the dLGN (dorsal lateral geniculate nucleus). This increase was visible from 3 weeks after lesioning on and persisted until 32 weeks after induction of the retinal lesions. The degeneration of the retinal ganglion cell axons provoked a proliferation of reactive astrocytes in the central part of the dLGN, as visualized by GFAP (glial fibrillary acidic protein) immunostaining, which slowly subsided between 20 and 32 weeks after lesioning. In area 17, we failed to find significant changes in GAP-43 expression, despite evidence that the observed topographical reorganizations (Kaas et al., 1990, Science, 248, 229-231; Gilbert and Wiesel, 1992, Nature, 356, 150-152), are paralleled by sprouting of the long-range horizontal cortical connections (Darian-Smith and Gilbert, 1994, Nature, 368, 737-740). In conclusion, while GAP-43 expression after retinal lesions occurs in parallel with some signs of reorganization in the deafferented dLGN (Eysel et al., 1980, Brain Res., 181, 285-300), it does not accompany the topographical reorganization observed in the visual cortex. This suggests that GAP-43 expression is not necessarily associated with the recovery of visually evoked activity. We are currently examining the origin of the increased GAP-43 expression in the dLGN.

517.5

THE DORSAL LATERAL GENICULATE NUCLEUS OF THE VERVET MONKEY AFTER NEONATAL HEMISPHERECTOMY. [1,3]D. Boire*, [1,2]M. Herbin,[3]A. Ptito and [1,2,3]M. Ptito. [1]Neuropsychol. Unit and [2]CRSN, Université de Montréal and [3]MNI, McGill University, Montreal, CAN.

The effect of early hemispherectomy on the organization of the dorsal lateral geniculate nucleus (dLGN) was studied in the Vervet monkey. Retinal projections to the dLGN was studied using anterograde transport of HRP. Blocks containing the dLGN were cut in coronal sections and HRP was visualized with TMB and AHM as chromogens. Adjacent series of sections were prepared for cytochrome oxydase (CO) and acetylcholinesterase (AChE) histochemistry and neuronal cell bodies were visualized with Nissl staining. Anterograde transport of HRP following intraocular injections produced terminal labelling in a clear laminar pattern in both dLGN. Dense labelling was seen in the magnocellular layers particularly in their more lateral portion in the dLGN ipsilateral to the lesion. CO histochemistry also revealed more intense staining in the lateral portion of the magnocellular layers. Slightly less dense labelling was observed throughout the appropriate parvocellular layers. The contralateral dLGN was comparable to that of intact animals. The dLGN ipsilateral to the hemispherectomy was reduced 70% in size, distorted in shape and exhibited severe gliosis. In Nissl stained sections, laminar organization was elusive since very few neurons survived.Typical large magnocellular elements were practically absent and very dispersed parvocellular elements were observed. These results are discussed in terms of residual vision in the blind field following hemispherectomy.

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517.6

INTRINSIC AS WELL AS TARGET-DERIVED FACTORS ARE INVOLVED IN THE DETERMINATION OF ANOMALOUS RETINO-THALAMIC TERMINAL MORPHOLOGIES **Changying Ling*, Sonal Jhaveri and Gerald E. Schneider; Dept. of Brain & Cognitive Sciences, M. I. T., Cambridge, MA 02139.**

In normal adult hamsters, the lateral posterior nucleus of the thalamus (LP) receives a sparse retinal input with a special distribution pattern and distinct morphological features. Neonatal tectal lesions deprive retinal axons of their major target, and at the same time massively denervate the LP, resulting in a marked increase of retinal input to the LP (Schneider, '73). In order to determine whether the terminal morphology of retinothalamic axons is dicated by the retina or by its target substrate, we subjected newborn hamsters to surgical procedures known to produce a massive retinal projection to LP. Two months later, we examined the terminal fields and the morphological features of retinal axons in LP using cholera toxin subunit B (CT-B) as an anterograde tracer. Immuno-localization of the CT-B revealed the detailed morphology of retinal terminals. Our results show that the density and termination zones of abnormal retinal projections in LP vary depending on the time of the neonatal surgery, and on the degree of the denervation caused by the tectal lesion. Several different terminal types can be recognized. In the ventromedial LP, strings of varicosities which seem to envelop proximal dendrites are similar to terminals of the tecto-LP projection. By contrast, in the dorsolateral LP, terminals resemble Type R1 and Type R2 retinogeniculate terminals (Erzurumlu et al., '88). The segregation of distinct terminal types, resembling normal tectothalamic axons or normal retinogeniculate axons, indicates roles for both target derived and intrinsic factors in the specification of terminal morphology.

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517.7

EMERGENCE OF CLUSTERED EYE-SPECIFIC PATTERNS IN EXPERIMENTALLY INDUCED RETINAL PROJECTIONS TO THE FERRET AUDITORY THALAMUS. A. Angelucci*, K.S. Cramer and M. Sur, Department of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Neonatal deafferentation of the ferret medial geniculate nucleus (MGN) produces a pattern of clustered retinal projections scattered throughout the MGN in the adult. Clusters related to the ipsilateral eye are consistently adjacent to but spatially segregated from clusters related to the contralateral eye. By analogy with lamination in the LGN, clustering and/or eye-specific segregation in the novel projection may result from the refinement of an initially more diffuse projection. To investigate this possibility, we have examined the emergence of eye-specific clustering in the novel projection to the MGN.

Following MGN deafferentation at birth, ferret kits received injections of WGA-HRP in one eye and cholera toxin subunit B in the other eye one to two days before perfusion at 3, 5, 7, 14 and 27 days post-surgery (PS). The pattern of anterograde labeling in the MGN was examined. At PS3 and PS5, labeling was diffuse and extensive throughout the medio-lateral and antero-posterior extent of MGN. Retinal axons traversing the nucleus had few branches, and inputs from the two eyes overlapped extensively. At PS7, labeling was diffuse, although a tendency to cluster was apparent in some areas. At PS14, clusters were more apparent, but overlap between projections from the two eyes was still evident. By PS27, axonal arbors were more elaborate and nearly adult-like, and clusters had become denser and further restricted.

The progressive restriction of retinal projections into eye-specific clusters in the MGN occurs over the same time period as the formation of layers and sublayers in the normal LGN. These processes may thus share similar afferent-driven mechanisms. Clustering and eye-specific segregation in the MGN occur as retinal axon arbors aggregate into specific regions. This process may involve activity-dependent interactions between axons and their targets.

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517.8

INHIBITION OF NITRIC OXIDE SYNTHASE DOES NOT DISRUPT FORMATION OF EYE-SPECIFIC LAYERS IN THE FERRET LATERAL GENICULATE NUCLEUS. K. S. Cramer*, A. Angelucci, and M. Sur, Department of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Nitric oxide (NO) may act together with NMDA receptors in activity-dependent remodeling of connections. ON/OFF sublamination in the ferret lateral geniculate nucleus (LGN) requires NMDA receptor activation (Hahm et al., Nature 351:568-570, 1991) and nitric oxide (Cramer and Sur, Soc. Neurosci. Abst. 20:1470, 1994) Segregation of eye-specific layers during the first week appears not to require activation of postsynaptic NMDA receptors (Smetters et al., Brain Res. 658:168-178, 1994). NO synthase activity becomes detectable during the first postnatal week and peaks at about four postnatal weeks (Cramer et al., J. Comp. Neurol. 353:306-316, 1995). We have examined the role of NO in the formation of eye-specific layers.

Ferret kits received Nω-nitro-L-arginine (L-NoArg) or Nω-nitro-D-arginine methyl ester (D-NAME, an inactive isomer), 20 mg/kg/day i.p., from P1 to P7. This dose of L-NoArg has been shown to reduce NO synthase activity in the brain and inhibits ON/OFF sublamination. At P7, animals received injections of WGA-HRP in one eye and cholera toxin B subunit (CTB) in the other eye. The pattern of anterograde labeling in the LGN was assessed at P8. Eye-specific segregation in L-NoArg treated animals did not differ from that of normal or control animals. In all animals, the left and right LGN had a complementary pattern of labeling, and WGA-HRP and CTB staining were complementary within each brain. Treated animals did not differ from normal or control animals in weight gain or overall brain morphology.

These results suggest that NO is not involved in the segregation of eye-specific layers in the LGN. In ferret retinogeniculate development, NO appears to be required only when NO synthase levels are high in the LGN and when NMDA receptors are required for refinement of connections. *Supported by EY07023*

517.9

APP AND APLP2 LOCALIZATION IN THE DEVELOPING HAMSTER PRIMARY VISUAL PATHWAY. A.M. Confaloni*, A. W. Lyckman, G.Thinakaran, S.S Sisodia and K. L. Moya, INSERM U334 and CNRS URA 1285, SHFJ, CEA, 91401 Orsay, France; Ist. Sup. Sanità, 00161 Rome, Italy; Johns Hopkins Univ. Sch. Med., Neuropath. Lab., Baltimore, MD 21205.

The amyloid precursor protein (APP) and the amyloid precursor-like protein (APLP2) are related membrane proteins arising from a multigene family. Both of these proteins undergo considerable changes in their synthesis and axonal transport during development. Here we have used polyclonal antibodies specific for APP (CT-15) and APLP2 (D2-II, CT12) to study their localization in the developing hamster retinofugal pathway.

At P0 and P1, when many retinal axons are rapidly elongating, strong APP and APLP2 immunoreactivity (IR) was observed in the optic tract (OT) over the lateral geniculate nucleus (LGN). Within the LGN we observed numerous single fibers, some studded with varicosities. In the superior colliculus (SC), APP and APLP2 IR fiber bundles were present in the stratum opticum/superficial grey. No IR cell bodies were found in any brain region.

At P11 and P12, when retinal axons are arborizing and forming numerous synapses, the OT is lightly IR for APP and APLP2. In the LGN, the APLP2 antisera revealed clusters of IR puncta that appear to correspond to early retinal axon terminal types. In the P12 SC, APLP2 IR is concentrated in cell bodies while that of APP shows only a light neuropil presence.

In the adult LGN and SC, APP and APLP2 were concentrated in cell bodies while the neuropil was very light. The OT, was devoid of APP and APLP2 IR.

These results show that the developmental localization of APP and APLP2 follows a similar pattern. The proteins are distributed along fibers during rapid axon growth and then shift to the target neuropil during synapse formation. At later stages, APP and APLP2 concentrate in cell bodies. We suggest that APP and APLP2 may be subject to similar regulatory signals during development and in the adult.

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517.10

NMDA RECEPTOR SUBTYPES IN THE FERRET LGN DURING DEVELOPMENT. A. S. Ramoa*, E. Jakoi, and C. Gerwin. Dept. of Anatomy and Dept. of Neurology, Medical College of Virginia, VCU, Richmond, VA 23298.

The adult form and function of the lateral geniculate nucleus (LGN) arise after extensive modifications in circuit organization that, in the ferret, occur during the first postnatal month. We have shown recently that NMDA receptor-mediated responses of the retinogeniculate pathway change markedly during this critical period of development (J. Neurosci. 14:2098, 1994). These changes, which may be important in facilitating retinogeniculate remodeling, could result from age-related modifications in the molecular composition of the NMDA receptor. To examine this possibility, synaptic transmission and expression of mRNA in LGN neurons were studied during the first 3 postnatal months. Whole-cell recordings in the LGN slice preparation revealed that the NMDA receptor-mediated response was enhanced during the first postnatal month in both retinogeniculate and corticogeniculate pathways. Kinetic properties of the NMDA response as well as the ratio of NMDA/non-NMDA receptor-mediated response changed. Additionally, application of ifenprodil, which binds with higher affinity to heteromeric NR1/NR2B receptors than to NR1/NR2A receptors (Neuron 10:267, 1993), blocked more potently the synaptically induced NMDA response in LGN neurons during the first postnatal month than later in life. These findings suggest that the subunit composition of the NMDA receptor changes during development and subunit NR2B is predominant at early stages. To examine the mechanisms of these changes, complementary oligonucleotide probes to NR1A, NR2A and NR2B subunits were used in Northern analysis of total RNA isolated from the LGN. Ratios of mRNA encoding NR2A and NR2B subunits were unaltered during development, suggesting that the changes in NMDA receptor response properties reflect translational and/or post-translational regulatory mechanisms. (Supported by NSF)

517.11

CULTURED RAT VISUAL SYSTEM ON PLANAR ELECTRODE ARRAYS. A. Kawana*, H. Kamioka, P.J. Charlety, E. Maeda and Y. Jimbo, NTT Basic Research Laboratories, Atsugi-shi, Kanagawa, 243-01 Japan

Electrical activity plays a major role in sculpting synaptic circuits in the nervous system. One of the best studied examples of this is the mamalian visual system. However, the changes in electrical activity during the development of the visual system remain poorly understood, because of the difficulties in carrying out multisite, non-invasive recording. We have already described multisite, non-invasive monitoring of activity in organotypic cultures of the visual cortex using the planar electrode arrays (PEA) (P. Charlety et al ; Soc. for Neurosci., 1994 543.9). Here we describe the organotypic coculture of the major regions of the rat visual system on the PEA and observations on the changes in electrical activity during the development and interconnection of the networks.

64 planar electrodes embedded in the culture substrate were separated into three blocks. The distance of each block was 600 um. Slices (300 um) of visual cortex (VC) and retina were isolated from P1 rat and lateral geniculate nucleus (LGN) from E18 rat, and fixed on individual block of the substrate using coagulated chicken plasma. One day after initiating the culture, neurites grew extensively from the VC , while neurites of LGN and retinal ganglion cells grew out from the tissue at three days *in vitro* (3 DIV). Electrical activity was observed from the VC and LGN simultaneously after 5 to 6 DIV. The VC showed usually synchronized burstings from 7DIV which were observed to propagate to the LGN area and sometimes to the retinal area. As very little delay of the onset of the bursting was observed at LGN and retina, the activity in these regions may come from neurites grown out from the cortex. Activity of the cortex could not be evoked by the stimulation of the LGN. After 14 DIV, the electrical activity of VC and LGN could be evoked by the electrical stimulation from LGN and VC, respectively. These results suggest that it takes around 14 days to form functional synaptic connections between VC and LGN. The retina formed hardly any functional synaptic connections with LGN. One reason for this may be that the preferred target of the ganglion cells of the rat is not the LGN, but the superior colliculus.

517.12

SPATIO-TEMPORAL DISTRIBUTION OF EVOKED ACTIVITY ACROSS THALAMO-CORTICAL COCULTURES: A STUDY WITH VOLTAGE-SENSITIVE DYES. H.-P. Höpp*, C.G. Galizia and C.M. Müller
MPI für Entwicklungsbiologie, D-72076 Tübingen, Germany.

Cocultures of visual cortex with other brain tissues have demonstrated the formation of the specific afferent and efferent connections. Physiological measurements have confirmed the organotypic organisation of the cocultures. We performed fast optical recordings to determine the spatio-temporal distribution of evoked activity in thalamo-cortical cultures.

Cortical slices of 350µm thickness were taken from 4-6 day old rat pups and thalamic explants from E16 rat embryos. One or two thalamic explants were positioned close to the pial and/or white matter side of a visual cortical slice. Cocultures were then maintained for 7 to 42 days on Millipore culture dishes at an air-medium interface. The cultures were stained for 10-30min with 1-50µM RH795 or Di-4-ANEPPS (Molecular Probes Inc.). Recordings were carried out using a 464 element photodiode array read out at 630Hz. Electrical stimuli of 50-200µs duration and 50-700µA intensity were applied via tungsten stimulation electrodes into the thalamic explants

The optical response typically encountered in the cortex was a depolarizing transient peaking 12-16ms after the stimulus. Across cortical layers highest amplitudes and rates of rise for this component as well as shortest delay times were frequently found in layer 4. Double components with distinct "gaps" between their individual delay times were observed over the apposition zones of the cocultured explants. Applying a paired pulse paradigm, we found depression of the second response, possibly due to the presence of feed-forward inhibitory interactions as described in-vivo. Suppression of the transients was also demonstrated by a similar paired pulse paradigm employing 2 spatially separate stimulation electrodes and was found to depend critically on the duration of the inter-stimulus interval (ISI) used: at ISI 55ms suppression was minute, whereas at ISI 15ms it was almost complete. At ISI 0ms (= coincident stimuli) positive cooperativity was observed.

Our experiments confirm and extend previous findings on the layer-specific spatio-temporal dynamics of synaptic activity in thalamo-cortical cocultures.

518.1

DETECTION OF VISUAL PATTERNS IN DYSTROPHIC RCS RATS FOLLOWING RPE TRANSPLANTS. P.J. Coffey[1], L. Hetherington[1], S.J. Whiteley[2], T.M. Litchfield[2], R.D. Lund[2] AND S.R. Wright*[1] [1]Dept of Psychology, University of Sheffield, England, [2]Institute of Ophthalmology, London, England, [3]Dept. of Biomedical Sciences, University of Sheffield, England.

Recent work has shown that injections of retinal pigment epithelial cells (RPE) into the sub-retinal space of the dystrophic Royal College of Surgeon (RCS) rat can reduce the degeneration of photoreceptors seen in these animals. The goal of this procedure was to stop degeneration and improve visual performance in these animals. To date, an improvement in ERG's following this procedure has been documented, but no behavioural study has shown an improvement in visual detection or acuity. In order to assess whether this would be an appropriate clinical procedure for the related human condition of age related macular degeneration (ARMD), we examined visual pattern detection in the RCS rat after implantation of RPE cells.

Three groups of animals were used in this experiment, dystrophic RCS rats with sub-retinal RPE injections, dystrophic RCS rats and non-dystrophic RCS rats. The response of these animals to visual change in the environment were recorded in open field arenas in which a part or all of the visual environment could be altered without changing non-visual features. The rats were placed into the arenas for 5mins on two separate occasions with an interval of 5mins between each placement. The animals locomotor activity was recorded on video tape. Non-dystrophic RCS rats increased their locomotor activity when there was a change in the whole visual environment, but specifically when the change was from an unpatterned to a patterned environment. Dystrophic RCS rats locomotor activity did not change when the visual environment was altered indicating that they did not detect (ie. see) a change in the visual environment. However, dystrophic RCS rats, which had previously received a subretinal injection of RPE cells, locomotor activity did increase following a change in the visual environment.

These preliminary results suggest that injections of RPE cells into the eye of the dystrophic RCS rat can improve visual pattern detection.

518.2

SYNAPTIC BASIS OF TRANSPLANT DRIVEN PUPILLARY LIGHT REFLEX IN NEONATAL AND ADULT HOSTS

D.D.A.Lawson and R.D.Lund*, Neural Transplant Program, Institute of Ophthalmology, Bath Street, London EC1V 9EL, UK.

Intracerebral retinal transplants have been shown to drive a pupillary light reflex (PLR) in rats (Klassen and Lund, PNAS USA 84, 6958-60: 1987), although the synaptic basis of this behaviour has yet to be examined in detail. Here we investigated the ultrastructure of the olivary pretectal nucleus (OPN), believed to be the main relay station for optic afferents in the PLR, thus enabling a comparison of host visual circuitry with that established using retinal grafts.

Embryonic (E12-E14) retinae were transplanted over the midbrain of both neonatal (P1) and immunosuppressed adult rats, the hosts surviving for a minimum of 4 months or 10 weeks respectively. Secondary tracing techniques (anterograde degeneration and/or HRP) were then employed, and the OPN studied with EM in association with control material.

Both groups displayed a transplant-driven PLR, with neonatal recipients demonstrating graft terminals throughout the entire OPN. These were involved in the intricate synaptic configurations typical of the normal optic input to the nucleus, with some sharing the same dendrites as the host projection. Adult recipients, however, had only 1/10th the number of transplant terminals by comparison, with the majority of innervation concentrated in the myelinated nuclear periphery.

These results indicate that whilst the developing CNS can accomodate complex transplant integration, both an inhibitory environment and existing neural pathways may compromise adult plasticity. It is nevertheless evident that sufficient circuit reconstruction does occur to enable behavioural adaptation, possibly via a modification of normal adult synaptic patterns.

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518.3

EXPRESSION OF SYNAPTOPHYSIN IN THE RAT RETINA FOLLOWING VARIOUS TRANSPLANTATION STRATEGIES. A.A.Vugler[1], P.J. Coffey[1], R.D. Lund[2] AND M. Vidal-Sanz*[3]. [1]Dept of Psychology, University of Sheffield, [2]Institute of Ophthalmology, England, [3]Dept of Ophthalmology, University of Murcia, Spain.

Previous studies using either fetal retinal transplants on to the tectum of neonates and adults, or peripheral nerve grafts to convey regenerating retinal axons to the tectum of adults, indicate that the newly established retino-tectal connections can mediate functional responses in the host animal. Thus, it is important to understand the degree to which normal synaptic organisation within these retinas is preserved. In the present study we used synaptophysin immunohistochemistry (SYN-IR) to compare patterns of synaptic organization in normal retinas to: i) retinas implanted over the tectum of neonatal and adult rats, and; ii) retinas with axons regenerating along peripheral nerve grafts.

In normal retinas, synaptophysin immunoreactivity (SYN-IR) was detected in both the inner (IPL) and outer (OPL) plexiform layers. Also, there was a third lightly stained band of variable intensity at the level of the outer limiting membrane. This pattern of expression was consistent amongst all normal retinas examined. In retinas implanted into neonates, the pattern of SYN-IR was very similar to that of the normal retina with two distinct plexiform layers positively stained. However, there was no weakly stained third band and the OPL appeared more diffuse and less intensely stained than in normals. In retinas implanted into adult rats, the pattern of SYN-IR was not as distinct as that observed either in normal retinas or in retinas implanted into neonates. Specifically, the distinction between IPL and OPL was not as clear, although some laminal organization was preserved.

The differential expression described above may reflect a number of possibilties including the age of the host recipient, placement of transplant and the reduced connectivity to host visual systems seen in adult transplants. This reduced level of synaptic organization may reflect functional differences between adult and neonatal transplants. These preliminary results might suggest a possible reduction in the synaptic organisation of transplants when placed into an adult recipient.

518.4

ORGANIZED EMBRYONIC RETINAL TRANSPLANTS TO NORMAL OR LIGHT-DAMAGED RATS. R.B. Aramant* and M.J. Seiler. Dept. of Ophthalmology and Dept. of Anatomy, University of Louisville School of Medicine, Louisville, KY 40292.

The aim of this study was to replace degenerated photoreceptors with embryonic retinal transplants forming parallel retinal layers like a normal retina.

Pieces of embryonic rat (E16-20) retinas were transplanted into retinas of adult normal or light-damaged Long-Evans rats. Donor retinas were labelled before transplantation with bromo-deoxy uridine (BrdU). Retinas, embedded in gelatin or collagen; or unembedded, were cut into pieces (~0.7 mm wide, up to 2 mm length). They were delivered with minimal distortion to the subretinal space by a custom-made instrument with a flat plastic nozzle, using an anterior approach.

Transplants placed with the right polarity, with their photoreceptors towards the host RPE, developed parallel layers with photoreceptor inner and outer segments. Outer segments stained intensely for rhodopsin. However, transplants placed in a reversed orientation, with their photoreceptors facing the host photoreceptors or the inner nuclear layer, did not develop outer segments. BrdU-immunoreactive donor cells could be clearly distinguished from host cells. Transplants to light-damaged retina integrated well with no glial barriers between graft and host.

In conclusion, it is possible to transplant intact pieces of fragile embryonic retina which retain the capability to develop into organized layers. In transplants with parallel layers, differentiating photoreceptors need to be in contact with host RPE to develop outer segments.

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518.5

DISTRIBUTION OF TRANSPLANT-DERIVED RETINAL GANGLION CELLS THAT PROJECT TO THE OLIVARY PRETECTAL NUCLEUS

M.J. Young* and R.D. Lund, Neural Transplant Program, Dept. of Pathology, Institute of Ophthalmology, Bath Street, London, U.K.

The olivary pretectal nucleus (OPN) is known to be the retinorecipient nucleus responsible for the pupillary light reflex in rats. We have recently shown that the retinal projection to the OPN arises largely from the ventral hemiretina, or superior visual field (Young et al., Soc. Neurosci. Abstr., **20**, 771:1994). Here we have examined the pattern of retinal ganglion cells that project to the OPN in animals that have received intracerebral embryonic retinal transplants at birth. It is known that these grafted retinae are capable of eliciting a graft-mediated pupillary light response in the host eye, but the pattern and type of retinal ganglion cells contributing to this response have not yet been examined.

Neonatal Lister-Hooded rats received an embryonic (E13) retina over the region of the superior colliculus. The right eye was removed at the time of transplantation, which favours the innervation of the left OPN by the grafted tissue. Animals were tested at 2-3 months of age for the presence of a graft-mediated pupillary light reflex, and the left OPN of responding animals (as well as that of non-responding control animals) labelled with crystals of Di-Asp. The animals were allowed to survive for 6-7 days, and were then perfused and the host retina whole-mounted, while the injection site and grafted retinae were cut at 100 µm on a vibratome. Both host and grafted retinae were then viewed under fluorescence illumination, and the distribution of labelled retinal ganglion cells determined. It was found that, like the normal two-eyed animals, the labelled cells in the host retinae were largely distributed in the ventral retina. The labelled cells in the grafted retinae, however, showed no obvious preference for location, and were found throughout the grafted retinae. The cell types of retinal ganglion cells projecting to the OPN were found to be similar in both host and transplanted retinae.

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518.6

CO-TRANSPLANTATION OF RETINAL PHOTORECEPTORS AND PIGMENT EPITHELIUM IN RATS. B. Juliusson[1], K. Arnér[1], T. van Veen[2] and B. Ehinger[1]*. [1]Dept. of Ophthalmology, Univ. of Lund, S-22185 Lund, Sweden and [2]Dept. of Zoology, Univ of Göteborg, S-41390 Göteborg, Sweden.

The purpose of this investigation was to combine certain dissection and transplantation techniques in order to be able to transplant a coherent sheet of photoreceptor neurons and retinal pigment epithelium to a subretinal site in rats. Whole eyes from 8-14 days old pigmented rats were incubated in enzyme solution. After enzyme treatment, the retina with adhering pigment epithelium could be dissected. The retina was then embedded in gelatin and the inner retina was removed by vibratome sectioning. From the remaining photoreceptor layer and retinal pigment epithelium, small square pieces (1.5x1.5 mm) were cut out for transplantation. These pieces were inserted in a custom made transplant carrier consisting of a flattened plastic tube with a plastic piston inside. Adult Sprague-Dawley rats were used as recipients. The transplant carrier was inserted through a window in the cornea and advanced past the lens through the zonula fibers and into the subretinal space, were the transplant was delivered. After one week, the transplants were examined by hematoxylin and eosin staining and immuno-cytochemistry on histological sections. The transplanted retinal pigment epithelium could be seen as a layer of cells at an appropriate location. In transplants, in which the neural retina and pigment epithelium remained appositioned, proper layering was achieved and both rods, S-cones and M-cones were found.

This method provides the possibility to transplant a coherent sheet of both photoreceptor cells and retinal pigment epithelium to the subretinal site in rat eyes.

518.7

FETAL SPINAL CORD TRANSPLANTS MEDIATE ANATOMICAL REORGANIZATION AND SUPPORT THE DEVELOPMENT OF TARGET REACHING AND COORDINATED POSTURAL ADJUSTMENTS AFTER CERVICAL SPINAL CORD INJURY IN NEONATAL RATS. P.S. Diener* and B.S. Bregman, Georgetown University School of Medicine, Washington, DC 20007.

After upper cervical spinal cord overhemisection (HX) in the neonatal rat, skilled reaching fails to develop and associated postural adjustments are disrupted. The aim of this study was to determine if, in the presence of a fetal spinal cord transplant (TP), skilled forelimb movements will develop. Three day old rats received cervical spinal cord HX (C3), with (N=13) or without (N=8) a transplant of fetal cervical spinal cord (E14); unoperated pups (N=9) served as controls. All pups were examined daily from birth to one month of age using a behavioral protocol assessing reflexes, postural and forelimb motor skills. They were also trained and tested as adults for 4 weeks to perform goal-directed reaching tasks. Rats in the HX group failed to develop target-reaching and displayed poor postural control during movement. The HX + TP group developed both target-reaching and associated compensatory postural adjustments. Neuroanatomical tracing techniques were used to examine the reorganization of CNS pathways that may influence these movements. Axotomized segmental (DRG), corticospinal and brainstem spinal neurons projected axons throughout the host cord and transplant, but were dramatically reduced in the HX groups' spinal cord tissue. FluoroGold injected into the lumbar cord (L2) retrogradely labeled long propriospinal neurons located throughout the cervical (C1-C4) and brachial cord (C5-T1) in the HX + TP group. Propriospinal neuronal label was diminished in most spinal cord sections throughout C3/C4 in the HX groups, but was preserved in segments C5-T1. Acquisition of target-reaching and accompanying postural adjustments require a precise integration of segmental, intersegmental and supraspinal input to propriospinal and motor neurons over many spinal cord levels. We suggest that the transplants support the re-establishment of a more balanced input onto both propriospinal and motor neurons, permitting the development of skilled forelimb activity after neonatal spinal cord injury. Supported by NIH NINDS NS27054.

518.8

TRANSPLANT-MEDIATED IMPROVEMENT OF LUMBAR REFLEX EXCITABILITY FOLLOWING CHRONIC THORACIC CONTUSION SPINAL CORD INJURY IN THE RAT. Thompson*, F.J., Parmer, R., Mirandi, T.P., Reier, P.J. Depts of Neuroscience and Neurosurgery, University of Florida Brain Institute, Gainesville, Florida 32610.

The aim of this study was to test the capacity of fetal spinal cord (FSC) transplants to improve the functional control of lumbar spinal reflex excitability following chronic contusion spinal injury of the midthoracic spinal cord.. Based on the previously demonstrated robust changes in rate-modulation of the lumbar monosynaptic reflex excitability following midthoracic contusion and their relevance to the development of spasticity, analysis of these reflex measures was selected to evaluate the potential for intraspinal fetal grafts to improve the functional control of reflex excitability. The transplant treatment consisted of an intraspinal injection of E-14 FSC tissue into the cavity of the contusion lesion three months post injury. We analyzed lumbar monosynaptic reflexes in animals six months following midthoracic contusion injuries. The benefit of the treatment was determined by comparing reflex measures in animals which received treatments to those recorded in contused-untreated animals. In addition, reflex analysis was performed in normal animals and time-matched glucose-saline controls. Analysis of the tibial monosynaptic reflex in animals with midthoracic contusion injuries revealed significant alterations in rate-depression throughout the range of test frequencies. The responses observed in the graft survival group (C-180T) were markedly improved over those observed for any other injury group, particularly in the lower range of the test frequencies (i.e. 1-5 Hz). For example, at 1 Hz, a significant difference between the values observed in the transplant group were significantly more normal than observed in the contused only group (ANOVA, $p < 0.05$, post hoc t-test). Anatomical study revealed graft survival in 13 of 35 animals. Surviving grafts included some in which well integrated nearly full thickness restoration of the spinal cord replaced the original lesion cavities. (Sup. by SCRF-1226-01, PVA)

518.9

FETAL SPINAL CORD TRANSPLANTS ENHANCE HINDLIMB BEHAVIORAL FUNCTION IN SPINALLY TRANSECTED ADULT RATS. Y.Itoh,* T.Sugawara, F.Mori, and M.Kowada Department of Neurosurgery, Akita University School of Medicine, Akita City, Akita 010, JAPAN

Spinal cord transection in adult mammals is not only associated with paraplegia but also produces various somatic and autonomic dysfunctions which can cause death within days after lesioning. Intraspinal transplantation of embryonic CNS tissues has been used in an attempt to induce regeneration after spinal cord injury. In this study we placed embryonic spinal cord transplants in spinally transected adult rats and evaluated the extent of locomotor recovery of the hindlimbs. After a T8 laminectomy, adult Sprague-Dawley rats received intraspinal transplants of E14 spinal cord into a complete transection cavity and the dural matter was then sutured with 10-0 nylon stitches. After surgery, manual bladder expression was performed every 12 hours. One month later hindlimb behaviors on the treadmill were videotaped, videocaptured into IBM-PC, and analyzed kinematically. Bladder control became automatic by 7 days in all rats. About 70% of the transplanted rats survived 1 month after surgery. There were neither spasms nor choreoathetoid movements. Although the hindlimbs of the animals generally did not contribute to overground treadmill locomotion, some responses such as hindlimb supporting reactions and bipedal treadmill locomotion were occasionally seen. Embryonic spinal cord transplants therefore enhance recovery of some hindlimb locomotor responses after midthoracic spinal cord transection in adult rats and may provide a milieu that promotes reconstruction of interrupted spinal cord circuits.

518.10

ARCUATE NUCLEUS TRANSPLANTS REDUCES HYPERREACTIVITY IN THE STARTLE RESPONSE IN NEONATALLY MSG-TREATED RATS. F.J. Yang* and M-T. Romero Department of Psychology, State University of New York at Binghamton, Binghamton, NY. 13902

Neonatal treatment with monosodium glutamate (MSG) in rodents produces degeneration of the hypothalamic arcuate nucleus (AN) and other circumventricular areas. Hyperreactivity to environmental stimuli is also observed in these animals. In the present study 18 female rats were treated with a low dosage of MSG (0.4mg/g) or saline (SC) on postnatal days 2,4,6,8,10. On postnatal day 12, 4 of the MSG-treated animals received hypothalamic transplants containing the arcuate nucleus. The other 6 received sham surgery. Saline injected rats were also given AN grafts or sham surgery. At the end of the experiment, brains were processed for immunocytochemistry. Preliminary results indicate that neonatal MSG treatment reduced habituation rate and induced hyperreactivity in the acoustic startle response. AN grafts restored habituation and reduced hyperreactivity in MSG-treated animals to control levels. Immunocytochemical results demonstrate that all grafted tissue was viable and expressed NPY and tyrosine hydroxylase. Given that corticotropin releasing factor(CRF) seems to be involved in the modulation of the startle response, it is possible that AN grafting restructured the CRF input to the pituitary destroyed by MSG. Further studies need to address this hypothesis.

518.11

MORPHOLOGICAL ASSESSMENT OF LONG-TERM FETAL BASAL FOREBRAIN ALLOGRAFTS IN FORNIX TRANSECTED CEBUS MONKEYS. **Y.-T. Liu, E.J. Cochran*, L. Martel, and J.H. Kordower, Center for Brain Repair and Department of Neurological Sciences, Rush Presbyterian Medical Center, Chicago Ill. 60612.**

Grafts of cholinergic basal forebrain neurons survive and reverse memory deficits observed in animal models of Alzheimer's Disease. The present study examined the morphological properties of fetal monkey basal forebrain autografts placed in the hippocampus of Cebus monkeys. Ten young adult Cebus monkeys underwent unlateral aspirative lesions of the fornix using an open microsurgical approach. Ten-fourteen days later, monkeys received implants of solid E25-70 day Cebus monkey basal forebrain into two sites in the hippocampus. The age of the donor was determine via ultrasound and the coordinates for the implant site was determine using MRI guidance. These monkeys were sacrificed between 6 and 18 months post-transplantation. Six additional young adult Cebus monkeys underwent fornix transections but did not receive implants and served as control. Control monkeys displayed comprehensive loss of cholinergic innervation as visualized by acetylcholinesterase (AChE) staining and immunohistochemical staining for ChAT and the low affinity p75NGF receptor. Surviving grafts were found in all monkeys up to 18 months following transplantation except for those monkeys receiving implants from fetuses >55 days in gestation. Grafts from younger fetuses provided extensive cholinergic reinnervation to the hippocampus and complete homotypic reinnervation was achieved in many cases. Interestingly, the expression of all markers of cholinergic basal forebrain neurons (ChAT, AChE, p75NGFr, and galanin) was similar within each case, except for the monkey sacrificed 18 months post-transplantation. In this animal, numerous AChE, p75NGFr and galanin-containing neurons were observed. However, there was a paucity of ChAT-ir cells. These data indicate that cholinergic basal forebrain neurons can survive long-term in the nonhuman primate brain. (Supported by NS25655)

518.12

IMMUNOLOCALIZATION OF METHIONINE-ENKEPHALIN IN THE DEVELOPING TRANSPLANTED OLFACTORY BULB. D.L. Greene[1,2]*, J.N. Kou[1], and L.E. Westrum[1]. Dept. of Neurological Surgery[1], The Univ. of WA, Seattle WA 98195; Dept. of Surgery[2], The Univ. of AZ, Tucson AZ 85724.

The rat olfactory bulb (OB), with its unique architecture, connectivity, and continuously regenerating olfactory nerve (ON), has been extensively studied as a model for neuronal plasticity and transplantation. To further understand the mechanisms of transplantation, we are investigating the localization of neurotransmitters that are expressed by specific cell populations within the OB. Previously it was shown that methionine-enkephalin (Met-ENK) localizes to the periglomerular (PG) cells and fibers, and granule cells (GC) and their dendrites. These cells are all inhibitory interneurons, intrinsic to the OB, making Met-ENK a marker to follow the development and organization of TX OB neurons. Pregnant dams were injected with [^{3}H] thymidine to label the donor OBs. Neonatal rats received a unilateral OB transplant (TX) from donor embryonic rats (15 days gestation) in the site from which the host OB had been previously removed. One, two, and greater than four weeks post-transplantation, rats were anesthetized and perfused with 4% paraformaldehyde. Cryostat sections of the TX OB were incubated with a polyclonal antibody to Met-ENK, and ultimately reacted with DAB for visualization. One week after transplantation (n=3), the TX OBs were not connected with the host forebrain, but multiple PG and GC -like cells, reactive for Met-ENK, were visible. Two weeks after transplantation (n=3), the TX OBs were connected by a small fiber bundle to the host forebrain, and the PG and GC-like cell population, reactive for Met-ENK, increased. In addition, multiple large neurons, possibly excitatory mitral/tufted-like cells, were reactive for Met-ENK. Greater than four weeks after transplantation (n=5) the TX OBs were fully connected with the host forebrain, and the level of Met-ENK reactivity was even greater with multiple PG-like cells and fibers, GC-like cells and dendrites, and mitral/tufted-like cells labeled. These results show that TX OBs contain enkephalinergic inhibitory interneurons independent of connectivity and organization; yet with increasing growth and connectivity Met-ENK levels are upregulated such that excitatory OB neurons exhibit detectable levels in addition to normally reactive inhibitory neurons. Supported by AHA fellowship #92005090 and NIH # NS09678. LEW is an affiliate of the CDMRC.

518.13

A STEREOLOGICAL STUDY OF THE SYNAPTIC ORGANIZATION OF FASCIA DENTATA TRANSPLANTS J. Zimmer* and T. Sørensen, Dept. of Anatomy & Cell Biology, Univ. of Odense, Denmark

The synaptic differentiation of intracerebral, rat fascia dentata transplants was analyzed by the new disector stereology in order to precisely estimate, for different grafts locations, the total number of synapses and subtypes per granule cell and their dendritic distribution. Neonatal dentate tissue were grafted to 3 locations in newborn rats: the subarachnoidal space (isolated grafts), the fascia dentata (homotopic grafts with host brain perforant path innervation) and the neocortex (heterotopic grafts with neocortical innervation). After 100 days the dentate grafts and the fascia dentata from normal controls were processed for EM, and semithin and ultrathin sections cut for light and electron microscopic stereological analysis of granule cell numbers, volume of dentate molecular layer and synapse numbers and types. Other grafts with corresponding locations were used to confirm the host perforant path and neocortical innervation by lesion-induced, electron dense axonal degeneration. All grafts contained the normal types of synapses found in fascia dentata. Expressed per granule cell the homotopic (n=3) and the heterotopic grafts (n=7) had a normal or near normal total number of synapses.The isolated grafts displayed a 35% decrease. A group of homotopic grafts with 50 days survival showed a slight, but not statistically significant hyperinnervation.The 50 day old homotopic grafts, the heterotopic intracortical and the isolated grafts showed immature traits by not displaying the normal increase in total synapse numbers and the normal decrease in shaft synapses from the middle to the outer third of the molecular layer.- Using disector stereology we have found that grafted dentate granule cells can become structurally and numerically almost normally innervated by neocortical afferents, and that even homotopically located dentate grafts undergo long-term synaptic reorganization along the distal dendrites.

518.14

HOMOTOPIC AND HETEROTOPIC E17 CORTICAL TRANSPLANTS PLACED IN NEONATAL RAT ROSTRAL CORTEX MAINTAIN AXONAL PROJECTIONS APPROPRIATE TO THE HOST CORTICAL LOCALE: EVIDENCE FROM ANTEROGRADE TRACING EXPERIMENTS USING BIOTINYLATED DEXTRAN. B. Shi* and B. B. Stanfield, Laboratory of Neurophysiology, NIMH, NIH Animal Center, Poolesville, MD 20837.

Earlier work using retrograde axonal tracers showed that both homotopic and heterotopic (occipital) E17 fetal cortical transplants placed in neonatal rat rostral cortex extend and maintain projections appropriate to the rostral cortex. This led to the notion that regional features of cortical areas are not rigidly specified at E17. Recently, a study using an anterograde tracer reported quite different results; namely that such heterotopic transplants extend projections appropriate to their region of origin and fail to project to rostral cortical targets such as the spinal cord. We have used the anterograde tracer biotinylated dextran to reexamine this issue.

Pieces of rostral or occipital E17 fetal cortex were placed into lesion cavities in the rostral cortex of newborn rats. Biotinylated dextran was iontophoretically injected into the transplants at 5 weeks of age. Only cases with injections confined to the transplants were included in the study. In both transplant types, clusters of labeled neurons are seen within the transplants and labeled axons enter the host brain. The distribution of labeled axons arising from the two types of transplants is similar: Both transplant types project to the sensorimotor thalamus, to the deep layers of the superior colliculus and to the spinal cord; and neither type projects to visual thalamus, to visual cortex or to the superficial layer of the superior colliculus. However, some differences between the projections of the two transplant types are seen. More labeled axons are seen in the spinal cord and fewer in the deep layers of the superior colliculus in cases with homotopic transplants, and the axons in the spinal cord also extend further caudal in these cases. These results confirm the earlier retrograde tracer findings and indicate that while the overall projections of the transplants are similar, there may be subtle and quantitative differences that may reflect an emerging commitment to regional fate at E17. This is consistent with behavioral differences recently found in animals with the two types of transplant.

518.15

PRESERVATION OF *trk* A IMMUNOREACTIVITY IN THE MEDIAL SEPTAL AREA OF FORNIX TRANSECTED RATS BY FETAL HIPPOCAMPAL GRAFTS. R. H. Baisden*, C. C. Seymour and M.L. Woodruff, Dept. of Anatomy & Cell Biology, J. H. Quillen College of Medicine, East Tennessee State Univ., Johnson City, TN 37614

trk A is a specific receptor for nerve growth factor (NGF). It contains a tyrosine kinase functional element and is present in medial septal nucleus/diagonal band (MS/DB) cholinergic neurons. Lesions of the fimbria-fornix prevent transport of NGF from the hippocampus to the MS/DB and produce loss of *trk* A and choline acetyltransferase (ChAT) in these neurons. Intraventricular NGF infusion prevents this loss (Venero et al, *Neuroscience, 59*, 797-815, 1994). Kromer et al. (*Brain Res., 210*, 153-171, 1980) demonstrated that transplants of fetal hippocampus placed into the cavity produced by surgical destruction of the fimbria-fornix will maintian ChAT and acetylcholine esterase (AChE) in medial septal neurons. The purpose of the present experiment was to determine if such transplants could sustain *trk A* receptors in MS/DB neurons in rat with fimbria-fornix lesions and, if so, the time course of the preservation. Twenty five adult male Long-Evans hooded rats were given bilateral aspiration lesions of the fimbria-fornix. Ten of these were killed by an overdose of sodium pentobarbital in pairs 6, 12, 24, 30 and 60 days after the lesion. The other 15 received transplants of E20 hippocampus. Three of these rats were killed each day on the same days as the lesion-only rats. Following perfusion with 4% paraformaldehyde the brains were removed and 40 μm frozen sections from the anterior septum through the anterior half of the hippocampus were retained and stained with thionin or processed using the avidin-biotin immunocytochemically for *trk* A or ChAT or histochemically for AChE. Both of the enzymes and *trk* A were preserved in rats given transplants, but were lost by day 12 in rats with lesions alone. (Supported by NIH Grant ES 04070-08 to MLW and by a Research Development Grant from ETSU to RHB).

518.16

ELECTROPHYSIOLOGICAL CHARACTERISTICS OF EXCITATORY SYNAPTIC CURRENTS OF PATCH-CLAMPED PURKINJE NEURONS GRAFTED ON AN ADULT CEREBELLUM. F. Tempia, M. Bravin and P. Strata*. Dept. of Human Anatomy and Physiology, University of Torino, I-10125, Torino, Italy.

We have recently shown that Purkinje neurons (PNs) from a cerebellar primordium grafted onto the surface of an adult uninjured cerebellar cortex receive functional synapses from the three main PN input fibers. In order to evaluate the extent of maturation of the functional properties of excitatory postsynaptic currents we have investigated their electrophysiological characteristics in more detail. Whole-cell patch-clamp recordings in rat tissue slices were obtained from 20 PNs migrated from the graft into the molecular layer of the host cerebellum. In the presence of a $GABA_A$-receptor antagonist, extracellular stimulation evoked graded and all or none currents typical of parallel and climbing fibers. With a paired stimuli protocol, graded currents showed an enhancement of the second response while all or none currents showed a depression, as described in the normal cerebellum. To investigate the nature of postsynaptic receptors, firstly we applied the AMPA/kainate receptor antagonist CNQX (10 μM): as in normal PNs both types of postsynaptic currents were blocked. Secondly, we studied their current-voltage relationship to establish whether such receptor channels were of the linearly conducting-low Ca^{2+} permeable type or of the inward rectifying-highly Ca^{2+} permeable type expressed by cultured and probably by embryonic PNs. Both parallel and climbing fiber-mediated currents had a linear I-V relation with a reversal potential close to 0 mV, giving further support to their mature nature. In conclusion, all tested electrophysiological properties of migrated PNs were of the normal adult type. In addition, we recorded 17 PNs still located in the graft. They shared all characteristics of migrated PNs except the amplitude of climbing fiber responses, that failed to increase to normal adult values at late stages after grafting.

MEMBRANE COMPOSITION AND CELL-SURFACE MACROMOLECULES I

519.1

COMPARISON OF *IN VITRO* CELL CULTURE OF RAT HIPPOCAMPAL, SPINAL CORD, AND CORTICAL NEURONS ON ARTIFICIAL SURFACES. J.J. Hickman*[1], K.E. Foster[1], D.R. Jung[1], A.E. Schaffner[2], and J.L. Barker[2]

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We compared the development of embryonic rat hippocampal, spinal cord, and cortical neurons on artificial surfaces, *in vitro*, in serum-containing and serum-free media. The artificial surfaces are composed of Self-Assembled Monolayers (SAMs) on glass cover slips attached via silane linkages. SAMs with a variety of functional groups were studied, including -COOH, $-NH_2$, phenyl, -SH, and $-CH_3$. Surface composition, both before and after culture, was determined by X-ray Photoelectron Spectroscopy (XPS). The results will be analyzed using a multivariant analysis package. In some cases, the surface greatly contributed to both initial adhesion and long term survival. Some surface effects were found to vary according to cell type.

519.2

CELL-SURFACE MOLECULES IMPORTANT TO THE MAINTENANCE OF RAT CEREBELLAR PURKINJE CELLS IN CULTURE. V. Dumon and M. Docherty*, Depts. of Physiology and Pharmacology, Royal Free Hospital School of Medicine, London NW3 2PF, UK.

We are particularly interested in a GABAergic cell-surface molecule related to Glutamate Decarboxylase (GAD) which may play an important role in cellular recognition when associated with Immunoglobulin-superfamily molecules (Baekkeskov et al, Nature 347, 151-156, 1990). We wish to investigate the importance of this molecule and its association with Thy-1, and related proteins, using Purkinje cells prepared from the cerebella of 20 day old rats. The conditions required for isolation and maintenance of cells with tertiary dendritic trees have been established, using a total of 110 Sprague-Dawley rats. Sagittal slices (0.5mm thick, 10 per cerebellum) were cut by hand using a razor blade and incubated for 60 min at 37°C in a solution containing 20mM pipes di-potassium, 240mM sucrose, 10mM KCl, 25mM glucose, 6mM $MgCl_2$, 1mM $CaCl_2$, 2 mM pyruvate, gassed with 100% O_2, pH 7 (sucrose buffer). Slices were then exposed to 0.2% pronase E (wt/vol sucrose buffer) for 40 min at 37°C. Extensive washing removed the enzyme before trituration. Cells were grown under a variety of conditions (different substrates, different media) and their survival monitored for more than 20 days. Immunohistochemical analysis of the Purkinje cells present in these cultures has revealed many diversities within this cell population. We have examined these subpopulations in more detail using single-cell RT-PCR. Single identified cells were removed from the culture dish using glass pipettes of internal diameter 40μm. After lysis of the cell, mRNA was extracted onto magnetic beads bearing oligo-dT and subjected to reverse transcription and subsequent PCR using either rTth (Promega) or Tth (Boehringer Mannheim) and a variety of defined primers. PCR products were identified using Southern blotting. This culture system has allowed us to correlate the expression of Thy-1 and various forms of GAD with survival capacity.

519.3

EFFECT OF POTASSIUM CONCENTRATION ON (NA,K)-ATPASE IN NON-NEURONAL CELLS GROWN IN CULTURE. L.H.Farber*, Q.Wang, D.A.Riley and D.J.Miletich. Dept. of Anesthesiology, University of Illinois at Chicago, College of Medicine, Chicago, IL 60612.

While hypokalemia (low blood potassium) is a condition of some importance to clinicians, it holds some curiosities for the basic researcher as well. Among these is the differential effect of low potassium on different organ systems in the body. Of particular interest is the fact that while hypokalemia leads to tissue levels of potassium and the enzyme (Na,K)-ATPase that are decreased in heart and skeletal muscle, these levels remain unchanged in the brain (Azuma *et al.*, Am. J. Physiol. 260:C958-C964, 1991; Albrecht and Miletich, submitted). We have endeavored to further elucidate this matter by investigating individual cell types in culture. Cells in primary cultures were switched to serum-free media containing 1 mM, 3.5 mM or 5.0 mM potassium for 36 hours. Total cellular RNA was isolated from these cells and assayed for (Na,K)-ATPase message by Northern Blot analysis. Extremely low levels of potassium led to an increased expression of the α-subunit of the (Na,K)-ATPase in astrocytes. Moderate decreases in potassium levels, however, did not alter the level of expression. The results will be discussed along with their implications for the differential effects of hypokalemia in intact organisms.

519.4

USE OF CERAMIDE GLYCANASE IN CELL CULTURE: EFFECT ON MYOGENESIS. K.C. Leskawa*, M.A. Niebauer, W.I. Shaikun and L.D. Cambron. Dept. Anat. Sci. & Neurobiol., Univ. Louisville, Louisville, KY 40292

Ceramide glycanase from the leech *(Hirudo medicinalis) has been used to* liberate oligosaccharides from glycosphingolipids (GSLs), including the gangliosides, for carbohydrate structural studies. Here, we report that this enzyme can also be employed in cell culture studies to probe GSL function. Clonal skeletal muscle cells were treated with 2 mU/ml of ceramide glycanase in complete media (containing 10% serum). Myoblast membrane fusion, which normally leads to the formation of multinucleated myotubes, was drastically inhibited. Analysis after 24 hr incubation revealed approximately 70% hydrolysis of gangliosides and 80% hydrolysis of neutral GSLs. When myoblasts were treated with ceramide glycanase for 2 days, and then transferred to control media, the ability to fuse gradually returned, myotube formation lagging 2 days behind control cultures. This suggests that during this recovery period the cells express new GSLs at the cell surface, where they play a role in the membrane fusion event. This is presently being examined directly by using surface saccharide labeling techniques.

When non-glycosylated sphingoids were studied, it was found that treatment of the myoblasts with ceramide glycanase resulted in extremely high increases in ceramide, sphingosine and sphingosine-1-phosphate. All of these compounds are believed to operate as second messengers. While our initial hypothesis is that myoblast surface glycosphingolipids are involved directly in the membrane fusion event (via recognition by surface glycosyltransferases), this new data suggest that GSL-derived second messengers could be involved in a very different signalling event during myogenesis. Supported by NINDS grant NS21057.

519.5

EFFECT OF ACUTE GANGLIOSIDE EXPOSURE ON ^{3}H-DOPAMINE ACCUMULATION & RELEASE BY SYNAPTOSOMES.

M. Medrano, R. Cabeza, D. Moss* and L.N. Irwin. Dept. Biol. Sci., Univ. of Texas at El Paso, TX 79968

Reports in the literature suggest that gangliosides may be excitatory and that they may increase neurotransmitter release. To address the possibility that this altered excitability may affect ^{3}H-dopamine (^{3}H-DA) storage or release, we investigated the effects of acute ganglioside exposure on ^{3}H-DA accumulation and release. Synaptosomes from rat brain striatum and cortex were prepared and exposed to 37 µg/ml of a ganglioside mixture for 0, 30, 60 and 90 min. The accumulation of ^{3}H-DA was assessed after 30 min. of exposure to the neurotransmitter. Synaptosomes were resuspended in a normal Krebs or high potassium Krebs (35mM) solution for 15 min., to assess basal and stimulated release of ^{3}H-DA, respectively. Although our results indicate that there is no significant effect of gangliosides on accumulation, basal release or stimulated release of ^{3}H-DA, an effect on stimulated release by K^+ cannot totally be ruled out because the $[K^+]$ was close to maximal.

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519.6

DIFFERENTIAL MEMBRANE INTERACTIONS OF CORTISOL AND ESTRADIOL: IMPLICATIONS FOR CYTOSOLIC RECEPTOR ACTIVITY.

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Both cortisol and estradiol are lipophilic steroids which interact with the cell plasma membrane, in addition to binding to cytosolic receptors. To explore the interactions of these steroids with phospholipid multilamellar vesicles, we determined their membrane-based partition coefficients and their time-averaged locations within the phospholipid bilayer. In the absence of cholesterol, the (Kp[mem]) values for cortisol and estradiol were 250 and 5,000, respectively. In the presence of a physiological concentration of cholesterol (0.4:1 cholesterol/phospholipid mole ratio), the Kp[mem] values for both cortisol and estradiol were reduced by 50%. The latter is consistent with the finding of Jacobsohn *et al* (Biochim Biophys Acta 1195:131; 1994). In washout experiments, the dissociation of cortisol from the membrane was significantly greater than that of estradiol (> 50% vs. < 5%).

One-dimensional electron density profiles generated by small-angle x-ray diffraction (13Å resolution) indicated that these steroids occupy discrete, time-averaged locations in the lipid bilayer. Estradiol, a nonpolar steroid, was concentrated near the center of the membrane hydrocarbon core, the region with the greatest molecular volume. In contrast, cortisol, a more polar steroid, was concentrated near the phospholipid headgroups, a charged region of high molecular density.

These findings support a model of steroid partitioning to discrete locations in the cell plasma membrane prior to binding to cytosolic receptors. The membrane interactions of these steroids 1) are influenced by membrane lipid composition, particularly cholesterol content, 2) may modulate their ability to bind to and activate their specific intracellular receptors, and 3) may influence the structure-activity characteristics of membrane-bound proteins.

519.7

SHRINKING- AND SWELLING-INDUCED NEURONAL MEMBRANE DYNAMICS MONITORED BY CONFOCAL MICROSCOPY. CE Morris*, Mills LR Loeb Institute, Ottawa Civic Hosp, Ottawa K1Y 4E9* & Playfair Institute, Toronto Western Hosp, Toronto M5T 2S8

Subjected abruptly to shrinking stimuli, neurons internalize membrane in the form of vacuole-like dilations (VLDs) readily apparent by light microscopy. Abrupt swelling stimuli, by contrast, promote externalization of neuronal membrane (see Wan et al 1995 & Reuzeau et al 1995, both J Memb Biol 145: May issue). VLD formation and reversal are insensitive to wide variations in intracellular and extracellular ionic conditions; tension rather than chemical changes attendant on shrinking and swelling may therefore be what triggers and/or drives these membrane dynamics. Although we primarily used snail neurons, VLDs were elicited in cultured day 17 embryonic hippocampal neurons and glia and a variety of other cells. Confocal microscopy on living cells was used to study the provenance of membrane associated with VLDs.

The membrane dye, DiI, applied as an ethanol solution, partitioned almost exclusively into plasma membrane. VLDs, elicited by a shrinking stimulus immediately after DiI application, stained with DiI. When VLDs were made to reverse (via a second swelling stimulus), DiI-stained VLD membrane "everted" to the plasma membrane. In the absence of a reversing stimulus, VLDs could be seen to pinch off and become true vacuoles. These osmomechanically elicited membrane dynamics were observed repeatedly at discrete sites and were restricted to the substrate-adherent surface.

In mouse neurons, as in molluscan neurons, bath-applied rhodamine-dextran entered VLDs as they formed and was expelled during VLD reversal. *Supported by NSERC Canada research grants to CEM and to LRM.*

519.8

HETEROPHILIC NCAM-MEDIATED ADHESION OF TRANSFECTED CELLS TO AGRIN FROM EMBRYONIC CHICK BRAIN. B. A. Murray*, S. D. Storms, A. S. Kim, B.-H. T. Tran and G. J. Cole†. Dept. of Developmental and Cell Biology and Developmental Biology Center, Univ. of California, Irvine, CA 92717-2300, and †Neurobiotechnology Center and Dept. of Cell Biology, Neurobiology and Anatomy, The Ohio State Univ., Columbus, OH 43210.

The neural cell adhesion molecule NCAM mediates adhesion by homophilic and heterophilic mechanisms, and heparan sulfate proteoglycans (HSPGs) have been suggested to be heterophilic NCAM ligands. The acetylcholine receptor clustering protein agrin is a heparan sulfate proteoglycan that has been shown to bind to purified NCAM. In this study, transfected mouse L cells expressing the 140 kilodalton isoform of chicken NCAM adhered to purified agrin adsorbed to a nitrocellulose substratum, and the adhesion was inhibited by a monoclonal antibody specific for chicken NCAM. Digestion experiments with heparinase and heparitinase indicated that the heparan sulfate sidechains of agrin were required for adhesion. Two brain HSPGs, agrin and the antigen recognized by the 6C4 monoclonal antibody, were detected immunologically in adhesive fractions prepared from brain membrane extracts by ion exchange chromatography, and depletion of these HSPGs with specific monoclonal antibodies inhibited the adhesion of the transfected cells to these fractions. In contrast, the HSPG perlecan was present in one of the adhesive fractions, but immune depletion using an anti-perlecan monoclonal antibody did not affect adhesion. Immune depletion and chondroitinase ABC digestion experiments suggested that chondroitin sulfates (presumably on proteoglycans) are not involved directly in adhesion, but they may modulate adhesion to HSPGs. Our experiments support the view that NCAM can mediate the adhesion of cells to agrin and that NCAM is a multivalent adhesive molecule whose function is affected both positively and negatively by interactions with several different types of extracellular matrix and cell surface molecules. (Supported by NSF grant IBN-9220156.)

519.9

THE DROSOPHILA NEURAL CELL ADHESION MOLECULE NEUROGLIAN INDUCES THE POLARIZED ASSEMBLY OF THE ANKYRIN CYTOSKELETON IN S2 CELLS. M. Hortsch[1]*, C. Liu[1], D. Homer[1], G. McVicar[2], S. Dissanayake[2], and R. Dubreuil[2]. [1]Dept. of Anatomy & Cell Biol., Univ. of Michigan, Ann Arbor, MI 48109, [2]Dept. of Pharm. & Physiol. Sci., Univ. of Chicago, Chicago, IL 60637

Sites of cell-cell adhesion are known to be important sites of cytoskeleton interaction with the plasma membrane. Cell adhesion molecules (CAMs) as well as cytoskeletal elements are often segregated into specialized regions of cells, such as the dendritic or axonal processes of neurons. We recently obtained evidence that Drosophila neuroglian, a member of the L1-CAM family, directly interacts with ankyrin and recruits the assembly of an ankyrin-linked membrane skeleton in S2 tissue culture cells. Ankyrin links integral membrane proteins to the actin-spectrin-based membrane skeleton in a range of different cell types and is often concentrated within restricted membrane domains. A direct protein interaction between ankyrin and neuroglian was demonstrated using the yeast two-hybrid system. In Drosophila S2 cells ankyrin is found in the cytoplasm of untransfected cells, but is specifically recruited to sites of cell-cell contact in neuroglian expressing cells. Neuroglian appears to be activated by its extracellular adhesive function so that ankyrin selectively associates with sites of cell-cell contact and not with other regions of the cell surface. These results indicate that a neural CAM can transmit positional information directly to the ankyrin-spectrin matrix. The polarization of the membrane cytoskeleton could induce the recruitment of additional signaling molecules to sites of cell-cell adhesion and might play an important role in the induction of physiological responses to cell adhesion, such as the CAM-dependent outgrowth of neurites.

519.10

IMMUNOCYTOCHEMICAL ANALYSIS OF RECEPTOR PROTEIN TYROSINE PHOSPHATASE-κ EXPRESSION IN THE NEWBORN AND ADULT RAT CNS. G.I.Botchkina, I.Sap, T. Li, Y.-P. Jiang and J.M. Musacchio.* Dep. of Pharmacology, NYU Medical Center, NY10016

RPTP-κ is a recently described receptor-like protein tyrosine phosphatase, the extracellular domain of which mediates homophilic cell-cell adhesion (Sap et al., 1994, Mol. Cel. Biol., 14:1). We have shown previously by in-situ hybridization analysis that the level of expression of RPTP-κ mRNA is higher in the developing than in the adult CNS (Jiang et al., 1993, Mol. Cel. Biol., 13:2942). Antiserum 116 was generated against a synthesized peptide, corresponding to residues 60 to 76, and then was affinity purified. Primary antibodies with peptide preadsorbtion were used as a control.

In newborn rats, the highest level of RPTP-κ immunoreactivity was seen within the cell bodies and their processes in the ventricular and subventricular zone, choroid plexus cells and ependymal cilia. Some intensely stained migrating cells were scattered far from the ventricular zone. Both, newborns and adults, display large number of moderately immunostained cells through the brain and spinal cord gray matter.

In adults, the dot-like IR was abundant throughout the dorsal horns of the spinal cord, all rostro-caudal subdivisions of the trigeminal nucleus, and within the area postrema, gracile and cuneate nuclei. Many neurons in the dorsal root and trigeminal ganglia have moderate granular cytoplasmic immunostaining. Several neuronal populations within the brainstem, hippocampal formation and Purkinje cells in the cerebellum express moderate to low level of RPTP-κ IR.

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519.11

PUTATIVE CD9-ASSOCIATED PROTEINS AND LIGAND/RECEPTOR IN S-16 SCHWANN CELLS AND PERIPHERAL NERVE MEMBRANE EXTRACTS. M. Hadjiargyrou*, Z. Kaprielian, K-O. Cho and P.H. Patterson. Division of Biology, California Institute of Technology, Pasadena, CA 91125

Previous work has shown that CD9 associates with various other membrane proteins in non-neural cells including the integrins GPIIb-IIIa, VLA, and $\alpha_6\beta_1$, small GTP-binding proteins, L1, and the diphtheria toxin receptor. To investigate whether CD9 also associates with other membrane proteins in neural cells, we used the anti-CD9 monoclonal antibody (mAb), B2C11, in immunoprecipitations with 1% CHAPS extracts of membranes from ^{35}S-methionine-labeled S-16 Schwann cells. Proteins of 130 kD and 16 kD specifically co-precipitate with CD9. If S-16 cells are extracted with 1% Brij-96, we find 30 kD and 34 kD proteins, as well as the 130 kD band, co-precipitate with CD9. De-glycosylation experiments indicate that the 130 kD protein resides on the cell surface.

We have previously shown that mAb B2C11 induces adhesion, proliferation and migration of Schwann cells. We speculate that the site recognized by B2C11 on CD9 is a functional epitope and may be a binding site of a natural ligand. To begin a search for such a ligand, we mapped the B2C11 epitope to the large extracellular domain of CD9 using fusion proteins and chimeric antibodies (CD9/IgG). Utilizing western analysis, we find that a chimeric antibody containing both CD9 extracellular domains binds a 70 kD antigen in sciatic nerve membrane extracts.

519.12

A NEURAL CHONDROITIN SULFATE PROTEOGLYCAN THAT IS SIMILAR, BUT NOT IDENTICAL, TO THE CAT-301 ANTIGEN. C.E. Lander*, H.J.L. Fryer and S. Hockfield. Section of Neurobiology, Yale Univ. School of Medicine, New Haven, CT 06510

Monoclonal antibody Cat-301 recognizes a chondroitin sulfate proteoglycan (CSPG) associated with the surface of a subset of neurons in the mammalian CNS. Because not all neurons are labeled by Cat-301, we hypothesized that other, related molecules would be expressed with distributions different from that of Cat-301. To test this hypothesis, we used cesium chloride gradient fractions enriched in CSPGs as an immunogen for the production of new antibodies. One of the antibodies generated with this immunization strategy, Cat-315, recognizes a proteoglycan related, but not identical, to the Cat-301 CSPG.

The Cat-315 antigen co-migrates with the Cat-301 antigen and undergoes a similar shift in mobility following digestion with chondroitinase ABC. Immunodepletion experiments indicate that the antigens recognized by these antibodies represent overlapping, yet distinct, sets of CSPGs. Another difference between the antibodies is that while both antibodies recognize bovine cartilage aggrecan, only Cat-315 recognizes aggrecan purified from rat chondrosarcoma.

Histologically both Cat-315 and Cat-301 bind to the surface of subsets of neurons with a similar lattice-like pattern. The two antibodies label overlapping populations of neurons. In rat, both antibodies stain neurons in the superior colliculus and the deep cerebellar nuclei. However, Cat-301 stains many spinal cord neurons, including motor neurons, while Cat-315 labels few spinal cord neurons. Further, while Cat-301 labels very few neurons in the rat cerebral cortex, a large number of cortical neurons are labeled by Cat-315.

Thus, some neurons that lack Cat-301 on their surfaces express a similar CSPG that is recognized by Cat-315. This suggests that the antigens recognized by Cat-301 and Cat-315 belong to a family of related CSPGs which may serve similar functions for different populations of neurons. Supported by EY06511.

519.13

DEVELOPMENTAL CHARACTERIZATION OF THE CAT-315 CHONDROITIN SULFATE PROTEOGLYCAN. H.J.L. Fryer, C.E. Lander, and S. Hockfield*. Section of Neurobiology, Yale Univ. School of Medicine, New Haven, CT 06510

Monoclonal antibody Cat-315 was produced using an immunization strategy designed to generate antibodies to molecules related to the Cat-301 proteoglycan. Like Cat-301, Cat-315 recognizes a developmentally-regulated, high molecular weight chondroitin sulfate proteoglycan (CSPG) that, in the adult, is associated with the surface of a subset of neurons in the mammalian CNS. Immunohistochemical, biochemical and cell culture experiments indicate that Cat-315 is synthesized early in development and is subsequently glycosylated and transported to the cell surface.

The pericellular Cat-315 staining is first seen at postnatal day 14; however, immunoreactivity is detected as early as embryonic day 15. At the light microscopic level, staining at embryonic and early postnatal ages appears diffuse and/or intracellular. By postnatal day 7, staining is clearly associated with neurons, but this staining is intra- rather than pericellular. In cultures of dissociated E15 rat cortex, Cat-315 stains an intracellular antigen in cells with a neuronal morphology.

On Western blots of E15 rat cortex homogenates Cat-315 recognizes a 550kd band. The apparent molecular weight of the antigen recognized by Cat-315 increases between E15 and adulthood. This increase is due to chondroitin sulfate addition, as chondroitinase ABC digestion of P0 or adult homogenates shifts the size of the immunoreactive band to the molecular weight of the antigen present in E15 cortical homogenates.

Together, these data suggest that the Cat-315 CSPG is synthesized at embryonic ages and that, as development proceeds, the antigen is glycosylated and subsequently transported to neuronal cell surfaces. Supported by EY06511.

519.14

MODULATION OF DSD-1-PROTEOGLYCAN EXPRESSION BY GROWTH FACTORS O. Schnädelbach*, C. Mandl and A. Faissner. Dept. of Neurobiology, University of Heidelberg, D-69120 Heidelberg

DSD-1-Proteoglycan (DSD-1-PG) is a chondroitin sulfate proteoglycan of 1000 kD Mr and comprises a core protein of 350-400 kD Mr which carries the DSD-1-epitope, a specialized glycosaminoglycan structure specifically recognized by monoclonal antibody (mAb) 473HD. DSD-1-PG is produced by immature glial lineages *in vitro* and *in vivo* and, purified from postnatal mouse central nervous system (CNS), promotes neurite outgrowth of embryonic day 18 rat hippocampal neurons, suggesting that it is involved in neuron-glia interactions.

DSD-1-PG displays maximal expression perinatally and is largely downregulated in adult CNS. The CSPG is upregulated in CNS stab wounds and, in addition to its role during development, might thus contribute to CNS plasticity. In order to understand the regulatory basis of DSD-1-PG expression an ELISA based on mAb 473HD was established and used to measure proteoglycan content in cell culture systems. The glial precursor cell line Oli-neu was chosen as model system and cultivated in the presence or absence of various factors. From all growth factors tested so far, TGF-ß1 significantly upregulated DSD-1-PG levels during a 24 hrs assay. TGF-ß1 increased DSD-1-PG released into in the culture medium by 538% and the CSPG contained in detergent extracts of the cell monolayer by 547%. In contrast, the fraction of DSD-1-positive cells remained unchanged, while their total number was reduced when compared to untreated controls.

From these data we conclude that TGF-ß1, a growth factor which is present both in astrocytes and microglia in CNS stab wounds, might be an effector of DSD-1-PG upregulation upon CNS damage.

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519.15

GLIAL CELLS IN THE NORMAL ADULT RAT HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM CONTINUE TO EXPRESS THE EXTRACELLULAR GLYCOPROTEIN, TENASCIN-C. D.T. Theodosis*, K. Pierre, M. A. Cadoret, M. Allard, A. Faissner[1] and D. A. Poulain INSERM U 378 Univ. Bordeaux II, F33076 Bordeaux, France and [1]Neurobiology, Univ. Heidelberg, D-69120 Heidelberg, Germany.

Neurons and glial cells of the hypothalamo-neurohypophysial system (HNS) undergo reversible structural changes in response to stimulation throughout life, a plasticity associated with changes in the number of synapses and the morphology of neurohypophysial axons. The HNS continues to display several 'embryonic' features that may allow such plasticity, including the expression of adhesion molecules associated with neurohistogenesis, such as polysialylated NCAM and the F3 glycoprotein (Theodosis & Poulain 1993 Neuroscience 57,501). We here describe the expression of another glycoprotein linked to neurite outgrowth in the developing and lesioned CNS, the extracellular matrix molecule, tenascin-C (TN-C). Immunocytochemistry with antibodies specific for different parts of TN-C revealed high levels of immunoreactivity throughout the adult rat HNS. Electron microscopy of immunoperoxidase-stained sections showed that the reaction predominated in extracellular spaces and on glial surfaces; in the neurohypophysis, it was also visible on axonal surfaces. In tissues from colchicin-treated rats, the reaction filled the cytoplasm of astrocytes and tanycytes in the hypothalamus and pituicytes in the neurohypophysis, indicating that, as in developing tissues, TN-C is secreted by all glial cells of the HNS. Immunoblots of tissue extracts from different regions of the system displayed immunoreactivities in the molecular weight range of 220/240 kD. No significant differences in levels of TN-C immunoreactivity were detected in relation to age, sex or physiological state, which indicates that the expression of TN-C is a permanent feature of the mammalian HNS throughout life. Whether it is implicated in the activity-dependent structural plasticity of the system remains to be determined.

519.16

AN EXTRACELLULAR MATRIX GLYCOPROTEIN ASSOCIATED WITH NEUROMUSCULAR JUNCTIONS AND PERIPHERAL NERVES Stephanie H. Astrow*, Minh Thuoc T. Nguyen, & Chien-Ping Ko, Dept. of Biol. Sci., Univ. of Southern California, LA, CA 90089-2520.

The extracellular matrix (ECM) at the neuromuscular junction (NMJ) is a repository for molecules involved in synapse formation and maintenance. To identify such molecules, we generated monoclonal antibodies against *Torpedo* ECM. One of these antibodies, 6D7, recognizes the synaptic region of frog NMJs. Immunocytochemical labeling of NMJs with 6D7 is continuous with labeling of preterminal axons. Immuno-electron microscopy indicates that 6D7 labeling is localized to the ECM primarily around the terminal Schwann cells at the NMJ. The ECM surrounding peripheral nerves is also immunopositive for 6D7 with labeling found at both myelinated and nonmyelinated axons. Denervated sartorius muscles have little 6D7 immunolabeling at NMJs two weeks following injury; after reinnervation, the intensity of immunolabeling is comparable to controls. Thus, expression of the 6D7 epitope at the NMJ is innervation-dependent. In the tails of *Xenopus* tadpoles, 6D7 staining colocalizes with the chevron-like arrangement of AChRs at NMJs. On immunoblots of a detergent-soluble extract of frog peripheral nerve, 6D7 recognizes a major band of Mr. 127 kD. Treatment of this extract with N-glycanase results in greater mobility of the major immunoreactive band while treatment with O-glycanase abolishes mAb 6D7 reactivity, suggesting that the 6D7 antigen is a glycoprotein and that the epitope is an O-linked sugar moiety. Taken as a whole, the characteristics of the 6D7 antigen do not correspond to a previously identified synaptic molecule. The notion that the 6D7 antigen may be a novel component of the synaptic ECM is currently being addressed by sequence analysis.

[Supported by NIH grant 17954.]

519.17

Identification and Characterization of the Human Extracellular Matrix Molecule Restrictin, R.L. Ackley, R.A. Reid, R. Madison*, J.J. Hemperly Dept. of Molecular Biology, Becton Dickinson Research Center, P.O. Box 12016, Research Triangle Park, NC 27709 USA.

We have characterized a new protein of the human extracellular matrix that appears to be the homolog of the chicken restrictin and rat janusin matrix proteins. Within the protein coding region, the nucleotide and deduced amino acid sequences of human restrictin are 88% and 93% identical to the chicken restrictin sequence, respectively. As seen in the rat and chicken molecules, human restrictin comprises a number of epidermal growth factor-, fibronectin Type III-, and fibrinogen-like domains. DNA probes for human restrictin detect an approximately 12 kb mRNA in fetal and adult human brain but not in fetal heart, liver, lung or kidney.

Antisera were developed against a fragment of human restrictin (consisting of a portion of the fibronectin Type-III repeats) expressed in bacteria. Using this antisera, we detected 160 kD and 180 kD bands in human brain extracts by Western blotting and demonstrated fiber tract staining in human and rat brain by immunohistochemistry. Surprisingly, we also detected restrictin in MatriGel, a commercial tissue culture growth substratum, and we have found human restrictin, unlike its rat and chicken homologs, to be present in peripheral nerve.

519.18

CHARACTERIZATION OF DYSTROGLYCAN-LAMININ INTERACTION IN PERIPHERAL NERVE. H. Yamada[1], A. Chiba[2], T. Endo[2], I. Kanazawa[3], K. P. Campbell[4], T. Shimizu[1] and K. Matsumura[1,*]. [1]Neurology & Neuroscience, Teikyo University School of Medicine, Tokyo 173, [2]Glycobiology, Tokyo Metropolitan Institute of Gerontology, Tokyo 173, [3]Neurology, Institute of Brain Research, Tokyo University School of Medicine, Tokyo 113, [4]HHMI, Physiology & Biophysics, University of Iowa College of Medicine, Iowa City, IA 52242.

Two of the dystrophin-associated glycoproteins, α- and β-dystroglycan (αDG and βDG, respectively), are encoded by a single gene and cleaved into two proteins by posttranslational processing. The peripheral nerve isoform of αDG with a molecular mass of 120 kDa binds laminin-2, the endoneurial laminin isoform comprised of the α2, β1 and γ1 chains. In congenital muscular dystrophy and *dy* mice deficient in laminin α2 chain, peripheral myelination is disturbed, suggesting a role for the dystroglycan-laminin interaction in peripheral myelinogenesis. To begin to test this hypothesis, we here characterized the DG-laminin interaction in peripheral nerve. αDG and βDG were co-localized with laminin-2 surrounding the outermost layer of myelin sheath of peripheral, but not central, nerve fibers, while Dp116 and utrophin were localized in the Schwann cell cytoplasm. αDG was extracted from the peripheral nerve membranes at pH 11, while βDG was not extracted at pH 11 or 12. βDG, which did not bind laminin in blot overlay, co-purified with αDG by wheat germ agglutinin and laminin affinity chromatographies. Our results indicate that αDG is an extracellular peripheral membrane glycoprotein which links βDG in the outer membrane of Schwann cell/myelin sheath with laminin-2 in the endoneurial basal lamina. We also present data which implicate glycosylation of αDG in the interaction with laminin. Based on these results, we propose a novel model of the molecular organization of the DG-laminin interaction in peripheral nerve.

519.19

LOCALIZATION OF α_5 INTEGRIN-LIKE IMMUNOFLUORESCENCE IN THE ADULT RAT HIPPOCAMPUS. L.S.Jones* and S.Grooms, Dept. of Dev. Biol. & Anat., Univ. So. Carolina, Sch. of Med., Cola., SC 29208.

Integrins are a superfamily of adhesion molecules that link the cytoskeleton with the extracellular matrix (ECM) and/or adjacent cells. These transmembrane proteins are heterodimeric, consisting of noncovalently linked α and β subunits. Because specific integrin subunits may be involved in synaptic remodeling that occurs during various forms of neuronal plasticity, we have used RT-PCR to verify the presence α_5-integrin mRNA in rat whole-brain extracts as well as in the hippocampus. We have further demonstrated α_5 integrin-like immunoreactivity in the adult rat brain using confocal microscopy. 250 micron vibratome sections were immunolabeled with a primary, anti-rat α_5 antibody and a secondary FITC conjugated, goat anti-rabbit antibody. Dense staining was noted on choroidal and ependymal cells where α_5-integrin may be localized along the basal lamina. The hippocampal pyramidal cell layer and the dentate granule cell layer were also highly immunopositive using this polyclonal α_5-integrin antibody. In fact, a pronounced band of intense α_5 integrin-like immunoreactivity was observed at the hilar surface of the granule cell layer. The localization of the α_5 antibody in the apical dendrites of CA1 pyramidal neurons created a striated pattern in stratum radiatum. A sharp band of positive immunoreactivity was also noted in stratum lacunosum moleculare. The pattern of α_5 integrin-like immunoreactivity in regions of high synaptic density suggest that integrins may contribute to the organization of dendritic and synaptic morphology.

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520.1

MOLECULAR CLONING AND CHROMOSOMAL ASSIGNMENT OF HUMAN BRAIN-TYPE PHOSPHODIESTERASE I/NUCLEOTIDE PYROPHOSPHATASE (PD-Iα) GENE. Hiroyuki Kawagoe, Osamu Soma, Junko Goji, Joji Inazawa,§ Hajime Nakamura, and Kimihiko Sano*. Department of Pediatrics, Kobe University School of Medicine, Kobe 650 Japan, and §Department of Hygiene, Kyoto Prefectural Medical College, Kyoto 602 Japan

Phosphodiesterase I/nucleotide pyrophosphatase is a widely expressed ectoenzyme. This enzyme cleaves phosphosulfate, pyrophosphate, and phosphodiester bond. Although phosphodiesterase I/nucleotide pyrophosphatase activity was found in most of human organs and body fluids including blood, cerebrospinal fluid, bile, and seminal fluid, the physiological function of this enzyme has not been known. In a previous study, we have cloned brain-type cDNA for this enzyme from rat brain and designated PD-Iα. In situ hybridization analysis demonstrated that PD-Iα mRNA is predominantly localized in choroid plexus epithelial cells, pigment epithelial cells of the eye, and the cells in granular layer of the cerebellum. In this study we have isolated cDNA and genomic DNA encoding human PD-Iα. Human PD-Iα cDNA has a 2,589-nucleotide open reading frame encoding a polypeptide of 863 amino acids with a calculated M_r of 99,034. Northern blot analysis revealed that human PD-Iα transcript was present in brain, kidney, and placenta. The data-base analysis showed that human PD-Iα was identical with human Autotaxin (ATX), a novel tumor motility-stimulating factor, except that human PD-Iα lacks 156 nucleotides and 52 amino acids of human ATX. Human PD-Iα and human ATX are likely to be the alternative splicing products from the same gene. The 5' region of human PD-Iα gene contains four putative binding sites of transcription factor Sp1 without typical TATA or CAAT box and there is a potential octamer binding motif in intron 2. From the result of fluorescence in situ hybridization, human PD-Iα gene is located at chromosome 8q24.1. These results give a new insight into a physiological function of phosphodiesterase I/ nucleotide pyrophosphatase.

520.2

SURFACE PROTEIN PHOSPHORYLATION BY ECTO-PROTEIN KINASE C: ROLE IN CELLULAR HOMEOSTASIS. E. Kornecki*, A. Babinska, H-A. Yang and Y.H. Ehrlich. Department of Cell Biology and Anatomy, SUNY HSC at Brooklyn, NY 11203, and CSI/IBR Center for Developmental-Neuroscience, CUNY at The College of Staten Island, NY 10314.

Neurons and platelets release ATP that phosphorylates specific proteins on the cell surface. We have reported recently that this activity is catalyzed by several types of ecto-protein kinases (PKs), including enzymes with the catalytic specificity of novel and atypical isozymes of protein kinase C (Hogan et al., J. Neurochem. *in-press*). Ecto-PKC with a low Km for ATP (<1μM) was found to operate on the surface of nonstimulated cells, where it may play a role in cellular homeostasis. We tested this hypothesis in experiments conducted with human platelets. Normal hemostasis maintains platelets in a discoid shape and prevents them from aggregating spontaneously in the circulation. In the present study, we used a specific anti-PKC monoclonal antibody termed M.Ab.1.9. M.Ab.1.9 recognizes the catalytic domain of PKC and inhibits PKC activity without affecting other known protein kinases. Preincubation of intact human platelet suspensions with M.Ab.1.9 inhibited ecto-PKC activity. Concurrently, M.Ab.1.9 induced platelet aggregation and secretion. This platelet activation by M.Ab.1.9 was direct and did not require the addition of any known platelet agonists. We propose that constitutive ecto-PKC produces a steady-state of phosphorylation of surface proteins that plays an important role in the maintainance of homeostatic balance. Initial studies with primary neurons cultured from embryonic brain suggest that a similar mechanism plays a role in neuronal survival.

520.3

POLYPEPTIDES LOCATED IN THE PLASMA MEMBRANES OF SYNAPTIC JUNCTIONS AND PUNCTA ADHAERENS JUNCTIONS IN THE RAT BRAIN MAY BE ECTO-KINASES. R. S. Lasher* and P.F. Erickson†. Depts. of C & S Biology and Medicine†, Univ. Colorado Med. Sch., Denver, CO 80262.

Previous work, using the monoclonal antibody (MAb) F4 and rabbit monospecific antibodies to urea-soluble polypeptides from synaptic junctions, indicated that at least four of these polypeptides are associated almost exclusively with the plasma membrane (PM) of the synaptic junction (SJ) and puncta adhaerens (PA) junctions, and that they share a common extracellularly-oriented epitope [R.S. Lasher and P.F. Erickson. 1993. *Neurosci. Absts.* 19:701]. Characterization of these polypeptides has been expanded further by probing Western blots of 1 and 2-dimensional (D) gels of urea-soluble SJs with another MAb, 40-47:11. This MAb recognizes some portion of the peptide GTPEYLAPE, a conserved sequence in the catalytic domain of a number of protein kinases [C.H. Hagedorn, et al. 1990. *FEBS Letts.* 264:59]. In addition to this peptide, 40-47:11 reacts strongly with the catalytic subunit of cAMP-dependent protein kinase (PK) and with phosphorylase kinase, but only weakly with Ca+/calmodulin-dependent PK II. Probing 1- and 2-D blots with 40-47:11 indicates that it reacts with a large number of polypeptides, including those seen to react with MAb F4 and the monospecific rabbit antibodies. Electron microscopy of Vibratome sections of rat cerebellum (ABC method), as well as unfixed and fixed synaptosomes (ABC method and immunogold), indicates that 40-47:11 recognizes an extracellularly-oriented epitope associated with PMs of SJs and PA junctions. In addition, in prefixed tissue, immunoreactivity is associated with the presynaptic dense projections, synaptic vesicles and the postsynaptic density. Both ABC and immunogold methods indicate that the epitope is also associated with filaments which bridge the synaptic cleft. These data are consistent both with the observation that ecto-kinase activity may play a role in synapse formation [Y. Kuroda et al., 1992. *Neurosci. Letts.* 135:255], and with the role of phosphorylation/dephosphorylation in the maintenance of adhaerens junctions [T. Volberg et al., 1992. *EMBO J.* 11:1733]. (Supported by NSF IBN-9320823).

520.4

SYNVESICLIN: A NOVEL 27 KD PROTEIN THAT IS A COMMON COMPONENT OF SMALL CLEAR AND LARGE DENSE-CORE REGULATED SECRETORY VESICLES. C.-J. Jeng and E. S. Schweitzer*. Department of Neurobiology, UCLA School of Medicine, Los Angeles, CA 90025-1763.

As part of a search to identify new components of the secretory apparatus in neuronal cells, we have isolated a monoclonal antibody that recognizes a 27 kD vesicle-specific protein in PC12 cells. This 27 kD protein is an integral membrane protein that is found in both small clear synaptic-like vesicles and large dense-core catecholaminergic vesicles. All of the 27 kD protein can be immunoprecipitated by antibodies against another vesicle protein, SV2. Immunohistochemical analysis and Western blot data indicate that the 27 kD protein is present in adrenal medullary cells but not in adrenal cortical cells, and in gray matter of the cerebral cortex but not in white matter or cerebellum. It is also present in endocrine, but not exocrine, cells in the pancreas. These data suggest that this 27 kD protein, which we have named synvesiclin, is specifically expressed in neural and endocrine cells, where it is localized to regulated secretory vesicles.

We have used the monoclonal antibody against synvesiclin to screen a λgt11 expression library of PC12 cDNA, and have isolated an insert containing a partial coding sequence for a novel protein. A probe made from the insert hybridizes to both a 2.4 kb and a 3.9 kb transcript in PC12 cells RNA, but only to the 2.4 kb transcript in RNA from rat brain cortex. Using this same insert, we have re-screened the cDNA library to obtain the full-length cDNA. We are now sequencing these inserts to determine the complete primary sequence of synvesiclin.

520.5

NT75 IS A NERVE TERMINAL PROTEIN COMPLEX. T.C. Ritchie* and J.D. Coulter. Dept. of Anat. & Neuroscience Program, University of Iowa, Iowa City, IA 52242.

NT75, the S-7B8 antigen, is a neuron-specific 75 kDa membrane protein concentrated in nerve terminals in the superficial spinal dorsal horn and selected synaptic regions in brain. Although virtually all neurons are capable of expressing NT75, the protein is maintained at high levels only in certain populations of neurons. NT75 expression is differentially regulated in distinct neural systems in normal adults and is modulated in a subset of spinal neurons in response to adjuvant-induced hyperalgesia. During development, expression of NT75 is tightly correlated with synaptogenesis and the onset of synaptic transmission. NT75 was purified from rat brain membranes with serial ion exchange and immunoaffinity chromatography and preparative SDS-PAGE. When highly purified NT75 is subjected to reducing SDS-PAGE, the protein resolves primarily to two bands of Mr 29 (p29) and 17 kDa (p17). Densitometry shows NT75 to be composed of p29 and p17 in a molar ratio of 2:1. Monoclonal and polyclonal antibodies to purified p29 stain NT75, p29 and two intermediate bands on Western blots. Tissue staining of rat CNS with p29 antibodies is comparable to that exhibited by the S-7B8 antibody, which is more highly reactive to the NT75 complex. Biochemical studies show purified p29 has a tendency to form disulfide linked homopolymers. Partial sequence obtained to date indicates p29 is unique. (NS23783)

520.6

IMMUNOREACTIVITY FOR PLASMA MEMBRANE CALCIUM ATPASE IN EDINGER WESTPHAL SYNAPTIC TERMINALS. J.T. Fujii* & F.T. Su. Dept. of Anatomy & Cell Biology, Wayne State U. Sch. of Med., Detroit, MI 48201

The plasma membrane calcium ATPase pump (PMCA) is a high affinity calcium pump that is typically associated with the plasma membrane. Using the monoclonal antibody 5F10 (Affinity Bioreagents), we have examined PMCA expression in the neurons of the chick Edinger Westphal (EW) nucleus. Immunoreactivity for PMCA is undetectable in cell bodies located in the EW nucleus. However, PMCA immunoreactivity is clearly visible in EW synaptic terminals located in the ciliary ganglion. PMCA immunoreactivity resembles immunoreactivity for the synaptic vesicle antigen SV-2 and co-localizes with immunoreactivity for substance P and enkephalin. While PMCA immunoreactivity is most striking in EW calyciform terminals, it is also present in boutons.

The presence of PMCA immunoreactivity in EW synaptic terminals was confirmed using transmission electron microscopy. Ultrathin sections were cut of tissue stained as frozen sections using a biotinylated secondary antibody, HRP avidin complex, DAB, and silver intensification. Surprisingly, reaction product was primarily associated with clusters of synaptic vesicles rather than plasma membrane. Synaptic vesicles isolated from Torpedo electric organ also stain positive for PMCA immunoreactivity in immunodot assays. Control experiments show that Torpedo vesicles are immunoreactive for SV-2 and negative for SERCA (sarcoplasmic/endoplasmic reticulum calcium ATPase).

Supported by NIH RO1 EY09768.

520.7

ISOLATION OF A NOVEL cDNA CLONE, mc49, FROM TORPEDO ELECTRIC ORGAN USING AN IMMUNOFLUORESCENCE-BASED EXPRESSION CLONING METHOD. M.A. Nastuk*[1], S. Davis[2], G. D. Yancopoulos[2] and J.R. Fallon[1]. [1]Worcester Foundation for Experimental Biology, Shrewsbury MA 01545 and [2]Regeneron Pharmaceuticals, Inc., Tarrytown NY 10591.

We have identified a unique cDNA clone from a Torpedo electric organ library using modified expression cloning methodology. The procedure that we developed utilizes fluorescence immunocytochemistry to stain monolayers of transfected COS cells in an antibody- or ligand-based screening strategy. Positive COS transfectants are identified by immunofluorescent visualization of expressed epitope or ligand binding site. Positively staining cells are harvested with micropipettes; plasmid DNA is extracted, amplified, and re-transfected into fresh COS. Clonal cDNA can be obtained after two amplification rounds. Thus, this procedure is rapid as well as affording the opportunity to observe the subcellular distribution of the products of transfected genes expressed by single cells.

We applied the antibody-based version of our expression cloning method in our ongoing effort to characterize novel molecules involved in synaptic differentiation. We screened a cDNA library derived from Torpedo electric organ, a tissue rich in cholinergic synapses. Our probe was an antibody that recognizes a carbohydrate-containing epitope located on the candidate agrin receptor dystroglycan, as well as on several other synaptic proteins (Bowe et al., Neuron 12, 1994). We isolated and sequenced a cDNA clone encoding a polypeptide with a predicted molecular weight of approximately 49kD. The protein contains a putative signal sequence, a cysteine-enriched central region, and no apparent transmembrane domains. Database searches show that mc49 has limited homology to certain sulfotransferases. Northern blotting of various Torpedo tissues reveals that mRNA encoding mc49 is highly enriched in electric organ. Further characterization of the mc49 gene product is underway. Supported in part by NIH HD23924.

520.8

ISOLATION OF A TRANSMEMBRANE PROTEIN COMPLEX FROM THE CHOLINERGIC NERVE TERMINALS OF THE ELECTRIC ORGAN OF *N. BRASILIENSIS*. W.J. Sunderland and Steve Carlson*. Dept. of Physiology and Biophysics, University of Washington, Seattle, Wa. 98195.

The neuromuscular junction is a highly specialized cell-cell interface. The nerve terminal shows a polarized structure with synaptic vesicles collecting at presynaptic sites directly opposite of post-synaptic clusters of nicotinic acetylcholine receptor (NAChR). The intervening synaptic basement membrane contains unique components such as acetylcholine esterase, agrin and S-laminin. It has been hypothesized that proteins in the presynaptic membrane may interact with the synaptic basement membrane to establish the nerve terminal's polarity. We have adapted a method from Miljanich (1982), for the immunopurification of nerve terminals from the electric organ of *N. Brasiliensis*. We begin with the standard synaptosomal preparation which is far from pure as indicated by the presence of NAChR, an indicator of post-synaptic contamination, and laminin and indicator of extracellular matrix. We further purify nerve terminals by immunoprecipitating with an antibody directed to an epitope of the SV2 proteoglycan that appears on the nerve terminal surface. These immunopurified membranes show greatly reduced levels of NAChR, but still contain laminin. In addition it is possible to immunoprecipitate nerve terminals with an anti-laminin antibody. We conclude that laminin is bound to the surface of our immunopurified nerve terminals. The form of laminin found on the nerve terminal stains with both an anti-S-laminin anti-sera (GP-1) and with an anti-HNK-1 antibody. If we solubilize immunopurifed nerve terminals with TX-100, and immunoprecipitate with an anti-laminin antibody, we bring down the cytoskeletal protein spectrin. We conclude that our techniques have allowed us to isolate a transmembrane protein complex linking together the cytoskeleton and extracellular matrix at the site of the nerve terminal. Total staining of this protein complex reveals more than 30 proteins. We are currently looking for the transmembrane protein(s) involved in this linkage.

520.9

DISTRIBUTION OF CYTOSKELETAL PROTEINS TALIN, VINCULIN AND α-ACTININ IN NORMAL AND DENERVATED RANA CUTANEOUS PECTORIS MUSCLE. R.E. LINDSEY and D. C. LINDEN.* Department of Biology, Occidental College, Los Angeles, CA 90041.

The distribution of cytoskeletal proteins talin, vinculin and α-actinin was studied in normal and denervated Rana pipiens cutaneous pectoris muscle using monoclonal antibodies and fluorescently labeled secondary antibodies. These proteins co-localize with clustered acetylcholine receptors (AChRs) at the neuromuscular junction (nmj) and myotendinous areas. Our goal was to study the role of these molecules in the formation of AChR clusters and organization of the synaptic extracellular matrix (possibly via integrins).

Our results at innervated nmjs show localization of talin, vinculin and α-actinin within rhodamine-α-bungarotoxin-visualized AChR-enriched areas. In denervated muscles, there is localized staining of fluorescent rhodamine-α-bungarotoxin and antibodies against talin, vinculin and α-actinin at regions of former nmjs. The extent of co-localization at perijunctional and extrajunctional areas in denervated muscle is being determined. We speculate that the muscle itself directs these cytoskeletal molecules to synaptic or extrajunctional cluster areas. The motor neuron is then responsible for maintaining synaptic structure. This work was supported by grants from the NSF (BIR9300299) and the California Foundation for Biochemical Research.

520.10

MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF SAP97 FROM RAT BRAIN AND BACTERIA EXPRESSED RECOMBINANTS S. Kuhlendahl*, W.J. Chung, S. Reuver, C.C. Garner. Neurobiology Research Center/Department of Cell Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294.

The synapse associated protein SAP97 is a member of the MAGUK family of membrane associated guanylate kinase homologs that is recruited to synaptic junctions and other sites of cell-cell contact. SAP97 exists in both a cytosolic and membrane bound form. In the latter it is highly enriched in synaptic junctional preparations. We have isolated cDNA clones encoding six different alternatively spliced isoforms of SAP97. The carboxy-terminal region of all isoforms shows high homology to soluble low molecular weight (Lwt) guanylate kinases (GK). Lwt GKs convert GMP to GDP, and are required for the synthesis of RNA and DNA, and indirectly for assembly of microtubules, protein synthesis, vesicular trafficking, *ras* mediated signal transduction pathways, and utilization of cGMP as a second messenger. Finding SAP97 at the plasma membrane suggests that membrane bound GKs may perform some novel function. To address the question whether SAP97 encodes an active GK, nucleotide binding studies and in vitro GK assays were performed on both recombinant bacteria expressed variants as well as on SAP97 expressed in rat brain. The *E. coli* expressed SAP97 failed to show GK activity. In contrast GK activity is found in both the soluble and membrane fractions of synaptosomes. Finding GK activity in the synaptic junctional membrane fractions, where SAP97 is highly enriched, and where Lwt GKs are absent, suggests that SAP97 may be an active membrane bound GK.

520.11

DIVALENT CATION EFFECT ON INCORPORATION OF EXOGENOUS GANGLIOSIDES INTO RAT BRAIN SYNAPTOSOMES.

M. Ditmore, R. Cabeza and L.N. Irwin*. Department of Biological Sciences, University of Texas at El Paso, TX 79968.

Exogenous gangliosides are incorporated into brain slices *in vitro* to varying degrees as a function of the perfusing solution (Irwin and Cabeza, *Soc. Neurosci. Abstr.* 20: 708). When slices are incubated with low calcium, the amount of [^{3}H]-gangliosides incorporated into a stable membrane compartment is changed. To further elucidate the influence of divalent cations on the distribution of incorporation we incubated synaptosomes for 30 min with [^{3}H]-gangliosides, then exposed them for 1 h to varying calcium/magnesium ratios (0.1 mM [Ca^{++}]/3.6 mM [Mg^{++}] to 2.5 mM [Ca^{++}]/1.2 mM [Mg^{++}]). After rinsing with 10% fetal calf serum and 0.1% trypsin, synaptosomes were extracted with chloroform/methanol (2:1). Gangliosides were isolated from this lipid fraction by silicic acid chromatography and resolved by TLC into the five major mammalian brain gangliosides (M1, D1a, D1b, T1b, Q1b). Individual bands were scraped and counted by liquid scintillation. Incorporation of exogenous gangliosides was 32% less at 1.3 mM [Ca^{++}]/2.4 mM [Mg^{++}] than under normal conditions (2.5 mM [Ca^{++}]/1.2 mM [Mg^{++}]). This decrease was reversed at both higher and lower calcium/magnesium ratios, suggesting the possibility of distinct mechanisms -- one attributable to calcium, the other to magnesium. Different calcium/magnesium ratios did not differentially affect the membrane incorporation of individual gangliosides, suggesting that these mechanisms operate on all gangliosides equally. *Supported by RCMI Grant G12-RR-08124 from the National Center for Research Resources, NIH; and by the J. Edward and Helen M. C. Stern Endowment*

521.1

REGULATION OF THE HUMAN 5-HT2A RECEPTOR PROMOTER ACTIVITY BY cAMP, TPA AND DIALYZED SERUM. Q.S. Zhu* and J.C. Shih. Dept. of Mol. Pharm. and Tox., Sch. of Pharmacy, Univ. of Southern California, 1985 Zonal Ave., Los Angeles, CA 90033.

For the human 5-HT2A receptor gene, we have identified a 0.74 kb HaeIII/PvuII promoter fragment which consisted of two sub-fragments: the 0.35 kb HaeIII/BamHI fragment, and the 0.39 kb BamHI/PvuII fragment. Both fragments exhibited promoter activity in a transient transfection assay and may be two alternative promoters for the gene. The 0.39 kb fragment contained a CRE-like sequence 5' TGAAGTCA 3'. However, 8-Br-cAMP, a PKA activator, did not have significant effect on its promoter activity (124%). TPA, an activator of PKC, increased the promoter activity of the 0.39 kb fragment slightly (160%). Since no AP-1 binding sequence has been found in this fragment, the increase may be indirect. When the HeLa cells were grown with dialyzed fetal bovine serum instead of control serum, the promoter activity was significantly increased in both the 0.35 kb (246%) and the 0.39 kb (797%) fragments, consistent with radioligand binding data. When 5 μM of 5-HT was added to the dialyzed serum, the promoter activity was not reduced to that of the control, suggesting that this increase was not due to the loss of 5-HT in the dialyzed serum. Gel retardation assay showed that binding of a nuclear protein to the 0.35 kb fragment was decreased. The significance of this DNA-binding protein will be discussed. (Supported by NIMH grants R37 MH39085 (MERIT Award), K05 MH00796 (Research Scientist Award), R01 MH37020 and Welin Professorship).

521.2

REGULATION OF CALRETININ GENE EXPRESSION IN PRIMARY CULTURES OF CEREBELLAR NEURONS

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Calretinin (CR), an EF-hand calcium binding protein (homologous to calbindin D28k ~58%), is predominantly localized in discrete sets of brain neurons. Aside from binding calcium, no function has been found for this protein. CR appears early in embryonic rat, mouse, and chick cerebellum. Cerebellar granule cells (CGC), unipolar brush cells (UBC), Golgi and Lugaro (but not Purkinje) cells demonstrate intense CR staining. At postnatal day 8, rat CGC can be removed and maintained *in vitro*. In CGC cultures, CR is expressed in large (UBC or Lugaro) cells which constitute only a small proportion of the neurons in the preparation. From day 1 *in vitro* (DIV0-1), the quantity of CR mRNA rises and peaks at ~200% the initial level at DIV2-3. During DIV3-6 CR mRNA decreases to ~18% of the peak level and remains at this low level. We are using mouse CGC cultures to investigate the transcriptional regulation of the CR gene through analysis of its putative promoter region. Gel mobility shift assays will identify specific binding domains for factors in nuclear extracts, and transfected luciferase reporter vectors will detect potential neuron-specific regulatory domains for the mouse CR gene in CGC and other cultured cells.

521.3

MODULATION OF EXPRESSION OF THE RAT BRAIN CREATINE KINASE (BCK) GENE. G.R. Molloy[1], C.D. Wilson[2] and E.V. Kuzhikandathil[3] University of Delaware, Newark, DE[1]; Zeneca Pharmaceuticals Inc., Wilmington, DE[2] and University of North Carolina, Chapel Hill, N.C.[3]

Creatine kinases (CK) catalyze the reversible transfer of a high energy phosphate group between creatine phosphate and ADP to regenerate ATP in cell types which expend ATP rapidly. In *primary* rat brain cell cultures BCK mRNA levels are high in astrocytes and oligodendrocytes and low in neurons (J. Neurochem. ***59***: 1925-1932 [1992]). Here we asked if this pattern of BCK expression was characteristic of some established neuronal and glial cell lines and whether BCK expression could be modulated. BCK mRNA and protein were high in both the RT4 peripheral neurotumor *stem cell* RT4-AC36 and its glial cell derivative RT4-D6 but, conversely, were 10-fold lower in the neuronal derivative RT4-E5 and undetectable in the neuronal RT4-B8. Cell confluence increased BCK enzyme activity in RT4-D6 by 7-fold and in RT4-E5 by 4-fold; BCK protein and mRNA were also increased at high cell density. These increases were not solely due to an elevation in cAMP since forskolin and IBMX had no significant effect. Similarly, BCK mRNA was found to be extremely low in mouse neuroblastomas NS20Y and N1E-115 but 10-fold higher in NG108-15, a hybrid cell composed of a C1300 neuroblastoma and a rat C6 glioma. These results indicate that BCK expression is highest in cells displaying glial properties and may suggest BCK is expressed early during the differentiation of glial progenitor cells.

In addition, we show BCK mRNA is dramatically induced (>10-fold) by butyric acid (BA) in NIH 3T3 and Balb/c 3T3 fibroblasts. Induction is due to increased BCK transcription and not altered BCK mRNA stability. Induction is rapid (maximal in 6 hrs), does not require de novo protein synthesis and is specific since it does not occur with GABA, AABA or γ-OH-BA. This may aid the identification of the nuclear proteins responsible for repression of BCK.

521.4

REGION SPECIFIC EFFECTS OF HALOPERIODL AND CLOZAPINE ON NEUROTENSIN AP-1 BINDING PROTEINS IN THE RAT BRAIN M.R. Adams* and D.M. Dorsa Depts. of Pharmacology, and Psychiatry, Univ. of WA, Seattle WA 98195 and GRECC, VAMC, Seattle WA 98108.

Our labarotory has previously demonstrated that administration of typical antipsychotic drugs (APD) lead to a dramatic increase in the transcription of the neurotensin/neuromedin N (NT/N) gene in neurons of the dorsolateral striatum (DLST) and shell sector of the nucleus accumbens (sNA). On the other hand atypical neuroleptics have little effect on NT/N mRNA levels in the DLST, but nonetheless cause a significant increase in NT/N mRNA in the sNA. To further understand these region specific effects, in the present studies we have attempted to determine which nuclear transcription factors interact with the neurotensin promoter in the DLST and sNA under both basal and stimulated conditions. The upstream promoter of the the NT promoter contains several synergistically interactive response elements, including an AP-1 element. Using gel shift assays, we have determined that both DLST and sNA nuclear extracts from animals given saline injections contained little if any NT AP-1 binding activity. However, following haloperidol administration there is a significant induction of NT AP-1 binding activity in both the DLST and NA. Clozapine treatment only slightly induces protein binding to the NT AP-1 sequence in the DLST but induces a similar amount of protein binding to ththis element in the sNA as haloperidol. Thus, haloperidol and clozapine sharethe ability to influence AP-1 transcription factor expression in the sNA but not in the DLST. Since previous studies have suggested that the protein induced in the sNA is not c-fos but another fos related antigen, further studies using specific antisera will be required to identify the factors involved and whether the factors differ with acute and chronic APD treatment. Supported by the VA, NS20311 and MCBTG, T32GM07270.

521.5

GLUTAMATE RECEPTOR-INDUCED CREB PHOSPHORYLATION AND IMMEDIATE EARLY GENE EXPRESSION IN GLIAL CELLS OCCUR THROUGH PROTEIN KINASE C ACTIVATION. M. Pende* and V. Gallo. Lab. Cell. Mol. Neurophysiol., NICHD, NIH, Bethesda, MD 20892.

The intracellular events leading to gene expression after non-NMDA receptor stimulation were analyzed in primary oligodendrocyte progenitor cells (O-2A). Down regulation of PKC by phorbol esters (TPA 10 nM for 24 h) and pretreatment with bisindolylmaleimide (0.5-5 μM) blocked the increase of *NGFI-A* and *c-fos* mRNA levels induced by kainate (300 μM). Incubation with kainate caused 153% increase in the specific binding of [3H]phorbol ester to intact O-2A cells, indicating a translocation of PKC to the membrane. Within 5-10 minutes after stimulation of non-NMDA receptors by kainate, the transcription factor CREB was phosphorylated on serine-133, as assessed by immunoblot analysis with an antiserum specific for the phosphorylated form of CREB. TPA (100 nM) and the muscarinic receptor agonist carbachol (100 μM) had a similar effect on CREB phosphorylation. Surprisingly, forskolin (100 μM) and growth factors PDGF and bFGF (10 ng/ml) failed to induce CREB phosphorylation in O-2A progenitor cells. Bisindolylmaleimide (5 μM) completely counteracted the effect of kainate, TPA and carbachol on CREB. The specificity of bisindolylmaleimide as PKC inhibitor was tested in PC12 cells. Bisindolylmaleimide (5 μM) was able to antagonize only TPA-induced phosphorylation of CREB in PC12 cells, whereas the effects of forskolin, NGF and ionomycin remained unaffected. Immunoblot analysis with a pan anti-PKC antibody of cellular fractions from O-2A progenitors demonstrated PKC translocation to the nucleus after phorbol esters treatment. These results define a novel pathway for CREB activation in neural cells that is dependent on PKC.

521.6

ANTISENSE INHIBITION OF c-JUN BLOCKS NEUROTENSIN GENE INDUCTION IN PC12 CELLS. P.R. Dobner* and R.J. Harrison. Dept. of Mol. Genetics and Micro. and Prog. in Neurosci., Univ. of Mass. Med. Cent., Worcester, MA 01655. The tridecapeptide neurotensin (NT) likely has multiple signaling functions in the CNS, including the modulation of dopaminergic pathways. The NT promoter which contains AP-1, CRE and GRE motifs appears to integrate diverse signals resulting from stimulation by NGF, glucocorticoids, and adenylate cyclase activators in PC12 cells. Co-transfection experiments have provided evidence that c-Jun plays a key role in NT gene activation through interactions with the glucocorticoid receptor and one or more CREB/ATF factors. To examine the importance of c-Jun for NT gene induction by inducers, PC12 cells were co-transfected with either a c-Jun antisense expression vector (pCMV-NUJ) or several control plasmids together with a pNT-Luc reporter plasmid and subsequently treated with combinations of NGF, lithium, dexamethasone, and forskolin. Co-transfection of increasing amounts of pCMV-NUJ resulted in a marked dose-dependent attenuation of NT gene induction by all inducer combinations tested. The induction of pNT-Luc expression was inhibited by 60-70% at the highest input level of pCMV-NUJ tested. In contrast, neither the pCMV2 vector nor a construct driving the expression of a truncated c-Jun sense RNA (pCMV-ΔJUN) inhibited expression. Interestingly, co-transfection of a Jun B antisense construct (pCMV-NUJB) potentiated induction in a dose-dependent manner. These results provide strong evidence that c-Jun plays a key role in the activation of NT gene expression and indicate that Jun B may be involved in dampening induction.

521.7

Purification and Identification of Chronically Induced Fras (Fos-Related Antigens) B.T. Hope*[1], H.E. Nye[2], J.-S. Chen[2], N. Hiroi[2], and E.J. Nestler[2], [1] Tufts University School of Medicine, Boston, MA 02111, [2] Yale University School of Medicine, New Haven, CT 06508.

We and others have observed a set of putative transcription factors, called chronic Fras, which are gradually induced in rat brain specifically in those regions that are repeatedly activated by a specific drug or treatment, including cocaine, morphine, kainate and electroconvulsive seizures, tranylcypromine, haloperidol, apomorphine following 6-OHDA lesions, and these lesions alone. By 2D gel electrophoresis, we have identified four chronic Fras (MW 35-37 kDa) which are not significantly induced by acute treatment but accumulate to considerable levels with repeated treatments and then persist with a half-life of ~7 days. At least in some brain regions, chronic Fras heterodimerize with JunD to form a long-lasting AP-1 complex which we have shown to display different DNA binding characteristics compared to that of the acutely induced AP-1 complex. Thus, chronic Fras may be capable of causing long-lasting changes in gene expression that are unique to chronic, versus acute or control, conditions. Immunochemical analysis of the chronic Fras suggests a similarity to ΔFosB. However, their relative time courses for induction, and their migration by 2D gel electrophoresis, show that the chronic Fras are not ΔFosB *per se*. To positively identify the chronic Fras, we have purified these proteins to homogeneity using a combination of ion exchange chromatography and 2D gel electrophoresis. We are presently obtaining partial amino acid sequences from peptide digests of the purified proteins. Eventual identification of the corresponding cDNA sequences of the chronic Fras will allow future experiments to study a causal role for chronic Fras in biochemical and behavioral changes following chronic treatments.

521.8

PHARMACOLOGICAL CHARACTERIZATION OF COCAINE-INDUCED CHRONIC FRAS (FOS-RELATED ANTIGENS). H.E. Nye* and E.J. Nestler Lab of Molecular Psychiatry, Yale University School of Medicine, Dept.s of Psych. and Pharm., New Haven, CT 06508

Novel 35-37 kD Fos-like transcription factors are induced by chronic cocaine in the rat striatum (Str) and nucleus accumbens (NAc); regions implicated in many behavioral effects of this drug (*Neuron* 13:1235). In order to more completely characterize chronic Fra induction by cocaine, we examined the pharmacological aspects of this phenomenon. Chronic dose-response studies as well as manipulations of dosing intervals showed that a moderate dose of cocaine given at intermediate intervals for an intermediate period of time resulted in maximal chronic Fra induction in the Str, while the NAc tended to be more responsive to lower doses of chronic cocaine. We have also investigated the mechanism of chronic Fra induction by cocaine. Adminstration of the D1 antagonist, SCH 23390 (0.5mg/kg), prior to each of eight daily cocaine injections blocked chronic Fra induction in both the Str and NAc. The D2 antagonist, eticlopride (0.5 mg/kg), in the same paradigm induced chronic Fras when administered alone, but produced no further increase when combined with cocaine. Seven days of haloperidol (1 mg/kg) treatment also robustly induced chronic Fras in the striatum. Repeated co-administration of D1 (SKF 81297, 1 mg/kg) and D2 (quinpirole, 1 mg/kg) agonists, but not either agonist alone, increased chronic Fras in the Str. Finally, repeated administration of the specific dopamine uptake inhibitor, GBR-12909 (20 mg/kg), induced chronic Fras in the Str and NAc, while chronic blockade of other monoamine uptake systems (sertraline for serotonin, desipramine for norepinephrine) had no effect on these proteins. These studies provide evidence for dopaminergic mechanisms of chronic Fra induction by cocaine, and further characterize novel transcription factors that may underlie long-term changes in the brain elicited by cocaine.

521.9

REGULATION OF ΔFOSB AND CHRONIC FRAS BY ELECTROCONVULSIVE SEIZURE (ECS) AND COCAINE TREATMENTS. J.S. Chen*, N. Hiroi, M.B. Kelz, B.T. Hope, Y. Nakabeppu^ and E.J. Nestler, Laboratory of Molecular Psychiatry, Yale University School of Medicine, New Haven, CT; ^Dept of Biochem., Medical Institute of Bioregulation, Kuyshu University, Fukuoka 812, Japan.

Repeated administration of ECS, drugs of abuse and other agents has been shown to induce several long-lasting Fos-related antigens (Fra), named chronic Fras. The slow induction and persist expression of the chronic Fras suggest that these proteins may mediate some long-term biochemical changes in the brain. Although there are some similarities between the chronic Fras and ΔFosB in terms of immunoreactivity and apparent molecular weight on 1D gels, the identity of the chronic Fras is still unknown. In the present study, we analyzed the regulation of ΔFosB expression by ECS and cocaine treatments, and characterized the chronic Fras by 2-D gel Western blotting. ΔFosB mRNA and protein were rapidly and transiently induced in cortex by acute ECS and in striatum by acute cocaine treatment. Induction of ΔFosB mRNA peaked at 0.5 h and returned to control levels by 12 h after a single ECS treatment. ΔFosB protein was induced maximally at 1 h and disappeared by 24 h after the ECS treatment. This induction pattern is different from the slow and persistent induction pattern of the chronic Fras. In 2D gels, ΔFosB and the chronic Fras migrated at different positions. Since the chronic Fras do not migrate further to the acidic end of the IEF gel than ΔFosB, they are unlikely to be phosphorylated isoforms of ΔFosB. Therefore, chronic Fras appear to be novel Fos-related proteins. Lack of the C-terminal antigen of FosB, which constitutes the major transactivation domain, in these chronic Fras suggests that they may be negative regulators for AP-1 mediated transcription.

521.10

DISTINCTIVE ACTIVATION BY CHRONIC ELECTROCONVULSIVE SEIZURE TREATMENT OF TRANSCRIPTION FACTORS IN THE RAT BRAIN N. Hiroi*, J.-S. Chen, E. J. Nestler. Laboratory of Molecular Psychiatry, Yale University School of Medicine, 34 Park Street, New Haven, CT 06508

Electroconvulsive seizure (ECS) treatment is one of the most effective treatments for depression. We have previously demonstrated in the rat neocortex that chronic ECS induces transcription factors with apparent molecular weights of 35-37 kDa that were recognized as a doublet by antiserum raised against a conserved sequence of c-Fos and Fos-related antigens (FRAs) (M. Iadarola, NIH). We have now further characterized these "chronic FRAs" in various brain areas. Gel mobility shift assays showed that chronic ECS significantly induced proteins that bind to the AP-1 DNA consensus sequence in the prefrontal cortex, striatum, and amygdala and, to a lesser extent, in the hippocampus. Immunoblotting showed that the patterns of induction of c-Fos and the FRAs in the prefrontal cortex, striatum and amygdala by ECS were virtually indistinguishable. The AP-1 complex in the prefrontal cortex induced after chronic ECS was disrupted by antisera against FosB and against JunD. Preliminary coimmunoprecipitation studies showed that the FRAs are dimerized with Jun family member proteins in a region-specific manner. These results suggest that chronic ECS activates 35-37 kDa FRAs in many reward-related regions and that transcriptional activation of late-response genes in these brain regions by chronic FRA-Jun family heterodimers might underlie, at least partly, the therapeutic efficacy of ECS treatment.

521.11

HSV-MEDIATED F1/GAP43 GENE TRANSFER: EVIDENCE FOR F1/GAP43-mRNA INDUCTION AND KNOCKDOWN OF KAINATE-INDUCED F1-mRNA. A. Routtenberg*, P.A. Serrano, R.L. Neve[1] & I. Cantallops. Cresap Neuroscience Laboratory, Northwestern Univ., Evanston, IL 60208; [1]Harvard Medical School and Mclean Hospital, Belmont, MA 02178.

The herpes simplex virus (HSV-1) is an efficient vector for introducing foreign genes into postmitotic neurons (TINS, 1991, 14:428). Here we induce or knockdown F1/GAP-43 mRNA in rat granule cells which normally do not express this gene in the adult. We used HSV-F1/GAP43 cDNA to induce and HSV-mediated antisense to knockdown kainate-induced F1/GAP43-mRNA (see Cantallopes, this meeting).

In the anesthetized rat, extracellular population responses in the dentate gyrus evoked by stimulation of perforant path fibers were used to guide the injections into the hilus for uptake by mossy fiber terminals (see Serrano, this meeting). The HSV-F1/GAP43 vector was injected unilaterally into the hilus. 24-72h post-injection the brain tissue was prepared for in situ hybridization using the rat antisense RNA probe corresponding to bases 779-1295 of the F1/GAP43 cDNA. The HSV-F1/GAP-43 cDNA was successfully introduced into granule cells as its mRNA was detected in both the dorsal and ventral blades 1-3d after viral injection. In adjacent granule cells no F1/GAP-43 mRNA was detected.

Kainate induces F1/GAP43 mRNA expression in granule cells peaking 24h after its application. To test the effect of the F1/GAP43 antisense on kainate-induced F1/GAP43-mRNA in granule cells, the HSV-F1/GAP43 antisense vector was injected unilaterally into the hilus 24h prior to kainate treatment administered s.c. (10mg/kg). 24h after kainate treatment, in situ hybridization was carried out. The anti-sense injected hippocampus showed a reduction of kainate-induced granule cell F1/GAP43 mRNA as compared to control injection of the HSV vector containing no exogenous cDNA. Control injection of the HSV vector did not alter kainate-induced F1/GAP43 mRNA levels. Introduction of the F1/GAP43 gene or its antisense into granule cells of the adult rat may prove useful in elucidating both the sufficient and necessary roles, respectively, of F1/GAP43 in axonal growth in the in vivo, intact mammalian brain. Supported by grant MH25281-20 to A.R., NSF Post-doctoral grant to P.A.S., "La Caixa" Fellowship (Spain) to I.C. and HD24236 grant to R.L.N.

521.12

TRANSCRIPTION FACTORS AND SYNAPTIC PLASTICITY: PROTEIN F1/GAP-43 GENE EXPRESSION AND THE BASIC HELIX-LOOP-HELIX FAMILY. W.R. KINNEY, R.K. MCNAMARA[1], J. P. ROSENFELD[1], E. VALCOURT, AND A. ROUTTENBERG. CRESAP NEUROSCIENCE LABORATORY, NORTHWESTERN UNIV., EVANSTON, IL 60208; [1]UNIVERSITY OF FLORIDA, GAINESVILLE, FL 32610.

We have recently linked the induction of protein F1/GAP-43 and its mRNA in hippocampal granule cells by kainic acid (KA) to growth of their axons, the mossy fibers, in the adult rat. If this induction involves the F1 promoter/enhancer 5' flanking region, then it may be predicted that altered binding to specific recognition elements on the promoter will occur after KA administration. Analysis of recognition elements on the F1/GAP-43 gene revealed an arrangement, previously described in other genes (*EMBO J.*, 1994, 13:3580), of multiple adjacent E-box elements, which are recognized by the basic Helix-Loop-Helix (bHLH) family of transcription factors (TFs). The presence of hippocampal TFs specifically binding to the E-box recognition element was established by selective blockade of binding both by cold competition and by an antibody to an E-box TF, MyoD1. Using a micro-dissected preparation enriched in granule cells, specific E-box binding was significantly reduced in kainate-treated animals. This was observed at 24 and 72 hr, but not before (3, 6 hr) or after (96 hr), paralleling in a general fashion the time of protein F1/GAP-43 mRNA induction by kainate. Since it is obvious that other TFs would likely be involved in F1/GAP-43 regulation, the lack of perfect parallelism is consistent with this fact.

To determine the generality of this E-box regulatory event, we studied 4 other situations where F1 expression has been increased: NGF treatment of PC12 cells, unilateral hilar lesions, long-term potentiation after 1 hr, and postnatal rat hippocampal development. In all 4 cases, decreased E-box binding preceded increased F1/GAP-43 mRNA expression. Converging evidence thus leads us to propose that E-box binding in the hippocampus act as a negative regulator of protein F1/GAP-43 gene expression. Proteins of the bHLH family would then be important for regulating axonal growth during both postnatal development as well as adult synaptic plasticity.[Supported by a Research Council of Canada Post-doctoral fellowship to R.K.M. and a NIMH grant MH25281-2 to A.R.].

521.13

DIFFERENCES IN RAT AND MOUSE HIPPOCAMPAL F1/GAP-43: CONSTITUTIVE mRNA EXPRESSION, KAINATE INDUCTION IN GRANULE CELLS, PROMOTER ACTIVITY IN TRANSGENIC MOUSE. U. Namgung, R. McNamara[1], K.A. Paller[2]*, and A. Routtenberg. Cresap Neuroscience Laboratory, [2]Department of Psychology, Northwestern University, Evanston, IL 60208; [1]University of Florida, Department of Psychiatry, Gainesville, FL 32610-0256

The induction by kainate (KA) of F1/GAP-43 mRNA observed in adult rat hippocampal granule cells (see Cantallops et al., this meeting) was not observed in 3 different mouse strains. Though KA induced behavioral seizures similar to those in the rat, neither induction of F1/GAP-43 mRNA, nor subsequent mossy fiber sprouting observed in rat were detected in mouse.

The distribution of constitutive levels of F1/GAP-43 mRNA were noticeably different in mouse and rat. Quantitatively, F1/GAP-43 expression in rat was higher in CA3 than CA1 pyramidal cells, but equivalent in these two subfields in mouse. In CA3, F1/GAP-43 expression was significantly greater in rat than mouse, while in CA1, F1/GAP-43 expression in rat and mouse were equivalent.

These species differences may be related to F1/GAP-43 promoter activity. We studied this using a transgenic mouse (line 252) bearing a 6 kb rat F1/GAP-43 promoter/11 kb intron 1-lacZ reporter construct (J. Neurosci., 14:499, 1994). Reporter activity in hippocampal pyramidal subfields paralleled the distribution of rat F1/GAP-43 mRNA levels. No promoter activity was observed in granule cells paralleling its absence in both rat and mouse. Constitutive F1/GAP-43 expression pattern is thus determined by recognition elements present on the F1/GAP-43 promoter rather than transcription factors acting on recognition elements common to rat and mouse. But KA did not activate the rat promoter in transgenic mouse granule cells. Failure of KA to induce granule cell F1/GAP-43 expression in mouse may occur because necessary transcription factors are either absent or are not activated by KA. (Supported by MH25281-21)

521.14

CLONING AND ANALYSIS OF FOS-RELATED GENE FROM RAT HIPPOCAMPUS AFTER SYSTEMIC INJECTION OF KAINIC ACID. Z. Feng, G. Bing, Q. Qi, B. Wilson, M. K. McMillian, K. R. Pennypacker, M. Iadarola# and J.-S. Hong, Lab. Environ. Neurosci., NIEHS/NIH, Research Triangle Park, NC; Neurobiology and Anesthesiology Branch#, NIDR/NIH, Bethesda, MD

AP-1 transcription factors play an important role in gene regulation by environmental stimuli. Systemic injection of kainic acid (KA), a glutamate receptor agonist, induced both short-term and long-term increases of AP-1 transcription factors. The acute response of AP-1 factors such as c-fos and fos-related antigen (FRA) to KA treatment has been extensive studied. However, the identity of the long-term AP-1 factors which are induced by KA is still unknown. The purpose of this study was to clone and analyze these long-term inducible transcription factors. We constructed a cDNA library from rat hippocampus three days after KA treatment and immunoscreened the new transcription factors with a FRA antibody, which was raised against the conserved M peptide region within the c-fos protein. One of fourteen positive clones, M13, from two million screened colonies was selected. Western blot analysis showed that M13 fusion protein can be recognized by FRA antibodies, but not by c-Fos, Fra-1 and Jun D antibodies. A translated M13 product is recognized by FRA antibodies in vitro and show 65-kDa band on SDS gel electrophoresis. The predicted peptide sequence of M13 encodes a 62-kDa protein which contains a leucine zipper domain and an octate glutamine repeat domain suggesting that this protein functions as a transcriptional factor. M13 is a unique gene based on searching Genbank. AP-1 gel shift assays with M13 translation products indicated that M13 may be involved in the stabilization of the AP-1 complex in the transcriptional regulation.

521.15

RETINOIC ACID STIMULATES TRANSCRIPTION OF ENDOGENOUS α-CaM KINASE II mRNA IN PC12 CELLS. J. Chen and P. T. Kelly*. *Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, Texas 77030*

Transcription of the gene encoding α-CaM kinase II is very active in certain neurons in the mammalian brain. α-CaM kinase II expression increases dramatically during postnatal development in hippocampus and cerebral cortex, but remains low in cerebellum. These properties indicate that its transcription is carefully regulated during brain development and in the adult CNS. We have shown that the expression of an exogenous β-galactosidase (β-gal) reporter plasmid driven by the core promoter of the α-CaM kinase II gene (Olson, et al., PNAS, 92: 1659-1663, 1995) is extremely low in transfected PC12 cells under standard growth conditions. However, retinoic acid (25 μM) stimulates cellular differentiation and significantly increases α-CaM kinase II gene expression based on β-gal activity measurements. To further examine the regulatory effect of retinoic acid on α-CaM kinase II expression, normal PC12 cells were treated with retinoic acid and changes in endogenous α-mRNA levels were analyzed by RNase protection assays. retinoic acid treatment for 5 days increased transcription of endogenous α-CaM kinase II mRNA by 5-fold compared to GAPDH mRNA; increases were detected only 24 hours after retinoic acid addition. These results suggest that retinoic acid may play an *in vivo* role in regulating α-CaM kinase II gene expression during brain development and in the adult brain. (Supported by NIH grant NS22452.)

521.16

MECHANISMS CONTROLLING CALCIUM REGULATED GENE EXPRESSION IN NEURONS. Claire M. Johnson[1], Sangeeta Chawla[1], Caroline S. Hill[2], Richard Treisman[2], Hilmar Bading[1]. SPON: Brain Research Association. [1]MRC Laboratory of Molecular Biology, Cambridge CB2 2QH, UK, [2]Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, UK.

Calcium (Ca^{2+}) ions play a central role in the control of gene expression following electrical activation in neurons. Two major sites of Ca^{2+} entry into hippocampal neurons are N-methyl-D-aspartate (NMDA) receptors and L-type voltage sensitive Ca^{2+} channels. We are using primary neurons from the rat hippocampus and the rat pheochromocytoma cell line PC12 as model systems to study the mechanisms by which activation of different types of Ca^{2+} channels are coupled to the regulation of gene transcription. One of the earliest genomic consequences of Ca^{2+} influx into neurons is the transcriptional induction of the c-*fos* proto-oncogene. Because this transcriptional response occurs independently of the synthesis of new proteins it provides a powerful experimental system to study signalling to the nucleus.

Previous DNA transfection experiments using the wild type genomic c-*fos* gene and successive 5' promoter deletion mutants have identified a number of DNA regulatory elements important for signal-regulated transcription. These include the serum response element (SRE), the cyclic AMP response element (CRE) and the ets binding motif. However, the regulatory role of these elements in the context of the intact c-*fos* promoter is less clear. To investigate this, genomic c-*fos* DNA constructs containing mutations of specific regulatory elements placed in the natural sequence context of the entire promoter region have been transfected into PC12 cells and then tested for inducibility by Ca^{2+} as well as growth factor pathways. Furthermore, using microinjection of oligonucleotides carrying recognition sequences for specific DNA binding proteins we analysed the contribution of individual promoter elements to the regulation of the endogenous c-*fos* gene. We demonstrate that Ca^{2+}-activated transcription is controlled by several DNA regulatory elements. The principal target for growth factor signalling pathway in PC12 cells is the ets motif.

521.17

INTERLEUKIN-1ß AND PHORBOL ESTERS SYNERGIZE TO INDUCE PROSTAGLANDIN G/H SYNTHASE-2 (CYCLOOXYGENASE-2, PGHS-2) IN PRIMARY MURINE ASTROCYTE CULTURES. M. D. Kaplan, P. D Coleman, and M. K. O'Banion*. University of Rochester School of Medicine and Dentistry, Depts. of Neurobiology and Anatomy and of Neurology, Rochester, NY 14642.

Cyclooxygenase is known to be important in physiologic functions such as inflammation and pain modulation. Recently, an inducible form of this enzyme, prostaglandin G/H synthase-2 (PGHS-2), was discovered. PGHS-2 is induced by inflammatory mediators such as IL-1ß, as well as by agents known to stimulate protein kinase-C (e.g. phorbol esters like TPA), possibly through activation of the transcription factor NF-κB.

To better understand the mechanisms of prostaglandin production in the CNS, we have examined PGHS-2 activity and expression in primary astrocyte cultures. We demonstrate a synergistic increase in prostaglandin accumulation and PGHS-2 activity with the application of IL-1ß and TPA, as well as similar increases in message levels for PGHS-2 as demonstrated by Northern Blot analysis. The antioxidant pyrrolidine dithiocarbamate (PDTC), which is known to inhibit transcriptional activation by NF-κB, completely blocks prostaglandin production and PGHS enzymatic activity in IL-1ß and TPA treated astrocytes. Despite these effects, PDTC (60 μM) did not inhibit the accumulation of PGHS-2 mRNA in treated cells. These findings suggest that PDTC influences PGHS-2 activity by an as yet undefined mechanism. Further studies to identify the mode of action of PDTC as well as the transcription factors that dictate PGHS-2 expression are in progress. Supported by grants from the NIA (LEAD AG09016, R01 AG01121-14 and ADC P30 AG08665), Dentist Scientist Award K16 DE00159, and the Markey Foundation.

521.18

IDENTIFICATION OF A PHORBOLESTER-RESPONSIVE ELEMENT IN THE NEUROPEPTIDE GALANIN GENE AND SOME *trans*-ACTIVATING FACTORS. Å.Rökaeus,* K. Jiang and G. Spyrou . Lab. of Biochemistry I, MBB, Karolinska Institutet, S-171 77 Stockholm, Sweden.

The expression of the biologically active neuropeptide galanin (GAL) is elevated upon, nerve-stimulation, injury, HSV1-infection and during development, by mechanism(s) largely unknown. In the human neuroblastoma cell line (SKNSH-SY5Y), levels of GAL mRNA are elevated by phorbol myristate acetate (PMA). In an attempt to characterize the gene element(s) and the *trans*-activating factor(s) acting on the GAL-gene in response to PMA, plasmids containing ≤ 5 kb of the bovine GAL promoter fused to luciferase, were transfected into these cells. In doing so, we could functionally identify a PMA-responsive region in the GAL-promoter between 131 and 46 bp and by homology-sequence-search a cAMP-like responsive element (CRE-like; TGACGCGG). When an oligonucleotide harbouring this site was tested in an electrical mobility shift assay (EMSA) system, together with nuclear proteins isolated from the SY5Y-cells, two main PMA-inducible bands were observed. The binding in the main bands were reduced with unlabelled oligonucleotides in the following order of potency: CRE-consensus > CRE-like > AP1-consensus. The binding in all bands were abolished by an antibody that had been raised against rat CREB (recognizes several different proteins) and the binding in the bigger of the two main bands was reduced by antibodies against cJun and JunD. The data suggest that cJun and JunD together with members of the ATF/CREB family may be involved in the formation of these EMSA-complexes and hence these proteins may mediate increases of GAL-expression by phorbolesters.

521.19

DEVELOPMENTAL CHANGES IN ELECTROCONVULSIVE SHOCK-INDUCED EXPRESSION OF IMMEDIATE EARLY GENES IN RAT BRAIN. S.H. Jeong, H.Y. Jung, Y.H. Joo, U.G. Kang, S.C. Cho, J.B. Park, Y.S. Kim*. Depts. of Psychiatry & Biochemistry, Seoul National University, College of Medicine, Seoul, KOREA, 110-744

In order to study the developmental changes of the signal transduction system in the brain, we studied the changing pattern of immediate early gene (IEG) induction and cAMP Response Element Binding Protein (CREB) phosphorylation by electroconvulsive shock (ECS) in postnatal 7, 14, 21 day (p7, p14, p21) rats.

Sprague-Dawley male rats aged 7, 14, 21 days were sacrificed with appropriate time interval after single ECS, and hippocampal and cerebellar tissues were taken out. The induction of IEGs and the phosphorylation of CREB (p-CREB) were measured by Northern and Western blot respectively.

Neither *c-fos* nor *jun B* was induced in p7 rat by ECS, both in hippocampus and in cerebellum. But in p14 rats, significant inductions of *c-fos* and *jun B* were detected in both regions. In addition, IEG induction level increased further in p21 rats, so that the level was comparable with that of adult rats. In contrast, *TIS8* (*zif/268*) induction was significant even in p7 rats in both hippocampus and cerebellum, and reached the level of adult rats in p14 rats. But the induction level in cerebellum was far behind than that in hippocampus.

The amount of CREB measured by Western Blot was not different among rats of different age. But the amount of phosphorylated CREB (p-CREB) after ECS was gradually increased as maturation proceeds (p7<p14<p21).

We demonstrated that the response of neural cells to ECS with IEG inducion undergoes gradual changes with maturation of brain tissue, and the nature of these changes vary according to each type of IEG, and each brain region. In addition we demonstrated that the phosphorylation of CREB in response to ECS also undergoes gradual changes with maturation.

521.20

ESTROGEN PRODUCES DIFFERENTIAL AND GENDER-SPECIFIC REGULATION OF AP-1 DNA BINDING ACTIVITY IN RAT HYPOTHALAMUS AND PITUITARY. Y.-S. Zhu* and D.W. Pfaff. Lab. of Neurobiol. & Behav., Rockefeller Univ., NY 10021

It has been shown that AP-1 proteins are nuclear transcription factors that can function as a third message to regulate potential target gene expression such as for preproenkephalin (PPE). Estrogen has been shown to regulate AP-1 gene expression such as for c-fos in uterus. Using the electrophoretic mobility shift assay (EMSA), we have studied estrogen effects on regulation of AP-1 DNA binding activity in neuronal and non-neuronal estrogen-target tissues. Specific AP-1 binding activity in nuclear extracts was observed in hypothalamus (HYP), pituitary (PIT) and uterus of ovariectomized (OVX) female rats. This AP-1 binding was composed of at least c-fos and c-jun protein as demonstrated by supershift and immunodepletion assays. Treatment with estrogen (20 μg/rat) increased the levels of AP-1 binding in PIT and uterus of OVX females, whereas it decreased the level of AP-1 binding in HYP. These estrogen effects were visible at 15 min after treatment and were maintained for at least 72 h. These changes were not observed in striatum and cerebellum, and were much more prominent in OVX females than castrated males. Analysis of c-fos mRNA by slot-blot hybridization failed to show substantial changes in HYP and PIT. On the other hand, the same estrogen treatment produced rapid and significant increases in PPE mRNA in HYP of OVX females. These results show that estrogen produces differential alterations in AP-1 DNA binding in different estrogen target tissues and also suggest that changes in AP-1 DNA binding and PPE mRNA were not always correlated.

LONG-TERM POTENTIATION: PHARMACOLOGY II

522.1

TRANSGENIC MICE EXPRESSING MGLUR2 IN CA1 REGION OF HIPPOCAMPUS SHOWED DEFECTIVE LATE PHASE LTP. L. Wang[1]*, M. Mayford[2], and M. Zhuo[2]. [1]Integrated Program Cell., Mol., Biochem. Studies, [2]HHMI, Ctr. Neurobiol. & Behav., Columbia Univ., NY, NY 10032.

Synaptic plasticity in the hippocampus is a widely used model for studying cellular and molecular mechanisms of learning and memory. Long-term potentiation (LTP) in the CA1 region of hippocampus contains two different phases: an early phase LTP (E-LTP) lasting 1-3 hours, and a late phase (L-LTP) lasting more than 3 hours. L-LTP has been shown in pharmacological experiments to be dependent on cAMP and cAMP-dependent protein kinase. To study the function of cAMP in L-LTP genetically, we have used the CaM kinase II promoter to generate transgenic mice that express the mGluR2 gene in CA1 neurons. mGluR2 is a metabotropic glutamate receptor that is negatively coupled to adenylyl cyclase. Electrophysiological studies using these transgenic mice showed that E-LTP induced by a single tetanus was not affected. However, L-LTP induced by multiple stimulus trains was significantly decreased in transgenic as compared to wild-type mice. Post-tetanic potentiation, paired-pulse facilitation, long-term depression, and depotentiation were not significantly different in transgenic as compared to wild-type mice. Biochemical studies are in progress to evaluate the inhibitory function of mGluR2 on adenylyl cyclase in the CA1 region. The mGluR2 transgene should block increases in cAMP only when the receptor is activated by its ligand glutamate. Thus, the effect of the transgene on cAMP levels should occur only during synaptic activation. The mice will also be used to probe the effects of L-LTP in learning and memory.

522.2

1S, 3R-ACPD dose-dependently induces a slow onset LTP and facilitates NMDA-dependent LTP in the dentate gyrus in vivo. D. Manahan- Vaughan, W. Wetzel* and K.G. Reymann. Federal Institute for Neurobiology, Brenneckestr. 6, D-39118 Magdeburg, Germany.

1S,3R -1 -aminocyclopentane 2, 3 -dicarboxylic acid (ACPD) is a selective agonist of metabotropic glutamate (mGluR) receptor. It has been shown by others that application of ACPD triggers a slow onset potentiation of the Schaffer collateral-CA1 synapse in hippocampal slices. This study examined the effect of ACPD in the dentate gyrus when applied in vivo.

Male wistar rats underwent implantation of stimulating and recording electrodes into the granule cell layer of the dentate gyrus (DG). Following 5 day recovery, the field excitatory post-synaptic potential slope function (fEPSP) and population spike amplitude (PS) was measured from freely moving animals. Drug concentrations used were 40 μM, 80 μM and 10 mM ACPD, and 200 mM (RS) -α -methyl -4 -carboxyphenyl -glycine (MCPG) in an injection volume of 5 μL. Drugs were applied via a cannula implanted the intracerebral ventricle.

Weak tetanisation produced a short-term potentiation (STP) of fEPSP and PS which decayed to baseline values by 90 min. Application of 40 μM ACPD prior to tetanus produced a similar response, whereas 80 μM ACPD facilitated STP into a LTP which lasted over 24 h. MCPG, an mGluR antagonist, completely inhibited the effect by 80 μM ACPD on LTP. 40 and 80 μM ACPD had no effect on baseline when compared with vehicle injected controls. 4 mM ACPD induced a slow onset potentiation of both fEPSP and PS which developed by 60 min and reached a plateau by 150 min. This response was maintained for over 4.5 h. MCPG applied prior to ACPD completely inhibited this effect. These results confirm that pharmacological activation of mGluRs by ACPD facilitates LTP, and indicate that under certain conditions, mGluRs can bypass the NMDA component of LTP.

522.3

THE mGluR ANTAGONIST MCPG AND THE CALCIUM CHANNEL BLOCKER ALUMINUM INHIBIT TEA-INDUCED LONG-TERM POTENTIATION. B. Platt*[1] and K. G. Reymann[2]. [1] University of Leeds, Dept. of Physiology, Leeds LS2 9NQ, UK; [2] Federal Institute for Neurobiology, Dept. of Neurophysiology, PSF 1860, 39008 Magdeburg, FRG.

A brief application of the K^+ channel blocker tetraethylammonium induces a long-lasting potentiation in the CA1 region of hippocampal slices (TEA LTP). This potentiation is assumed to be due to activation of voltage-dependent calcium channels (VDCCs) as a consequence of a strong depolarization. We investigated here the involvement of metabotropic glutamate receptors (mGluRs) and VDCCS in TEA LTP utilizing the mGluR antagonist (+)-α-methyl-4-carboxyphenyl-glycine ((+) MCPG) and the VDCC blocker aluminum (Al) on TEA LTP. Experiments were performed on rat hippocampal slices, field potentials (fEPSPs) were evoked by stimulation of the Schaffer collaterals and extracellularly recorded in the stratum radiatum of the CA1 region. TEA LTP was induced by application of 25 mM TEA for 7 min, which caused a stable potentiation. Sixty min after TEA application, the fEPSP slope was enhanced by 50%. Further control experiments revealed that TEA LTP is attenuated by the VDCC antagonist nifedipine (10 μM) but also by the N-methyl-D-aspartate (NMDA) antagonist D-2-amino-5-phosphonovaleric acid (D-AP5; 25 μM). TEA LTP was completely blocked when Al (2.7 μg/ml ≅ 100 μM) was coapplied with TEA, i.e. only a brief and depressed potentiation occured and the fEPSP slope declined back to baseline values within 45 min. For (+) MCPG (200 μM), a significant attenuation of TEA LTP was observed. Sixty min after TEA application the fEPSP slope was only potentiated by 18%.

Our data suggest that three components contribute to TEA LTP: one mediated by VDCCs, one dependent on NMDA activation and an additional one based on the activation of mGluRs.

522.4

INDUCTION OF SYNAPTIC POTENTIATION IN THE RAT HIPPOCAMPAL SLICE BY ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS IN THE PRESENCE OF ARACHIDONIC ACID D.R. Collins and S.N. Davies (SPON: Brain Research Association) Department of Biomedical Sciences, Marischal College, University of Aberdeen, Aberdeen, AB9 1AS, Scotland.

We have previously shown that induction of long-term potentiation (LTP) in rat hippocampal slices from which the CA3 region has been removed requires the activation of both N-methyl-D-aspartate (NMDA) and metabotropic glutamate receptors. Here we have tested the hypothesis that this reflects a requirement for NMDA receptor mediated activation of phospholipase A_2 and release of arachidonic acid during activation of metabotropic receptors.

Perfusion of neither 1S,3R-aminocyclopentane-1,3-dicarboxylic acid (ACPD, 50μM), nor arachidonic acid (10μM), for 5min induced any potentiation of the field EPSP recorded from 400μm thick transverse hippocampal slices. However, co-perfusion of ACPD and arachidonic acid consistently evoked a long lasting potentiation. This potentiation was not blocked by inclusion of D-2-amino-5-phosphonopentanoate (AP5, 40μM). Perfusion of the phospholipase A_2 activating peptide melittin (10μg/ml) alone for 5 or 10min had no long term effects, however, perfusion of ACPD immediately following melittin resulted in potentiation. The potentiation induced by co-perfusion of arachidonic acid and ACPD was prevented by the protein kinase C blocker hypericin (2μg/ml). Docosahexanoic acid (10μM) could not substitute for arachidonic acid in inducing potentiation.

The results indicate that activation of metabotropic glutamate receptors in the presence of either exogenously applied, or endogenously released arachidonic acid can induce synaptic potentiation, and that this potentiation relies at some stage on the activation of PKC.

522.5

THE REDOX STATE OF NMDA RECEPTORS DETERMINES WHETHER REPETITIVE STIMULATION EVOKES LTP OR LTD.

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Low frequency stimulation of the Schaffer collateral/commissural input to hippocampal area CA1 elicits long-term depression (LTD) of synaptic transmission while higher frequency stimulation yields long-term potentiation (LTP). NMDA receptors mediate both forms of plasticity. Here we show that redox modulation of the NMDA receptor determines whether a particular stimulation frequency results in LTD or LTP.

Brains isolated from young male rats were sliced on a vibrating microtome and maintained in an interface chamber at 35°C. In some experiments the slices were treated with the reducing agent dithiothreitol (DTT, 100 μM) during the last 30 minutes of the one hour incubation period following slice preparation. Exposing the slices to 5-5-dithio-bis-2-nitrobenzoic acid (DTNB, 1 mM) for 30 minutes oxidized the NMDA receptors. To induce plasticity, 250 stimuli were delivered at an intensity which initially evoked a 1 mV field EPSP. NMDA receptor activity was assessed by comparing the area of the EPSP elicited by a single stimulus to that evoked after 4 conditioning stimuli at 10 Hz.

Low frequency stimulation normally produces a slight depression of synaptic transmission. However, after chemical reduction by DTT, low frequency stimulation elicits modest LTP instead of LTD. DTNB completely reverses this effect of DTT. Similarly, following oxidation of NMDA receptors in naive slices, higher frequency stimulation causes LTD instead of LTP. These results support the idea that the magnitude of NMDA receptor activity determines whether repetitive stimulation evokes LTP or LTD.

522.6

SELECTIVE BLOCKADE OF INHIBITORY, BUT NOT EXCITATORY, POSTSYNAPTIC POTENTIALS AND THEIR LTP IN CA1 PYRAMIDAL NEURONS

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We previously reported that NMDA- dependent LTP of local inhibitory circuits can be elicited in hippocampal CA 1 pyramidal cells ($IPSP_A$ increase of 32.5 $\pm$ 24.7% in 8/10 intracellular recordings). To further evaluate this LTP, we limited feedforward excitation and inhibition by transecting the slice, leaving only the alvear pathway intact. Tetanic stimulation of the alveus lead to an LTP of the $IPSP_A$ of 43 $\pm$ 21 % in 8/12 neurons tested in agreement with our previous experiments. LTP of the IPSP was inhibited in control and transected slices by the NMDA antagonists APV, PCP, MK 801 and the endogenous substance, NAAG. A comparison of the dose - response relationship of APV for inhibiting excitatory LTP by stratum radiatum (SR) stimulation vs. inhibitory LTP by stratum oriens (SO) stimulation showed a 10- fold smaller IC_{50} for inhibitory LTP (IC_{50} (SR)= 5.75 $\pm$ 1.11μM; IC_{50} (SO)= 0.57 $\pm$ 1.13μM; n= 6 at each concentration tested: 50- 0.3 μM). A similar difference in sensitivity was observed for NAAG. The amplitude of alvear- evoked IPSP's was also sensitive to APV. At 0.4μM APV (approximately the IC_{50} for inhibitory LTP suppression), APV reduced the IPSP by 11 $\pm$ 3 % (n=4), about ½ of the maximal reduction elicited by 25 μM APV of 27 $\pm$ 8.5 % (n=4). We conclude that small doses of NMDA antagonists, including the endogenous partial antagonist, NAAG, are sufficient to interfere with inhibitory circuits while leaving excitation intact. Selective impairment of inhibitory circuits and their ability to undergo LTP, by low doses of antagonists, may lead to increased network activation and to specific deficits in information processing in the hippocampus, consistent with cognitive abnormalities described in PCP and ketamine induced psychosis and in schizophrenia.

522.7

LONG TERM POTENTIATION IN THE PREFRONTAL CORTEX FOLLOWING MANIPULATION OF DOPAMINERGIC SYSTEMS.

J.S. Bondar*, C.A. Chapman, R.J. Racine. Dept. of Psychology, McMaster University, Hamilton, Ontario, L8S 4K1.

Neocortical long term potentiation (LTP) has been difficult to induce in chronic preparations. Recently, our lab has been able to induce potentiation reliably after 10 days of administering high frequency pulse trains. In this study, monopolar field potentials were recorded in the rat prefrontal cortex in response to test pulses (0.1 ms, 40-1259μA) delivered to the corpus callosum. After three baseline tests, three groups were formed and each was administered drug injections (i.p.) 15 minutes prior to delivery of the pulse trains (30 trains of 8 pulses at 300Hz, 1259 μA) each day for 10 days. One group received 1.0 mg/kg apomorphine (agonist), another group received 1.0 mg/kg haloperidol (antagonist), and the control group received an equivalent volume of the sterile water vehicle. Evoked field potentials were recorded for an additional 4 week period following completion of the stimulation phase. Field potentials demonstrated two positive-going components. The early component was depressed at all intensities for the control animals 1 day after the last set of trains but had recovered to near baseline levels after 4 weeks. A similar depression has been found in our other neocortical LTP experiments, but it is correlated with increased discharge of cortical cells. A larger depression was observed in the apomorphine group that also returned to baseline by 4 weeks. The haloperidol group showed the largest depression and only partial recovery. For the second component, a small increase in amplitude was observed in the control group at 1 day post trains with a modest recovery after 4 weeks. The apomorphine group, however, demonstrated a larger enhancement that persisted 4 weeks after delivery of the pulse trains. In contrast, the haloperidol group showed a depression in this component with a return to near baseline levels after 4 weeks. These results demonstrate that long term potentiation can be induced in the prefrontal cortex, and that the potentiation can be modified by pharmacological manipulation.

522.8

DIFFERENTIAL EFFECTS OF PACAP ON SYNAPTIC TRANSMISSION OF RAT HIPPOCAMPAL SLICE.

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Pituitary adenylyl cyclase-activating polypeptide (PACAP) , a neuropeptide originally isolated from ovine hypothalamus, regulates hormone release and promotes differentiation or survival of cultured neuroblast. PACAP-38 stimulates neurite outgrowth of PC-12 cells, and increases cytosolic free calcium concentration in cultured rat hippocampal neurons. The PACAP type-I receptor, specific for PACAP, is positively coupled to adenylyl cyclase and phospholipase C. The type-II receptor, which does not discriminate between PACAP and vasoactive intestinal polypeptide, is coupled to only adenylyl cyclase. In this report we present that PACAP-38 induced long-lasting modification of synaptic transmission of rat hippocampal slice. Bath application of PACAP (1 μM) decreased both the initial slope of field e.p.s.p. at Schaffer collateral-CA1 synapses and the amplitude of population spikes of CA1 pyramidal neurons (mean percent of control: e.p.s.p., 87.6 $\pm$ 2.3 %, n = 24, $p < 0.005$; population spikes, 88.6 $\pm$ 3.6 %, n = 15, $p < 0.01$). In contrast, the synaptic strength in dentate gyrus was enhanced by the exposure to the same concentration of PACAP (e.p.s.p., 120.1 $\pm$ 3.5 %, n = 10, $p < 0.005$; population spikes, 142.3 $\pm$ 7.4 %, n =8, $p < 0.005$, in the presence of 5 μM bicuculin). These effects were sustained more than 2 to 3 hours and the magnitude of these effects was concentration-dependent (0.1-10 μM).

522.9

MODULATION OF LONG-TERM POTENTIATION IN THE HIPPOCAMPAL CA1 REGION BY CHOLECYSTOKININ.

D. Balschun*, and K. G. Reymann. Department of Neurophysiology, Federal Institute for Neurobiology, Brenneckestr. 6, POB 1860, D-39008, Magdeburg, Germany.

The physiological role of sulphated cholecystokinin octapeptide (CCK-8S) in the hippocampus is not well understood. The hippocampus represents the brain region in which long-term potentiation (LTP), a model of learning and memory in higher vertebrates, is most thoroughly investigated. We examined, whether (CCK-8S) may be involved in the induction and maintenance of hippocampal LTP. To address this issue, field excitatory postsynaptic potentials (fEPSP) and population spikes (PS) were recorded in the CA1 region of the rat hippocampal slice preparation and two different forms of N-methyl-D-aspartate (NMDA)-dependent LTP were employed. A weak LTP was induced by a single theta-pulse stimulation (TPS; 10 pulses at 7 Hz) and a strong LTP by a triple theta-burst stimulation (TBS; 10 bursts of 4 pulses, 100 Hz. interburst interval 200 ms, at 2 minute intervals). CCK-8S and the highly selective CCK_B-receptor antagonist PD 135158 were bath-applied 40 - 50 min prior to tetanization and up to 10 min thereafter.

TPS elicited a potentiation of the fEPSP slope and the PS amplitude lasting for about 1 hour. The application of 500 nM CCK-8S led to a higher magnitude of potentiation of the fEPSP slope and the PS amplitude and a marked protraction of potentiation. PD 135158 (1 μM) decreased the potentiation of the PS amplitude but had no influence on LTP of the fEPSP slope. TBS evoked a robust LTP remaining at increased levels for at least 4 hours. Application of 1 μM PD 135158 did not change the initial potentiation of the fEPSP-slope but caused a marked impairment of the maintenance. In contrast, PD 135158 led to a higher magnitude of potentiation of the PS amplitude shortly after potentiation, but exerted no influence on the maintenance of potentiation. The results suggest that CCK-8S may modulate long-term changes in glutamatergic synaptic transmission of the hippocampus via activation of CCK_B-receptors.

522.10

FACILITATORY ROLE OF SOMATOSTATIN VIA MUSCARINIC CHOLINERGIC RECEPTOR IN THE GENERATION OF LONG-TERM POTENTIATION OF POPULATION SPIKES IN THE DENTATE GYRUS *IN VIVO*.

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Accumulating evidence indicate that somatostatin is involved in the cognitive function. A brief tetanic stimulation to a monosynaptic excitatory pathway in the hippocampus induces long-term potentiation (LTP) of a synaptic response both *in vivo and in vitro*, which is considered to be a cellular basis of learning and memory process. Recent studies revealed that somatostatin enhanced the LTP in mossy fiber-CA3 pathway of the guinea pig hippocampus *in vitro*. In the present study, we investigated whether somatostatin modulates the generation of LTP in rat perforant path-dentate gyrus synapse *in vivo*. Male Wistar rats 8 - 9 weeks old were anesthetized with urethane and α-chloralose. A bipolar electrode was placed in the entorhinal cortex to stimulate the medial perforant path, and the evoked potential was extracellularly recorded from the granule cell layer of the dentate gyrus. A weak tetanic stimulation (20 pulses at 60 Hz) of the perforant path induced only marginal LTP. When somatostatin (5 - 500 ng/rat) was injected intracerebroventricularly (i.c.v.) 20 min prior to the tetanus, the intensity of LTP increased dose-dependently. Synaptic potential evoked by a low-frequency test simulation, however, was not altered by somatostatin. We next tested whether LTP-augmenting effect of somatostatin is mediated by cholinergic activation, because somatostatin was demonstrated to promote acetylcholine release in rat hippocampal slice. Pirenzepine (50 nmol/rat), a muscarinic M_1-receptor antagonist, did not affect the weak tetanus-induced LTP by itself. But when it was coapplicated with the somatostatin (50 ng/rat) 20 min before tetanus, it completely abolished the LTP-augmenting effect of somatostatin. These result suggest that somatostatin facilitates the generation of perforant path-dentate gyrus granule cell LTP via activating the muscarinic cholinergic receptor *in vivo*.

522.11

LTP IN HIPPOCAMPUS BY HISTAMINE, C-AMP AND PKA.
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The action of histamine on rat hippocampal CA1 pyramidal-cells in vitro was investigated in 0.2 mM Ca^{2+}/4 mM Mg^{2+}. Histamine (HA; 5 min; 0,1-10μM) caused, in 85% of the cells, a dose-dependent increase in firing rate which was characterized by a quickly rising and reversible (I), a longer lasting (II, ca. 30 min) and a later phase (III, starting after ca. 40 min). Cimetidine but not mepyramine prevented all excitations, impromidine mimicked them. Equal concentrations of histamine were more effective than impromidine. 8-Br-cAMP and the PKA-activator SP-cAMP also mimicked phases II and III. The adenylyl-cyclase-inhibitor SQ 22,536 or the PKA-inhibitor RpcAMP blocked them. APV significantly attenuated the late phase (III) indicating an indirect (secondary) NMDA-dependence. H1-receptor activation caused a depression of firing and seemed to potentiate H2-receptor actions. H3-receptor activation by R-alpha-methyl-histamine was ineffective on firing rate. All three phases of histaminergic excitation in the hippocampus are mediated by H2-receptors. Only the long lasting (II) and the later phase (III) depend on AC and PKA. The histaminergic system is involved in the regulation of behavioral state and can potentiate hippocampal transmission for prolonged periods of time. Supported by DFG and HFSP.

LONG-TERM POTENTIATION: PHARMACOLOGY III

523.1

ETHANOL AFFECTS HYPOTHALAMIC NEURONS PROJECTING TO THE HIPPOCAMPUS THAT INHIBIT DENTATE GRANULE CELL LTP.
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In previous studies we demonstrated that ethanol inhibition of hippocampal granule cell long term potentiation (LTP) is mediated by angiotensin II (AII) and the inhibition can be blocked by losartan, a specific AII AT_1 receptor antagonist (Wayner, et al. *Pharmacol. Biochem. Behav.* 45:455-464, 1993). The purpose of the present study was to demonstrate that this low dose ethanol inhibition of dentate granule cell LTP induction is mediated by lateral hypothalamic (LH) afferents that project to the granule cells. Anterograde tract tracing following horseradish peroxidase administration into the dentate granule cells revealed clearly filled neurons in the LH similar to those stained for AII (unpublished data). In urethane anesthetized rats, we have compared the effects of ethanol infusion, 6.0 μl/30 min, by means of an open ended push-pull cannula, in both the LH and the dentate gyrus, on dentate granule cell LTP. Results demonstrate a dose dependent inhibition of LTP induction when the LH is perfused, which can be blocked by losartan. Four doses of ethanol were used: 5, 10, 20, and 30 mM. There was no effect when the dentate gyrus was infused with 30 mM ethanol and normal LTP in the granule cells was observed.

523.2

IMPAIRMENT OF CA1 HIPPOCAMPAL LTP IN MICE WITH REDUCED CALBINDIN D_{28K} EXPRESSION.
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Calbindin D_{28K} (CaBP) is strongly expressed in the CA1 pyramidal neurons of the hippocampus but the role of this calcium-binding protein is unknown. Behavioral studies have shown impaired performances of spatial learning tasks in CaBP deficient transgenic mice. Here, we report that the maintenance of long-term potentiation (LTP) is altered in hippocampal CA1 area in these mice.

Hippocampal slices were prepared from homozygous transgenic or wild-type control mice according to conventionnal methods. Field potentials (fEPSP) were induced by electrical stimulation of CA1 afferent fibers and recorded in stratum radiatum using extracellular electrodes. No difference in the basal properties of the fEPSPs were observed between wild and transgenic mice: amplitude and slope of control fEPSPs, paired-pulse facilitation of fEPSPs, fiber volley amplitude and the ratio of fEPSP slope to fiber volley amplitude were comparable in both populations. A robust LTP following high frequency stimulation was observed in wild-type mice. In contrast, in most homozygous mice, a potentiation of the fEPSP developped but lasted for only 20 to 30 min. These results suggest that CaBP is necessary for the maintenance but not for the induction of LTP in the CA1 area. We are now studying LTP in the CA3 region where CaBP-immunoreactivity is present presynaptically in the mossy fibers. The comparison between the results in CA1 and CA3 fields where CaBP is present post- and presynaptically respectively, will give informations on the role of CaBP with respect to its neuronal localization.

523.3

SYNAPTIC PLASTICITY IN DISSOCIATED CHICK CEREBRAL NEURONS INDUCED BY Mg^{2+}-FREE MEDIUM
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To analyze the molecular mechanism of long-term enhancement of synaptic transmission, we have developed a dissociated cell culture system. Using whole-cell path clamp technique, we have demonstrated long-lasting increase of amplitude of spontaneous excitatory post synaptic currents (SEPSCs) induced by Mg^{2+}-free medium. This enhancement was inhibited by the addition of TTX, CNQX and AP5 in the Mg^{2+}-free medium, suggesting it depends on neural activity. Inhibitor of protein and RNA synthesis also inhibited the enhancement. Analysis of miniature EPSCs in the presence of TTX revealed that the enhancement accompanies the increase in frequency of miniature EPSCs. Thus, the enhancement is thought to be due to the increase of the number of transmitter release site and/or synaptic sites. Furthermore it was also demonstrated that the similar long-lasting potentiation was caused by exposing neurons to conditioned Mg^{2+}-free medium which was collected after induction of the enhancement and adjusted the Mg^{2+} concentration at the same in normal extra cellular recording solution. This result suggests that some factors which promote the potentiation of SEPSCs are released to the medium.

523.4

A selective neuronal nitric oxide synthase inhibitor blocks LTP induction and spatial learning in the rat
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Nitric oxide (NO) is a transmitter which has been suggested to play a role in memory formation. A novel selective NO synthase inhibitor, 7-nitro indazole (7-NI), has been shown to inhibit neuronal NO synthase by 85 % (30mg/kg *i.p.*) while having no effect on blood pressure. 7-NI produced amnesic effects both in a water maze and in an 8-arm radial maze (30mg/kg *i.p.*, Wistar rats, n=16). Latency as well as distance was greater in the 7-NI group in the water maze while swim speed was not affected. Latency, working memory (WM) and reference memory errors were also higher in the 7-NI group in the 8-arm maze. At the end of the second training day, no difference in performance between 7-NI and control groups was visible anymore. A change of baited arms caused a difference between groups in latency and WM errors. The quadrant analysis transfer test in the water maze showed a difference between 7-NI and control groups with a difference in the numbers of annulus crossings. Learning of a visual discrimination task was not affected. In the CA1 region of the anaesthetised rat, 7-NI (30mg/kg *i.p.*) prevented induction of LTP 30 min after injection in 6 out of 6 animals. 4 h after injection, when most of the drug is washed out, LTP was inducible again in 3 out of 3 animals. Injecting 7-NI along with L-arginine (225 mg/kg) concentrations prevented the block of LTP induction (6 out of 6). We conclude that neuronal NO synthase activity plays a role in LTP induction and in learning and memory formation in the rat.

523.5

MONOAMINE DEPLETION AFFECTS SYNAPTIC PLASTICITY IN CA1 REGION OF HIPPOCAMPAL SLICE BY c-AMP-DEPENDENT MECHANISM.

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In order to determine whether or not biogenic amines play a significant role in synaptic plasticity, we have compared the levels of field EPSP potentiation and depotentiation in control (C) and monoamine-depleted (D) rats. The depletion was achieved by 5 mg/kg reserpine injection 24 hours prior to experiment and by adding tyrosine hydroxylase inhibitor into the perfusion media. Depletion significantly decreased potentiation by 25-45% (40 min. after a single tetanus; 100 Hz, 1 sec). Depotentiation after low frequency stimulation (2 Hz for 10 min., 5 min. after the tetanus) was not decreased in D slices.

One role of biogenic amines is to increase c-AMP production through D1 dopaminergic or β-adrenergic receptors. To test the role of c-AMP, we applied water soluble derivative of adenylate cyclase activator, forskolin (10 μM) for 5 min immediately before the tetanus to both C and D slices. Forskolin application did not affect baseline response in C or D slices. However it did significantly increase the magnitude of potentiation and decreased the level of depotentiation in D slices, resulting in values similar to control. In the C slices, on the other hand, effect of forskolin was the opposite: decrease of potentiation and increase of depotentiation levels.

This results suggest that under normal conditions, biogenic amines released during stimulation increase the magnitude of potentiation by a c-AMP -dependent mechanism.

523.6

FORSKOLIN-INDUCED POTENTIATION IN HIPPOCAMPAL DENTATE GYRUS INCREASES PHOSPHORYLATION OF CREB. I.M. Sarvey* and P.J. Voulalas. Dept. of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

A variety of stimuli activate pathways of signal transduction that utilize calcium and cAMP as their second messengers, resulting in an increase in gene expression in cultured cells. In the dentate gyrus, forskolin, which directly activates adenylyl cyclase, induces a long-lasting (> 1h) increase in excitatory synaptic transmission. We have investigated the ability of forskolin to (1) potentiate dentate responses and (2) produce changes in the phosphorylation state of the cAMP-response element binding protein (CREB) in the *in vitro* hippocampal slice. Electrophysiological analyses of forskolin-treated dentate "mini" slices were accompanied by Western blot analyses of extracts of the mini slices. Forskolin (50μM) was bath-applied to mini slices and samples were removed 5, 10, 15 and 20 min after application of forskolin, and 20 min after wash-out of the agent. Forskolin increased the slope of the dendritic epsp to >140% of baseline levels (n=4), and increased phosphorylation of CREB above basal levels. Phosphorylation of CREB on Ser^{133} peaked and declined within 20 min of forskolin application. This time course of activation of CREB by phosphorylation correlates with the time course observed in cultured cells and implicates CREB as a mediator of events whose signal originates at the cell membrane and culminates in transcription of genes required for long-lasting potentiation. Supported by NIH NS23865.

523.7

GLUTAMATE RECEPTOR STIMULATION INCREASES THE PHOSPHORYLATION OF PRE- AND POSTSYNAPTIC PKC SUBSTRATES IN HIPPOCAMPAL SLICES. G.M.J. Ramakers*, P. Pasinelli, W.H. Gispen and P.N.E. De Graan. Rudolf Magnus Institute for Neurosciences, P.O. Box 80040, 3508 TA Utrecht, The Netherlands. (SPON: European Neuroscience Association)

Activation of protein kinase C (PKC) is thought to be an important step in long-term potentiation (LTP). Both pre- and postsynaptic PKC appear to be involved, because LTP induction in the CA1 field of the hippocampus increased the phosphorylation state of the presynaptic protein B-50 (a.k.a. GAP-43, F1) as well as that of the postsynaptic protein RC3 (a.k.a. neurogranin, BICKS) by an NMDA receptor dependent mechanism (Ramakers et al., J. Biol. Chem., in press). In this study we investigated the effect of glutamate receptor (GluR) activation on pre- and postsynaptic PKC activity, by measuring the in situ B-50 and RC3 phosphorylation. Rat hippocampal slices (450 μm) were labelled with $^{32}P_i$ (100 μCi/ml) for 90 min at 30° C and the in situ phosphorylation state of B-50 and RC3 was determined after quantitative immunoprecipitation and SDS-PAGE by phosphorimaging. Treatment with Glu increased the phosphorylation of B-50 and RC3 in a concentration- and time-dependent manner. This 1 mM Glu-induced increase in B-50 and RC3 phosphorylation could be inhibited by MCPG (500 μM), an antagonist for the metabotropic GluR (mGluR). Antagonists for the ionotropic glutamate receptors, CNQX (50 μM), an AMPA receptor antagonist, and APV (100 μM), an NMDA receptor antagonist, hardly affected the Glu-induced increase in RC3 and B-50 phosphorylation.

Our results show that activation of mGluR increases the phosphorylation state of both pre- and postsynaptic PKC substrates. These findings indicate an important role for mGluRs in regulating the phosphorylation state of both pre- and postsynaptic PKC substrates in the maintenance phase of LTP. (This work was supported by an ENP grant of the ESF).

523.8

DIFFERENTIAL EFFECTS OF DEPOLARISING STIMULI ON THE PHOSPHORYLATION STATE OF PRE- AND POSTSYNAPTIC PKC SUBSTRATES B-50 (GAP-43) AND RC3 (NEUROGRANIN). P. Pasinelli, G.M.J. Ramakers, M. van Beest, W.H. Gispen and P.N.E. De Graan* Rudolf Magnus Institute for Neurosciences. P.O. Box 80040, 3508 TA Utrecht, The Netherlands.

RC3 (a.k.a. neurogranin, BICKS) and B-50 (a.k.a. GAP-43, neuromodulin, F1) are neuronal members of a new protein family sharing an amino acid sequence, the IQ domain, containing a PKC phosphorylation site and a calmodulin (CaM)-binding domain. Recently, we have shown that induction of LTP induced an increase in the in situ phosphorylation of both proteins (Ramakers et al., J. Biol. Chem., in press). In the present study we investigated the effect of various depolarising stimuli on the phosphorylation state of B-50 and RC3. Rat hippocampal slices (450 μm) were labelled with $^{32}P_i$ (100 μCi/ml) for 90 min at 30° C and the in situ phosphorylation state of B-50 and RC3 was determined after quantitative immunoprecipitation and SDS-PAGE by phosphorimaging. Treatment of slices with K^+ (30 mM) or the K^+-channel blocker 4-Aminopyridine (100 μM) increased the phosphorylation state of B-50 without affecting RC3 phosphorylation. To verify that RC3 phosphorylation can be modulated under our experimental conditions, we treated slices with the phorbolester 4-α-phorbol 12,13 dibutyrate (PDB) or the phosphatase inhibitor okadaic acid. PDB induced a concentration- and time-dependent increase in both RC3 and B-50 phosphorylation, which could be prevented by PKC inhibitors. Okadaic acid (10^{-6} M) increased both B-50 and RC3 phosphorylation in a time- and concentration-dependent manner, indicating that PP1 and PP2A are involved in the regulation of the in situ phosphorylation state of both proteins. Cyclosporin A, an inhibitor of phosphatase PP2B, had no effect on basal B-50 or RC3 phosphorylation. In vitro PKC phosphorylated RC3 was dephosphorylated by endogenous okadaic acid sensitive phosphatases, as well as by purified PP2B. Our data show that RC3, like B-50, is an in situ PKC and PP1/2A substrate. Depolarising stimuli, which increase presynaptic B-50 phosphorylation, do not affect postsynaptic RC3 phosphorylation, indicating that the phosphorylation state of these proteins is differentially regulated. (This work was supported by an ENP grant of the ESF).

523.9

MODULATION OF PERTUSSIS TOXIN-SENSITIVE G-PROTEINS BY RC3/NEUROGRANIN

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RC3/neurogranin is a postsynaptic, forebrain-enriched protein distinguished by an "IQ" motif containing overlapping sites for PKC phosphorylation and calmodulin-binding (in the absence of Ca^{2+}). RC3 has also been implicated in synaptic plasticity based on its phosphorylation by PKC during NMDA receptor-mediated LTP in CA1 of hippocampus. Thus, it is essential to identify the functional consequences of RC3's phosphorylation, specifically its postsynaptic protein-protein interactions. Here we use two different experimental approaches to show that RC3 enhances pertussis toxin (PTX) effects on G-proteins. First in functional studies, RC3 increases the PTX sensitivity of serotonin-evoked IP3/Ca^{2+}-dependent Cl^- currents in *Xenopus* oocytes co-expressing $5HT_{2C}$ receptors with RC3 (control $5HT_{2C}$ receptor, 323±65 nA, n=5; $5HT_{2C}$ receptor + PTX, 260±24.2 nA, n=4,T=0.821, P<0.4) (control $5HT_{2C}$ receptor + RC3, 371±61.5 nA, n=5; $5HT_{2C}$ receptor + RC3 + PTX, 135±51.4 nA, n=4, T=2.84, P<0.025). Second, biochemical studies show that a full-length RC3 synthetic peptide (RC3-78, 0.001-10 μM), when PKC-phosphorylated, increases PTX-catalysed ADP-ribosylation of Go/Gi in hippocampal membrane-fractions (1.4-2.5 fold). The results together suggest that RC3 interacts with Go proteins and possibly Gi proteins in dendritic spines in forebrain neurons, where it may modulate the G-protein coupling of metabotropic glutamate receptors to phospholipase Cβ and IP3/Ca^{2+} production in response to NMDA receptor activation during LTP, and potentially in learning and memory.

523.10

METABOLIC INACTIVATION OF ASTROCYTES IMPAIRS HIPPOCAMPAL LTP IN VITRO Paul H.M. Kullmann* and Christian M. Müller. Max-Planck-Institute for Developm. Biol., 72076 Tübingen, Germany

Increasing evidence suggests intimate glia-neuronal interrelations. Thus, astrocytes respond to a variety of neurotransmitters and affect neuronal excitability, e.g. due to their role in ion, pH, and transmitter homeostasis. The functional links of these glia-neuronal interactions are still poorly understood. We therefore investigated whether astroglia are implicated in neuronal transmission and the induction or expression of long-term potentiation (LTP) in rat hippocampus. We recorded field-EPSP's to stimulation of two separate afferent pathways to the CA1 region of hippocampal slices of 4-8 week old rats. One afferent was used to monitor the capability of each slice to express stable LTP upon theta-burst stimulation (TBS, 3 bursts of 2 s applied within 20 min). Thereafter 2 mM Fluoroacetate (FA), which selectively inhibits the astroglial Krebs cycle was added to the superfusate. After at least 1 h of FA-application the second pathway was tetanized. In about 80% of the slices tested, TBS resulted only in short-term potentiation which disappeared within 1 h. However, LTP-induction by pairing low-frequency stimulation with coincident intracellular current injection showed a comparable occurence and amplitude of potentiation in control and FA-treated slices. This indicates that metabolic inactivation of astrocytes does not interfere with the manifestation of LTP, but rather with an early step in the induction cascade. Intracellular recordings revealed no significant differences in the response-amplitudes elicited by TBS in control solution and after FA-treatment. Thus, the effect on LTP is not simply due to a transmitter depletion caused by a lack of the astroglia-derived glutamate precursor glutamine. Current studies assess the possibility that astroglial intoxification may selectively reduce NMDA-mediated responses, or enhance GABAergic response components. Both effects could account for the observed failure of TBS-induced LTP versus a successful pairing-induced LTP. (supported by DFG Mu 908 / 2-2)

523.11

ADHESION MOLECULES & HIPPOCAMPAL PLASTICITY: EVIDENCE THAT A SELECTIVE, DELAYED CHANGE IN NEURAL CELL ADHESION MOLECULE-180 ($NCAM_{180}$) IS ASSOCIATED WITH LTP MECHANISMS.

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The NCAM family of adhesion molecules consists of three main isoforms of 180, 140, and 120 kDa, some of which are enriched in postsynaptic densities (Persohn et al., J. Comp. Neurol. 288:92, 1989) and synaptosomal membranes (Bahr et al., Brain Res. 628:286, 1993). Interactions between NCAMs have been implicated in memory consolidation (Doyle et al., J. Neurosci. Res. 31:513, 1992) and, recently, in LTP (Lüthl et al., Nature 372:777, 1994). To determine if NCAM isoforms are modified by certain synaptic plasticity mechanisms, experiments utilized long-term hippocampal slice cultures in which NCAMs and other synaptic and structural proteins are stable for at least two months (Bahr, J. Neurosci. Res. in press, 1995).

Brief stimulation of the hippocampal slice cultures with NMDA and rapid AP5 quenching, a paradigm shown previously to produce LTP (Thibault et al., Brain Res. 476:170, 1989), generated a gradual increase in $NCAM_{180}$ on immunoblots. The optical density X area for the 180 kDa band was 5-50% greater than that in sister cultures treated without NMDA ($p = 0.01$, paired t-test, n=17). The NMDA-induced enhancement of $NCAM_{180}$ immunoreactivity was more evident at 2 hours than 10 min post-stimulation, and within-sample analyses demonstrated that no corresponding change in $NCAM_{140}$ occurred. Moreover, the delayed increase was blocked by prior AP5 treatment, and was a transient change as evidenced by a decay phase during the 6-18 hour period post-stimulation. The NCAM modulation required a short receptor stimulation duration (10-30 sec) since extended exposures to NMDA induced a decrease instead of an increase in $NCAM_{180}$, an effect that was also AP5-sensitive. These results support hypotheses which implicate reorganization of synaptic morphology in LTP, and further suggest that such mechanisms involve adhesion molecules (supported by AFOSR 89-0383 and NSF IBN-9220156).

523.12

ADHESION MOLECULES & HIPPOCAMPAL PLASTICITY: INTEGRIN-TYPE MATRIX RECOGNITION CONTRIBUTES TO THE STABILIZATION OF LTP, POSSIBLY INVOLVING THE RGDS-SELECTIVE MATRIX RECEPTOR *F55*.

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Synapse modulation, selection, and formation processes are frequently invoked to explain certain types of synaptic plasticity and memory encoding. Correlates of such processes are evident during neurodevelopmental events that involve at least two classes of adhesion molecules. The first is a subgroup of the immunoglobulin superfamily known as neural cell adhesion molecules (NCAMs) and which have recently been implicated in long-term potentiation (Lüthl et al., Nature 372:777, 1994). The second is the integrin family of matrix receptors whose antagonists RGDS (Staubli et al., Behav. Neural Biol. 53:1, 1990) and GRGDSP (Xiao et al., NeuroReport 2:461, 1991) inhibit LTP stabilization. Studies (Grooms et al., Exp. Neurol. 122:253, 1993) have also indicated that hippocampal synapses contain integrin-like proteins that bind RGDS peptides (e.g., *F55*; Bahr & Lynch, Biochem. J. 281:137, 1992).

The present study tested if integrins operate during a different LTP stage than that determined for NCAMs, and whether candidate LTP stabilizers are identified with RGDS-affinity chromatography. Hippocampal slices treated with RGDS-peptides starting 10 min after LTP induction (n=13) exhibited control-level LTP (n=17) for 30 min but then gradually decayed by 40% ($p < 0.01$). In contrast, Lüthl et al. demonstrated that post-induction dissociation of NCAMs did not destabilize previously established potentiation. RGDS-peptides also eluted the 55 kDa *F55* and faint 27 and ~120 kDa proteins from GRGDSP-sepharose; *F55* was labeled by antibodies to the $\alpha_5\beta_1$ integrin and co-migrated with synaptophysin during synaptosome isolation. Similar proteins were eluted with GAVSTA which interacts with the RGDS region of matrix proteins; correspondingly, the GAVSTA peptide also blocked stable LTP by 51% ($p < 0.001$; n=16). Thus, integrin-type matrix interactions via *F55* may be involved in the functional reorganization of synaptic contacts (AFOSR 89-0383).

523.13

CALPAIN-MEDIATED PROTEOLYSIS AND LTP: IS CLEAVAGE OF THE SPECTRIN-BASED CYTOSKELETON DURING INDUCTION A PREREQUISITE FOR STABILIZATION?

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The structural correlates of LTP suggest that cytoskeletal modifications are involved in LTP stabilization. Calpain 1, a Ca^{++}-dependent protease with multiple cytoskeletal substrates, is present in dendrites and has an activation threshold within the range (low μM) of Ca^{++} concentrations achieved postsynaptically during high frequency stimulation. To investigate the potential role of calpain-mediated cytoskeletal cleavage in LTP, we sought to determine if calpain-mediated proteolysis is triggered by theta-burst stimulation (TBS), and if activation of the protease is necessary for the stable expression of potentiation. Using an antibody to the C-terminal calpain cleavage product of spectrin, we found that application of TBS to Schaffer-collateral axons in cultured hippocampal slices results in the accumulation of spectrin breakdown products (BDPs) in post-synaptic elements. Labelling occurred in the form of scattered puncta and immunostained dendritic processes in the stratum radiatum. The extent of these effects was correlated with the amount of TBS delivered using a six-point rating scheme ($r=0.73$); slices receiving low frequency stimulation exhibited uniformly lower BDP staining ($p < 0.001$). Cultured slices were also treated (5 days, 25 μg/ml) with antisense or sense oligonucleotides directed at calpain 1 mRNA. Antisense treatment reduced calpain 1 expression by approximately 50% as measured by activity and immunoblot assays. Following treatment, we attempted to induce LTP in antisense and control slices. The short-term effects of TBS (< 10 min) were not different between groups. However, EPSPs measured 30 min post-TBS were significantly less potentiated in antisense slices compared to controls (108±3.9% vs. 133±6.3% of baseline; $p = 0.003$, n=10 each). These results indicate that a cytoskeletal element, spectrin, is cleaved by calpain in response to LTP-inducing stimulation, and that this event may be a prerequisite for stabilizing mechanisms (supported by grants AFOSR 92-J-0307 and NIH AG00538 to G.L. and C.G.).

POTASSIUM CHANNEL STRUCTURE, FUNCTION AND EXPRESSION III

524.1

SUBSTITUTION OF G-PROTEIN BINDING DOMAINS ON THE C-TERMINUS ALTERS PORE PROPERTIES OF GIRK1 :

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Previously we have identified a region on the C terminus of GIRK1(CCα) capable of participating in the formation of coiled-coils and which we hypothesize is a G$\beta\gamma$ binding domain. To test our hypothesis, we created a chimeric channel in which CCα was replaced by the $\beta\gamma$ binding site from the C terminus of βARK. Ile 331 was replaced by Arg and Asn 384 was replaced either by Arg or Gly. Co-expression of GIRK-βARK I331R/N384G with M2 yielded an inwardly rectifying Icarb 25% of wild type current amplitude. N384R produced a non-functional mutant. We observed that 4% of Icarb was sensitive to block by 100 μM Ba^{++} in the external solution for GIRK-βARK I331R/N384G as opposed to the 98% block observed for GIRK-C. A significant leftward shift in the current-voltage relationship was observed for GIRK-βARK I331R/N384G. The current activation kinetics were also altered by substitution of the βARK G$\beta\gamma$ binding domain for CCα. The time course of current activation for GIRK1 has three components: instantaneous, fast (τ_1), and slow (τ_2). The initial instantaneous component of activation is absent in GIRK-βARK I331R/N384G. In addition, τ_1 is 9.4 times slower and τ_2 is 5.3 times slower than what is observed for GIRK1. The desensitization of wild-type currents typically observed with prolonged exposure to carbachol is replaced by a 200% potentiation of the carbachol induced currents in the GIRK-βARK chimeras. The dramatic changes observed in the biophysical properties of this chimera as compared to GIRK1 indicate that this region may not only be a G$\beta\gamma$ binding site critical for channel activation, but that it may also be part of the pore itself.

524.2

A NOVEL INWARD RECTIFIER POTASSIUM CHANNEL EXPRESSED PREDOMINANTLY IN GLIAL CELLS.

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We have isolated a novel inward rectifier K^+ channel predominantly expressed in glial cells of the central nervous system. Its amino acid sequence with a Walker type-A ATP-binding domain exhibited 53 % identity with ROMK1 and approximately 40 % identity with other inward-rectifier K^+ channels. We designate this new clone as K_{AB}-2 (the second type of inward-rectifying K^+ channel with an ATP-binding domain). *Xenopus* oocytes injected with cRNA derived from this clone expressed a K^+ current which showed classical inward rectifier K^+ channel characteristics. *In situ* hybridization showed K_{AB}-2 mRNA to be expressed predominantly in glial cells of the cerebellum and forebrain. Therefore, the K_{AB}-2 channel is, for the first time, identified as a glial cell inward-rectifier potassium channel, which may be responsible for K^+ buffering action of glial cells in the brain.

524.3

CLONING AND LOCALIZATION OF TWO BRAIN-SPECIFIC INWARDLY RECTIFYING POTASSIUM CHANNELS. Noam A. Cohen*, David S. Bredt, David J. Liss, and Solomon H. Snyder. Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Recently, a new family of genes encoding inwardly rectifying K^+ channels have been cloned. Utilizing a PCR based cloning strategy, with degenerate primers designed against conserved regions of the inwardly rectifying K^+ channels, two new members of the family have been identified in rat. Northern blot analysis of these clones demonstrates that they are localized selectively to the brain and are designated Brain Inwardly Rectifying K^+ (BIRK) channels. No expression is detected in kidney, spleen, liver, heart, or skeletal muscle. BIRK1 occurs in the brain at high density and is particularly enriched in the brainstem. BIRK2 is far less abundant and is most prominently expressed in the cerebral cortex, with lower levels in the corpus striatum and hippocampus. *In situ* hybridization for BIRK1 reveals neuronal distribution with highest densities of BIRK1 expression occurring in the area postrema, and lower densities in specific brain stem nuclei, including the vestibular nuclei, the motor and spinal trigeminal nuclei, and the ventral cochlear nucleus. *In situ* hybridization for BIRK2 also displays a discrete localization consistent with a neuronal distribution with highest levels in the forebrain. High levels of expression occur in the cerebral cortex with the most prominent labeling in the superficial layers. In the hippocampus, the dentate gyrus is most prominently labeled with levels in the CA-1-CA-3 regions being substantially less intense. Immunoprecipitation of BIRK1 reveals a band of approximately 70 kDa. Treatment of the immunoprecipitated protein with glycosidases increases the mobility of BIRK1 to a band of approximately 64 kDa.

524.4

GENOMIC STRUCTURE OF THE HUMAN G PROTEIN ACTIVATED POTASSIUM CHANNEL HGIRK1. †# O. Schoots*, $ T. Voskoglou and †$ H.H.M. Van Tol # Rudolf Magnus Institute, Utrecht, The Netherlands; $ Dept. of Pharmacol., Univ. of Toronto; †Clarke Inst. of Psychiatry, Toronto, Ont. Canada, M5T 1R8.

HGIRK1 is a human inwardly rectifying potassium channel which is opened by free G protein βγ dimers generated through the activation of various G protein linked receptors. This activation is mediated through a membrane-delimited pathway that does not involve cytoplasmic intermediates. We studied the genomic structure of HGIRK1. Southern blot analysis of human genomic DNA with a 2.9 kb HGIRK1 cDNA as a probe indicated the presence of at least two introns. Screening of a human genomic library with this cDNA resulted in the isolation of several positively hybridizing clones. Analysis of these clones showed that the gene contained three exons and two introns. The total size of the HGIRK1 gene exceeds 65 kb. In the exons three polymorphisms were found as compared to the HGIRK1 cDNA clone. The transcription initiation site was established by rapid amplification of the 5' end of the HGIRK1 cDNA and S1 nuclease protection assays. Sequences upstream of the mRNA transcription start site were analysed for promoter activity. Promoter constructs with β-galactosidase and luciferase reporter genes were made and tested in various cell lines.

524.5

INWARDLY RECTIFYING K^+ CHANNELS ARE LOCALIZED IN MICROVILLI OF SCHWANN CELLS AND MAY PARTICIPATE IN K^+ BUFFERING. H. Mi[1], T.J. Deerinck[2], M. Jones[1], M.H. Ellisman[2], T.L. Schwarz[1]*. [1]Dept. of Molecular and Cellular Physiology, Stanford Univ., Stanford CA 94305 and [2]Dept. of Neurosciences, UCSD, La Jolla CA 92093.

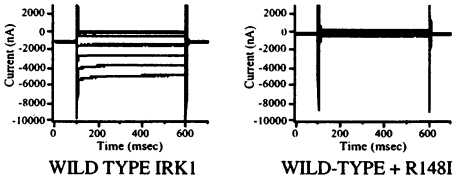
The presence of K+ channels in Schwann cells suggests that these cells may participate actively in action potential propagation in the peripheral nervous system. One previously suggested possibility is the buffering of extracellular K+ to prevent accummulation of K+ during axonal repolorization. An inwardly rectifying K+ channel that could mediate this effect has been recorded in Schwann cell membranes near the node of Ranvier.

We have identified two inwardly rectifying K+ channels, IRK1 and IRK3, that are expressed in adult rat Schwann cells. Immunocytochemistry with a polyclonal antibody localized these channels to nodes of Ranvier in sciatic nerve. Immunoelectron microscopy demonstrated that these K+ channels are highly concentrated in microvilli (also called nodal processes) that protrude from the surface of the Schwann cell at the node and fill much of the nodal cleft. This localization is distinct from that which we had previously observed for the delayed rectifier channel Kv1.5; Kv1.5 is also concentrated near the node, but it is not abundant in the microvilli. These microvilli have a large surface area and are well situated for buffering extracellular K+. The presence withing these structures of channels that are capable of permitting K+ influx at resting membrane potentials lends support to the K+ buffering hypothesis and indicates that IRK1 and IRK3 are likely to mediate this effect. The restricted distribution of these channels underscores how tightly channel localization is controlled in cells and how specialized the membrane domains of Schwann cells are for proper function.

524.6

CO-INJECTION OF PORE MUTANTS WITH WILD TYPE IRK1 BLOCKS CHANNEL EXPRESSION IN *XENOPUS* OOCYTES J.L. Kugler*, W.W. Xie, L.H. Philipson, and D.J. Nelson, University of Chicago, Chicago, IL 60637.

We have identified a critical residue in the pore region of the inward rectifier IRK1, Arg148, which when mutated to Met, Ile, Asn, His, Glu, or Ala, produces non-functional channels in *Xenopus* oocytes. To investigate the ability of these non-expressing pore mutants to act as dominant negatives, we co-injected them with wild type mRNA. When mRNA coding for the R148H and R148I mutants was injected at 1:1 with wild type mRNA, channel function was blocked. Co-injecting non-expressing C-terminally truncated channels had no effect on wild type currents. This indicates that 1) the site-directed mutants are being properly synthesized, and 2) the mutants are coassembling with the wild-type subunits and are blocking function at some point. We hypothesize that this residue may be interacting with Glu139 to form a structurally stabilizing salt bridge, possibly between adjacent subunits.



WILD TYPE IRK1 WILD-TYPE + R148I

Currents recorded in 50 mM K^+ by TEVC of *Xenopus* oocytes.

524.7

G PROTEIN-ACTIVATED INWARDLY RECTIFYING POTASSIUM CHANNELS EXPRESSED IN *XENOPUS* OOCYTES ARE INHIBITED BY ANTISENSE cDNA. M. U. Ehrengruber, L. Byerly*, P. Kofuji, Y. Xu, C. Lin, H. A. Lester and N. Davidson. Div. Biology, Caltech, Pasadena, CA 91125.

G protein-activated inwardly rectifying potassium channels (GIRKs) play a crucial role in determining cellular excitability. GIRK1 was originally cloned from rat atrium where it occurs as a heteromultimer with CIR, a member of the GIRK1 gene family. The family members GIRK1, GIRK2, and GIRK3 are found in the brain. We plan to use antisense cDNA plasmids to construct recombinant adenovirus for *in vivo* suppression of GIRKs, in order to identify their role in neuronal synaptic transmission. In preparation for these experiments, GIRKs were cloned into expression plasmids, injected together with the m2 acetylcholine receptor into the nucleus of *Xenopus* oocytes, and tested functionally using the two-electrode voltage clamp technique. We also constructed plasmids carrying antisense cDNA for GIRKs. To monitor the efficiency of nuclear injection, Shaker potassium channel cDNA was coinjected. Injection of GIRK1 or GIRK2 resulted in acetylcholine-sensitive GIRK currents which strongly correlated with Shaker currents. The ratio of GIRK2 currents at -120 mV to Shaker currents at 40 mV was 0.044, while it was only 0.013 when an equal amount of antisense GIRK2 was also injected. We are now testing different sense/antisense ratios, antisense cDNA lengths, GIRKs, and GIRK combinations. Our results demonstrate that specific antisense cDNA is efficient in suppressing GIRK currents, and that nuclear injection of *Xenopus* oocytes is useful to test antisense cDNA eukaryotic expression vectors in anticipation of studies applying antisense knockout of cDNA function in neurons and other cells. Supported by the Swiss National Science Foundation, NIMH, NIGMS, and HFSP.

524.8

MONITORING CHANGES IN PROTON METABOLISM ENABLES A FUNCTIONAL ANALYSIS OF INWARDLY RECTIFYING POTASSIUM CHANNELS EXPRESSED IN YEAST. K.M. Hahnenberger[1], S. Kurtz[2], T. Hoshi[3] and D.L. Miller[1]*. [1] Molecular Devices Corporation, Sunnyvale, CA 94089. [2] Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543. [3] Dept. of Physiology, University of Iowa, Iowa City, IA 52242.

It has previously been shown that expression of mammalian cardiac inwardly rectifying K channels and a member of the Shaker superfamily of K channels from *Arabidopsis thaliana*. complement a defect in potassium uptake in yeast strains lacking both high and low affinity potassium transporters. In yeast, K is taken up in a 1:1 ratio with extrusion of protons. The plasma membrane H-ATPase establishes a highly negative electrical potential across the membrane, which presumably maintains the inwardly rectifying K channels in the open state.

We have utilized the tight coupling between potassium uptake and proton extrusion in yeast to analyze the function of inwardly rectifying K channels using microphysiometry. The microphysiometer is a silicon sensor-based instrument which measures the rate at which cells acidify their external environment and can be used to detect small changes in proton fluxes across the plasma membrane. Exposure of yeast cells expressing a guinea pig cardiac IRK1 homologue or the *A. thaliana* KAT1 gene to channel blockers such as Cs and TEA results in a rapid and transient decrease in proton flux. Removal of the channel blockers results in a transient increase in acidification rates. The effectiveness of the channel blockers varied; the strain expressing the inward rectifying K channel exhibits a greater response to Cs while the strain expressing the plant Shaker channel was more affected by TEA. This system can be used to perform mutational analysis of ion channel function and to identify compounds which modulate ion channel activity.

524.9

PARTIAL CLONING AND CHARACTERIZATION OF A TWO-TRANSMEMBRANE DOMAIN K^+ CHANNEL IN CHICK. C.W. Lu* and S. W. Halvorsen. Dept. of Biochem. Pharmacol., SUNY at Buffalo, Buffalo, NY 14260.

While the function of ATP-sensitive potassium channels (K^+ATP) in controlling insulin release in pancreas has been described, little is known about their roles in the nervous system or muscle. Recently, K^+ channels (cKATP-1/CIR, uKATP-1) containing two putative transmembrane domains were cloned from rat heart. Whether they are K^+ATP or muscarinic K^+ channel components is still unresolved. We are interested in investigating the regulation of expression and function of these channels in the developing chick heart and nervous system. Degenerate oligonucleotide primers were designed from homologous regions of the N-terminal and pore-forming regions of cKATP-1 and other two-transmembrane domain K^+ channels and used for RT-PCR with total RNA isolated from embryonic chick heart. A 0.44 kb product was obtained whose DNA sequence showed the encoded peptide had 90% identity with cKATP-1. This DNA fragment (C1t3KATP) represented 1/3 of the putative full reading frame. Northern blot analysis using ^{32}P-labeled C1t3KATP revealed a 2.7 kb message in ciliary ganglion, heart, leg and brain (in the lowest to highest expression order).

We are examining the regulation of this chick K^+ channel by electric activity, metabolic states and ligands associated with K^+ channel actvivity.

524.10

G-PROTEIN α-SUBUNIT SELECTIVITY OF A CLONED INWARDLY RECTIFYING K^+ CHANNEL (GIRK) EXPRESSED IN *XENOPUS* OOCYTES. G.R. Seabrook*, N.A. Brown, D. Weinberg[2], G. Milligan[3], and G. McAllister. Merck Sharp & Dohme, Terlings Park, Eastwick Rd, Harlow Essex, CM20 2QR U.K. [2]Merck Research Laboratories, Rahway, NJ 07065 and [3]Univ. Glasgow, Glasgow, G12 8QQ, Scotland., U.K.

This study investigated the selectivity of G-protein α subunits for G-protein gated inwardly rectifying K^+ channels (GIRK) expressed in *Xenopus* oocytes using conventional two-electrode voltage-clamp recording techniques. The cDNA encoding GIRK was subcloned from a rat brain library and co-expressed with the cDNA for either G-protein α_o, αi1, αi2, or αi3 subunits, as well as one of a series of different G-protein linked neurotransmitter receptors. These included the human $D_{2(short)}$ and human NPY_1 receptors. Cells were voltage-clamped at -80 mV and after equilibration in high external K^+ (96 mM) to reverse the K^+ ion gradient (e.g. Dascal *et al.*, 1993 P.N.A.S. 90, 10235-10239) the ability of receptors to activate inward currents was examined. No effects of D_2 or NPY_1 receptor agonists were observed in control experiments (GIRK + alternative receptor). Co-expression with G-protein α subunits differentially increased the efficiency of receptor mediated activation of GIRK channels depending upon the species of receptor involved. Thus it was unlikely that these variations were due to differences in efficiency of subunit expression, and most probably reflects the preferences of cloned receptors for different second messenger pathways.

524.11

5-HT-INDUCED INHIBITION VIA G PROTEINS OF AN IRK CHANNEL CLONED FROM RAT BASOPHILIC LEUCEMIA CELLS. E. Wischmeyer, E. Dißmann, K.-U. Lentes* and A. Karschin. Max-Planck-Inst. for biophysical Chemistry, 37077 Göttingen, FRG.

The basophilic leucemia cell line RBL-2H3 exhibits a robust inwardly rectifying K^+ current, I_{KIR}, which features steep voltage dependence and is under the inhibitory control of an as yet unidentified signaling pathway. We have isolated by PCR amplification from RBL cDNA an IRK-type channel (RBL-IRK1) which showed 94% base-pair identity to the mouse IRK1 (s. Wischmeyer et al., 1995, Eur. J. Physiol. 41:809). When transiently transfected in COS cells, the expressed 25 pS RBL-IRK1 channels gave rise to currents with single channel and macroscopic properties identical to those in RBL cells. Intracellular perfusion for 10 min with GTPγS in the whole-cell mode of patch-clamp recording suppressed I_{KIR} by 50-80%. When COS cells were cotransfected with 5-HT1A receptors, application of 100 µM 5-HT (in 12/24 cells) also induced a current inhibition by 40.3±21.8% which could be mimicked by the selective 5-HT1AR agonist 8-OH DPAT and antagonized by 100 µM pindolol. More consistent and more pronounced current inhibition resulted from the simultaneous administration of 5-HT and the membrane-permeable 8-bromo-cAMP. Whereas 8-bromo-cAMP (100 µM) alone did not consistently affect I_{KIR}, coapplication with 50 µM 5-HT induced a block by 90.6±9.3% in 80% of the cells. The time constants of onset and relaxation from block were equally slow (τ~5 s). Thus, recombinant IRK-type channels in COS cells may be negatively controlled by a signaling process that possibly involves PKA-dependent phosphorylation.

524.12

EXPRESSION AND CELLULAR LOCALIZATION OF SIX MEMBERS OF THE GIRK AND IRK FAMILY OF INWARDLY RECTIFYING K^+ CHANNELS. C. Karschin, E. Dißmann[1], W. Stühmer and A. Karschin[1]* MPI for Experimental Medicine and [1]Biophys. Chemistry, 37077 Göttingen, FRG.

The activity of inwardly rectifying K^+ channels is either controlled by the action of G proteins (GIRK-type) or is primarily dependent on the membrane potential (IRK-type). Molecular cloning has shown that each subfamily consists of several closely related members encoded by separate genes. In situ hybridization for 2 channels and Northern blot analysis indicate that GIRK1 and IRK2 subunits may exist in the rat brain and GIRK2, GIRK3, IRK1 and IRK3 in the mouse brain. This study provides evidence for the differential expression of all six subunits in the adult rat brain. In addition to the molecular cloning of the rat homologues from a brain cDNA library, we have performed an in situ hybridization study to evaluate the mRNA expression and cellular localization. Using specific [^{35}S]-labeled oligonucleotide probes directed to non-conserved regions, we detected wide distribution with partly overlapping expression of the mRNAs of GIRK1,2,3 and IRK1,2,3. The distribution patterns of the GIRK subunits were very similar with high levels of expression in the cerebellum, olfactory bulb, cortex, thalamus and hippocampus. Marked differences existed in thalamic, hypothalamic and brainstem nuclei, e.g. both GIRK1 and GIRK2 were strongly expressed in the septum, where GIRK3 was absent. In contrast, IRK subunits were more differentially expressed: all mRNAs were abundant in dentate gyrus, caudate putamen, olfactory bulb and piriform cortex. IRK1 and IRK3 were restricted to these regions, but they were absent from most parts of the thalamus, cerebellum and brainstem, where IRK2 was predominently expressed. Overlapping expression in the same cells may point to functional consequences if certain channel subunits assemble as heteromultimers, as has been evidenced recently.

524.13

MUSCARINIC MODULATION OF INWARD RECTIFIER CURRENT IN RAT SYMPATHETIC NEURONS. Hong-Sheng Wang, Jane E. Dixon and David McKinnon* Dept. of Neurobiology and Behavior, State University of New York, Stony Brook, NY 11794

Intracellular recordings were made from rat sympathetic neurons in isolated superior cervical ganglia (SCG), celiac ganglia (CG), and superior mesenteric ganglia (SMG). Neurons were classified as either 'phasic' or 'tonic' based on their response to a maintained depolarizing current stimulus. An inward rectifier current was differentially expressed in phasic and tonic neurons. The inward rectifier conductance was 6.4 times larger in tonic neurons than in phasic neurons. The conductance was blocked by low concentrations of external Ba^{2+} and Cs^+ ions. The inward rectifier appeared to contribute to the resting membrane potential of tonic neurons.

The inward rectifier current was down-regulated by synaptic input. Repetitive stimulation of the input nerve trunk suppressed the inward rectifier conductance by 50-70%. The inhibition had a slow onset of 20-30 seconds, suggesting a second messenger mediated pathway. This synaptically mediated suppression was blocked by 1 µM atropine, and can be mimicked by bath application of muscarine. Application of 20 µM muscarine produced an average 78 ± 1.4% inhibition of the current. From dose-response curves for muscarine a mean dissociation constant of K_D = 1.95 ± 0.2 µM was determined. Schild plot analysis using the competitive antagonists pirenzepine and himbacine indicated that the effect of muscarine was mediated by the M1 class of muscarinic receptors. By down-regulating the inward rectifier, synaptic input probably increases the excitability of tonic neurons.

We have examined the expression of inward rectifier potassium channel genes in the sympathetic ganglia to determine which gene product underlies this current. We have also begun to reconstruct this neuromodulatory system in Xenopus oocytes to determine the molecular mechanisms involved.

524.14

IDENTIFICATION AND LOCALIZATION OF CA^{++}-ACTIVATED K^+ CHANNELS IN SCIATIC NERVES. R.M. Harris-Warrick[1]*, H. Mi[2], L. Inman[2], T.J. Deerinck[3], T.L. Schwarz[2], M.H. Ellisman[3]. [1]Dept. of Neurobiology and Behavior, Cornell Univ., Ithaca, NY 14853, [2]Dept. of Molecular and Cellular Physiology, Stanford Univ., Stanford CA 94305 and [2]Dept. of Neurosciences, UCSD, La Jolla CA 92093.

A large family of Ca^{++}-activated K^+ channels is encoded by the *slo* gene through alternative splicing. In order to understand the physiology of Schwann cells and myelinated nerve, we have studied the expression of this family of channels in peripheral nerve. By RT-PCR and hybridization screening of cDNA libraries of adult rat sciatic nerve, we cloned homologues of *slo* that were more than 95% identical to the murine *slo*. Though we had anticipated that this tissue might present a simpler array of gene products than the brain, we found instead that sciatic nerve RNA contained numerous alternatively spliced variants. There are three different regions in the channel that are subject to alternative splicing. Splice sites A and B were reported in both mouse and human *slo*. In rat sciatic nerve, splice site A has three forms with inserts of 0, 3, or 58 amino acids, and splice site B has two forms with either 0 or 26 amino acids. The third region, which was also seen in human *slo*, has two variants with either 0 or 4 amino acids (RSRK). We raised a polyclonal antibody against a peptide from the carboxyl terminal of the channel that is predicted to see all the forms of this channel. Immunocytochemistry revealed that the channel proteins are in Schwann cells and are associated with canaliculi that follow the outer surface of the cells. They are also relatively concentrated near the node of Ranvier in the Schwann cell outer membrane. The staining pattern is quite similar to what we previously reported for the voltage dependent K^+ channel Kv1.5. Electron microscopic studies are in progress to characterize the ultrastructural localization of the channels.

524.15

Modulation of large conductance Ca^{2+}-activated K^+ channel activity by charged lipids: structural requirements. Alison L. Clarke, Steven Petrou, John V. Walsh, Jr. and Joshua J. Singer*. Dept. Physiology, U. of Massachusetts Medical School, Worcester, MA 01655.

In a previous study carried out in toad stomach smooth muscle cells we showed that the features of the fatty acid (FA) required for its activation of a 50 pS K^+ channel were both a negatively charged head group and a sufficiently hydrophobic acyl chain (Petrou *et al.*, JGP, 103:471). In the present study we investigated whether similar structural features are required for modulation of the activity of a different K^+ channel; the large-conductance (270 pS) Ca^{2+}-activated K^+ channel (CAK) in the smooth muscle cells from the rabbit pulmonary artery (RPA). Three classes of analogs, structurally related to short and medium chain length FAs, as well as some naturally occurring lipids (10-50 μM) were applied to inside-out and outside-out patches. Compounds in which the negatively charged carboxylic acid head group is replaced with a negatively charged sulphonate group (alkyl sulphonates) mimic the action of FAs, increasing channel activity. Palmitoyl lysophosphatidate, a negatively charged naturally occurring lipid, also increased channel activity. Alkyl amines, in which the negatively charged head group is replaced with a positively charged ammonium or trimethylammonium group and the endogenous, positively charged lipid, sphingosine, suppressed channel activity. n-Alkanols, like dodecanol, which possess a neutral head group had no effect on channel activity. Because the CAK channel of the rabbit pulmonary artery is gated by both voltage and Ca^{2+}, the possibility that these charged lipids may act by changing the local $[Ca^{2+}]_i$ or the electric field within the membrane by affecting membrane surface charge needed to be addressed. FAs and FA analogs were effective in Ca^{2+} free solutions (0 mM Ca^{2+}, 5 mM or 20 mM EGTA) so that FA action via effects on local $[Ca^{2+}]_i$ seems to be ruled out. Moreover, responses were obtained in experiments in which FAs and other analogs were applied to patches in high ionic strength (330 mM KCl) bath and pipette solutions, to "shield" surface charge. This study shows that the structural features of FAs required for activation of the toad 50 pS K^+ channel are shared by the CAK channel of the RPA. The mechanism of FA action on the CAK channel appears to be direct suggesting that FAs and other naturally occurring lipids, like sphingosine and palmitoyl lysophosphatidate, may act as endogenous second messengers on this channel. NIH DK 31620.

524.16

MOLECULAR GENETIC STUDIES OF THE HUMAN CA^{2+}-ACTIVATED K^+ CHANNEL α AND β SUBUNITS. N. Godinot[1], P. Reinhart[3], A. Baktha[1], N. Shepherd[1], S. Stinnett[1], W. Kimmberly[1], R. Mertz[2], C. D. Foster[2], and J. Tseng-Crank[1]*. [1]Dept. of Molecular Genetics and [2] Dept. of Cell Physiology, Glaxo Research Institute, RTP, NC 27709 and [3]Dept. of Neurobiology, DUMC, P.O. Box 3209, Durham, NC 27710.

The Ca^{2+}-activated K^+ channels (K_{Ca}) regulate various physiological functions such as muscle contraction and neurotransmitter secretion by direct coupling of intracellular Ca^{2+} to membrane potential. We studied tissue specificity of channel isoforms and possible linkages of channel genes to inherited diseases in order to define a role for this channel in therapeutic exploitations. We have cloned the K_{Ca} channel α and β subunits from a human brain cDNA library and human genomic libraries. Both subunits are encoded by single genes, with multiple intron/exon structures for the α subunits and at least 2 introns interrupting the coding sequences of the β subunit. The α subunit is widely distributed, being found in virtually all tissues, while expression of the β subunit is restricted to smooth muscles and a few brain regions. Coexpression of both subunits may, therefore, offer functional specificity of the K_{Ca} channels in smooth muscles. We defined human chromosomal localizations by Southern hybridization to human-hamster somatic hybrid cell lines and by FISH analysis. The α subunit is located at chromosome 10q22.3 and the β subunit is located at chromosome 5q34. We are currently characterizing suitable microsatellite markers to carry out disease linkage analysis.
Work supported in part by NIH grant #NS31253 to PHR.

524.17

cSLO, A CALCIUM-ACTIVATED POTASSIUM CHANNEL GENE EXPRESSED IN CHICK'S COCHLEA. G.-j. Jiang, M. Zidanic, C. Griguer and P.A. Fuchs*, Dept. Physiol., U. Colorado Sch. Med., Denver, CO 80262.

Large conductance calcium-activated potassium channels (maxi-K^+ channels) contribute to electrical tuning of auditory hair cells in several nonmammalian vertebrate species. $I_{K(Ca)}$, flowing through maxi-K^+ channels, appears late during cochlear development in chicks, coincident with a period of rapid functional maturation. We have screened a cDNA library derived from hatchling chick's cochlear duct for clones with homology to *mslo*, a mouse brain homologue of the *Drosophila* gene *slowpoke* that codes for large conductance calcium-activated potassium channels (*mslo* provided by Dr. L. Salkoff, Washington U.). A full-length clone (3.9 kb) was isolated and sequenced and found to have 97% identity at the amino acid level with *mslo* over the S1-S6 transmembrane domains. In the extended carboxy terminus characteristic of slowpoke transcripts the degree of identity dropped to 93%. At the very 3' end *mslo* extends 53 amino acids further than *cslo*. *cslo* is better matched to the recently described human brain maxi-K channel *hbr*1 (Tseng-Crank *et al.*, 1994, *Neuron* 13:1315) at the 5' and 3' ends where both sequences diverge from that of *mslo*. Like *hbr*1, *cslo* contains no inserts at splice sites 1-4. It will be of interest to learn if transcription of a *cslo* variant underlies the developmental acquisition of $I_{K(Ca)}$. Supported by NIDCD DC 00276 and the National Organization for Hearing Research.

524.18

CLONING AND CHARACTERIZATION OF THE β-SUBUNIT OF A HUMAN MAXI-K CHANNEL Kimberly Folander* and Richard Swanson
Dept. of Pharmacology, Merck Research Labs, West Point, PA 19486

Large conductance Ca-activated potassium (maxi-K) channels have been demonstrated to be composed of two subunits (α and β). cDNAs encoding the β-subunit of a human maxi-K channel were isolated by screening a human aorta cDNA library and the deduced amino acid sequence is ~85% identical to the bovine protein. The gene encoding the beta subunit was mapped to human chromosome 5q34-q35 using both hybridization to DNAs derived from a panel of somatic cell hybrids and the identification of CEPH human megaYACs encoding this gene that were previously mapped to this region. Transcription of the gene was demonstrated, by Northern analysis, in a variety of human tissues with the highest levels in uterus, aorta, small intestine, colon, prostate, testis, and ovary. In most cases, the ratio of the α- and β-subunit mRNAs detected on Northern blots was equivalent from tissue to tissue. However, in several cases, the ratio varied significantly. For example, in brain and skeletal muscle, there was a preponderance of the α-subunit RNA whereas in heart, lung, and kidney, there was more β than α. These results suggest that coassembly of the α- and β-subunits may not be obligatory (ie., inclusion of the β-subunit might represent a molecular mechanism for the generation of functional diversity in maxi-K channels) or that the synthesis of functional channels in the brain, for example, may be limited by the amount of the β-subunit. Furthermore, the abundance of β-subunit mRNA in tissues such as heart suggests that this subunit might also be a structural component of other types of K^+ channels.

524.19

DIFFERENCES IN CALCIUM SENSITIVITY OF TWO HUMAN BK CHANNEL ALTERNATIVE SPLICE VARIANTS. S.I. Dworetzky*, J.T. Trojnacki, M.C. McKay, C.G. Boissard, J.E. Starrett and V.K. Gribkoff. CNS Drug Discovery, Dept. 405 Bristol-Myers Squibb Co., Wallingford, CT 06492.

Several lines of physiological evidence exist for the diversity of BK channels in native cells, yet to date, only 1 gene encoding the large conductance calcium-activated potassium channel has been cloned. Alternative exon splice variants, regulatory subunits, and the existence of additional family members are some of the possibilities that could explain the differences observed in calcium sensitivities, pharmacology, single channel conductance, and channel kinetics. We have cloned several *hSlo* splice variants and report on the calcium and pharmacological sensitivities of two isoforms. There exists two splice exons at junction A, 1 at junction B, and 1 at junction C. Splice exons A1 (3 aa), B1 (27 aa) and C1 (4 aa) are identical to the *mSlo* exons. The A2 splice exon, which codes for a 29 aa insert, is only 62% homologous to the equivalent *mSlo* exon. The two variants, *hSlo* (no splice exons) and *hSloA2* (containing the A2 exon) were investigated using the two-electrode voltage clamp configuration in oocytes and inside-out single channel recordings from transiently transfected HEK 293 cells. The current-voltage relationships of oocytes expressing *hSlo* and *hSloA2* were virtually identical under control conditions, as was their response to the benzimidazolone BK opener NS1619 and the specific peptidergic BK blocker iberiotoxin. The single channel conductance of the *hSlo* and *hSloA2* channels were both approximately 285 pS. However, a significant difference in their sensitivity to calcium was observed; the *hSloA2* channels were significantly more sensitive to calcium. These results demonstrate that the inclusion of the A2 exon confers an increased calcium sensitivity to the *hSlo* channel, although the functional consequences of this is not yet known.

525.1

CRITICAL RESIDUES FOR DETERMINING DIFFERENCES IN SINGLE CHANNEL CONDUCTANCE OF TWO CYCLIC NUCLEOTIDE-GATED ION CHANNELS. D.T. Liu, Z.-P. Sun, and S.A. Siegelbaum*. Integrated Program in Cellular, Molecular, and Biophysical Studies, Depts. Pharmacol., & Ctr. Neuro. & Behav., HHMI, Columbia University, N.Y., NY 10032

The single channel conductance of the catfish olfactory cyclic nucleotide-gated (CNG) ion channel (OLF, 83pS) is larger than that of the bovine retinal CNG ion channel (RET, 30pS). The H5 region determines the difference in single channel conductance between RET and OLF by determining the pore diameter (Goulding et al., Nature 364:61-64, 1993). In the H5 region, RET and OLF differ in only three amino acid residues: RET contains a serine, leucine, and threonine at positions 350, 351, and 364, respectively, whereas OLF contains cysteine, phenylalanine, and methionine at the corresponding positions. To determine which residues are critical in determining channel conductance, each of the three residues in RET was mutated individually to the corresponding residue in OLF. Mutant cRNA were injected into *Xenopus* oocytes, and single channel recordings were then obtained. When threonine 364 was changed to a methionine, the RET single channel conductance increased from 30pS to about 43pS. When leucine 351 was changed to a phenylalanine, the single channel conductance increased from 30pS to about 48pS. Thus, each of these residues by itself was not able to fully convert the conductance of RET to OLF (83pS). Studies of the serine 350 to cysteine mutant, double mutants, and the triple mutant are presently being performed.

525.2

CONSTRUCTION OF A REPLICATION DEFECTIVE HERPES SIMPLEX VIRUS TYPE 1 VECTOR ENCODING A I_A POTASSIUM CHANNEL. D.R. Beers,* L. Gui and J.L. Noebels. Dept. Neurology, Baylor Coll. Medicine, Houston, TX 77030.

The efficient introduction of genes into terminally differentiated cells is a powerful new method to study neuronal physiology. Herpes simplex virus type 1 (HSV-1) vectors can infect and persist in neurons, and therefore offer the ability to stably transfect foreign genes. Deletion of viral ribonucleotide reductase (RR^-) renders HSV-1 incapable of replicating in post-mitotic neurons. To modify membrane excitability by selectively overexpressing specific ion channels, an RR^- HSV-1 vector was constructed that encodes a genetically engineered non-inactivating I_A K^+ channel subunit. The insert T7-C210, encoding the I_A K^+ channel subunit with a modified 5' untranslated CITE sequence and a T7 amino acid idiotypic tag sequence (for the immunocytochemical localization of the expressed gene product), was excised from the plasmid pCITE-1 by *Eco*RI and *Not*I digestion. pKHFΔ, containing a 10.1 kb HSV-1 fragment that removes 90% of the large subunit of RR coding region, was linearized by digestion with *Bgl*II. The recessed 3'-ends of both T7-C210 and pKHFΔ were filled in with Klenow and then blunt-end ligated forming the plasmid $pK^+KHF\Delta$. Orientation of the K^+ channel subunit was determined by *Bam*HI and *Hind*III digestion. $pK^+KHF\Delta$ was linearized with *Eco*RI and then transfected into VERO cells with lipofectamine. Following a 5 hour incubation, the transfected cells were infected with hrR3, a HSV-1 virus encoding the *lacZ* gene at the RR locus. The recombinant virus that had lost the *lacZ* sequence due to homologous recombination was detected by screening progeny virus for white plagues against a background of blue plaques in the presence of X-Gal. This vector or another constructed identically that encodes a mutant K^+ channel subunit and forms a dominant-negative phenotype when combined with wild-type K^+ channel subunits, may up- and down-regulate, respectively, K^+ currents and membrane excitability in transfected cells. *Supported by National Multiple Sclerosis Society.*

525.3

SPECIFIC INTERACTION OF VOLTAGE-GATED K^+ CHANNEL α- AND β-SUBUNITS EXPRESSED IN TRANSFECTED CELLS. K. Nakahira*, G. Shi, S. Hammond, K. J. Rhodes and J. S. Trimmer, Dept. Biochem. and Cell Biol., SUNY at Stony Brook, Stony Brook, NY 11794 and CNS Disorders, Wyeth-Ayerst Res., Pearl River, NY 10965.

To investigate the specific interaction of voltage-gated K^+ channel α- and β-subunits, we have expressed the rat Kvβ1 and Kvβ2 K+ channel β-subunit polypeptides either alone or in the presence of α-subunits by transfection of mammalian cells. Kvβ1 and Kvβ2-transfected cells expressed polypeptides of molecular mass 44 kDa and 38 kDa, respectively, similar to the size predicted from the deduced primary sequence and the same size as proteins detected in rat brain by specific antibodies. Unlike Kvβ2, immunofluorescent localization of Kvβ1 showed intense fibrous staining of transfected cells, and Kvβ1 was found to partition into the detergent insoluble fraction of these cells. These findings suggested a unique association of Kvβ1 with a component of the cytoskeleton.

Co-immunoprecipitation of α- and β-subunits expressed in co-transfected cells showed the existence of specific subunit associations. Kvβ1 was co-immunoprecipitated with Kv1.1, Kv1.2, Kv1.3, Kv1.5 and Kv1.6, but not with Kv2.1 and Kv3.1. Kvβ2 showed the same binding specificity. This suggested restricted association of these β-subunits within the *Shaker* (Kv1) family of mammalian K^+ channels and the existence of a conserved molecular mechanism of α/β subunit association.

525.4

CYSTEINE OXIDATION MODULATES GATING OF Kv2.1

Juan M. Pascual* and Arthur M. Brown. College of Physicians and Surgeons, Columbia University, New York, NY 10032 and Rammelkamp Center, Case Western Reserve University, Cleveland, OH 44109.

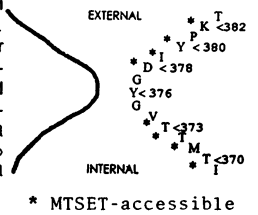
We have superfused methylmethanethiosulfonate (MMTS) to oocytes expressing Kv2.1 channels (WT). MMTS is uncharged and reacts with cysteinyl sulfhydryls transferring a S-methyl group. MMTS caused a pronounced slowing of current activation and inactivation. This effect was absent in Kv3.1 channels. Charged MMTS analogs had no effect on Kv2.1 (*Pascual* et al., Neuron in press). To identify the MMTS target area we engineered three Kv2.1 constructs: **a)** a 351-amino acid C-terminal deletion that removes all 4 cysteines at the C-terminus, **b)** a mutant channel that combines C232A+C393S+C394S, replacing the only three cysteines in the transmembrane core region and **c)** a 139-amino acid N-terminal deletion that eliminates the first 6 cysteines at the cytoplasmic N-terminus. The first two constructs gated similarly to WT channels and were sensitive to MMTS. Only the N-terminal deletion produced slowly gating channels (*VanDongen* et al., Neuron 5, 1990) that were insensitive to MMTS. We propose that reduced cysteine residue(s) at the N-terminus are closely associated with the gating machinery of Kv2.1. Supported by NIH grants NS23877 and HL37044 to AMB.

525.5

RESIDUES LINING THE INTERNAL MOUTH OF Kv2.1

C. C. Shieh, J. M. Pascual[1], A. M. Brown* and G. E. Kirsch. Rammelkamp Center, Case Western Reserve University, Cleveland, OH 44109 and [1]College of Physicians and Surgeons, Columbia University, New York, NY 10032.

We have applied the substituted cysteine-accessibility method (SCAM) developed by Karlin and coworkers (*Akabas* et al., Science 258, 1992) to residues 369-383 in the P region of Kv2.1 (WT). Application of charged methanethiosulfonate ethyltrimethylammonium (MTSET) to the cytoplasmic surface of excised macropatches irreversibly reduced currents through channels containing the substitutions T370C, T372C, T373C and V374C. WT, I369C, M371C and other residues at the external channel mouth were unaffected by MTSET. Taken together with previous SCAM results in which residues including and beyond D378C were accessible to external MTSET (*Pascual* et al., Soc. Neurosci. Abstr. 20, 1994), we infer that the narrowest, MTSET-inaccessible portion of the pore is lined by residues included in the G375-Y376-G377 triplet. Supported by NIH grants NS29473 to GEK and NS23877 and HL37044 to AMB.



525.6

MUTATIONS IN THE S4 REGION OF A JELLYFISH POTASSIUM CHANNEL. N. G. Grigoriev, J. D. Spafford, W. J. Gallin and A. N. Spencer* Dept. of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada, T6G 2E9 and Bamfield Marine Station, Bamfield, B.C., Canada, V0R 1B0.

In most known *Shaker* K+ channels, the putative voltage sensor (S4 segment) has seven conserved, positively charged amino-acids positioned at every third residue. However a primitive, *Shaker*-like potassium channel (*jShak1*) from the hydrozoan jellyfish, *Polyorchis penicillatus* has only six positively charged residues in the S4 segment. Mutations were made in *jShak1* to determine the functional importance of the position of an additional seventh charge, and the total length of the S4 segment. Two types of mutants were constructed. One type involved insertion of 3 residue motifs and the other involved replacement of single residues. Evidence from expressed mutants suggests that the voltage sensitivity of *jShak1* depends primarily on the position of charges on the helix and not on the total number of charges in the S4 segment. Supported by NSERC Research Grant A0419.

525.7

REGULATION OF VOLTAGE DEPENDENCE OF THE POTASSIUM CHANNEL KAT1. L. Marten, D.J. Henneger, and T. Hoshi*. Dept. of Physiology and Biophysics, The University of Iowa, Iowa City, IA 52242.

The KAT1 channel currents are observed in response to hyperpolarization even though its structural organization is similar to those of depolarization-activated K^+ channels. We examined how the voltage-dependent gating of KAT1 could be manipulated by expressing the channel in *Xenopus laevis* oocytes.

When recorded in the inside-out configuration, the KAT1 channel gradually runs down. This is caused by a large shift of the voltage-dependent activity to more hyperpolarizing potentials. We searched for factors which could prevent or even reverse the rundown. Since the intracellular carboxyl segment of the channel contains several putative phosphorylation sites, we studied effects of various agents that affected the phosphorylation state of the channel. Intracellular adenosine triphosphate (ATP), but not the nonhydrolyzable ATP-analog AMP-PNP, often at least partially reversed the rundown. However, none of the agents examined alone so far reliably prevented the rundown phenomenon.

Mutations in the S4 segment, the putative voltage sensor, also affected the apparent voltage-dependent gating of KAT1. Based on the S4 sequence comparison among KAT1, *Shaker* and *eag*, we focused on S168, S180, and R176. Although the voltage dependence of the mutants shifted dramatically to a more positive direction (up to 100 mV), the mutations, however, did not markedly alter the steepness of the apparent macroscopic G(V) curve. One possible interpretation is that the KAT1 S4 domain is not coupled tightly to the voltage-dependent gating and that other channel segments are also involved in the apparent activation of KAT1. (Supported by HFSP, NIH)

525.8

PROTEIN DOMAINS MEDIATING INTERACTIONS BETWEEN VOLTAGE GATED POTASSIUM CHANNELS AND MEMBRANE ASSOCIATED GUANYLATE KINASES E. Kim* and M. Sheng, Howard Hughes Medical Institute, and Dept. of Neurobiology, Mass General Hospital and Harvard Medical School, Boston, MA 02114

At different subcellular locations, K^+ channels are likely to interact with distinct intracellular cytoskeletal or signalling molecules. Using the yeast two-hybrid system, we have identified members of the *discs large* (dlg) family of membrane associated guanylate kinases as proteins that interact specifically with the shaker subfamily of voltage-gated K^+ channel subunits. Deletional and site-directed mutagenesis have defined a peptide sequence in the C-terminal intracellular domain of the K^+ channel subunit and a specific domain in the dlg-related proteins, which mediates the interaction.

525.9

THE PORE OF VOLTAGE-GATED POTASSIUM CHANNELS AS SEEN THROUGH THE EYES OF DENDROTOXIN. R. G. Sorensen*. Dept. Medicine, Div. Environmental Medicine and Toxicology, Jefferson Medical College, Philadelphia, PA 19107.

α-Dendrotoxin is a member of a homologous family of polypeptides isolated from the venom of the green mamba, Dendroaspis angusticeps, that have been shown to block various voltage-dependent potassium channels of the central and peripheral nervous systems. Furthermore, the dendrotoxins are specific blockers of the Shaker-like, Kv1.x class of cloned mammalian potassium channels. Previously, I have expressed recombinant α-dendrotoxin in bacteria from a synthetic gene encoding the primary sequence of the polypeptide. Using site-directed mutagenesis of the synthetic gene, mutant dendrotoxins are being prepared. These toxins are subsequently tested for the ability to block Kv1.1 that has been expressed in Xenopus oocytes. Changes in the affinity for block of Kv1.1 as a result of the amino acid substitution are used to identify those residues that are required for the dendrotoxin polypeptide to bind to the outer aspect of the external vestibule of the ion pore, and thereby, block voltage-dependent potassium channels. These data will be further discussed with respect to the 3-dimensional structure of dendrotoxin in order to build upon the proposed model of the external vestibule of voltage-dependent potassium channels derived from results using charybdotoxin and various other potassium channel blockers. These results will also be discussed with respect to the ability of dendrotoxin and the mutant polypeptides to block additional members of the Kv1.x class of potassium channels. (Supported by NIH grant NS31670.)

525.10

IDENTIFICATION AND EXPRESSION OF CHARIBDOTOXIN-BINDING SITES IN CULTURED RAT SKELETAL MUSCLE. S.R. Sampson*, A. Bak and S. Viqdor-Alboim, Otto Meyerhoff Center, Dept. of Life Science, Bar-Ilan Univ., Ramat-Gan, Israel 52900.

High-conductance, charybdotoxin (ChTx) -sensitive K^+ (Maxi-K) channels are one of several types of K^+ channels found in excitable cells such as nerve and skeletal muscle. These channels play an important role in restoration of the membrane potential following an action potential. While much as been learned about the properties of individual channels, little is known about the factors regulating their expression in excitable cells. We have investigated the expression of ChTx-sensitive K^+ channels in cultured skeletal muscle by using a polyclonal antibody raised in rabbits against ChTx (Almone Labs., Jerusalem). Crude homogenates were prepared of cultures made from limb muscles of 1-2 day-old rat pups. Identification of ChTx binding sites was done with western blotting techniques. After SDS-PAGE and electrophoretic transfer of proteins to Immobilon-P membranes, the membranes were blocked by incubation in TBS containing 3% gelatin and incubated in TBST buffer containing 10 nM ChTx for 30 min. After removal of this solution and washing of the membranes, they were incubated overnight with antibody buffer containing rabbit polyclonal anti-ChTx. A distinct band of apparent molecular weight 31 kDa was observed. When membranes were incubated with ChTx and then exposed to anti-PKCα instead of anti-ChTx, the 31 kDa band was not seen. Similar findings were obtained when the membranes were exposed to an antibody against the KV1.5 K channel (courtesy of Dr. J. Trimmer). Expression of the ChTx binding sites was found to increase with development of muscle cells in culture but was not dependent on fusion of individual myoblasts to multinucleated myotubes. These data suggest that the ChTx antibody recognizes ChTx bound to a site corresponding to the β-subunit of the ChTx-sensitive K^+ channel, which is also of 31 kDa apparent molecular weight. (Supported by the Harvett-Aviv Neuroscience Fund and the Ben and Effie Raber Neuroscience Research Fund.)

525.11

THE *SEIZURE* GENE OF *DROSOPHILA MELANOGASTER* ENCODES A POTASSIUM CHANNEL OF THE H-ERG TYPE. XinJing Wang*, Elaine R. Reynolds, Peter Deák and Linda M. Hall. Department of Biochemical Pharmacology, SUNY at Buffalo, Buffalo, NY 14260-1200.

Mutations in the *seizure* (*sei*) locus cause a temperature-induced hyperexcitability (seizures) followed by paralysis (Jackson et al., *J. Neurosci.* **5**:1144, 1985). Position cloning based on RFLP mapping has shown that the *seizure* gene product is a potassium channel subunit which is the *Drosophila* homolog of H-Erg, a member of the *eag* potassium channel family from humans (Warmke et al., *PNAS* **91**:3438, 1994). Two transcript size classes produced by *sei* are expressed in a tissue and developmentally distinct fashion. Genomic sequencing of 6 mutant alleles shows that the phenotype of hyperexcitability followed paralysis is caused by the loss of, or a change in, this potassium channel subunit leading to a problem in repolarization following an action potential. Five null alleles are all recessive showing that the complete loss of one allele can be compensated by the remaining normal copy. Homozygous null alleles are viable, but hyperexcitable. At least one null allele leads to sodium channel down-regulation as measured electrophysiologically (O'Dowd & Aldrich, *J. Neurosci.* **8**:3633, 1988) and by saxitoxin-binding. A missense mutation in the *sei* subunit causes a dominant phenotype suggesting that *sei* potassium channels are composed of multiple subunits and the missense form of the protein acts to "poison" channel function.

525.12

CLONING, EXPRESSION AND DISTRIBUTION OF KV4.3, A NEW MAMMALIAN SUBUNIT OF A-TYPE, LOW-VOLTAGE-ACTIVATING POTASSIUM CHANNELS. P. Serôdio*, E. Vega-Saenz de Miera and B. Rudy, Dept. Physiology & Neuroscience and Dept. Biochem., NYU Med. Ctr., N.Y. 10016

The mammalian Shal homologs (KV4 subfamily) are K^+ channel proteins that express transient outward currents in *Xenopus* oocytes. Antisense hybrid-arrest experiments in oocytes injected with rat brain poly (A) RNA have demonstrated that the KV4 channels are components of a 4-AP sensitive, fast-activating, fast-inactivating A-current, with a range of activation below the threshold for Na^+ action potential generation ("subthreshold" A-current or I_{SA}). This same I_{SA} is specially pronounced in oocytes injected with rat poly-A preparations from hippocampus, thalamus or cerebellum (Serôdio et al. J.Neurophysiol, 72, p.1516-1529). Here we report the characterization of a new mammalian Shal-related protein (KV4.3) with high sequence conservation in the transmembrane portion (over 90%) as well as at the N- and C-terminals (80% and 60% respectively) to other reported members of the KV4 subfamily. Injection of KV4.3 cRNA into *Xenopus* oocytes generates a transient A-type potassium current. Consistent to the previously reported characteristics of KV4.1 and KV4.2, the currents generated by KV4.3 in oocytes can be modified to resemble whole-brain I_{SA} by co-injection with poly (A) RNA treated with antisense oligonucleotides which arrest the expression of I_{SA} or with a 2-4Kb rat brain poly (A) RNA fraction which does not express any detectable current on its own. Notwithstanding the high sequence and functional conservation of KV4.3 as compared to other Shal K^+ channels, the distribution of KV4.3 observed in rat brain by *in situ* hybridization, differs markedly from other members of this subfamily. In some brain areas KV4.2 and KV4.3 overlap but in others their distribution seems to be complementary, although the currents generated by these two cDNAs injected in oocytes are very similar.

525.13

DEVELOPMENTAL EXPRESSION OF KV3.2, KV3.1 AND GIRK K^+ CHANNEL PROTEINS IN THE MAMMALIAN CNS. E. Bueno[1], H. Yang[1], A. Ponce[1], D. H. P. Lau[1], A. Chow[1], S. Chen[1], G. Rameau[2*], C. Sekirnjak[3], M. E. Martone[3], M. Ellisman[3], S. Chen[1], D. Hillman[1], B. Rudy[1,2] and W. Thornhill[4]. [1]Dept of Physiology and Neurosciences and [2]Dept. of Biochemistry, New York University Medical Center, NY 10016, [3]Dept. of Neurosciences, UCSD, La Jolla, CA, [4]Dept. of Physiology and Biophysics, Mount Sinai Medical Center, NY.

Antibodies raised against peptides corresponding to sequences of two Shaw-related K^+ channel subunits (KV3.1 and KV3.2) and a component of G-protein activated inward rectifier channels (GIRK1) were used to analyze the distribution of these subunits in the brain. Each of these proteins is expressed in specific neuronal populations of the adult rat brain. KV3.1 is found in the somatic and/or axonal membrane of cerebellar granule cells, the reticular thalamic nucleus and various neuronal populations involved in the processing of auditory signals, as well as in parvalbumin-containing interneurons in the neocortex, the hippocampus and the caudate-putamen. KV3.2 proteins have an axonal distribution in some neuronal populations such as thalamic relay neurons and somatic in others such as neurons of the globus pallidus, red nucleus, deep cerebellar nuclei and a subset of neurons in the cortex and the hippocampus distinct from that expressing KV3.1. GIRK1 proteins are present in somas and dendrites of cerebellar granule cells, pyramidal cells of the hippocampus, and others. GIRK1 proteins appear to be present also in axons and terminals such as in thalamocortical projections. KV3.2 and GIRK subunits have a localization consistent with a role in the regulation of the efficacy of the thalamocortical synapse. These cellular and subcellular patterns of expression are not seen in young rats and are not fully established until about two to three weeks of postnatal development. Supported by a Grant in Aid from the American Heart Association and NIH grant NS 30989.

525.14

DEVELOPMENTAL EXPRESSION OF THE POTASSIUM CHANNEL SUBUNIT KV3.1 IN PARVALBUMIN CONTAINING INTERNEURONS OF THE RAT HIPPOCAMPUS. J. Du*[1], M. Weiser[2], B. Rudy[2] & C. J. McBain[1], [1]NICHD-LCMN, NIH, Bethesda, MD 20892. and [2]Dept. Physiol. & Neurosci. NYU Medical Ctr. New York, NY 10016.

In situ hybridization has shown that the voltage-dependent K^+ channel subunit Kv3.1 is localized in interneurons of the hippocampus (Weiser *et al.* J. Neurosci. 14, 1994). Using an antibody raised against the C-terminus of Kv3.1, we have investigated the developmental expression of Kv3.1 in the hippocampus using immunostaining and Western blot techniques. Kv3.1 expression was first observed in tissue derived from P8 (12μm sections). The cell number per section reached a maximum by P14 (64.0 ± 3.6 cells/section) and was sustained through both P20 (61.2 ± 6.3) and P40 (57.8 ± 4.7) (N=4 animals). The Kv3.1 protein was expressed predominantly in cells at the borders between st. pyramidale and st.oriens and st. radiatum (28.2%), in the subiculum (33.8%), and the dentate hilus (22.4%). In contrast, only a small number of immuno-positive cells were in st. oriens-alveus (8.9%) and st. radiatum-lacunosum (6.5%) (N=4 animals). Double staining of Kv3.1 and antibodies to GAD or parvalbumin showed that every Kv3.1-positive cell was both GAD and parvalbumin-positive. Approximately 80% of all parvalbumin-immunoreactive cells were Kv3.1-positive. Western blot analysis of hippocampal membrane vesicles confirmed that Kv3.1 expression started at P8. The membrane protein content of Kv 3.1 increased through P10 to P40, despite the observation that the Kv3.1-positive cell number per section remained constant after P14. Electrophysiological recordings were made from cells subsequently shown to be Kv3.1-positive. These cells fired spontaneous, brief action potentials with a prominent AHP consistent with their interneuron morphology. Interestingly, parvalbumin-positive cells are resistant to damage in a number of seizure models. It is possible that Kv3.1 channels contribute to the survival of these cells by virtue of their activation at extreme depolarizing potentials occurring during seizure episodes.

525.15

THE NEURAL-SPECIFIC EXPRESSION OF THE Kv3.1 POTASSIUM CHANNEL GENE IS DIRECTED BY UPSTREAM REGULATORY ELEMENTS L. Gan, S. Hahn, T.M. Perney* and L.K. Kaczmarek Dept. of Cellular and Molecular Physiology and Dept. of Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06510.

The Kv3.1 potassium channel gene has been shown to be largely expressed in brain, especially in neurons that are capable of firing action potentials at high frequencies in response to synaptic inputs. Many neurons in brain stem nuclei express Kv3.1 at very high level. To investigate mechanisms that lead to the specific expression pattern of the Kv3.1 gene *in vivo*, we have generated transgenic mice in which the 4.3 Kb of the 5' flanking region of the Kv3.1 gene is linked to the E. Coli lacZ reporter gene. We have shown that the overall expression pattern of the transgene is similar to that of the endogenous Kv3.1 gene. In particular, neurons in auditory brainstem and hippocampal interneurons express high levels of the transgene.

We have also studied genetic elements involved in the cell-specific expression of the Kv3.1 gene using transient transfection assays. A series of promoter fragments of the Kv3.1 gene with successive 5' deletions were linked to the chloramphenicol acetyl transferase reporter gene and transfected into various types of cell lines. Our results reveals that the Kv3.1 gene promoter is active in PC12 cells, but not in non-neuronal types of cells. Our data also suggest that both silencing and enhancing elements are involved in the neural-specific expression of this gene.

525.16

IMMUNOHISTOCHEMICAL LOCALIZATION OF Kv1.1, Kv1.2, Kv1.4, Kv1.6, Kvβ1 and Kvβ2 K+ CHANNEL POLYPEPTIDES IN RAT HIPPOCAMPUS. M.M. Monaghan*, J.S. Trimmer and K.J. Rhodes, CNS Disorders, Wyeth-Ayerst Res., Pearl River, NY 10965 and Dept. Biochem. and Cell Biol., SUNY, Stony Brook, NY 11794.

In the present study we used immunohistochemical techniques to localize Shaker K^+ channel α- and β-subunit polypeptides in the rat hippocampal formation. Polyclonal and monoclonal antibodies specific for the rat Kv1.1, Kv1.2, Kv1.4, Kv1.6, Kvβ1 and Kvβ2 subunit polypeptides were examined for specificity on immunoblots of rat brain membranes and by immunofluorescence in COS cells transiently transfected with the corresponding cDNAs. For immunohistochemistry, sections of paraformaldehyde fixed rat brain were reacted in 0.010 M $NaPO_4$ buffer containing 3% normal serum, 0.1% triton X-100, and the primary antibodies diluted 1:200-1:5000. Binding of the primary antibodies was visualized using the ABC Elite peroxidase method (Vector) and a nickel-enhanced DAB procedure.

Within the hippocampal formation, many of these K^+ channel subunits were concentrated within the projection fields of the perforant path. Specifically, there was a dense band of immunoreactivity for Kv1.1, Kv1.2, Kv1.4, Kv1.6, Kvβ1 and Kvβ2 in the middle third of the dentate molecular layer, and a similarly dense band of immunoreactivity for the four α-subunits, but not Kvβ1 or Kvβ2, in the molecular layer of CA1-CA3. In the CA3 subfield, immunoreactivity for Kv1.1 and Kv1.4 was concentrated in the mossy fiber zone, and in the CA1 subfield, immunoreactivity for Kv1.1 and Kvβ1 was concentrated in stratum radiatum. Interestingly, immunoreactivity for Kvβ2, but not the α-subunit polypeptides, was contained within the apical dendrites of hippocampal pyramidal cells. Taken together, these data suggest that the individual afferent, intrinsic, and efferent hippocampal projection systems express voltage-gated K^+ channels with varied and distinct subunit compositions and electrophysiological properties.

525.17

EXPRESSION OF VOLTAGE-GATED K^+ CHANNEL β-SUBUNIT mRNAs IN ADULT RAT BRAIN AND PERIPHERAL TISSUES: *IN SITU* HYBRIDIZATION AND NORTHERN ANALYSES. S.P. Nawoschik*, N.X. Barrezueta, K. Nakahira, L.E. Schechter, J.S. Trimmer and K.J. Rhodes, CNS Disorders, Wyeth-Ayerst Res., Pearl River, NY 10965 and Dept. Biochem. and Cell Biol., SUNY, Stony Brook, NY 11794.

Recent cloning of ß-subunits associated with voltage-gated K^+ channels and their co-expression with α-subunits has revealed that the gating and conductance properties of the expressed channels can be profoundly affected by the presence of a ß-subunit polypeptide (Rettig et al., Nature 369:289, 1994). We examined the expression of two ß-subunit polypeptides, Kvß1 and Kvß2, in rat brain and peripheral tissues using Northern and *in situ* hybridization analyses. Riboprobes directed towards unique regions of the rat Kvß1 and Kvß2 sequences were prepared by the PCR using the corresponding full length cDNAs as templates, and used to probe Northern blots of mRNA extracted from rat brain and peripheral tissues (Clontech) as well as tissue sections of fresh-frozen rat brain.

Analysis of Northern blots indicated Kvß1 and Kvß2 are predominantly expressed in brain, with lower levels of Kvß2 expression in spleen, heart and lung. Analysis of *in situ* autoradiograms revealed that Kvß1 and Kvß2 are widely and heterogeneously distributed in rat brain. Moreover, the relative abundance of Kvß1 and Kvß2 mRNA varied across brain regions. For example, Kvß1 expression was greatest within the caudate-putamen, nucleus accumbens, and CA1 pyramidal cells, with intermediate levels in Purkinje cells and brainstem motor structures. Kvß2 expression was greatest in neocortex, hippocampus, and entorhinal cortex, with intermediate levels in striatum, thalamic relay nuclei and the cerebellum. Comparison of ß-subunit expression with that for *Shaker* α-subunits suggested that Kvß1 mRNA was co-distributed predominantly with Kv1.4 mRNA, and Kvß2 mRNA was co-distributed predominantly with Kv1.2 mRNA.

525.18

AXONAL SUBCELLULAR LOCALIZATION OF A POTASSIUM CHANNEL SUBUNIT IN RAT BRAIN: KV3.1B. C. Sekirnjak, R. Komesu, M. E. Martone, E. Bueno⊗, M. Weiser*⊗, B. Rudy*⊗, and M. H. Ellisman. San Diego Microscopy and Imaging Resource, Dept. of Neuroscience, Univ. of California at San Diego, La Jolla, CA 92093-0608 and ⊗Dept. of Physiology and Neuroscience, New York Univ. Medical Center, New York, NY 10016.

We have analyzed the localization of the voltage gated K^+ channel protein Kv3.1b in rat brain at the light- and electron microscopic level, using Kv3.1b-specific antibodies. The investigation was carried out in the cerebellum, neocortex and hippocampus. We found the protein to be present in cerebellar granule cell axons (parallel fibers), in lower levels in proximal dendrites, and in cytoplasmic patches of the somata. Cell bodies and axonal terminals of a population of neocortical interneurons were found to be labeled. In the hippocampus we observed labeling in somata, processes, and synaptic terminals of certain nonpyramidal cells. Fluorescence double-labeling with parvalbumin and calbindin was used to demonstrate colocalization of Kv3.1b in cortical and hippocampal interneurons and establish the identity of these cells. Our immunocytochemical analysis is consistent with a predominantly somato-axonal localization of the protein in the areas under investigation (Weiser et al, J. Neurosci., in press). We are currently comparing this distribution with that of other members of this large family of potassium channels. Preliminary results with Kv4.2 indicate that this channel subunit colocalizes with Kv3.1b in cerebellar granule cells. However, Kv4.2 shows a somatodendritic localization, in contrast to the somato-axonal distribution of Kv3.1b.

525.19

THE EFFECTS OF INNERVATION ON THE DEVELOPMENTAL EXPRESSION OF Ca^{2+}-ACTIVATED K^+ CURRENTS IN EMBRYONIC PARASYMPATHETIC NEURONS ARE NOT ACTIVITY-DEPENDENT. Priva Subramony* and Stuart E. Dryer, *Program in Neuroscience, Florida State University, Tallahassee, FL 32306,*

Chick ciliary ganglion (CG) neurons that develop *in situ* in the absence of preganglionic innervation fail to express Ca^{2+}-activated K^+ currents ($I_{K[Ca]}$), but the mechanism of this effect is unknown (see *J. Neurosci.* 14: 3156, 1994). One possibility is that the effects of innervation are dependent upon synaptic activation of CG neurons. Alternatively, nerve terminals may secrete a differentiation factor unrelated to synaptic transmission. In the present study, chick embryos were treated chronically with mecamylamine, a potent neuronal nicotinic receptor antagonist, in order to block synaptic activation of CG neurons *in ovo*. Mecamylyamine (5 mg/kg) was applied repeatedly throughout the developmental stages where functional chemical synapses are present within the CG (E6-13). With this protocol, afferent preganglionic nerve terminals are still present. As reported previously by several investigators, chronic blockade of synaptic transmission *in ovo* resulted in a large increase in naturally-occurring cell death. The resulting decrease in ganglion size was the same as that produced by complete ablation of the preganglionic nucleus early in development. However, the $I_{K[Ca]}$ in E13 mecamylamine-treated CG neurons was indistinguishable from that of saline-treated controls. This suggests that the effects of the preganglionic afferent innervation on $I_{K[Ca]}$ expression *in situ* are mediated by a nerve terminal-derived differentiation factor and are not dependent upon intact cholinergic synaptic transmission. NS-32748.

525.20

REGULATED EXPRESSION OF A-TYPE POTASSIUM CURRENT IN PC12 CELLS. E. A. Liles and A. L. Willard*. Dept. of Physiology, University of North Carolina, Chapel Hill, NC 27599-7545.

Calcium influx is an important consequence of neuronal electrical activity. It is essential that excitable cells express a quantitatively appropriate balance of ion channels in order to achieve Ca influx that is right for cellular survival and function. Therefore regulation of ion channel expression is crucial. Both the duration and amplitude of action potentials are partially controlled by K currents. We have used chronic depolarization of cultured neural cells as a model for studying activity-dependent regulation of the expression of voltage-dependent ion channels.

PC12 cells were differentiated by treatment with NGF for 7-21 days prior to treatments with medium containing 25 mM KCl, which depolarizes them to -40 mV. This treatment leads to reduced Ca influx during electrical activity in at least two ways. Ca channel expression is decreased and, as we report here, expression of a rapidly activating and inactivating, 4-aminopyridine-sensitive (A-type) K current is dramatically increased following treatments with 25 mM KCl as brief as 24 hours. Confirming prior findings (Hoshi & Aldrich, 1990), almost no (1/19) untreated cells had detectable A current. In contrast, 27/37 cells treated with 25 mM KCl expressed this current. To test whether influx of external Ca is required to trigger expression of A current, we examined the effects of nitrendipine, which blocks L-type Ca channels. Nitrendipine reduced the expression seen after 48 hours of 25 mM KCl treatment from 75% of cells to <15% of cells.

We hypothesize that this is a novel regulatory mechanism by which excitable cells protect themselves against excess Ca influx during electrical activity. It will be interesting to examine the transduction pathways that control this change. Supported by an NIH research grant.

ACETYLCHOLINE RECEPTOR: NICOTINIC—ACETYLCHOLINE-EFFECTS OF NICOTINE ON SPECIFIC BRAIN REGIONS

526.1

NICOTINIC RECEPTOR ACTIVATION POTENTIATES GLUTAMATERGIC SYNAPTIC TRANSMISSION IN CULTURED HIPPOCAMPAL NEURONS. K.A. Radcliffe and J.A. Dani*. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

The muscle form of the nicotinic acetylcholine receptor (nAChR) found at the neuromuscular junction is well studied, but the role of neuronal forms (NnAChRs) is less well understood. Using a new quantitative approach, we recently demonstrated that the calcium influx through nAChRs is significant, suggesting that nAChRs could participate in synaptic plasticity processes (Vernino et al. (1994), *J. Neuroscience* 14: 5514-24). In the present experiments, we have used whole-cell patch clamp electrophysiology to study synaptic transmission in 15-25 day old hippocampal cell cultures. Cells were plated onto small islands of substratum to limit process outgrowth and the number of neurons contributing input to a given cell. In the presence of atropine, tetrodotoxin, and cadmium, applications of ACh (1-3mM) or nicotine (0.5mM) activated a current with rapid kinetics that caused a subsequent increase in the frequency of miniature spontaneous synaptic currents (mSSCs) at glutamatergic synapses. The mechanism for enhancement of glutamatergic mSSCs is nicotinic, not muscarinic, because atropine did not block the effect and the effect was seen with the specific agonist nicotine. In some cases, the potentiation of glutamatergic mSSC frequency was seen on islands of substratum that were extremely small, ensuring the presence of autapses. Thus, glutamatergic autapses can be potentiated if the neuron displays a current in response to nicotine application. In addition, the effect depends upon external calcium, suggesting that activation of nAChRs produces a presynaptic calcium influx that initiates the potentiation of glutamatergic mSSCs. This work was supported by NIH grant NINDS NS 21229.

526.2

NICOTINE UPREGULATION OF NICOTINE BINDING SITES IN NGF-DIFFERENTIATED PC12 CELLS: CORRELATION WITH NICOTINE-INDUCED ACh RELEASE. R. Widstrom, J.S. Aguilar, and E.L.M. Ochoa*. Department of Pediatrics, University of California at Davis, Davis, CA 95616 and Department of Pharmacology, University of Illinois at Chicago, Chicago, Illinois 60612.

Chronic nicotine upregulates nicotine binding sites that control neurotransmitter release from nerve terminals. We used intact PC12 cells as a model on which to test the hypothesis that nicotinic receptor upregulation coincides with desensitization of nicotinic receptor function. Cultures were: controls (C); exposed to one micromolar nicotine for one day (N); exposed to 50 ng/ml for 7 days (NGF), or NGF-treated and subsequently exposed to nicotine for one day at 1 micromolar concentration (NGF/N). Nicotine was removed prior to the assays. Nicotine increased the specific binding of [^{3}H]-nicotine (54% at 80 nM drug in N with respect to C). Differentiation of PC12 cells with NGF increased binding (205%) over C. A further increase in [^{3}H]-nicotine binding occured in NGF/N (347% of C; 142% over NGF). Binding of [^{3}H]-nicotine was also determined in membranes prepared from NGF, and NGF/N PC12 cells. At both 40 nM and 150 nM [^{3}H]-nicotine, specific binding was increased by 36 % in NGF/N as compared to NGF. To test whether desensitization of nicotinic receptor function accompanies upregulation, we measured [^{14}C]-Ach release upon acute nicotine challenge (50 micromolar) using [^{14}C]-choline and an extraction technique that selectively measures released Ach. In either C or N, an acute nicotine challenge did not release ACh above basal levels, contrasting with the two-fold increase in ACh release induced by nicotine in NGF. NGF/N cells had a statistically significant increase in the basal release of ACh upon nicotine challenge over NGF. However, these cells did not respond to a further nicotine challenge. Our data suggest that nicotine upregulates [^{3}H]-nicotine binding sites in NGF differentiated cells and that these sites may be responsible for the elevated basal release of ACh. This may explain the nicotine-induced desensitization of ACh release seen upon nicotine challenge. *This research was supported by funds provided by the Cigarette and Tobaco Surtax Fund of the State of California through the Tobacco-Related Disease Research Program of the University of California, Grant Number 3RT-0098.*

526.3

NICOTINE STIMULATED RELEASE OF [^{3}H]NOREPINEPHRINE FROM CULTURED LOCUS COERULEUS CELLS. K.A. Gallardo*, C.G. Griffith, F.M. Leslie. Department of Pharmacology, College of Medicine, University of California, Irvine, CA 92717.

The structure and function of neuronal nicotinic acetylcholine receptors (nAChR) are of primary importance in elucidating the effect of nicotine on the central nervous system (CNS). Morphological and molecular studies of these pentameric ion channels in the adult rat brain indicate their composition to be solely α and β subunits, of which numerous subtypes exist. Upon stimulation of central nAChRs diverse cellular responses are observed which appear to be related to the phenotype of the neuron being expressed, such that activation by nicotine can stimulate the release of various neurotransmitters with variable efficacy depending on the cell type being examined. Whether this differential release of neurotransmitter is due to receptor subunit heterogeneity is not known. There is presently conflicting evidence regarding the effect of nicotine on norepinephrine (NE) release. We have further examined this issue using primary dissociated locus coeruleus (LC) cells in culture. Cells were harvested from rostral rhombencephalon of E15 Sprague-Dawley rat embryos, cultured for 4 days, then pre-loaded with radiolabeled NE. Following a washout period, the effect of nicotine on [^{3}H]NE release was examined in both calcium-containing and calcium-free media. Preliminary data indicate that nicotine stimulates NE release from embryonic LC cells in a dose-dependent manner. This nicotine effect is independent of extracellular calcium. Prior exposure of the cells to nicotine induces subsequent desensitization of the nicotinic response. These data suggest that there are nicotinic receptors on LC cells which stimulate neurotransmitter release by a novel mechanism. Ongoing studies involve a more complete pharmacological characterization of the nAChR by examining LC cell responses to various agonists and antagonists. *PHS grant #19319 and 30109.*

526.4

THE EFFECTS OF ORAL NICOTINE ADMINISTRATION ON BRAIN NICOTINIC RECEPTORS AND RESPONSIVENESS TO NICOTINE. J.A. Sparks, K. Agee and J.R. Pauly*. Dept. of Pharmacology, Medical College of Georgia, Augusta GA 30912-2300.

Our laboratory is using a mouse model to study the effects of in utero nicotine exposure on brain development. Since glucocorticoid hormones have been shown to regulate the number of brain nicotinic receptors as well as behavioral responsiveness to nicotine, it is important to chronically expose pregnant mice to nicotine in a non-stressful manner. We have tested whether chronic nicotine delivery via the drinking solution produces tolerance to nicotine and alterations in brain nicotinic receptors consistent with studies that have measured these parameters following other methods of nicotine delivery. Female C57BL/6 mice were given water, 2% saccharin, or nicotine in 2% saccharin (50, 100 or 200 μg/ml) as a drinking solution for 30 days. Plasma cotinine levels were determined by ELISA and ranged between 0-200 ng/ml of plasma. Nicotine-treated animals developed dose-dependent tolerance to the locomotor depressant and hypothermic actions of nicotine. Brain nicotinic receptors, as measured by [^{3}H]-cytisine and [^{125}I]-alpha bungarotoxin autoradiography, were increased following chronic nicotine exposure. These data suggest that chronic oral niocotine exposure is appropriate for long term, non-invasive delivery of this drug. Supported by DA-08443 and the Callaway Foundation.

526.5

INTRACELLULAR RECORDING OF NICOTINIC RESPONSES IN SUBSTANTIA NIGRA PARS COMPACTA (SNc) NEURONS OF THE RAT. E.M. Sorenson* and S.T. Kitai, Department of Anatomy and Neurobiology, University of Tennessee, College of Medicine, Memphis, TN 38163.

SNc neurons express mRNA for the α_3, α_4, α_5, β_2, and β_3 subunits of the nicotinic receptor. High affinity ^{3}H-nicotine binding in the SNc indicates that some of these subunits may be localized on neuronal cell bodies in the SNc. Furthermore, extracellular recording has shown that nicotine increases the firing rate of SNc neurons. Using intracellular recording in the brain slice, we want to determine whether dopaminergic SNc neurons directly respond to nicotinic agonists. Most neurons have responded to bath superfusion of 10-100 μM nicotine with a depolarization and a decrease in input resistance. The depolarization was usually accompanied by an increase in cell firing. Immunocytochemical labeling of the neurons for biocytin, injected during recording, and tyrosine hydroxylase (TH), the synthetic enzyme for dopamine, revealed that of the cells localized all were TH positive. This included the cells that did not appear to respond to nicotine. We conclude that most TH neurons in the SNc depolarize in the presence of nicotine. Whether the nicotinic receptors in the SNc are pre- or postsynaptic is being further investigated as well as the possibility that some TH neurons are unresponsive due to nicotinic receptor desensitization. This work is supported by USPHS grants NS 20702 and NS 26473.

526.6

SELECTIVE ACTIVATION OF RAT EXTRAHYPOTHALAMIC CRH-CONTAINING NEURONS BY NICOTINE. K.M. McAllen, S.G. Matta, J.D. Valentine, S. Nicol*, and B.M. Sharp, Minneapolis Medical Res Fndn and Dept Medicine, Hennepin County Medical Center and Univ of Minnesota, Minneapolis, MN 55404

cFos is expressed in CRH+ neurons in the hypothalamic paraventricular nucleus (PVN) in response to iv nicotine. While PVN CRH responses are characterized, regions outside the PVN have CRH-containing neurons that may well be responsive to nicotine. To study this, immunocytochemical identification of CRH+ neurons and/or cFos+ nuclei was done in adult male rats that received saline or nicotine (0.045 mg/kg/30s or 0.09 mg/kg/60s) iv. The low dose of colchicine (7 μg, 16 h previously) permitted some visualization of CRH in cell bodies, but minimized cFos expression. In the bed nucleus of the stria terminalis, cFos increased (3 fold) in response to both doses of nicotine, but the number of double-labeled cells was unaffected. In the central nucleus of the amygdala, cFos again showed a dose-dependent increase; in addition, the number of double-labeled neurons increased 4 fold. In contrast, in the dorsal raphe, cFos+ cells decreased dose-dependently (20 and 45%), while the number of double-labeled cells decreased (60%) only in response to the higher dose. In the locus coeruleus, only the higher dose of nicotine stimulated an increase in both cFos+ (4 fold) and cFos+/CRH+ (3 fold) neurons. In the parabrachial nucleus, cerebellum and cortex double labeling was not seen. The differential sensitivity and responsiveness of CRH neurons throughout the brain to nicotine indicate that CRH may mediate some of the CNS effects of nicotine. (Supported by DA03977)

526.7

EFFECTS OF BRIEF INFUSIONS OF NICOTINE ON EXPRESSION OF cFOS IN SPECIFIC POPULATIONS OF NEURONS WITHIN BRAINSTEM REGIONS. S.G. Matta*, J.D. Valentine, J. Pomonis and B.M. Sharp, Minneapolis Medical Research Foundation and Departments of Medicine, Hennepin County Medical Center and University of Minnesota, Minneapolis, MN 55404

Previous studies from this laboratory have shown dose-dependent induction of cFos immunoreactivity within catecholaminergic regions of the rat brainstem in response to 10-15 sec iv injections of nicotine. The present experiments were performed to identify the peptidergic neurons potentially involved in ACTH secretion that may respond to nicotine (administered at a constant infusion rate of 0.045 mg/kg/30 sec for 30 or 60 sec). Nicotine dose-dependently induced cFos in NTS-A_2, NTS-C_2, A_1 and LC, but not in C_1; A_2 and LC showed 2.5 fold enhancements to the 30 sec infusion. These two regions and C_2 showed between 3.5-4 fold induction to the longer infusion. In A_2, the absolute number of neurons double labeled for cFos and either activin, galanin or NPY were relatively unaffected by nicotine. In contrast, nicotine increased cFos+/NPY+ neurons in C_2, and in LC both galanin and NPY neurons showed increases in double labeling; again, in both regions most of the nicotine-induced cFos expression did not occur in double (+) neurons. In summary, regionally selective expression of cFos in peptidergic neurons located in brainstem catecholaminergic regions was observed in response to nicotine. Nevertheless, most of the neurons activated to express cFos were unidentified. (Supported by DA03977)

526.8

NORNICOTINE-EVOKED [^{3}H]DOPAMINE RELEASE FROM RAT STRIATAL SLICES: DESENSITIZATION OF THE RESPONSE TO NORNICOTINE AFTER PRIOR EXPOSURE TO NICOTINE. L.P. Dwoskin*, L.H. Teng, S.T. Buxton, and P.A. Crooks. College of Pharmacy, University of Kentucky, Lexington, KY 40536.

Previous studies demonstrate that superfusion of rat or mouse striatal synaptosomes with low, inactive concentrations of nicotine results in desensitization of the response to a subsequent nicotine exposure (Rowell and Hillebrand, 1994; Grady et al, 1994). Nornicotine is an active nicotine metabolite and tobacco alkaloid present in brain after peripheral nicotine administration. However, its contribution to the CNS effects of tobacco smoking has not been characterized. In order to determine if nornicotine evokes dopamine (DA) release by a similar mechanism as nicotine, we determined if cross desensitization to nornicotine would occur following a pre-exposure to nicotine. In the present study, rat striatal slices preloaded with [^{3}H]DA were superfused for 60 min in the absence or presence of nicotine (0.1 μM). During this period, nicotine-evoked [^{3}H]DA release returned to basal levels. Then, both control and nicotine pre-exposed slices were superfused with nornicotine (10 μM) for 60 min. In control slices, nornicotine evoked [^{3}H]DA release. In the nicotine pre-exposed slices, however, no response to nicotine or nornicotine was detected. Thus, nicotine pre-exposure resulted in desensitization to the response to nicotine and cross-desensitization to the response to nornicotine, suggesting that these alkaloids act via a common mechanism. (Supported by a grant from the Tobacco and Health Research Institute, Lexington, KY).

526.9

A PRESYNAPTIC MODULATORY ROLE FOR THE α-BUNGAROTOXIN-SENSITIVE NICOTINIC ACETYLCHOLINE RECEPTORS (nAChRs) IN RAT OLFACTORY BULB NEURONS. E.S. Rocha[1,3], M. Alkondon[1], D.R. Burt[1*], and E.X. Albuquerque[1,2]. Depts. [1]Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201; [2]Lab. Mol. Pharmacol. II, IBCCF, Univ. Fed. Rio de Janeiro, RJ, Brazil, 21944.

Functional nAChRs sensitive to blockade by α-bungarotoxin (α-BGT) have been characterized from both hippocampal and olfactory bulb (OB) neurons of the rat (*J. Pharmacol. Exp. Ther.* 265: 1455, 1993; *Neurosci. Lett.* 176: 152, 1994). We used the whole-cell patch-clamp technique on cultured OB neurons to investigate one of the possible physiological roles of α-BGT-sensitive nAChR in the brain. ACh had differential effects on different OB cell types. In small (≈ 10 μm) OB neurons, pulse (0.1-1 sec) applications of ACh, via a U-tube, elicited whole-cell currents whose peak amplitude increased as a function of the concentration of ACh (0.03 - 3 mM) and was blocked by methyllycaconitine (MLA, 1 nM). In larger (15-20 μm) OB neurons, application of ACh to the cells was followed by bursts of miniature excitatory postsynaptic currents (MEPSCs), even though ACh was unable to evoke whole-cell current. In a 3-sec time period following the pulse application of ACh (>300 μM) to the larger neurons, MEPSCs were observed in all trials, whereas at concentrations <100 μM failures were observed. The MEPSCs showed a reversal potential near 0 mV and were independent of action potentials. The frequency of MEPSCs was substantially reduced by MLA (1 nM) or by the AMPA-receptor antagonist CNQX (10 μM). MEPSCs were not observed when ACh was applied to the neurons in a Ca^{2+}-free and $MgCl_2$ (2 mM)-containing external solution. . These findings indicate that the activation of the MLA-sensitive nAChR results in the release of glutamate which in turn activates AMPA type glutamate receptors possibly at dendro-dendritic synapses in OB neurons. (USPHS Grants NS25296, ES05730, & T32-ES07263).

526.10

EVIDENCE THAT NORNICOTINE ACTS TO RELEASE DOPAMINE FROM RAT STRIATAL SLICES BY A NICOTINIC RECEPTOR-MEDIATED MECHANISM. L.H. Teng*, S.T. Buxton, P.A. Crooks and L.P. Dwoskin. College of Pharmacy and Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40536.

Previous results demonstrate that nornicotine, an active nicotine metabolite and tobacco alkaloid, evokes the release of dopamine (DA) from rat striatal slices in a concentration- and calcium-dependent manner (Dwoskin et al, 1993). To determine if the effect of nornicotine is mediated by a nicotinic receptor, the ability of mecamylamine (MEC, a noncompetitive nicotinic receptor antagonist) and dihydro-β-erythroidine (DHβE, a competitive nicotinic receptor antagonist) to inhibit the effect of nornicotine was investigated. MEC (0.01-100 μM) and DHβE (0.01-10 μM) did not evoke [^{3}H]DA release themselves. However, the high concentration of DHβE (100 μM) evoked [^{3}H]DA release, and therefore, was not used in antagonism studies. MEC and DHβE inhibited [^{3}H]DA overflow evoked by 10 μM nicotine. The highest concentration of MEC (100 μM) and of DHβE (10 μM) inhibited the response to nicotine by 90% and 75%, respectively. In comparison, MEC was effective in inhibiting the response to low concentrations of nornicotine (1-10 μM). However, MEC was not effective against the high concentrations (100 μM - 3 mM) of nornicotine. DHβE (10 μM) inhibited 80 to 90% of the response to nornicotine (1-10 μM). Similarly, the effect of the high concentrations of nornicotine (>100 μM) was not antagonized by DHβE. Thus, concentrations of nornicotine less than 100 μM activate MEC- and DHβE-sensitive nicotinic receptors to evoke DA release from rat striatal slices. (Supported by a grant from the Tobacco and Health Research Institute, Lexington, KY).

526.11

REGULATION OF nACHR β4 SUBUNIT GENE EXPRESSION BY NERVE GROWTH FACTOR. C.B. Bigger, M. Hu, and P.D. Gardner*. Center for Molecular Medicine, Institute of Biotechnology, Univ. of Texas Health Science Center, San Antonio, TX 78245.

Nicotinic acetylcholine receptors (nAChR) belong to a multigene family (α2-α9, and β2-β4) that is expressed in distinct compositional, temporal and spatial patterns within the adult mammalian CNS. The functional diversity and differential expression of individual subunit genes is most likely generated by distinct molecular mechanisms. Our laboratory has focused upon the characterization of the regulatory mechanisms governing the expression of the β4 subunit gene during neuronal differentiation using the PC12 cell line as a model system. PC12 cells undergo differentiation into non-replicating sympathetic-like neuronal cells upon stimulation with NGF. We and others have shown that the steady-state levels of β4 mRNA increase approximately 5-fold upon NGF stimulation of PC12 cells as determined by Northern blot analysis. This up-regulation occurs during the late stages of neuronal differentiation and is independent of cAMP-dependent-protein kinase A activation. Transient transfection experiments and nuclear run-off assays have demonstrated that up-regulation of the β4 subunit gene occurs in part by transcriptional activation. Consistent with these data, inhibition of transcription with Actinomycin D (Act.D) did not result in a decrease over time in steady-state levels of β4 mRNA in non-stimulated versus NGF-stimulated PC12 cells, suggesting that post-transcriptional mechanisms involving β4 mRNA stability do not play a major role in NGF induction of β4 subunit gene expression. Furthermore, steady-state levels of β4 mRNA remained constant up to ten hours in the presence of Act. D in non-stimulated PC12 cells. Therefore, these data indicate that a major control point of β4 subunit gene expression during neuronal differentiation of PC12 cells occurs at the level of transcriptional activation. Further characterization of the promoter elements that confer NGF-inducibility are underway. (This work was supported by NIH grant NS30243.)

526.12

DIFFERENTIAL REGULATION OF THE α-BUNGAROTOXIN-BINDING NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNIT IN DEVELOPING NEURONAL CELLS BY K^+ DEPOLARIZATION AND NMDA RECEPTOR ACTIVATION. S.A. Berman*, M. Didier, M. Xu, P. Almaguer and S. Bursztajn, Lab. for Molecular Neuroscience, McLean Hosp/Harvard Med. Sch. Belmont, MA 02178.

We investigated the expression of α-BTX-binding nAChRs on mouse cerebellar granule cells grown under trophic conditions consisting of either chronic K+ (30 mM) depolarization or NMDA receptor stimulation. In cerebellar cultures, 125I-α-BTX bound to a single saturable class of sites with a Kd identical to its binding on the immunoisolated α7 nAChR subunit. Combined autoradiographic and immunocytochemical studies revealed that 25-30% of glutamatergic neurons, but not glial cells, displayed a somatic and neuritic localization for these sites. The presence of the NMDAζ1 glutamate receptor subunit was also visualized on neurons expressing this αBTX-binding. In High K+ cultures α-BTX binding exhibited a transient pattern, increasing during synaptogenesis reaching a plateau at 10-14 days in vitro and decreasing thereafter. In contrast, when cultures were maintained in a low K+ (12.5 mM) medium, the neuronal survival was reduced and the relative amount of 125I-α-BTX binding decreased dramatically. Chronic NMDA treatment of cerebellar cultures in presence of low K+ promoted long-term cell survival and maturation as well as upregulated the 125I-α-BTX binding during the first week in vitro as observed in high K+ condition. However, the ontogeny of α-BTX binding sites in NMDA-treated cells did not display the later transient increase which occurs in high K+ culture conditions. This apparent upregulation of nAChRs in the presence of high K+ appeared to be the result of an increase in the number of granule neurons binding α-BTX and an elevation of the total number of α-BTX binding sites per cell. To show further that the α-BTX binding sites represent α7 nAChRs, RNA from cerebellar granule cells was amplified by RT-PCR and this product sequenced showing a very high homology with the rat α7 nAChR cDNA. Studies using specific molecular probes for the mouse α7 subunit are in progress to determine whether K+ depolarization or NMDA receptor activation regulate α7 nAChR mRNA.

526.13

COMPARISON OF DOPAMINE RELEASE AND RUBIDIUM EFFLUX FOR ACTIVATION AND DESENSITIZATION BY NICOTINIC AGONISTS. Sharon R. Grady*, Michael J. Marks, Scott F. Robinson, and Allan C. Collins. University of Colorado, Boulder, CO 80309.

Nicotinic agonists stimulate the release of [^{3}H]dopamine (DA) from striatal synaptosomes and the efflux of $^{86}Rb^+$ (Rb) from thalamic synaptosomes. Both responses desensitize upon long-term exposure to nicotine. The results reported here compare DA release and Rb efflux elicited by six nicotinic agonists: L-nicotine, D-nicotine, acetylcholine, carbachol, cytisine, and epibatidine. P2 fractions were prepared from C57BL/6 female mice, loaded with DA (striatum) or Rb (thalamus), and exposed to nicotinic agonists for 3-10 min. Maximal responses and desensitization rates were measured. All six agonists elicited concentration-dependent increases in DA release or Rb efflux and in the desensitization rates for both processes. Epibatidine was the most potent agonist tested (EC_{50} = 3 nM for DA and 11 nM for Rb), while carbachol was the least potent (EC_{50} = 8.8 μM for DA and 32.5 μM for Rb). Although it was active at low concentrations, cytisine was the least efficacious compound tested for both measures. DA release and Rb efflux differed in the relative affinities, maximal responses, and desensitization rates determined for the six agonists. Furthermore, the residual rate of DA release was substantially higher than the rate of Rb efflux observed during prolonged exposure to each agonist. Treatment with low concentrations of the agonists induced concentration-dependent decreases in subsequent responses to 10 μM nicotine. The IC_{50} values measured for each agonist for DA release, Rb efflux and [^{3}H]nicotine binding were highly correlated, but not identical. The two-state model of Katz and Thesleff adequately explained the results obtained for both DA release and Rb efflux. However, the kinetic differences observed for these two functional responses suggests that the receptors mediating the responses are similar, but not identical. Supported by DA-03194 and DA-00116.

ACETYLCHOLINE RECEPTOR: NICOTINIC EXPRESSION AND TREATMENT EFFECTS

527.1

GENE STRUCTURE AND cDNA MUTATION SCREENING OF α7 NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR IN CONTROL AND SCHIZOPHRENIC SUBJECTS

C. Drebing*, A. Davis, J. Hopkins, M. Barnhart, J. Logel, R. Freedman and S. Leonard. Departments of Pharmacology and Psychiatry, University of Colorado Health Sciences Center and Veterans Administration Medical Center, Denver, CO 80262.

Genomic structure of the α7 nicotinic receptor was obtained by Extra Long PCR using rTth polymerase (Perkin-Elmer) and DNA purified from human postmortem brain. Sequence of the DNA in both schizophrenic and control populations showed evidence of polymorphisms. A YAC clone of α7 has also been obtained; Southern blotting confirmed the presence of α7 coding sequence and chromosome mapping confired the presence of the YAC on human chromosome 15. The cDNA sequence of α7 was obtained for mutation screening from RNA purified from cycloheximide treated lymphoblast cell lines by ectopic (illegitimate) PCR. Comparison of the sequence of control and schizophrenic cDNA with that of a neuroblastoma cell line, SHSY5Y, and a clone obtained from a normal hippocampal brain library, showed conservative polymorphisms were present in both populations.

527.2

COMPARISON OF THE REGIONAL DISTRIBUTION OF α7 NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNIT mRNA AND [^{125}I]-α-BUNGAROTOXIN BINDING IN HUMAN POSTMORTEM BRAIN. S. Leonard*, C. Breese, C. Adams, J. Logel, C. Drebing, Y. Rollins, and M. Barnhart. Dept. of Pharmacology, University of Colorado Health Sciences Center and Veterans Administration Medical Center, Denver, CO 80262.

The neuronal α7 nicotinic acetylcholine receptor is a member of the ligand-gated ion channel gene family and is the major α-bungarotoxin (αBTX) binding receptor in mammalian brain. We have determined the regional localization of α7 mRNA expression and [^{125}I]-α-bungarotoxin binding in 40 regions dissected from human postmortem brain. Frozen tissue sections were hybridized *in situ* with a human brain α7 cRNA probe, and compared with [^{125}I]-αBTX autoradiography in matched tissue sections. Although not abundantly expressed, α7 expression was principally localized to neurons in sensory and motor nuclei, such as hippocampus, geniculate, and the reticular thalamic nucleus (RTN), but was seen at lower levels in other brain regions. [^{125}I]-αBTX binding was seen on neuronal processes, as well as to cell bodies. While α7 mRNA expression and [^{125}I]-αBTX were co-localized in some of the brain regions examined, there were several regions where the localization of α7 mRNA and [^{125}I]-αBTX binding sites did not coincide. In the hippocampus, where α7 hybridization was moderately abundant, most cells were only weakly labelled with [^{125}I]-αBTX and only rare non-principal neurons with processes were heavily labelled. Additionally, in the RTN, there were only a few α7 mRNA positive cells, but extensive [^{125}I]-αBTX labelling of interwoven and fused cellular processes over the entire nucleus was observed. The anatomical localization of the α7 nicotinic acetylcholine receptor implicates this receptor in a number of sensory and sensory motor processes in the human brain.

527.3

POTENTIATION OF ACH-EVOKED CURRENTS THROUGH α4+β2 AND α5+α4+β2 NACHRS: LOCALIZATION OF THE Ca^{+2}-REGULATORY SITE. J.A. Ramirez-Latorre and L.Role*. Center for Neurobiol. & Behavior, Columbia University. 722W 168th St. New York, NY 10032.

To identify sequences responsible for Ca^{+2}-mediated potentiation of nAChR currents we have studied macroscopic and single-channel currents in oocytes injected with the RNA for the combinations of α4+β2 and α5+α4+β2. Increased $[Ca^{+2}]_{ext}$ alters nAChR channels by i) decreasing single channel conductance, ii) increasing mean open time and iii) increasing NPo. The net effect of these changes is a potentiation of macroscopic ACh-evoked currents by Ca^{+2}. Previous experiments show that the Cys reactive reagent MTSEA interferes with Ca^{+2} regulation of both macroscopic and single nAChR channel currents. Access to the reaction site for MTSEA appears to be restricted since the larger derivatives (MTSET) have no effect. When each Cys in the N-terminal domain and two within the M1-M2 loop of α4 regions were mutated to gly to examine potential MTSEA reaction sites, a single residue -Cys 243- within the M1-M2 loop was identified as crucial. We hypothesize C-243 is sufficiently close to the Ca^{+2} regulatory site that MTSEA block of Ca^{+2} regulation serves as a reporter for the molecular interactions of this site with Ca^{+2}, since the mutant is still regulated by Ca^{+2}. The sequence around C-243 is a reasonnable candidate site for Ca^{+2} regulation, in view of local negative charge(α4: M1-LPSECGEK-M2).The alteration of the proposed Ca^{+2} pocket by α5 is of particular interest since α5 contributes another negative charge to the region. Our studies also suggest that reduction of Cys243 by MTSEA, decreases the Ca^{+2} permeability of α4β2 nAChRs. The α7 nAChR is highly permeable to Ca^{+2} and yet the sequence in this region differs only by the substitution of a Ser for C-243. It seems likely that changes in this region are connected to Ca^{+2}-nAChR interactions. Supported by NS22061 to L.R.

527.4

COMPENSATORY CHANGES IN nAChR CHANNEL EXPRESSION FOLLOWING ANTISENSE OLIGONUCLEOTIDE-MEDIATED SUBUNIT DELETION: α5 MINUS AND α7 MINUS nAChR CHANNELS

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Previous studies have demonstrated that α3 subunit is a key component of nAChR channels in peripheral autonomic neurons (studies from Berg, Linstrom, Jacob and colleagues as well as our laboratory). Recent studies using antisense oligonucleotide-mediated deletion show that α5 and α7 subunits also participate in native nAChR channels (see abstracts by Yu et.al., 1993,1994). In the course of these studies we found that treatment of neurons with antisense oligonucleotides to α3, α5 or α7 decreased subunit protein levels and deleted specific AChR channel subtypes, but also resulted in the expression of novel ACh-gated currents with distinct biophysical and pharmacological profiles.

The current study indicates that α5 and α7 may "compensate" for one another since the antisense-mediated deletion of one of these subunits results in the expression of channel subtypes with the pharmacological and/or biophysical properties of the other. Previous studies indicate that α5 containing nAChRs are large γ, slowly desensitizing channels that are ~ 100x less sensitive to ACh (see abstract by Ramirez et al., 1993). Deletion of α7 produces novel high conductance channels and results in a biphasic dose response curve. One component of the curve has an EC50 similar to that of the control, while the other component has an EC50 that is ~ 20 fold higher than that of first component. Likewise, deletion of the α5 subunit results in ACh-evoked currents that are blockable by α7-bungarotoxin. Since α7 is the only α-BGT sensitive subunits normally expressed by these cells, this suggests that α7 subunit is compensating in α5-minus neurons. Alternative interpretations, e.g., that increased expression rather thanincreased assembly of novel subunits are being examined. (supported by NIH22061 to LR)

527.5

CHRONIC NICOTINE TREATMENT UP-REGULATES α3 ACHRS AND α7 ACHRS EXPRESSED BY THE HUMAN NEUROBLASTOMA CELL LINE SH-SY5Y.

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The human neuroblastoma cell line SH-SY5Y expresses a variety of neuronal nicotinic receptor (AChR) subunits which include α3, α5, α7, β2, and β4. Chronic treatment with nicotine, but not d-tubocurarine or mecamylamine, induced a 600% increase in the number of α3 AChRs but only a 30% increase of α7 AChRs. Mecamylamine and nicotine are not synergistic in causing receptor up-regulation. The nicotine concentration dependence, time course, and extent of AChR upregulation are different from those of α4β2 AChRs in M10 cells. Scatchard analysis indicated that after chronic nicotine treatment the AChRs do not change their affinity for nicotine and α-bungarotoxin. Northern blot analysis showed that chronic nicotine exposure does not change the mRNA level of α3 or α7 subunits. These results indicate that up-regulation of α3 and α7 AChRs after chronic nicotine treatment occurs posttranscriptionally, as it does in M10 cells. (Supported by grants from NINDS, CSTR, CTR, and MDA).

527.6

[^{3}H]EPIBATIDINE BINDS TO NON-α4/ß2 HIGH AFFINITY NICOTINIC RECEPTORS IN IMR-32 CELLS. M.I. Dávila-García*, R.A. Houghtling and K.J. Kellar. Department of Pharmacology, Georgetown University School of Medicine, Washington, DC 20007.

IMR-32 neuroblastoma cells were established back in 1970 by Tumilowicz from human neural crest cells. These cells bind [^{125}I]α-BTX with high affinity and [^{3}H]Ach with low affinity. They have also been shown to express transcripts for α3, α5 and ß4 nicotinic receptor subunits, which is consistent with their ganglionic origin. [^{3}H]Epibatidine ([^{3}H]EB) is a high affinity ligand for neuronal nicotinic acetylcholine receptors. We have recently demonstrated that this ligand binds two high affinity sites in adult rat forebrain with Kd values of 14.7 and 360 pM and that these sites appear to be present in nearly equimolar concentrations. One of these sites is the α4/ß2 receptor, which binds other high affinity agonist radioligands. In the present study we examined [^{3}H]EB binding in IMR-32 cells. Since they do not appear to contain α4/ß2 receptors, [^{3}H]EB binding in these cells could help to determine the possible identity of the second site in brain. We found that IMR-32 cells contain two high affinity sites neither of which is labelled by [^{3}H]cytisine even at high concentrations (20nM). These two sites seem to be nicotinic in nature since nicotine competes effectively against [^{3}H]EB for binding at both sites. Curare displaces the radioligand effectively ($IC_{50} \approx 2$uM), but di-hydro-ß-erythroidine, mecamylamine and hexamethonium are not very effective competitors. Nicotine treatment for 8 days increases the number of [^{3}H]EB binding sites in IMR-32 cells in a dose dependent manner starting at concen-trations as low as 1μM. Thus, we found that these cells, which do not bind [^{3}H]cytisine and hence probably do not contain α4/ß2 nicotinic receptors, may have a third subtype of receptor which binds [^{3}H]EB with high affinity. At least one of these non-α4/ß2 nicotinic sites is regulated by nicotine.

This work was supported by NIH grants AG009973 and DA06486 to KJK and DA05417 to MIDG.

527.7

Elimination of Putative Phosphorylation Sites On Nicotinic Acetylcholine Receptors Affects the Rate of Recovery From Rapid Onset Desensitization. K. Paradiso, R.W.Kullberg[2], and P. Brehm*

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Fast perfusion of acetylcholine (ACh) to excised patches from *Xenopus* oocytes injected with RNA encoding *Xenopus* muscle nicotinic acetylcholine receptors was used to study sensitivity to ACh and kinetics of desensitization. Dose-response curves place estimates of EC-50s at 4.2μM for the embryonic receptor type (αβδγ) and 9.4μM for the adult receptor type (αβδε). Despite differences in EC-50, no corresponding difference was found in the onset rate of desensitization between the two receptor types. Application of 100μM ACh resulted in rapidly decaying inward currents which were fit by a single exponential function with an average time constant of ~60ms for both the αβδγ and the αβδε receptors. The time-dependence of recovery for αβδγ receptors, measured with paired pulses, was best fit with two exponentials corresponding to a fast (1.7 ± 0.5s, mean ± s.d.) and a slow (23.4 ± 9.2s) component of recovery. To determine whether phosphorylation affects kinetics of desensitization, wild type αβδγ receptors were compared to receptors in which the PKA-dependent phosphorylation sites were mutated from serine to alanine on the α, δ, and γ subunits. The time constants of desensitization onset were similar for both the wild type and the mutant receptors as were the time constants of recovery. However, the fast time constant of recovery accounted for only 38% of the recovery in the mutants compared to 77% in wild type αβδγ receptors. Thus, the overall recovery from desensitization is slower for mutant receptors supporting the proposition that receptor phosphorylation is a potential mechanism by which postsynaptic responses can be modulated. The fast onset of desensitization and slow recovery further suggests that at active synapses only a fraction of the total receptors are available for activation. Consistent with this idea, application of 2ms pulses of ACh to wild type αβδγ receptors, at frequencies between 10 and 40Hz, results in a progressive decrease in response ending in a steady state, as predicted by our measured onset and recovery rates.

527.8

CLONING OF cDNA'S ENCODING PROTEINS THAT INTERACT WITH THE 43 KD PROTEIN. E.T. Fung*, Bill, and R.L. Huganir. Department of Neurosciences, Johns Hopkins University School of Medicine. Baltimore, MD 21205.

During development of the neuromuscular junction, the nicotinic acetylcholine receptor (nAChR) clusters in the post-synaptic membrane. While the mechanisms by which this process occur are largely unknown, some of the molecules involved in the process have been identified. One of these is the 43 kD protein, a post-synaptic membrane-associated molecule. Expression of this protein in heterologous systems leads to formation of patches of 43 kD protein at the cell surface, and co-expression with nAChR leads to the formation of patches containing both 43 kD protein and nAChR. More recently, it has been shown that 43 kD protein can cluster each of the subunits of the nAChR individually. A knowledge of the proteins with which 43 kD interacts would be critical to the understanding of the mechanisms by which it functions. Traditional approaches such as immunoprecipitation have failed to identify those proteins. As an alternative, we have turned to the yeast two-hybrid system to identify cDNA's for proteins with which 43 kD might interact. This system has proven to have a high level of sensitivity and specificity. In addition, it provides direct sequence information. Screening of a cDNA library yielded 16 positives. Sequencing and characterization of these clones is underway.

527.9

DMXB, AN AGONIST SELECTIVE FOR THE α-BUNGAROTOXIN BINDING SITE, TRANSIENTLY NORMALIZES AUDITORY GATING IN THE DBA/2 MOUSE.

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DBA/2 mice, an inbred mouse strain with reduced numbers of hippocampal α-bungarotoxin (α-BTX) binding sites, consistently fail to inhibit their response to the second of 2 identical auditory stimuli, 0.5 sec apart. These mice produce hippocampal midlatency evoked potentials of similar magnitude to both clicks, unlike other strains (e.g. C3H, C57Bl, ST/b) which show a diminished response to the second stimulus. Previous studies in anesthetized rats have shown a loss of auditory gating with central administration of α-BTX. We have shown that systemic nicotine injections (3 mg/kg, sc) produce a transient normalization of gating in the DBA/2 mice. Here, we report that DMXB, an agonist selective for the α-BTX binding site, also produces normalization of gating in these mice. 5 min interval recordings of hippocampal auditory evoked potentials were made prior to and after a 1.0 mg/kg, sc, injection of DMXB. DMXB produced a rapid normalization of gating which was brief in duration as compared to nicotine. These data suggest that the α-BTX-sensitive subtype of the nicotinic receptor modulates auditory gating in the DBA/2 mouse.

527.10

COMPARATIVE LEVELS OF NICOTINIC $\alpha_4\beta_2$/[^{3}H]CYTISINE AND α_7/[^{125}I]α-BUNGAROTOXIN RECEPTORS IN AGED MEMORY-IMPAIRED AND MEMORY-UNIMPAIRED LONG EVANS RATS.

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It is now established that nicotinic receptors, likely of the $\alpha_4\beta_2$ sub-type are markedly decreased in Alzheimer's Disease (AD) brains. Nicotine and related agonists have been shown to facilitate attentional (Rochford et al. this meeting) and cognitive behaviors in various mammals, including man. Together, this leads to pilot clinical trials with nicotinic agonists in AD. However, less is known about the α_7 sub-type which is also expressed in the brain and has high affinity for the toxin α-bungarotoxin (α-BTX). The aim of the present study was to compare the status of $\alpha_4\beta_2$- and α_7 -like nicotinic receptors in an animal model that assesses cognitive impairments, the aged memory-impaired (AI) and aged memory-unimpaired (AU) Long Evans rats (Quirion *et al.* J.Neurosci. 15:1455, 1995). Apparent levels of $\alpha_4\beta_2$- and α_7-like nicotinic sites were evaluated by quantitative receptor autoradiography using [^{3}H]cytisine (10nM) and [^{125}I] α-BTX (1.5nM) as ligand, respectively. As reported earlier in young animals (Clarke *et al.* J.Neurosci. 5:1307, 1985), the distribution of these two classes of nicotinic receptors is segregated and relatively complementary. No significant changes in [^{3}H]cytisine binding levels were detected between adult (6 month old) and 24 month old AI and AU rats. Similar results were obtained for specific [^{125}I] α-BTX binding sites, except for significant increases in the CA1 subfield of the hippocampus and the accessory olfactory bulb (AOB) in the AU vs young groups, and significant decreases in the dentate gyrus and AOB of AI vs AU animals. Therefore, it appears that both $\alpha_4\beta_2$ and α_7 nicotinic receptor sub-types are not markedly altered during the normal aging process. The α_7 /[^{125}I] α-BTX sites seem to be more sensitive to the effects of aging however, their contribution to the emergence of the cognitive deficits in the rat remains to be fully elucidated. Supported by the MRCC.

527.11

NEURONAL NICOTINIC RECEPTOR mRNA ENCODING α4 SUBUNIT IS DIFFERENTIALLY EXPRESSED IN RAT BRAIN.

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The purpose of this study was to quantitate neuronal nicotinic receptor (nNR) α4 subunit mRNA in adult and three-week old Sprague-Dawley CD rat brain. 50 μg total RNA from adult or three-week old rat hippocampus (hippocampus +septum), cortex and thalamus was size-fractionated and transferred to nitrocellulose. Northern hybridization was performed via an α-^{32}P dCTP-labelled 200 bp probe encoding the non-conserved 3' intracellular loop of nNR α4-1 subunit cDNA. Total RNA was normalized for gel loading via γ-^{32}P ATP-labelled 28S rRNA. All data were quantified via Molecular Dynamics intensity units as the ratio of α4 mRNA to 28S rRNA. Preliminary studies have identified three transcripts homologous to the α4-1 cDNA. The sizes of these transcripts were 8.5, 4.2 and 2.4 kb, and their quantities were similar in both age groups. All three transcripts were present in the cortex and thalamus. Virtually no 8.5 kb signal was detected in the hippocampus. The ratio of 8.5 kb mRNA to 28S was higher in the thalamus than in the cortex. The ratio of the 4.2 kb transcript to 28S was greater in the thalamus than in the cortex or hippocampus, which had similar ratios. The ratio of the 2.4 kb fragment to 28S was higher in the thalamus than in the cortex, and much lower in the hippocampus. These findings correlate well with both the putative existence of multiple transcripts encoding the α4 subunit in rat brain, as well as the high abundance of α4 mRNA in the thalamus and cortex in comparison to the hippocampus. The overall goal of this study is to determine the effects of prenatal nicotine exposure on the postnatal development of Sprague-Dawley CD rats, with analyses on the quantity and distribution of nNR subunits at various postnatal time points.

527.12

CODISTRIBUTION OF NICOTINIC ACETYLCHOLINE RECEPTOR α3 AND β4 SUBUNIT mRNAs DURING RAT BRAIN DEVELOPMENT

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Neuronal nicotinic acetylcholine receptors (nAChR) are pentameric ion channels, that mediate fast ligand gated currents. Whereas central [^{3}H]nicotine binding sites consist predominantly of α4 and β2 subunits, other subunits are also expressed in rat brain and form functional heteromeric nAChR in expression studies. In rat peripheral ganglia α3 and β4 have been shown to form nAChR; however, this combination has not been reported in rat CNS. A previous study reported very limited expression of α3 and β4 mRNAs during early postnatal development. We have used *in situ* hybridization with sensitive ^{35}S-labeled full-length riboprobes to further examine codistribution of α3 and β4 mRNA in developing rat brain. Our results show, that both subunits have a distinct distribution pattern during development, with expression intensity stronger and more widespread than in the adult. Several areas showed codistribution of both mRNAs. In the telencephalon α3 and β4 mRNAs were found in frontal, primary visual and retrosplenial cortex with peak expression during the first, second and the third postnatal week, respectively. In the CA3 region of the hippocampus, there was codistribution of α3 and β4 mRNAs during the first and second postnatal week, with α3 but not β4 declining thereafter. In the caudate putamen, α3 and β4 mRNAs were found in scattered, large neurons throughout postnatal development. In the diencephalon, codistribution of α3 and β4 mRNAs was limited to the medial habenula which had the most intense expression for both subunits in neonates and throughout development. In the mesencephalon α3 and β4 mRNA expression was detected in the dopaminergic nuclei substantia nigra pars compacta and ventral tegmental area throughout development. However, β4 hybridization was weak in the SN, where several other nAChR subunits were also localized. In the pons both α3 and β4 mRNAs were detected in the adrenergic nucleus locus coeruleus and several brain stem nuclei many of them cholinergic. The codistribution of α3 and β4 mRNAs in different brain areas during development suggests that they form a distinct neuronal nAChR subtype which may be involved in rat brain development and neurotransmitter release. Coexpression studies are currently underway to determine whether α3 and β4 mRNA are expressed in the same cell. *Supported by PHS grant # NS30109*

527.13

α-CONOTOXIN EI: A NEW ANTAGONIST OF NICOTINIC ACETYLCHOLINE RECEPTORS.

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α-Conotoxin EI was purified from milked venom of the Atlantic fish-hunting cone snail *C. ermineus* by RP-HPLC, based on it's ability to produce muscle paralysis in fish and mice. The sequence was confirmed by LSI-MS and total synthesis. Disulfides were determined using partial reduction with TCEP. Although EI has a cysteine spacing analogous to α-conotoxins PnIA and PnIB, and a disulfide connectivity analogous to α-conotoxins MI and SI, it's sequence differs significantly from all known α-conotoxins. Functional effects of EI were investigated *in vivo* and *in vitro*, and compared with MI. MI is approximately 11 times more toxic to mice than EI. α-Conotoxin EI displays two distinct affinities of 11 ± 2.4 nM and 350 ± 62 nM (32-fold difference) for the two acetylcholine-binding sites on nicotinic receptors from mouse muscle BC_3H-1 cells. In contrast, α-conotoxin MI displays two distinct affinities of 0.55 ± 0.21 nM and 25 ± 5.2 μM (45,000-fold difference). Despite their different sequences and different affinities at the two acetylcholine-binding sites, both α-conotoxin EI and MI display higher affinity for the acetylcholine-binding site near the α/δ subunit interface. The apparent affinities and site preference of α-conotoxin EI for *Torpedo* nicotinic receptors were also determined.

527.14

A NEW α-CONOTOXIN WHICH TARGETS α3β2 NICOTINIC ACETYLCHOLINE RECEPTORS.

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We have isolated a 16-amino acid peptide from the venom of *Conus* (a marine snail) which potently blocks the α3β2 neuronal subtype of nicotinic acetylcholine receptors (nAChRs). This peptide was identified by electrophysiologically screening venom fractions against cloned nicotinic receptors expressed in *Xenopus* oocytes. The peptide's structure, which has been confirmed by mass spectrometry and total chemical synthesis, differs significantly from those of all previously isolated α-conotoxins. Disulfide bridging however is conserved. The toxin blocks the response to acetylcholine in oocytes expressing α3β2 nAChRs with an IC_{50} of 5-10 nM. Preliminary studies indicate that the peptide is 2 to 3 orders of magnitude less active in blocking ACh-responses of all other tested αxβy nAChRs. We have recently reported the isolation and characterization of α-conotoxin ImI, which selectively targets the (homomeric) α7 subtype of neuronal nAChRs. Other α-conotoxins selectively target the muscle subtype of nAChR. Thus, it is increasingly apparent that α-conotoxins represent a significant resource for ligands with which to probe structure-function relationships of various nAChR subtypes.

527.15

AN *IN SITU* HYBRIDIZATION STUDY OF mRNA EXPRESSION FOR α5 AND α6, PUTATIVE NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNITS, DURING POSTNATAL DEVELOPMENT OF THE RAT BRAIN. B.J. Morley and H.K. Happe*. Laboratory of Neurochemistry, Boys Town National Research Hospital, Omaha, NE 68131.

Two members of the nicotinic acetylcholine receptor subunit gene family, α5 and α6, have not been demonstrated to form functional receptors when expressed in combination with other receptor subunits in oocytes. Their function in the CNS is unknown. In adults their distribution is more restricted but overlaps the distribution of α4 and α7 mRNAs, which encode subunit components of the high-affinity agonist receptor and the α-bungarotoxin receptor, respectively. We have used *in situ* hybridization with [^{35}S]-labeled riboprobes to examine expression patterns in developing rat brain. Expression of α5 mRNA is much higher at day 10 than in adults for most brain regions. In cortex early expression of α5 mRNA in layers I, IV and VI contrasts with adults where α5 is apparent only in layer VI. The interpeduncular n. differs from other brain regions expressing α5 as increases, rather than decreases, in mRNA expression occur during the late postnatal period. Expression of α6 mRNA in medial habenulae and locus coeruleus is at near adult levels early in postnatal development while expression of α6 mRNA increases 2-3 fold in ventral tegmentum and substantia nigra compacta. Our results indicate that the α5 subunit, like the α7 subunit, may have a role during the primary period of synaptogenesis as levels are high before day 10 and decline while synaptic interactions are being formed. For α6 subunits the increase in mRNA expression occurs after other nicotinic subunits have reached adult levels and therefore may be involved in later developmental modulation of receptor properties or subunit composition.

527.16

AGE-RELATED CHANGES IN THE EXPRESSION OF NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNIT ALPHA-4 IN THE MOUSE BRAIN REVEALED BY IMMUNOHISTOCHEMICAL LOCALIZATION. R. Kulmer, L.C. Gahring, and S.W. Rogers*. S.L.C. VA-GRECC, and Human Molecular Biology and Genetics Program, University of Utah, Salt Lake City, UT 84112.

Antibodies to neuronal nicotinic acetylcholine receptor (nAChR) subunit α4 were prepared in rabbits to bacterial fusion proteins. These antibodies exhibit subunit specificity and they immunoprecipitate high affinity ^{3}H-nicotine binding sites (Rogers, et al., J. Neurosci. 12:4611, 1992; Flores et al., Molec. Pharmacol. 41:31, 1992). We have used these antibodies to localize nAChR α4 staining in the mouse brain. Overall staining resembles the distribution of high affinity nicotine binding sites and nAChR α4 RNA. Staining is in neuronal cell bodies, putative dendrites, and it is particularly robust in some white matter tracks such as the stria terminalis suggesting that both pre- and post-synaptic receptor localization may occur. Comparisons of the immunoreactivity pattern between brains from young (3 months, N = 9) and aged (24-28 months, N = 8) CBA/J mice were made. Our findings suggest that there is a marked reduction in the number of immunoreactive cells in the pyramidal layer and stratum oriens of CA1 of the hippocampus of aged mice. Furthermore, the number of dendritic arbors and axonal collaterals extending into the stratum radiatum from the pyramidal layer of these mice is diminished. These results suggest that changes in nAChR α4-related expression and distribution occur in defined subregions of the mouse brain during aging. *Supported by NIH grant AG04418 and the American Federation for Aging Research.*

527.17

DIFFERENTIAL EXPRESSION OF NEURONAL NICOTINIC RECEPTOR SUBUNIT mRNAs AMONG INDIVIDUAL INTRACARDIAC PARASYMPATHETIC NEURONS IS REVEALED BY SINGLE CELL RT-PCR K. Poth*, J. Cuevas, T.J. Nutter, D.J. Adams, C.W. Luetje, Department of Molecular and Cellular Pharmacology, University of Miami, Miami, FL, 33101.

Neuronal nAChRs are formed by various subunit combinations, each with distinct pharmacological and biophysical properties. In parasympathetic neurons cultured from rat intracardiac ganglia, 3 distinct single channel conductances have been observed (Adams and Nutter,1992,*J. Physiol.* (Paris) 86: 67-76). Whole cell currents exhibit several different rank orders of potency for nicotinic agonists (Nutter and Adams, unpublished). Thus, individual intracardiac parasympathetic neurons may express different arrays of nAChR subunits. We have used RT-PCR to gain insight into the neuronal nAChR subunit mRNA expression patterns of these neurons. The cytoplasms of individual intracardiac parasympathetic neurons were extracted using patch pipets and subjected to RT-PCR. The nAChR subunit mRNA expression pattern differed among individual neurons, ranging from relatively simple (for example: α3, α5, β4, or α3, α5, β2) to complex (for example: α2, α3, α4, α7, β2). The α3 subunit was nearly always expressed, while other subunits such as α5, α7 and β3 were clearly expressed in some neurons and not in others. Thus, differential nAChR subunit expression may account for multiple channel classes and different pharmacological profiles observed in individual parasympathetic neurons from rat cardiac ganglia. [Support: NIH DA-08102, AHA-FL 92GIA/849 and 92II/4 (CWL), NIH HL-35422 (DJA)]

ACETYLCHOLINE RECEPTOR: NICOTINIC EXPRESSION

528.1

PROPERTIES OF TRANSGENIC NICOTINIC ACETYLCHOLINE RECEPTORS STABLY EXPRESSED IN HUMAN CELLS AS HOMOOLIGOMERS OF WILD-TYPE OR MUTANT RAT OR CHICK α7 SUBUNITS. E. Puchacz*[1], J.-L. Galzi[§], B. Buisson[†], D. Bertrand[†], J.-P. Changeux[§] and R.J. Lukas[1]. [1]Barrow Neurological Institute, Phoenix, AZ 85013 USA, [§]Pasteur Institute, Paris, France and [†]Central Medical University, Geneva, Switzerland.

Transfected cells of the SH-SY5Y human neuroblastoma or of the native nAChR-null SHEP-1 human epithelial clonal lines have been obtained that stably overexpress/express specific 125-I-labeled α-bungarotoxin binding sites. The radiotoxin binding sites appear to represent nicotinic acetylcholine receptors (nAChR) formed as homooligomers of α7 subunits derived from rat or chick wild-type or mutant transgenes introduced into SH-SY5Y or SHEP-1 cells. In all stably transfected cell lines expressing wild-type transgenes tested to date, functional responses are observed that are sensitive to blockade by native α-bungarotoxin or methyllycaconitine and that exhibit fast kinetics for channel opening and desensitization. Transfectants are positively selected based on hygromycin resistance, and levels of expression of transgenes can be manipulated by variation of hygromycin concentration in culture medium. The efficiency of recovery of stable transfectants seems to be diminished in cells that express mutant α7 transgenes that are predicted to code for proteins that form slowly-desensitizing nAChR of high Ca^{2+} permeability, perhaps suggesting that expression of mutant nAChR is cytotoxic. These stably transfected cells are ideally suited for elucidation of basic principles of ligand-gated ion channel structure and function using the simplest of all possible prototypes, a functional homooligomer. Funded in USA by NIDAL and STRC.

528.2

FUNCTIONAL PHARMACOLOGICAL PROFILES OF DIVERSE NICOTINIC ACETYLCHOLINE RECEPTOR SUBTYPES: MECHANISMS OF ACTIVATION OR INHIBITION. R.J. Lukas, L. Lucero*, C.M. Eisenhour, A. Brockey, and M. Bohayets. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

Our previous work has shown that clonal cell lines of different derivation are excellent models for studies of fundamental properties of diverse, naturally-expressed nicotinic acetylcholine receptor (nAChR) subtypes. Here we report that agonist potencies and efficacies of agents such as nicotine, cytisine, epibatidine, and suberyldicholine vary across nAChR subtypes and to some degree across species for a given nAChR subtype based on studies testing function of human muscle-type nAChR expressed in cells of the TE671/RD clonal line or of mammalian ganglionic nAChR (probably containing α3 and β4 subunits) expressed in cells of the IMR-32 or SH-SY5Y human neuroblastomas or the PC12 rat pheochromocytoma. Antagonistic potencies of agents such as d-tubocurarine, pancuronium, methyllycaconitine, mecamylamine, and various local anesthetics, snake toxins, or marine toxins also vary across nAChR subtypes, as do mechanisms (competitive vs. non-competitive inhibition) of nAChR block. Moreover, agents such as verapamil and dihydropyridines block nAChR subtypes with potencies that rival those for blockade of voltage-gated Ca^{2+} channels. These findings validate usefulness of clonal cells for studies of nAChR structures and functions and for elucidation of potencies and mechanisms of action of nicotinic compounds that may be clinically useful, e.g., to alter smoking habits, in cognitive enhancement, etc. Funded by NIDAL, STRC, CTR, APDA.

528.3

INTERACTIONS OF TACHYKININS WITH DIVERSE NICOTINIC ACETYLCHOLINE RECEPTOR SUBTYPES. C.M. Eisenhour and R.J. Lukas*. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

Substance P (SP) has been reported by several investigators to affect functional responses and/or ligand binding of some nicotinic acetylcholine receptor (nAChR) subtypes. Here we show that SP is the most potent of the tachykinins (TK) tested (SP, neurokinins A and B, eledoisin) as an acutely-acting inhibitor of function of muscle-type ($\alpha1\beta1\gamma\delta$ subunit-containing) nAChR from cells of the TE671/RD human clonal line or of ganglionic ($\alpha3\beta4$ subunit-containing) nAChR from cells of the SH-SY5Y human neuroblastoma or the PC12 rat pheochromocytoma. The concentrations of SP or the other TK required to affect nAChR function ($IC_{50} \geq 1$ μM) are much lower than the concentrations shown to have little-if-any effect on radiolabeled nicotinic ligand binding to muscle-type nAChR or $\alpha3\beta4$-nAChR or to ganglionic nAChR containing $\alpha7$ subunits that also are found on SH-SY5Y or PC12 cells. No evidence was obtained for high (nM) affinity SP binding sites in these cells that could be coupled to second messenger production or for effects of chronic TK treatment on nAChR expression. These and other results are consistent with the conclusions of Stafford et al., (Molec. Pharm. 45: 758, 1994) that SP acts directly to affect nAChR function, probably by targeting sites on $\beta4$ (and $\beta1$) subunits remote from the agonist and competitive antagonist binding domains. Funded by STRC.

528.4

SENSITIZATION OF THE MUSCLE NICOTINIC ACETYLCHOLINE RECEPTOR IN TE671 CELLS BY THE COMPETITIVE ANTAGONISTS VECURONIUM AND ROCURONIUM. F. Van Huizen*, C. Sünnen and D. Schaap. Organon Teknika, Bioscience Research Unit, PO Box 84, 5280 AB Boxtel, The Netherlands

The TE671 cell line was used to study the desensitization and sensitization of the muscle nicotinic acetylcholine receptor (nAChR). Carbachol (CCh) stimulated the influx of $^{86}Rb^+$ into the cells with an EC_{50} of $5.5*10^{-5}$M. This influx was blocked by the competitive antagonists vecuronium and rocuronium with IC_{50} values of $4*10^{-8}$ and $8*10^{-7}$ M, respectively. Pre-incubation of the cells with CCh caused a time- and dose-dependent desensitization of the CCh-induced $^{86}Rb^+$-influx into the cells. After 20 min, 60% of control stimulation was left, while after 60 min only 20% was left. Binding of [^{125}I]α-bungarotoxin (α-BTX) was decreased with 20% after 60 min pre-incubation with 1 mM CCh.

In contrast, pre-incubation with low concentrations of the competitive antagonists vecuronium bromide or rocuronium bromide caused a sensitization of the $^{86}Rb^+$-influx. One hour pre-incubation of the cells with 1 μM vecuronium bromide or rocuronium bromide increased the control stimulation with 60% and 160%, respectively. Only a 5% increase in [^{125}I]α-BTX binding was observed, which excludes receptor upregulation. It is known that the nAChR exists in equilibrium between the resting state and the desensitized state. Since competitive antagonists bind preferably to the resting state, this will shift the equilibrium in that direction and sensitize the receptor.

528.5

ERBB3 AND ERBB2/NEU TRANSDUCE THE EFFECT OF HEREGULIN ON ACETYLCHOLINE GENE EXPRESSION IN MUSCLE: DIFFERENTIAL EXPRESSION AT THE ENDPLATE. N. Altiok, J.-L. Bessereau and J.-P. Changeux*. Neurobiologie Moléculaire, Département des Biotechnologies, Institut Pasteur, 75015 Paris, France

The motor neurons modulate acetylcholine receptor (AChR) gene expression in skeletal muscle by two signalling pathways: the transmitter-evoked depolarization of muscle membrane inhibits AChR genes transcription throughout the myofiber presumably via activation of a serine/threonine kinase, while the transcription rates of AChR genes in the synaptic region are increased by nerve-derived trophic factors including AChR-inducing activity (ARIA). To gain further insight to these interactions we characterized the receptor of heregulin (HRG)/ARIA in muscle. We showed that HRG increases AChR α-subunit mRNA levels via tyrosine phosphorylation of ErbB3 and ErbB2/neu in chick, rat and mouse myotubes. The protein tyrosine phosphatase inhibitor, pervanadate potentiated the responses to HRG that were in turn blocked by the tyrosine kinase inhibitor erbstatin, indicating the relevance of tyrosine phosphorylation to these events. The effects of HRG were inhibited by enhanced cellular serine/threonine phosphorylation which has been implicated in the repression of AChR genes by electrical activity. Immunocytochemical analysis of adult rat muscle revealed that while ErbB2/neu and ErbB4 are distributed in a diffuse pattern along the myofiber membrane, ErbB3 expression is exclusively restricted to the endplate suggesting its involvement in synapse-specific transcription of AChR genes by HRG/ARIA.

528.6

DISSOCIATION BETWEEN ANTINOCICEPTION AND ANTI-INFLAMMATORY EFFECTS OF NICOTINE IN THE FORMALIN TEST IN RATS. A. A. Houdi*, M. Welch and C. Diaz. College of Pharmacy and THRI, University of Kentucky, Lexington, KY 40546.

Nicotine may selectively alter sensitivity to certain classes of pain stimuli. In this study, we examined the central and peripheral antinociceptive properties of nicotine in rats using the formalin-test. It has been suggested that the transient early phase of pain (0-5 min) after formalin is due to the direct effect on sensory receptors, whereas the tonic late phase (15-45 min) is due to an inflammatory response. In control groups, formalin solution (0.5%) injected subcutaneously (sc) into the surface of one hind paw of male Sprague-Dawley rats (300 ± 30 gm, body wt.) pretreated with saline produced a characteristic biphasic pain response. Subcutaneous nicotine administration inhibited this formalin pain response in a dose-dependent (0.1-2 mg/kg) manner. Intracerebroventricularly (icv) administered nicotine inhibited formalin pain response of the first phase at lower dose (4 μg/rat) and the first phase and partially the second phase at higher doses (20 & 40 μg/rat). The lower dose of icv nicotine was ineffective when given sc. Nicotinic antagonist mecamylamine (0.5 mg/kg, sc) antagonized the sc nicotine effect, whereas hexamethonium (5 mg/kg, sc), a nicotinic antagonist which does not readily cross the blood brain barrier, partially blocked the response only to the second phase of nicotine's effect. Hexamethonium given icv produced analgesia to the first phase of pain only and did not affect nicotine response given centrally. Pretreatment with an opioid receptor antagonist (naloxone 1 or 2 mg/kg, sc), or nitric oxide synthase inhibitor (N^G-nitro-L-arginine methyl ester, sc or icv) had no effect on the analgesic properties of subcutaneous nicotine (1 mg/kg). These results indicate that nicotine possesses analgesic activity toward two kinds of pain which may be differently modulated; a short-lived pain caused by the direct effect of formalin on sensory receptors followed by a longer lasting pain due to inflammation. This analgesic effect of nicotine is not mediated by endogenous opioids or nitric oxide systems. Supported by KTRB.

528.7

REGULATION OF α-BUNGAROTOXIN RECEPTOR EXPRESSION IN DEVELOPING NEURONS. C. Brumwell, M.S. Levey*, O.C. Ikonomov and M.H. Jacob. Worcester Fdn. for Biomed. Res., Shrewsbury, MA 01545. Cellular and molecular mechanisms that regulate synapse differentiation on neurons are still largely undefined. Chick ciliary ganglion (CG) neurons express two different classes of nicotinic cholinergic receptors, acetylcholine receptors (AChRs) and α-bungarotoxin receptors (α-BgtRs), that are segregated into distinct domains in synaptic regions. AChRs are predominantly localized in the specialized postsynaptic membrane, whereas α-BgtRs are restricted to perisynaptic regions of the neuron surface. We have recently established that the expression of AChRs is differentially regulated by innervation and target tissue interactions during synapse formation. We have now determined that these cell-cell interactions also regulate α-BgtR expression.

α-BgtR $\alpha7$ subunit mRNA levels per neuron increase 3.4-fold during synapse formation and maturation, from E8 to E14, as determined by quantitative RT-PCR. Surgical removal of the preganglionic neurons or postganglionic target tissues prior to synaptogenesis causes CG $\alpha7$ mRNA levels to decline 1.6-fold and 2.2-fold, respectively. Interestingly, the effects of input- and target-deprivation are not additive. In contrast to $\alpha7$, β-tubulin mRNA levels are not affected by these manipulations. Similarly, α-BgtR protein levels are specifically reduced by input- or target-deprivation as determined by histochemical labeling. Thus, innervation and target tissues regulate the expression of neurotransmitter receptors that are excluded from postsynaptic membranes. The absence of additive effects suggests input and target tissues either provide a similar factor(s), or different factors that activate a common step in the signal transduction pathway regulating $\alpha7$ transcript levels. Supported by NIH 21725, and the Fairlawn Foundation.

528.8

A MAJOR DETERMINANT OF COMPETITIVE ANTAGONIST SENSITIVITY OF NEURONAL NICOTINIC RECEPTORS IS LOCALIZED TO RESIDUES 55-63 OF THE β2 SUBUNIT. S.C. Harvey*, C.W. Luetje Department of Molecular and Cellular Pharmacology, University of Miami, Miami, FL 33101.

The β subunit contribution to the sensitivity of neuronal nAChRs to the competitive antagonist neuronal bungarotoxin (NBT) has been localized to the first 80 residues of β2 (Wheeler et al,1993,*FEBS Lett.* 332:139). Determinants of sensitivity to both NBT and the structurally distinct competitive antagonist, dihydro-β-erythroidine (DHβE), are located within this region (Harvey and Luetje,1994,Soc.Neurosci.Abstr. 20:1134). We constructed additional β subunit chimeras to more closely map these determinants. Receptors formed by α3 and β2 were sensitive to 100 nM NBT (96.6±1.6% block), while α3β4 was insensitive. At an EC20 ACh concentration, α3β2 was 50 fold more sensitive to DHβE (IC50≈400 nM) than α3β4 (IC50≈20 μM). 3 μM DHβE effectively blocked α3β2 (90.0±4.6% block) but had little effect on α3β4 (13.1±5.1% block). Receptors formed by β2-54-β4 were indistinguishable from α3β4 (5.7±26.0% block by NBT, 19.2±3.9% block by DHβE). Receptors formed by β2-63-β4 were blocked 86.4±5.6% by NBT and 81.0±5.5% by DHβE. Receptors formed by β2-73-β4 were blocked 89.6±1.4% by NBT and 90.1±3.1% by DHβE. Receptors formed by β2-80-β4 were blocked 97.8±2.0% by NBT and 93.3±0.5% by DHβE. These results show that the major determinant for both NBT and DHβE sensitivity lies within sequence segment 55-63. Mutation experiments are in progress to identify the critical residues within this region. [Support: NIH DA-08102, AHA-FL 92GIA/849 and 92II/4].

528.9

NICOTINE ENHANCES THE PEPTIDERGIC EPSC AND POTENTIATES TRANSMISSION IN BULLFROG SYMPATHETIC GANGLIA

P. Jobling*, W-X Shen and J.P. Horn. Department of Neurobiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

Synaptic release of LHRH by preganglionic terminals excites bullfrog sympathetic neurons through suppression of M-current and activation of a second current. We have studied the effects of nicotine upon synaptic currents, responses to exogenous LHRH, and M-current, when recorded under one- and two-electrode voltage clamp. Bath application of 3 μM nicotine evoked a steady inward current of 0.95±0.57 nA (n=9). Nicotine enhanced the peptidergic EPSC in 6 of 9 cells by 55±36% (x±sd) and the inward current evoked by exogenous LHRH in 5 of 6 cells by 54±23%. M-current relaxations measured after hyperpolarizing voltage steps (-25-40 mV to -50-60 mV) were decreased by 19±14% (n=9, $p<0.01$), although the total current measured at the end of the voltage step increased in 3/9 cells.

Extracellular recordings of peptidergic afterdischarges from rami communicantes and simultaneous recording of membrane potential from individual B and C neurons revealed that peptidergic transmission was potentiated at nicotine concentrations of 250 nM-1μM with little associated membrane depolarization.

The results imply that activation of ligand-gated nicotinic channels can modulate synaptic events controlled by G-protein coupled receptors. Supported by NIH NS21065 and a Postdoctoral Fellowship (PJ) from the AHA, PA Affiliate.

528.10

THE BINDING SITE OF RAT AND CHICK FORMS OF THE NICOTINIC RECEPTOR SUBUNIT ALPHA 7 IS DISTINGUISHED BY THE PIP SERIES OF CHOLINERGIC AGONISTS.

R.W. Vazquez, G.A. Weiland* and R.E. Oswald. Dept. Pharmacology, Cornell Univ., Ithaca, NY 14853 USA.

A structurally related, semirigid series of cholinergic agonists where synthesized (based on 1,1-dimethyl-4-acetylpiperizinium, PIP; Carter & Oswald, *Biophys. J.* 65:840-851, 1993) and used to study activation and channel blockade of the nicotinic acetylcholine receptor (nAChR).

R =	
H	HPIP
CH_3	EthylPIP
CH_2CH_3	PropylPIP
$CH_2CH_2CH_3$	ButylPIP
$CH_2CH_2CH_2CH_3$	PentylPIP
$CH_2CH_2CH_2CH_2CH_3$	HexylPIP

Patch clamp experiments using BC_3H1 cells expressing the mouse muscle form of the nAChR, showed that, at low concentrations (1-2 μM), the PIP series were potent agonists, with little difference in activation properties. At high concentrations (mM), the series displays structure- and voltage-dependent noncompetitive channel blockade. Current efforts have focused on the agonist properties of the PIP series for the neuronal forms of nAChR, chick and rat α7. Using the oocyte expression system with a two-electrode voltage clamp, the PIP series displayed a discontinuous agonist profile for chick α7. Short chain derivatives activated the channel fully; however, agonist activity was lost when the chain was lengthened from PropylPIP to ButylPIP. This was found to be due most likely to the channel activation properties, rather than voltage-dependent channel blockade, as the longer chain PIP compounds failed to activate the channel at positive membrane potentials under conditions for which outward current was easily observed with ACh and shorter chain PIP derivatives (EDTA injection into the oocyte). Under identical conditions, the entire PIP series could produce currents in oocytes injected with the rat α7 subunit. A related agonist (DMPP) has been shown to be a weak partial agonist of the chick α7 nAChR but a full agonist for the rat α7 nAChR (Gerzanich & Lindstrom, *Mol. Pharmacol.* 45:212-220, 1994). These set of experiments indicate that the PIP series can be used as sensitive probes of the nAChR binding site.

528.11

AGONIST SENSITIVITY OF NICOTINIC ACETYLCHOLINE RECEPTORS IN RAT VAGUS NERVE.

K. Eskesen* and V.S. Levy. Novo Nordisk A/S, Health Care Discovery, Neuroscience, Novo Nordisk Park, DK-2760 Måløv, Denmark.

A pharmacological characterization of the ligand-gated acetylcholine receptor present in the axons of the rat vagus nerve was sought by studying the potency and efficacy of a series of nicotinic agonists on desheated vagus nerve using the "grease-gap" technique. Characteristic bell-shaped concentration response curves can be obtained from large depolarizations of the vagus nerve. The EC_{50} values for these responses to 5 agonists when given in the rank order of potency are: epibatidine: 0.03 μM, lobeline: 0.9 μM, nicotine: 6 μM, cytisine: 19 μM and DMPP: 24 μM. DMPP is the most and lobeline the least efficacious of the compounds tested with maximal responses of 250% and 40%, respectively, of the maximal response to nicotine. The efficacy of epibatidine, nicotine and cytisine was close to equal. There is, however, an additional component of the depolarization induced by agonists of nicotinic acetylcholine receptors. This depolarization is of lower amplitude and observed at concentrations 2-3 decades lower than the EC_{50}-values calculated, indicating the presence of high affinity receptors. The results do not give any clear indication as to which subtype of the neuronal nicotinic receptors is present, since there is no agreement with the rank order of potency of agonists we have tested on the vagus nerve with data published for subtypes expressed in oocytes from xenopus Laevis. Rather it indicates that we obtain responses from more than one subtype and possibly combinations of subunits different from the ones tested in oocytes.

528.12

ACTIVATION OF NICOTINIC ACETYLCHOLINE (ACh) RECEPTOR CHANNELS MOBILIZES CALCIUM FROM CAFFEINE-SENSITIVE STORES IN FURA-2 LOADED CULTURED RAT MYOBALLS.

J. F. Fiekers* and T.J. Heppner, Dept. Anatomy & Neurobiology., Univ. Vermont Coll. Med. Burl., VT 05405.

Measurement of the kinetics of $\{Ca^{2+}\}_i$ in response to activation of ACh receptor channels was performed using dual excitation microspectrofluorometry in fura-2-loaded rat myoballs. Receptor-activated currents were also recorded using the whole cell configuration of the patch clamp technique. Agonist-induced currents, as well as increases in $\{Ca^{2+}\}_i$, were blocked by prior exposure of myoballs to α-bungarotoxin. Short applications of agonist (<1 sec) produced a rapid transient increase in $\{Ca^{2+}\}_i$ which usually returned to baseline levels. However, prolonged applications of agonists (>10 sec) produced a fast transient followed by a long-lasting plateau. The individual components of the $\{Ca^{2+}\}_i$ signal were unrelated to activation of voltage-gated calcium channels because the responses were retained in the presence of voltage-gated calcium channel blockers. Both responses, however, were dependent on extracellular calcium. Caffeine (10mM, 10 sec) produced a rapid increase in $\{Ca^{2+}\}_i$ which initially was markedly prolonged in the presence of ryanodine. Successive applications of caffeine reduced the $\{Ca^{2+}\}_i$ response in the presence of ryanodine. Plateau, but not transient, responses of ACh-activated $\{Ca^{2+}\}_i$ responses were reduced following ryanodine. These results suggest that activation of ACh receptor channels plays an important role to increase $\{Ca^{2+}\}_i$ by (1) influx of external calcium through the receptor-channel complex and (2) intracellular release via caffeine-sensitive stores. (Supported by MDA).

528.13

MODULATION OF THE ACTIVITY OF THE NICOTINIC α3β4 RECEPTOR SUBTYPE BY ALCOHOL.

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Previous electrophysiological studies examining interactions between alcohol and cloned nicotinic acetylcholine receptor subunits expressed in *Xenopus* oocytes have shown that the α7 receptor subtype is strongly inhibited by alcohol. In contrast, the effect of alcohol on other neuronal subtypes containing the β2 subunit, including α3β2, are marginal, (de Fiebre et al., 1994, P14, Abstracts, International Symposium on Nicotine, Montreal, Canada). However, in oocytes in which the α3β4 combination was expressed from RNA or DNA, co-application of 30 mM ethanol with 1-10 μM nicotine or acetylcholine resulted overall in a mean inhibition of the agonist-induced inward current of -2.9 ± 5.1%, (± S.E., n=21, range= -73.4 to +23.5%; Ba^{++} replacing Ca^{++} in the recording solution). 100 mM ethanol potentiated the response by 33.8 ± 5.1%, (n=12, range = +0.3 to +56.7%); and 300 mM ethanol gave a potentiation of 83.7 ± 19.1%, (n=13, range = -20 to +216.4%). The varied response to alcohol, especially at lower concentrations, suggests multiple mechanisms of receptor regulation which may contribute to synaptic plasticity in brain and ganglia. The sensitivity of receptors containing α3 and β4 subunits to clinically relevant concentrations of ethanol may also be involved in the interactive processes of addiction to alcohol and nicotine. We wish to acknowledge the support of the M.R.C. (U.K.), The Royal Society, S.H.E.R.T., the Strathclyde Molecular Biology Lab and the technical contributions of Fiona Kempsill, Dr. Diane Dixon and Angela Garman.

528.14

Peptidyl Prolyl Isomerase Activity of Cytoplasmic Cyclophilin in the Maturation of Homo-oligomeric Receptors.

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We have recently shown that maturation of homo-oligomeric ligand-gated ion channels, α7 nicotinic and type 3 serotonin ($5HT_3$) receptors is blocked by the widely used immunosuppressant cyclosporin A (CsA). This effect is most likely to be due to the selective blockade of a ubiquitous polypeptide called cyclophilin A (CyPA) that is present in high concentrations within neurons. The cyclophilins (CyPs) are a family of helper molecules involved in the folding of cellular proteins. They catalyse the cis-trans interconversion of prolyl peptide bonds through their peptidyl prolyl isomerase activity (PPIase), and may also serve as molecular chaperones in protein folding. Their role in CsA-induced immunosuppression is shown to be indirect through the inhibition of calcineurin.

In the present study we first provide conclusive evidence that CsA-induced blockade of homo-oligomeric receptor expression in *Xenopus* oocytes is not mediated through the indirect calcineurin-dependent mechanism. A non-immunosuppressive CsA analog SDZ211-811 that selectively blocks the PPIase activity of CyPs but does not cause inhibition of calcineurin blocked receptor expression as effectively as CsA. Second, we show that the CsA-induced reduction in receptor expression is due to the blockade of the cytoplasmic CyP, CyPA. Overexpression of CyPA but not endoplasmic reticulum resident CyP, CyPB caused a reversal of the CsA effect on receptor expression. This reversal is not simply due to the buffering of CsA by CyPA because overexpression of a mutant CyPA which is deficient in high affinity CsA binding activity also produces a reversal of the CsA effect. This mutant has a tryptophan to phenylalanine substitution at position 121 (W121F) in CyPA. This result also indicates that the ability of CyPA to bind CsA with high affinity is not important for its action in receptor maturation. A CyPA double mutant that has a histidine to glutamine change at position 126 which causes a ~200-fold reduction in the PPIase activity of CyPA also produced a reversal of the CsA effect. The latter result suggests that either the PPIase activity of CyPA is not required for the maturation of homo-oligomeric receptors or that minimal PPIase activity is sufficient to produce significant functional receptor expression. To determine which one of these two possibilities holds true for the action of CyPA we have made another CyPA mutant (CyPAR55A) that shows a ~1000-fold reduction in PPIase activity compared to the wild-type. These results will enable us to clarify whether the physiological role of CyPA in oligomeric receptor maturation is that of a PPIase or that of a molecular chaperone.

Supported by grants from NIH (JP) and a MDA fellowship (SAH).

528.15

RATE CONSTANTS IN ACETYLCHOLINE RECEPTOR DEGRADATION Rufeng Xu and Miriam. M. Salpeter* Department of Neurobiology and Behavior, Cornell Univ., Ithaca, NY 14850

Degradation of muscle acetylcholine receptors(AChRs) is believed to proceed via internalization, and subsequent digestion in lysosomes (Devreotes and Fambrough, Cold Spring Harbor Symp Quant Biol 40: 237, 1976; Libby et al., Cell 19: 481, 1980; Fumagalli et al., J. Neuropath & Expt. Neurol. 41: 567, 1982). However, no detailed analysis of the rate constants involved in this internalization and exocytosis is available. We used proteinase K (PK), an enzyme previously used to cleave surface molecules (Stoorvogel et al., J. Cell Biol. 108:2137, 1989; Görne-Tschelnokow et al., EMBO J. 13:338, 1994), to determine PK-sensitive and PK-resistant AChRs in innervated mouse diaphragm muscles. The AChRs on mouse diaphragms were labeled with ^{125}I-α-bungarotoxin *in vivo* and, at different times after labeling,the muscles were removed, washed and counted. This established that the degradation rate of the total muscle receptors had a $t_{1/2}$ ~ 10.5 days. At each post-labeling time point, the same muscle samples were digested in PK,and the PK-resistant residual radioactivity counted. After subtracting the PK-resistant counts from the total counts, a PK-sensitive compartment was established. The results were compared with a mathematical model which assumed an internalization rate constant, k_i and an exocytosis rate constant, k_o. The amount of receptors remaining in the PK- sensitive and resistant compartments at different times after labeling was consistent with a k_i=0.0673 ($t_{1/2}$ ~ 10.3 days) and a k_o=0.693 ($t_{1/2}$ ~ 1 day). Rate constants in denervated muscles and the effect of agents that influence degradation rate will be reported. Supported by NIH NS 09315 and 2 T32 GM07469.

528.16

NICOTINIC RECEPTORS ACTIVATION ON CENTRAL AUTONOMIC PREGANGLIONIC NEURONES IN RAT SPINAL CORD SLICES. C.Dominguez-Perrot*, A.Bordey and P.Feltz. Institut de physiologie, URA 1446 CNRS. 21 rue R.Descartes 67000 Strasbourg.

Nicotinic acetylcholine receptors (nAChRs) on sympathetic preganglionic neurones (SPNs) of the region near the central canal were studied using the whole-cell patch-clamp technique in neonate rat transversal spinal cord slices (200-250 µm).

Visualization under Nomarski optics and labelling with Lucifer Yellow in the recording pipette allowed to identify recorded cells as SPNs. Central SPNs are localized in the dorsal part of the central region (CR). they are fusiform in shape, with axes ranging from (20 to 25µm)*(9 to 12.5µm) and dendrites mediolaterally orientated. They exhibit a firing frequency ranging between 1-6 Hz which declines during the recording. Under current-clamp conditions. pressure ejection (1 mM. 100 msec) of the nicotinic receptor agonist. 1,1-dimethyl-4-phenyl-piperazinium (DMPP) induced a depolarizing response and increased the firing frequency of SPNs. Under voltage-clamp conditions. in SPNs of IML and CR. DMPP induced a small inward current ranging from -4 to -15 pA with an increase in noise. The current-voltage (IV) relationship for DMPP-induced current exhibited a reversal potential around 0 mV and a strong outward rectification. The nAChRs responses were blocked by mecamylamine, d-tubocurarine (d-TC) but were insensitive to hexamethonium, toxin F and methyllycaconitine (MLA). Moreover, superfusion of tetrodotoxin (1 µM). CNQX (10 µM). cadmium (100 µM) did not abolish the DMPP-induced current which confirmed a direct effect of DMPP onto SPNs. At the peak of the current, DMPP induced the discharge of excitatory postsynaptic currents (EPSCs) or increased their discharge rate for 1-2 min following the DMPP application. These EPSCs were completely abolished by CNQX. We concluded that SPNs of the IML and CR are endowed with a functional nicotinic acetylcholine receptors. Moreover glutamate-containing terminals project onto SPNs.

528.17

IDENTIFICATION AND CHARACTERIZATION OF TYROSINE PHOSPHORYLATED PROTEINS FROM THE ELECTRIC ORGAN OF *T. CALIFORNICA*. S. Balasubramanian and R.L. Huganir*, Dept. of Neuroscience, Howard Hughes Med. Inst., Johns Hopkins Univ. School of Med., Baltimore, MD, 21205.

Tyrosine phosphorylation has been implicated in regulation of the synthesis, distribution and gating properties of the nicotinic acetylcholine receptor (nAChR). To better understand these processes, we are attempting to identify additional proteins which are tyrosine phosphorylated at the cholinergic synapse.

The electric organ of *T. Californica* is abundant in nAChR and nAChR associated proteins, and was therefore chosen as our protein source. Phosphotyrosine containing proteins were isolated by immunoaffinity chromatography using antiphosphotyrosine antibodies. Polyclonal antibodies were then generated against the tyrosine phosphorylated proteins and used to screen a λgt11 expression library made from electric organ cDNA. Most of the clones identified in this manner represented the nAChR itself, which has previously been shown to be tyrosine phosphorylated (Qu et al., 1990). Of the 70 remaining clones 12 encoded type III intermediate filament from *T. Californica*; 11 encoded the 90 kDa heat shock protein; 9 encoded non-erythroid alpha spectrin; 39 clones were novel. We are presently characterizing the possible role of tyrosine phosphorylation in the regulation of these proteins.

528.18

EFFECT OF EPIDERMAL GROWTH FACTOR, INTERLEUKIN-6 AND CHOLINERGIC LIGANDS ON THE TRANSCRIPTION OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNIT GENES BY HUMAN THYMIC CELLS. Mirta Mihovilovic[1,*], Yun Mai[1] and Allen D. Roses[1,2]. [1]Department of Medicine, Division of Neurology and [2]Department of Neurobiology. Duke University Medical Center, Durham, NC 27710.

Transcription of the α-3, α-5 and β-4 subunits of nicotinic neuronal acetylcholine receptor (AcChR) genes in thymocytes and thymic epithelial cells and immunohistochemical analyses using polyclonal anti-α-3 antibodies suggests that neuronal AcChRs similar to those expressed in ganglia are expressed in thymic cells. These receptors may be of importance in transducing signals delivered to the thymus through the autonomic nervous system.

Thymocytes express nicotinic neuronal AcChR transcripts at levels comparable to those of the thymic tissue as a whole, while epithelial cells express much lower transcripts levels. Expression of these transcripts in primary cultures of thymic epithelial cells is dependent on the presence of epidermal growth factor (EGF) and to a lesser extent on interleukin (Il-6). Thymic epithelial primary cell cultures bind monoclonal antibodies that recognize neurofilaments and in the presence of IL-6, develop thymic dendritic cell morphology that is concomitant with a decrease in keratin expression. Establishment of thymocytes in culture leads to lower levels of transcription for the α-3 and β-4 AcChR subunit genes but IL-6 delays this down regulation. In addition, long term exposure to both carbamylcholine and nicotine appears to promote α-3 AcChR gene transcription in cultured thymic epithelial cells. These results indicate that the thymic endocrine/ paracrine microenvironment is necessary to sustain AcChR subunit gene transcription by thymic cells and that cholinergic activity may modulate thymic AcChR gene transcription.

528.19

ON MECHANISMS OF NICOTINE-INDUCED UPREGULATION AND FUNCTIONAL INACTIVATION OF DIVERSE NICOTINIC RECEPTOR SUBTYPES. L. Ke*, C.M. Eisenhour, M. Bencherif[1], E. Puchacz and R.J. Lukas. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013 ([1]Currently at R&D, R.J. Reynolds Tobacco Co., Winston-Salem, NC 27102).

Our previous work has shown that chronic nicotine exposure induces upregulation (increases in radioligand binding sites) of muscle-type nicotinic acetylcholine receptors (nAChR), ganglionic nAChR containing α3 and β4 subunits or containing α7 subunits, CNS nAChR containing α4 and β2 subunits, and even transgenic nAChR in stably transfected cells lines expressing α7 or α4 and β2 nAChR subunits - all without altering nAChR subunit mRNA levels. We also have shown that chronic nicotine induces long-lasting functional inactivation (FI) of muscle-type and α3β4-nAChR, but also that temporal and dose dependencies for FI differ from those for nAChR upregulation. Here we report results of pharmacological studies that further distinguish nicotine-induced upregulation from FI, that illuminate possible mechanisms involved in these processes, that identify classes of drugs (forskolin, TMB-8, pancuronium) having high value in these types of studies, that help elucidate the complex effects of treatments with drugs such as cycloheximide on nAChR expression and sensitivity to nicotine treatment, and that reveal a wide range of effects of nicotine treatment on nAChR metabolism. Results are framed within a model emphasizing post-transcriptional effects of nicotine and state transitions of diverse nAChR subtypes. Funded by NIDAL, STRC, ADCRC.

528.20

Target induced Changes in nAChR -Mediated currents and Subunit Gene Expression Require Membrane Associated Factor(s).

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Development of transmitter sensitivity in sympathetic ganglia has been charted by measuring the progressive increases in the magnitude of ACh-evoked currents and the levels of the α3, α5, α7 and β4 subunit mRNAs (Moss and Role, 1992; Devay et al, 1994). These changes are induced by both preganglionic innervation and by target contact. Here we examined whether innervation of distinct sympathetic targets might differentially regulate nAChR expression. Chick sympathetic neurons, naive of preganglionic innervation or target contact (E9), were cocultured with either embryonic heart or kidney. ACh gated macroscopic and single channel currents were assayed in neurons that appeared to innervate the target cells. The expression of α3, α5, α7, β4 relative to actin was detected by quantitative RT-PCR. Innervation of embryonic heart by sympathetic ganglia neurons reduced the magnitude of ACh- evoked currents and increased the expression of α5 and α7. Cardiac myocyte- lysate mimicked the effect of target contact on whole cell current and subunit expression. However when sympathetic neurons were cocultured with heart in a transfilter (12 µ) chamber, the whole cell current decreased but mRNA levels were unchanged.

In similar experiments when embryonic kidney was used as a target, innervation of kidney explants increased ACh-evoked current and nAChR subunit expression in the sympathetic neurons. As with heart, the influence of kidney on nAChR expression could be mimicked by lysed target, but not by soluble target derived factors in the transfilter coculture assay. Supported by NS29071 to LR.

529.1

N-METHYL-D-ASPARTATE (NMDA)-INDUCED SEPTAL LESION PREVENTS THE RETENTION OF TOLERANCE TO ETHANOL (E) BY DESGLYCINAMIDE^9-[ARG^8]-VASOPRESSIN (DGAVP). P.H. Wu, J.F. Liu, A.J. Lança, A.I. Arifuzzaman, L. Grupp* and H. Kalant. Dept. Pharmacology, Univ. of Toronto, and Addiction Research Foundation, Toronto, Canada

Tolerance to E can be maintained by DGAVP (s.c.) acting on central vasopressin V_1 receptors. We have identified a neurocircuit underlying the action of AVP on tolerance to E, that consists of serotonergic, vasopressinergic, cholinergic and glutamatergic projections. Other evidence implicates septal neurons that have NMDA receptors. Male Sprague Dawley rats were anesthetized and injected with saline-1 mM CaCl, (S) or NMDA (50nM in S) into the medial *(M, 0.1 μl) or medial plus lateral septum (M+L, 0.3 μl) in 10 min. After 4 weeks* to recover from the surgery, they were tested for their initial sensitivity to E (1.8 g/kg, i.p.) on the moving belt, and were treated chronically with E until they showed tolerance to E. Then E treatment was stopped, S or DGAVP (10 μg/rat, s.c.) treatment began, and retention of tolerance was tested 9 days later. At the end of the experiment, the rats were treated with colchicine (1.2 mg/kg, s.c., 36 h) and perfused with fixative. Brain sections were cut and stained with FITC labelled antibodies for choline acetyl transferase (CAT) and GABA. All rats showed the same initial sensitivity to E and developed tolerance to E in the same time [$F(2,21)=0.08$, $p>0.93$]. DGAVP maintained tolerance in the S group but not in the M and M+L groups [$F(2,20)=3.77$, $p<0.04$]. Nissl staining of the sections showed marked depletion of cells bodies in the M and M+L of the NMDA-treated animals. Immunocytochemistry showed that GABA immunoreactivity was markedly decreased in the lateral septum. Our results indicate that the lateral septal GABA cells with NMDA receptors may be involved in the maintenance of tolerance to E by DGAVP. Supported by NIAAA grant RO1-08212-06.

529.2

NMDA EFFECTS ON RETINAL NEUROSTEROIDS. P. Guarneri[a]*, R. Guarneri[a], C.Cascio[b], R.Monastero[b], T.Piccoli[b], F.Piccoli[b], V.Papadopoulos[c], [a]IBS,CNR;[b]Inst. Neuropsych.,Palermo,Italy;[c]Dept.Cell Biol.,Georgetown Uv.,Washington D.C.,USA.

Neurosteroids, such as pregnenolone sulfate (P5-S), have been implicated in the NMDA-induced neuronal injury and the regulation of NMDA-gated and voltage-dependent Ca^{2+} currents. In the present study, we examined the effects of NMDA on retinal steroidogenesis both *in vitro* and *in vivo*. Retina, an useful model in degenerative studies, has been shown to synthesize neurosteroids (J.Neurochem. 1994, 63:86). Intact retina possess P450scc enzyme, mainly located at ganglion cells, and synthesizes the novo pregnenolone (P5) and P5-S. In addition, retina neurosteroid synthesis is under control of $GABA_A$ receptors (Brain Res. 1995.in press). To study the effects of NMDA in *in vitro* P5 synthesis, retina from adult rats was incubated in Hank's buffer in the presence of 1-100 μM NMDA and P5 metabolism inhibitors. P5 synthesis was stimulated by NMDA and this effect was reversed by MK 801 and EGTA. Both cGMP content and LDH activity, measured to monitor NMDA effects, increased. This increase was potentiated by elevating the extracellular Ca^{2+}. When NMDA was intraocularly injected, P5 and P5-S raised after 30 min. However prolonged treatments (12-16 hrs) decreased P5 levels only. In order to eliminate adrenal steroid interferences in retinal neurosteroid analysis, rats were treated with dexamethasone (DEX). Retinal P5 levels declined in DEX-treated rats compared with those of saline-treated group, whereas P5-S levels were unchanged. After prolonged exposure, when serum corticosterone levels were almost abolished, P5 levels were higher compared to those measured after short exposure, suggesting an activation of retinal P5 synthesis. DEX-treatment did not interfere with NMDA effects on P5 and P5-S. These results suggest that (i) NMDA regulates neurosteroid synthesis, maybe through stimulation of GABA release, and (ii) NMDA-stimulated P5-S formation may be involved in neurodegenerative events.

529.3

NMDA RECEPTOR MEDIATED BRAINSTEM LESIONS PRODUCED BY ACUTE THIAMINE DEFICIENCY IN RATS. S.X. Zhang* and P.J. Langlais. Dept. of Psychology, San Diego State University, and VA Medical Ctr., San Diego CA 92182

An acute bout of pyrithiamine-induced thiamine deficiency (PTD) in rats produces lesions within thalamus, mammillary bodies and brainstem which resemble Wernicke's encephalopathy. Evidence suggests that thalamic lesions involve glutamate-NMDA receptor mediated excitotoxicity (Langlais, *Metabolic Brain Disease* 10: 31-44, 1995). The present study examined the role of NMDA receptors in thiamine deficiency induced neuronal loss within several brainstem nuclei. Male Sprague-Dawley rats were treated daily with a thiamine-free diet and pyrithiamine hydrobromide (0.25 mg/kg/day, i.p.). Pairfed controls and PTD rats were administered either MK-801 (2mg/kg/day, i.p.) or saline beginning at an early, prelesion stage (approximately day 12) and ending with the administration of thiamine, 100 mg/kg, i.p., on day 15-16. After 5 days of recovery, the brains were removed and neuronal density measured. In the PTD-saline group, neuronal loss was greatest in the inferior olivary nucleus (IOD, 77% loss) followed by the locus coeruleus (LC; 61%), lateral vestibular (LV; 58%), medial vestibular (MV; 45%), lateral superior olivary. (LSO; 31%) and superior paraolivary (SPO; 22%) nuclei. MK-801 treatment was associated with a significant 20-25% increase in neuronal density in IOD, IOP, MVV, LV and LSO nuclei but had no effect on neuronal loss in LC or SPO nuclei. These observations suggest that NMDA receptors are involved in the pathogenesis of PTD-induced neuronal degeneration in some but not all brainstem nuclei. Supported by NINDS grant #NS 29481 and VA Merit Award to PJL.

529.4

CELL SIZE-DEPENDENT LOSS OF RETINAL GANGLION CELLS AFTER INTRAOCULAR NMDA- AND KAINATE-INJECTIONS IN ADULT RATS C.K.Vorwerk*[1], M.R.Kreutz[1], M.Brosz[2] and B.A.Sabel[1]
Otto-von-Guericke-University, Faculty of Medicine, [1]Institute of Medical Psychology, [2]Institute of Biostatistics, Leipziger St.44, Magdeburg 39120, Germany

The cell size distribution of retinal ganglion cells (RGC) surviving N-methyl-D-aspartate (NMDA) or kainate (KA) induced lesions was compared to uninjured RGCs in whole mounted rat retinae of adult rats. NMDA (20 nmoles) or KA (5 nmoles) was injected intraocularly. To determine the number and size of surviving RGC's, horseradish peroxidase (HRP) was injected into the contralateral superior colliculus 48 hours later . After an additional two days the retinae were dissected out, whole mounted, stained and analyzed for HRP-positive cells using a computer based image analysis system.
We analyzed 15,000 cells taken from uninjured controls, over 4000 NMDA treated cells and over 10,000 cells from KA treated rats. The control cell pool was divided into four size classes with equal numbers of cells in each class (3750 cells). The mean cell diameter for each class was 4.7, 8.0, 10.8, 16.2 μm. The cells from NMDA and KA treated rats were sorted into these four size classes, and this distribution compared to the expected (control) distribution. In class 3 (10.8 μm) 18% fewer cells were counted in NMDA retinae than expected when compared to controls, assuming both populations had the same distribution. Whereas 12-15% more cells were found in class 1 (4.7 μm) and class 4 (16.2 μm). A different pattern was seen in kainate treated animals, where in class 1 30% more cells were found compared to the control group. Interestingly, in class 3 and class 4 (larger cells) there were 15% fewer cells compared to control. The differential sensitivity of cell-size classes suggests that discrete subclasses of RGCs have different expression patterns of glutamate receptor subtypes and that larger RGCs are more sensitive to KA than NMDA injury.
Supported by DFG-Sa 433/3-1

529.5

MALONATE-INDUCED EXCITOTOXIC DEGENERATION OF THE DOPAMINERGIC NIGROSTRIATAL PATHWAY. B.P. Connop, R.J. Boegman, K. Jhamandas, R.J. Beninger and J.V. Milligan*. Departments of Pharmacology & Toxicology, Psychology, and Physiology, Queen's University, Kingston, Ont. K7L 3N6.

Recently, inhibition of mitochondrial function has been shown to produce excitotoxic damage in the brain via a mechanism involving indirect activation of N-methyl-D-apartate (NMDA) receptors. We have used malonic acid, an inhibitor of succinate dehydrogenase, to determine if it produces excitotoxic damage to the dopaminergic nigrostriatal pathway. In adult Sprague-Dawley rats, unilateral malonate infusions (0.5 μl) were made into the substantia nigra pars compacta and striatal tyrosine hydroxylase (TH) activity measured at 4 and 7 days post-infusion. Intranigral malonate, 0.25 and 0.5 μmol produced 16.2% and 62.5% decreases in striatal TH activity, respectively. A comparable decrease in striatal TH activity was seen at both 4 and 7 days post-infusion. Animals receiving malonate did not show excitation or seizures. In animals injected with 0.5 μmol malonate, systemic pretreatment with the NMDA receptor antagonist - MK801 (30 min pre- and 210 min post-infusion) - produced a 13.5% decrease in striatal TH activity. The present study shows that malonate can produce excitotoxic damage to the nigrostriatal dopamine pathway by indirect activation of NMDA receptors. (Supported by the Medical Research Council of Canada)

529.6

MANIPULATION OF MEMBRANE POTENTIAL MODULATES MALONATE-INDUCED STRIATAL EXCITOTOXICITY IN VIVO. J.G. Greene* and J.T. Greenamyre. Depts. of Neurobiology and Anatomy, Neurology, and Pharmacology, Univ. of Rochester Medical Center, Rochester, NY 14642.

Malonate is a competitive inhibitor of succinate dehydrogenase (SDH), the neurotoxicity of which is mediated indirectly by the NMDA-subtype of glutamate receptor. We have investigated the effects of pharmacological manipulation of membrane potential on the toxicity of malonate by using intrastriatal injections in rats and analysis of lesion volume. We report that a low dose of the Na-K ATPase inhibitor ouabain (0.1 nmol) that was not overtly toxic greatly exacerbated the toxicity of a low dose of malonate (0.6 μmol); this combined toxicity was blocked by the noncompetitive NMDA antagonist MK-801. Injection of a high concentration of potassium produced similar results to ouabain. The toxicity of a higher dose of ouabain (1 nmol) was largely prevented by MK-801. Co-injection of the K^+ channel activator minoxidil (4 nmol) attenuated the toxicity of 1 μmol of malonate by about 60%. These results indicate that membrane depolarization exacerbates malonate neurotoxicity and that membrane hyperpolarization protects against malonate-induced neuronal damage. We hypothesize that the effects of membrane potential on malonate toxicity are mediated through the NMDA receptor as a result of its combined agonist- and voltage-dependent properties.

529.7

ENHANCEMENT OF NMDA-INDUCED NEUROTOXICITY IN CULTURED HIPPOCAMPAL NEURONS FOLLOWING CHRONIC ETHANOL EXPOSURE
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It has been proposed that the adaptive mechanism by which ionotropic glutamate receptors cope with chronic inhibition by ethanol (EtOH) involves an increase in receptor function. This study demonstrates that chronic ethanol exposure results in an enhancement of NMDA receptor mediated neurotoxicity in cultured hippocampal neurons. In cultures of hippocampal neurons (2 wks *in vitro*), NMDA-induced neurotoxicity was examined following a 7 day chronic exposure to 100 mM EtOH (CEE) and in control cultures receiving no EtOH.

NMDA application induced cell death in dose dependent fashion in both control and CEE cultures. NMDA-induced cell death was significantly enhanced in CEE at all concentrations (50-250 μM) versus controls ($p < 0.003$, ANOVA). For example, NMDA-induced cell death at 50μM was 46±5% in control and 60±5% in CEE cultures; while at 250μM NMDA cell death was 82±2% in control and 93±2% in CEE cultures. Kainic acid induced cell death was not altered as a result of CEE ($p > 0.100$, ANOVA). Inclusion of APV (70μM) during the CEE treatment paradigm prevented the enhancement of NMDA-induced cell death. Furthermore, maintenance of EtOH through NMDA application retarded NMDA-induced cell death ($p < 0.001$, ANOVA). No significant change in total cell numbers (live + dead) was apparent following NMDA application in control vs. CEE cultures ($p > 0.970$, ANOVA). These data indicate that the increased toxicity involves selective effects on NMDA receptors and may reflect withdrawal-related processes.

It has been postulated that the enhancement of NMDA-induced neurotoxicity involves an enhanced increase in intracellular calcium influx through the NMDA receptor. We are currently examining intracellular calcium transients in CEE and control cultures. Supported by NIAAA Fellowship F31-AA05361 & AA08986.

529.8

NMDA TOXICITY IN STRIATAL CULTURES IS POTENTIATED BY KCl-INDUCED CHRONIC DEPOLARIZATION.
Q. Chen*, D.J. Surmeier and A. Reiner. Dept. Anat & Neurobiol., Univ. of Tennessee - Memphis, Memphis, TN 38163.

Cortical innervation plays an important role in the expression of NMDA receptor mediated excitotoxicity in striatum. Based on studies of cerebellar granule cells, it seemed possible that cortical input to striatum has this influence by chronic depolarization produced by cortical input. We therefore studied the influence of KCl depolarization on the development of NMDA receptor mediated vulnerability in striatal neurons.

Dissociated striatal neurons from E17 rat embryos were cultured for two weeks in Barrett's media containing either high (25 mM) or low (3 mM) KCl. The vulnerability of these neurons to NMDA receptor agonists including NMDA and quinolinic acid (QA) was examined by monitoring cell loss 24 hrs after 1 hr exposure. The neurons grown in high KCl had larger cell bodies and longer, thicker processes than neurons grown in low KCl. We found that 500 μM NMDA produced significant striatal cell loss in both high and low KCl treated cultures, but QA up to 5 mM had no significant toxic effect. The striatal neuron loss produced by 500 μM NMDA was significantly greater (47% loss) in the high KCl group than in the low KCl group (25%). In conclusion, our studies suggest that membrane depolarization produced by corticostriatal innervation could significantly potentiate NMDA receptor-mediated excitotoxicity in striatum. Chronically depolarized striatal cultures with strictly controlled glutamate content in the media may provide a more appropriate model to study the role of NMDA or other glutamate receptor subtypes in excitotoxicity in striatum. NS-19620, NS-28721 (AR); NS-28889 (DJS); and UT, Neuroscience of Excellence (QC).

529.9

GLUTAMATE RECEPTORS MODULATE IBOGAINE NEUROTOXICITY
E. O'Hearn* and M.E. Molliver. Dept. of Neuroscience & Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205

To test the excitotoxicity hypothesis of ibogaine-induced PKC damage we attempted to block glutamate receptors. GYKI 52466, a non-NMDA antagonist, was injected (10 or 30 mg/kg i.p.) before and twice after one dose of ibogaine (50 or 100 mg/kg i.p.). The treated rats (n=14) exhibited substantially greater loss of PKCs and microglial activation compared to rats given ibogaine alone. After both drugs, the bands of PKC loss were much wider and the degeneration extended to both paravermis and hemispheres. We then tested the effects of an NMDA antagonist. MK-801 (1 mg/kg i.p.) administered prior to ibogaine (50 or 100 mg/kg i.p.; n=7) markedly attenuated the degree of PKC loss caused by ibogaine alone. Neither GYKI 52466 nor MK-801 alone caused degeneration. These results demonstrate that ibogaine toxicity is profoundly modulated by glutamate antagonists. Enhancement of PKC loss after blocking non-NMDA receptors has several possible explanations. GYKI 52466 may have blocked excitation of cerebellar inhibitory neurons (basket, stellate, or Golgi cells), increasing PKC susceptibility to excitotoxic injury. Alternately, blocking non-NMDA receptors on inhibitory interneurons in brainstem may lead to increased firing in precerebellar neurons that activate PKCs. A third consideration (evidence lacking) is that vulnerability may increase due to an imbalance between AMPA/Kainate and metabotropic receptors. In contrast, the attenuation of PKC loss by MK-801 suggests that NMDA receptors are involved. MK-801 might reduce the activation of inferior olive by ibogaine (ibogaine binds to NMDA receptors: ref. Popik et al., 1994); alternately, excitation of other neurons (e.g., granule cells) via NMDA receptors, may normally contribute to ibogaine-induced PKC degeneration. These results suggest that neuronal connections and receptors other than those between climbing fibers and PKCs may contribute to ibogaine neurotoxicity.
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529.10

EFFECT OF CYCLOTHIAZIDE ON EXCITOTOXIC HIPPOCAMPAL INJURY IN THE IMMATURE RAT BRAIN.
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Excitatory amino acid receptor desensitization may be a mechanism to limit glutamate neurotoxicity. Cyclothiazide blocks glutamate receptor desensitization, therefore we investigated the effects of cyclothiazide on AMPA- and kainate-induced hippocampal injury in the immature rat. Under deep anesthesia, postnatal day (PND) 7 rats received intrahippocampal injections of AMPA (2.5 nmol/0.5μl) or kainate (10 nmol/0.5μl) alone, and in combination with cyclothiazide (2.5 nmol or 25 nmol/μl). Additionally, cyclothiazide (25 nmol/0.5 μl) was injected alone. Injury to the hippocampus was assessed one week later on coronal Nissl stained sections by comparison of regional cross-sectional areas of the dorsal hippocampus ipsilateral(I) and contralateral(C) to the injection site. Injury was calculated according to the formula 100 X (C-I)/C (percent damage). Cyclothiazide (25 nmol/0.5 μl) produced a mild injury to the hippocampus (5.7 ± 5.6% damage). AMPA (2.5 nmol/0.5 μl) produced 32.5 ± 6.7% damage, but cyclothiazide (2.5 nmol and 25 nmol/0.5 μl) coinjected with AMPA did not significantly alter the injury to the hippocampus. Kainate (10 nmol/0.5 μl) produced 15.7 ± 4.5% damage to the dorsal hippocampus. Coinjection of cyclothiazide increased kainate-mediated hippocampal injury (cyclothiazide 2.5 nmol, 23.6 ± 5.8% damage; cyclothiazide 25 nmol/0.5 μl, 33.5 ± 7.8% damage, $p < 0.05$, ANOVA). The results indicate that cyclothiazide enhances excitotoxic hippocampal injury in vivo. Supported by NIH grant NS01482.

529.11

AMPA RECEPTOR DESENSITIZATION MASKS RAPID GLUTAMATE EXCITOTOXICITY IN CULTURED MOUSE CORTICAL ASTROCYTES.
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Although cultured astrocytes express functional glutamate receptors coupled to increases in intracellular free calcium, they are generally resistant to excitotoxic cell death. One explanation is that glutamate receptor activation may be limited by rapid desensitization. To test this, we blocked AMPA receptor desensitization with a thiazide diuretic, cyclothiazide (CYZ), in cultured mouse Type I astrocytes.

In whole-cell recordings of astrocytes, brief applications of 300 μM kainate (KA) evoked small sustained inward currents, while 300 μM AMPA evoked small rapidly desensitizing inward currents. Both dramatically increased when desensitization was blocked by addition of 100 μM CYZ. A few astrocytes were injured after 24 hr exposure to 500 μM KA, but not to 500 μM AMPA or to 500 μM glutamate (GLU). However, with the coapplication of 10-100 μM CYZ, each of the glutamate agonists caused widespread (60-80%) cell death, occurring even within the first few hours. This dramatic effect with CYZ, which alone was not toxic, was concentration-dependent for each of the agonists (EC_{50} 30-100 μM) and was abolished by the further addition of a competitive non-NMDA antagonist, NBQX (30 μM). CYZ also enhanced AMPA-stimulated glutamate release into the bathing medium within 10 minutes after drug application. NMDA caused no injury even in the presence of CYZ. Concanavalin A (0.3 mg/ml), enhanced astrocyte toxicity during KA, but not AMPA, exposure and was blocked by NBQX. The AMPA receptor-mediated excitotoxicity with CYZ varied with the age of the astrocyte cultures; the maximal effect occurred around 2 weeks, but was absent by 4 weeks *in vitro*. Desensitization of astrocyte AMPA- and KA- subtypes of glutamate receptors limits excitoxicity and may influence astrocyte and neuron vulnerability in cerebral ischemia.
Supported by NIH NS32326, NS01543 (MPG), and NS01443 (KAY).

529.12

KAINATE-INDUCED DELAYED EXCITOTOXICITY IN ISOLATED RETINA.
Q. Chen, J. Olney, T. Almli, M. Wax*[1], M. Price and C.Romano[1]. Dept. Psych. and Ophthal.[1], Washington. U. St. Louis, MO 63110.

Delayed excitotoxic neurodegeneration occurs in response to brief periods of activation of glutamate receptors of either non-NMDA or NMDA subtype in isolated embryonic chick retina. Kainate is a powerful excitotoxin in this system, causing much more cell death (assessed by LDH release and histologically) than NMDA, AMPA, or glutamate. One mechanism for the high toxicity of kainate may be that while AMPA receptors desensitize rapidly in response to AMPA or glutamate, kainate responses are relatively non-desensitizing. Experiments were performed to test this hypothesis. First, GYKI 52446, an AMPA receptor-selective antagonist at the concentrations used, blocked kainate-induced delayed excitotoxicity, indicating that kainate toxicity was mediated through the AMPA receptor. Second, the effects of cyclothiazide, an agent that blocks desensitization, on excitotoxicity mediated by AMPA, glutamate, and kainate were determined. MK-801 was present to eliminate any NMDA receptor-mediated toxicity. Both glutamate- and AMPA-induced toxicity were greatly potentiated by cyclothiazide, whereas kainate-induced toxicity was not significantly affected. Third, co-incubation of the retina with glutamate (to desensitize AMPA receptors) and kainate decreased the toxicity of kainate. These results are consistent with the notion that the powerful toxic properties of kainate result in part from the non-desensitizing response of kainate at AMPA receptors. Desensitization does limit the neurotoxicity of the endogenous agonist glutamate and therefore this may serve as a physiological protective mechanism

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529.13

THE NON-NMDA ANTAGONIST LY300168 (GYKI 53655) ATTENUATES AMPA-INDUCED EXCITOTOXICITY IN NEONATAL RAT BRAIN. J.D.E. Barks*, P. Wang, X.-H. Liu. Dept. of Pediatrics, Univ. of Michigan, Ann Arbor MI 48109-0646.

Susceptibility to injury mediated by the excitatory amino acids (EAAs) NMDA and AMPA peaks in immature rat brain (Brain Res 459:200; *ibid.* 583:54). NMDA toxicity is consistently attenuated by many competitive and non-competitive NMDA antagonists; reported effects of the non-NMDA antagonists NBQX and GYKI 52466 on AMPA toxicity have been less robust. We tested the effect of the putative AMPA receptor desensitization-enhancer LY300168 (GYKI 53655, E Lilly) (2.5 mg/kg/dose i.p. x 3 doses 15, 120 and 240 min post-EAA, *vs.* vehicle) on excitotoxic injury in postnatal day 7 (P7) rats. P7 rats were injected with (S)-AMPA (2.5 nmol, n=14) or NMDA (5 nmol, n=27) into right hippocampus (HIP). Bilateral HIP volumes were estimated by image analysis on P12. Injection of AMPA (2.5 nmol) resulted in marked right HIP atrophy with pyramidal cell loss. LY300168 (n=7; 2 died, at 2h and 48h) significantly attenuated AMPA-induced right HIP injury [right HIP volume (mm^3, mean±sd): AMPA+vehicle 3.66±0.56; AMPA+LY 5.22±0.61; p=0.002, t-test]. Based on comparison with left HIP volume, 2.5 nmol AMPA resulted in 56±8% right HIP volume loss in controls, but only 43±7% after LY300168 (p<0.02, t-test). Injection of NMDA (5 nmol) resulted in moderate right HIP atrophy. LY300168 (n=14; 1 died after 48h) had no effect on NMDA-induced right HIP injury [right HIP volume (mm^3, mean ±sd): NMDA+ vehicle 7.58±1.08; NMDA+LY 7.30±1.71]. Mortality was not increased by LY300168 administration (3/20 vehicle-treated *vs.* 3/21 LY300168-treated). The data suggest that AMPA receptor desensitization modulates the response of immature brain to AMPA- but not NMDA-mediated injury, and that desensitization-enhancing strategies may be neuroprotective against EAA-mediated injury to the developing brain.

529.14

MODULATION OF HYPOXIC/HYPOGLYCEMIC NEURONAL INJURY BY DIFFERENT METABOTROPIC GLUTAMATE RECEPTORS. T. Opitz, and K. G. Reymann*, Department of Neurophysiology, Federal Institute for Neurobiology, Brenneckestr. 6, POB 1860, D-39008, Magdeburg, Germany.

To investigate the influence of metabotropic glutamate receptors (mGluR) on neuronal injury caused by cerebral hypoxia/ischemia, we employed an in vitro model of hypoxia/hypoglycemia. Hippocampal slices were transiently exposed to an oxygen- and glucose-free environment which causes a pronounced drop of both ATP and creatine phosphate, an anoxic depolarization, and an incomplete recovery of synaptically evoked population spike in the CA1 region after one hour (48.5 ± 3.6 % of baseline values). This recovery was used as a measure of neuronal viability.

In this model, we applied various agonists specific for different mGluR subtypes. The specific class 1 mGluR agonists trans-azetidine-2,4-dicarboxylic acid (trans-ADA) and 3,5-dihydroxyphenylglycine (DHPG) appeared to be highly protective, but only if it was applied 20 min before the hypoxia/hypoglycemia. An activation of class 2 metabotropic glutamate receptors by (2S,1'R,2'R,3'R)-2-(2,3-dicarboxycyclopropyl) glycine (DCG-IV), which inhibits adenylyl cyclase activity, led to a marked deterioration of the population spike recovery and even to a total prevention of the protective effect of the N-methyl-D-aspartate (NMDA) agonist D-2-amino-5-phosphonopentanoic acid.

In conclusion we could show that an activation of phospholipase C coupled (class 1) mGluRs prior to hypoxia/hypoglycemia exhibits a pronounced protective effect. In contrast to findings in NMDA toxicity in neuronal cultures we were able to demonstrate a detrimental effect by the activation of mGluRs negatively coupled to the adenylyl cyclase (class 2).

We wish to thank Drs. A. P. Kozikowski and H. Shinozaki for providing trans-ADA and DCG-IV, respectively.

529.15

AMYLOID PRECURSOR PROTEIN REGULATION BY IONOTROPIC AND METABOTROPIC GLUTAMATE RECEPTORS. A. Valerio*, A. Alberici, M. Paterlini, M. Grilli, P. Galli, M. Pizzi, M. Memo and P.F. Spano. Div. Pharmacol., Dept. Biomed. Sci. & Biotech., Brescia Univ. Medical School, Brescia, Italy.

The effects of the ionotropic glutamate receptor (iGluR) selective agonist N-methyl-D-aspartate (NMDA) on amyloid precursor protein (APP) levels were investigated in primary cultures of rat cerebellar granule cells. Neuron immunostaining with the anti-APP monoclonal antibody 22C11 (Boehringer Mannheim) was significantly increased 4 hours after a 15 min pulse with 100 μM NMDA. Immunoblotting of granule cell extracts using R37 antiserum, recognizing a C-terminal portion of APP, showed that all the intracellular full-length APP isoforms increased after the NMDA pulse in a dose-dependent manner. This effect was prevented by incubating the neurons in the presence of the selective metabotropic GluR (mGluR) agonist 1S,3R-ACPD. This phenomenon was related, in terms of doses and time, with the observed 1S,3R-ACPD-mediated protection on NMDA-induced granule cell death. Our results suggest that the GluR-mediated changes in APP content might participate in the control of neuronal viability. The relative abundance of different iGluR and mGluR subtypes could be of relevance in determining the neuronal response to excitatory amino acids in terms of both APP expression and survival.

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529.16

STIMULATION OF mGluR TYPES 1/5 NORMALIZE GLUTAMATE-INDUCED ALTERATION OF CALCIUM HOMEOSTASIS AND EXCITOTOXICITY IN CULTURED CEREBELLAR GRANULE CELLS. M. Pizzi*, P. Galli, O.Consolandi, V. Arrighi, M. Memo, P.F. Spano. Div. Pharmacol., Dept. Biomed. Sci. & Biotech., Brescia Univ. M , Brescia, Italy.

Stimulation of metabotropic glutamate receptor (mGluR) has been shown to counteract glutamate-mediated excitotoxicity in different neuronal cultures. In the present study the role of the mGluR on the control of neuronal calcium homeostasis was investigated in cerebellar granule cells. The mGluR agonist, tACPD, produced a transient increse of intracellular calcium concentration ([Ca^{2+}]i) which showed to be developmentally regulated, being maximal at 4 days in vitro (DIV). Moreover, tACPD significantly reduced the [Ca^{2+}]i rise produced by glutamate in the absence of external Mg^{2+}. This latter effect occured at experimental conditions superimposable to those producing neuroprotection. tACPD neither affected [Ca^{2+}]i elevation due to depolarizing concentrations of KCl (25 mM) nor the calcium entry evoked by the calcium ionophore A 23187 (10 μM). Inhibitory effect of tACPD was insensitive to K+ channel blockade operated by 2 mM tetraethylammonium (TEA). The capability of different mGluR agonists to reduce glutamate-increased [Ca^{2+}]i rise strictly correlated with their property to elicit neuroprotection. L-AP4 (500 μM), which stimulates mGluR4/R6/R7 subtypes, was inactive in preventing glutamate-mediated both disturbance of calcium homeostasis and neurotoxicity. tACPD inhibitory modulation of glutamate-evoked [Ca^{2+}]i rise and cell death appeared to be highly sensitive to protein kinase C blockade produced by bisindolylmaleimide (1μM) while was weakly affected by the cyclic AMP analogue dibutyrryl cyclic AMP. These data suggests that activation of mGluR1/R5 receptor subtypes reduce glutamate excitatory transmission by a mechanism involving protein kinase C activation.

529.17

Activation of class II of metabotropic glutamate receptors (mGluRs) inhibits the release of excitatory amino acids (EAAs) from slices and synaptosomes of rat hippocampus A. Poli,* P. Di Iorio , R. Lucchi, R. Ciccarelli , G. Battaglia^, F. Nicoletti and F. Caciagli . Dept. of Biology, Univ. of Bologna, Italy; Inst. of Pharmacology, Univ. of Chieti, Italy; ^Inst. of Pharmacology, Univ. of Catania, Italy; Dept. of Exp. Med., Pharmacology Section, Univ. of Perugia, Italy.

The release of EAAs seems to be modulated by mGluRs. In the hippocampus, mGluR1-5, mGluR2-3 (class II) and L-AP4-sensitive receptors have been found. We observed that the non selective mGluR agonist (1S,3R)-1-aminocyclopentane-1,3 dicarboxilic acid (1S,3R-ACPD 100 μM) reduced the electrically evoked release of endogenous EAAs from hippocampal slices. Conversely, the non selective mGluR antagonist (+)-α-methyl-4-carboxyphenylglycine (300 μM) enhanced the evoked output. Both (2S,1R,2R,£r)-(2,3-dicarboxycyclopropyl)-glycine (DCG-IV) and (2S,3S,4S)-α-(carboxycyclopropyl)-glycine (L-CCG-I), two agonists of mGluR2-3, caused a dose-dependent inhibition of EAA release from electrically triggered slices and K^+ stimulated synaptosomes. The inhibition was reverted by (2S,3S,4S)-2-methyl-2-(carboxycyclopropyl)-glycine, a new selective antagonist of mGluR2-3. DCG-IV and L-CCG I (1-10 μM) resulted to be 30-50 fold more potent than 1S,3R-ACPD. Surprisingly, L-AP4 did not affect EAA release from slices. In order to establish which transduction patway is involved in the activity of the mGluR agonists we observed that 1S,3R-ACPD, DCG-IV and L-CCG-I reduced the electrically stimulated cAMP formation in the slices but, as opposed to 1S,3R-ACPD and L-CCG-I, DCG-IV did not stimulate PPI hydrolysis.

529.18

ACTIVATION OF CLASS I METABOTROPIC GLUTAMATE RECEPTORS (mGluRs) ENHANCES NMDA TOXICITY IN CULTURED CORTICAL CELLS. V. Bruno*[1], G. Battaglia[1], A. Copani[1], G. Casabona[1], T. Knopfel[2], R. Kuhn[2], P. Dell'Albani[3], D.F. Condorelli[3] and F. Nicoletti[4]. [1]*Institutes of Pharmacology and* [3]*Biochemistry, University of Catania, and* [4]*Dept. Exp. Med. Biochem. Sci., University of Perugia, Italy;* [2]*CIBA-GEIGY, Basel, Switzerland.*

Mouse cortical neurons in mixed cultures express high levels of mGluR5, as indicated by both Northern analysis and immunocytochemistry. Expression of mGluR1a,b or c was low or absent. mGluR5 receptors were functionally active, as reflected by the ability of class I mGluR agonists, including quisqualate, 3,5-dihydroxyphenylglycine (DHPG) or trans-azetidine-2,4-dicarboxylic acid (t-ADA) to enhance inositol phospholipid hydrolysis. To study the influence of mGluR5 on neuronal degeneration, we have applied quisqualate, DHPG, or t-ADA either during or immediately after a toxic pulse with NMDA. In both cases, mGluR agonists increased the potency of NMDA in inducing neuronal death, although they were not toxic by themselves. Amplification of NMDA toxicity was prevented by inhibtors of protein kinase C (PKC), as well as by prolonged exposure to phorbol esters, a procedure which down regulates PKC. We conclude that activation of class I mGluRs (in particular mGluR5) in cultured cortical cells enhances excitotoxic neuronal death through a mechanism which involves PKC.

529.19

METABOTROPIC RECEPTORS IN EXCITOTOXICITY: (S)-4C3HPG PROTECTS AGAINST STRIATAL QUINOLINIC ACID LESIONS

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Striatal quinolinate lesions mimic many of the neurochemical and neuropathological characteristics of Huntington's disease, including the relative loss of medium spiny neurons with sparing of interneurons. Metabotropic glutamate receptors (mGluRs) have been implicated in this toxicity. In fact, mGluR5 is heavily expressed in striatal projection neurons and not in interneurons whereas mGluR2 is confined to cholinergic interneurons. We have used two phenylglycine analogs to investigate which receptors are involved in the excitotoxic process: (S)-4-carboxy-3-hydroxyphenylglycine ((S)-4C3HPG), a selective agonist of mGluR2 and antagonist of mGluR5; and (+)-alpha-methyl-4-carboxyphenylglycine ((+)-MCPG), an antagonist of both mGluR2 and mGluR5.

(S)-4C3HPG or (+)-MCPG was injected into the striatum of male rats with 100 nmol of quinolinic acid, and 2,3,5-triphenyltetrazolium chloride (TTC) was used for lesion analysis. Doses of 1000 nmol (S)-4C3HPG or (+)-MCPG alone were not toxic. When co-injected with quinolinic acid, 500 nmol of (S)-4C3HPG reduced lesion volume by 55% ($p<0.01$) while 1000 nmol reduced lesion volume by 91% ($p<0.01$). The same doses of (+)-MCPG had no effect on lesion size. These observations confirm the important role of mGluRs in excitotoxicity. At this time it is unclear whether protection is due to antagonist effects of (S)-4C3HPG at mGluR5 or agonist effects at mGluR2.

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529.20

THE METABOTROPIC GLUTAMATE RECEPTOR AGONIST, 4C3HPG, REDUCES KAINATE-INDUCED DEATH OF CORTICAL NEURONS EXPRESSING CALCIUM-PERMEABLE AMPA/KAINATE RECEPTORS.

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Metabotropic glutamate receptor agonists selective for the mGluR2/3 receptor subtype can reduce the overall neuronal death induced by NMDA, but not AMPA or kainate, in murine cortical cell cultures (Buisson and Choi, elsewhere this meeting; also see Bruno et al., Eur J Pharmacol 256: 109, 1994). Exposure times of several hours are required for either AMPA or kainate to destroy most cortical neurons, but a small subpopulation identified by kainate-activated cobalt uptake (a marker for AMPA or kainate receptors linked to calcium-permeable channels) exhibits heightened calcium influx and vulnerability to death induced by kainate exposures as brief as 10-60 min (Turetsky et al., Neurobiol Dis, 1:101, 1994; also unpublished data). Since kainate toxicity on these cobalt-positive neurons resembles NMDA toxicity on the general cortical neuronal population, we tested the hypothesis that the selective mGluR 2/3 agonist, S-4-carboxy-3-hydroxy-phenylglycine (4C3HPG) would indeed protect cobalt-positive neurons from kainate-induced death. 20 min exposure to 100 μM kainate in the presence of 10 μM MK-801 produced 73.9 % (±1.7 SEM, n = 14 cultures) loss of cobalt-positive cells, while causing little additional death in the general neuronal population. Adding 100 μM 4C3HPG during the kainate exposure reduced cobalt-positive cell loss to 62.7% (±2.7 SEM) (n=14, different at $P < 0.01$). This observation supports the idea that mGluR 2/3 receptor activation can reduce excitotoxicity associated with rapid calcium entry, whether that calcium entry is mediated by NMDA or calcium-permeable AMPA/kainate receptors.

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EXCITATORY AMINO ACIDS: EXCITOTOXICITY VII

530.1

BRIEF EXPOSURE TO AMPA INDUCES Ca^{2+}-DEPENDENT EXCITOTOXICITY SELECTIVE TO CA1 OF ORGANOTYPIC RAT HIPPOCAMPAL SLICE CULTURE, WHILE KAINIC ACID INDUCES "SLOW" NEUROTOXICITY IN CA3.

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Distinctive features of selective and delayed toxicity of the non-NMDA glutamate receptor agonists AMPA and kainic acid (KA) on subpopulations of pyramidal neurons are characterized using rat hippocampal slices, cultured from 9 day old Sprague-Dawley rats [Stoppini (1991) *J Neurosci Methods* **37**, 173-182] and studied after 14-21 days in vitro. Slices were exposed to AMPA at 35oC in Na^+-free sucrose buffer. Other slices were exposed to KA for 16 hours in serum-free growth medium. All cultures were restored to fresh culture medium for the recovery period. Some slices were pre-exposed for 15 min to one of the following antagonists: the selective, competitive AMPA receptor antagonist NBQX (10 μM), or LY-293558 (Lilly Corp, Indianapolis, IN; 10 μM) or the non-competitive AMPA antagonist GYKI 52466 (Lilly; 10 μM) or the selective KA antagonist gamma-D-glutamylaminomethyl sulfonic acid (GAMS; 500 μM). All studies included 5 μM dizlocilpine to block NMDA channels. Slices were stained with both propidium iodide 5 μg/ml and 5μM SYTO 11 Live Cell Indicator (Molecular Probes, Eugene, OR). Digital images were captured microscopically using rhodamine and fluorescein filture sets.

Results: N= 6 slices for each. Brief (30 min) exposure of hippocampal slice cultures to 5 μM AMPA caused Ca^{2+}-dependent death, 24 hours later, of pyramidal neurons preferentially in CA1. In contrast, 5 μM kainate required 16 hours of exposure and 2 days of recovery to exhibit toxicity, restricted to CA3 pyramidal neurons. AMPA or kainic acid at 1 μM were not toxic while AMPA or kainic acid at 10 μM caused death throughout the pyramidal cell layer (n=3). Agents that inhibit desensitization were not necessary to produce AMPA toxicity. Specific blockade by 3 different AMPA receptor antagonists attenuated AMPA but not kainate toxicity. Conversely, the kainate receptor antagonist GAMS (500 μM) blocked kainate but not AMPA toxicity. Control slices, without glutamate agonist or in Ca^{2+}-free buffer showed no toxicity.

530.2

GLUTAMATE-INDUCED CHANGES IN INTRACELLULAR FREE CALCIUM AND EXCITOTOXICITY IN CULTURED RAT CEREBELLAR GRANULE CELLS

Kathleen P. Platt & David R. Bristow SPON: Brain Research Association. School of Biological Sciences, University of Manchester, Manchester, UK.

Excitotoxicity has been implicated in the aetiology of a number of neurological disorders, however the molecular mechanisms underlying this have not been fully elucidated. In this study the susceptibility of rat cerebellar granule cells to glutamate-induced excitotoxicity was examined at 7 DIV using fluorescein diacetate/ propidium iodide staining. Neurons were exposed to glutamate (1 μM - 10 mM) for 30 minutes at 37°C and their viability was assessed 24 hours later. Glutamate induced a concentration-dependent neurotoxicity (ED_{50} 62 ± 34 μM). Experiments to determine the time course of glutamate-induced excitotoxicity suggested that the majority of neuronal death occurred within 4 hours after exposure to glutamate. To examine the role of calcium in excitotoxicity, intracellular free calcium ($[Ca^{2+}]_i$) levels were recorded from single cerebellar granule cells (7 - 8 DIV) loaded with fura-2/AM. The $[Ca^{2+}]_i$ was recorded in single neurons exposed to 1 mM glutamate for 30 minutes and throughout the ensuing 4.5 hours (26 ± 1°C). In 10% (8/78) of the neurons, $[Ca^{2+}]_i$ immediately rose to a sustained plateau (>1 μM) and failed to recover. The remaining 90% (70/78) of the cells buffered the glutamate induced $[Ca^{2+}]_i$ load back to near basal levels after the glutamate was removed. However, in 51% of these neurons (which showed a glutamate-induced $[Ca^{2+}]_i$ load/ min of 640 ± 57 nM (mean ± SEM, n = 36)), sustained increases in $[Ca^{2+}]_i$ were observed at various times over the next 4 hours. In contrast, the remaining 49% of these neurons (which showed glutamate-induced $[Ca^{2+}]_i$ load/ min of 406 ± 39 nM (mean ± SEM, n = 34)) maintained near basal $[Ca^{2+}]_i$ levels for the remainder of the experiment. This delayed calcium overload correlated with neuronal cell death as determined by either loss of fura-2/AM fluorescence or by failure to respond to a subsequent glutamate stimulus. These results support a relationship between the glutamate-induced calcium load and subsequent cerebellar granule cell death ($p < 0.01$ ANOVA with Scheffe's test).

530.3

AMPA-INDUCED LESION OF THE RAT GLOBUS PALLIDUS: QUANTIFICATION OF THE CALCIFIED AREAS AND OF THE ASTROGLIAL AND MICROGLIAL REACTIONS.

V. Petegnief, J. Saura and N. Mahy*. U. Biochemistry, Sch. of Medicine, Univ. of Barcelona, Spain.

A dose-response study of the formation of calcium deposits and associated astroglial and microglial reactions was performed after microinjection of 0.5 μl of a millimolar range of concentrations (0.54-21.6 mM) of AMPA in the rat ventral globus pallidus. At one month post-lesion, calcified formations were observed and quantified in the ipsilateral ventral globus pallidus, ventral pallidus, nucleus basalis of Meynert, substantia innominata, reticular thalamic nucleus and entopeduncular nucleus of the rats treated with 5.4, 10.8, 16.2 and 21.6 mM of AMPA. At the two higher doses, calcium deposits were also detected in the ipsilateral striatum at the lateral border of the globus pallidus and also in ventral nuclei of the thalamus. Calcium deposits were associated with astroglial and microglial reactions that reached statistical significance at the two higher doses. Monoamine oxidase B autoradiography, reflecting the astroglial reaction, showed a significant ($P < 0.01$) increase in the enzyme in the ipsilateral globus pallidus and caudate putamen. The microglial reaction was characterized by peripheral benzodiazepine receptor (PBR) autoradiography. Ipsilateral globus pallidus and reticular thalamic nucleus showed a significant ($P < 0.001$) increase in PBR radioligand binding. In conclusion, excitotoxic lesion of the rat globus pallidus with AMPA induced, in a dose-dependent manner, the formation of calcium deposits together with regionally different astroglial and microglial reactions. (Supported by FISss 94/1461 and BIOMED PL 931359).

530.4

RESTING AND KAINATE-INDUCED Ca^{2+} LEVELS MEASURED BY FURA-2 IMAGING OF INDIVIDUAL CA1 NEURONS AFTER GLOBAL ISCHEMIA IN THE RAT.

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Global ischemia in the rat induces a reduction in expression of GluR2 (the subunit that limits Ca^{2+} influx via AMPA receptors) in the hippocampal CA1 region prior to neurodegeneration. We hypothesize that Ca^{2+} permeable AMPA receptors may directly mediate $[Ca^{2+}]_i$ increases and thereby introduce $[Ca^{2+}]_i$ loads that contribute to delayed neuronal degeneration. We measured basal and kainate evoked $[Ca^{2+}]_i$ by optical imaging in voltage clamped CA1 pyramidal neurons in hippocampal slices. At -80 mV resting $[Ca^{2+}]_i$ did not differ significantly in ischemic vs. control neurons (143 ± 50 nM vs. 220 ± 36 nM). To eliminate the contribution of voltage- and NMDA receptor dependent Ca^{2+} influx during kainate (KA) application, which might mask changes directly due to AMPA receptors, the membrane potential was stepped from -80 to +20 mV in the presence of TTX (3 μM), nimodipine (10 μM), DL-APV (100 μM) and MK-801 (10 μM). This voltage step produced a large rise in $[Ca^{2+}]_i$ that was similar in ischemic and control neurons. However, recovery at 10 min was more complete in control than in ischemic neurons (0.8 ± 0.1 μM, n = 5 vs. 1.6 ± 0.4 μM, n = 6). Application of KA (300 μM, 60 s) at +20 mV produced an outward current and elevated Ca^{2+} levels in somata and proximal dendrites of neurons from both control and ischemic rats. These effects were blocked by CNQX. In a small sample of cells no significant difference in the $[Ca^{2+}]_i$ increase was found in 24 hrs post-ischemic vs. control neurons (41%, n = 2 vs. 45%, n = 4). Further experiments at 24 hrs and later time points are expected to shed light on the molecular mechanisms underlying delayed neuronal death after global ischemia.

530.5

Ca^{2+}-DEPENDENT NEUROTOXICITY: RELATIONSHIP BETWEEN SOURCE, INFLUX RATE AND $[Ca^{2+}]_i$ HOMEOSTASIS Y.M.Lu*, H.Z.Yin, J. Chiang and J.H. Weiss. Depts. of Neurology, U.C. Irvine, Irvine CA 92717.

Ca^{2+}-dependent glutamate neurotoxicity results after Ca^{2+} influx through any of three primary routes: NMDA channels, voltage sensitive Ca^{2+} channels (VSCC) and Ca^{2+} permeable AMPA/kainate channels (CPAK). Neurons posessing CPAK, (about 13% of neurons in cortical culture) can be identified by kainate stimulated Co^{2+} uptake (Co^{2+}(+) cells), and are unusually kainate vulnerable.

A close correlation was seen between Ca^{2+} influx rate (through different routes) and resultant injury, supporting the idea that agonist triggered influx rate is a primary determinant of injury: Exposures to NMDA (20 μM with 1.8 mM Ca^{2+} in the media) and kainate (100 μM kainate in 10 mM Ca^{2+}) that gave similar $^{45}Ca^{2+}$ influx rates, resulted after 6 hours in comparable levels of cortical neuronal injury. Also, kainate-triggered $^{45}Ca^{2+}$ influx rates into highly vulnerable Co^{2+}(+) neurons were estimated (using kainate "pre-exposures" to selectively destroy Co^{2+}(+) neurons) to be much greater that influx into other neurons lacking CPAK.

However, imaging studies suggested a generally poor relationship between influx and $[Ca^{2+}]i$. With 100 μM kainate exposures, despite the high estimated influx rate, mean $[Ca^{2+}]i$ responses in Co^{2+}(+) neurons were little different from those in other neurons. Similarly, NMDA exposures (20 μM) in 1.8 mM Ca^{2+}, despite triggering a much lower influx rate, caused $[Ca^{2+}]i$ responses similar to those seen with exposure in 10 mM Ca^{2+}. These observations suggest that sequestered Ca^{2+} (invisible to imaging) might be an important contributor to injury. However, with low (10 μM) kainate exposures Co^{2+}(+) neurons showed significantly greater mean $[Ca^{2+}]i$ responses than other neurons, perhaps reflecting very low rates of Ca^{2+} influx in cells lacking CPAK.

530.6

Zn^{2+} PERMEATES Ca^{2+} PERMEABLE AMPA/KAINATE CHANNELS AND TRIGGERS SELECTIVE NEURODEGENERATION. H.Z. Yin*, and J.H. Weiss. Depts. of Neurology and Psychobiology, U.C. Irvine, Irvine CA 92717.

Neurons possessing Ca^{2+} -permeable AMPA/kainate channels can be identified by a histochemical stain based on kainate stimulated Co^{2+} uptake (Co^{2+}(+) cells) and are unusually vulnerable to kainate toxicity. The divalent cation, Zn^{2+}, is synaptically released at many excitatory synapses, and may serve as a trans-synaptic messenger (it can permeate NMDA channels and voltage sensitive Ca^{2+} channels [VSCC]). We set out to determine whether Zn^{2+} permeates Ca^{2+} permeable AMPA/kainate channels.

When cortical cultures are exposed for 5 min to kainate (100 μM) in the presence of Zn^{2+} (300 μM), and the Zn^{2+} sensitive fluorescent dye, TS-Q, Zn^{2+} accumulation is seen in virtually all neurons. However, indicating Zn^{2+} permeability through Ca^{2+} permeable AMPA/kainate channels, if Na^+ ions are removed from the media (to prevent depolarization and activation of VSCC), TS-Q fluorescence was largely limited to the small (about 13%) subset of neurons that are Co^{2+}(+). We next exposed cultures to kainate (for 5 min as above) in Na^+ containing media but with a lower Zn^{2+} concentration (100 μM), and assessed injury 24 h later. Indicating a greater injuriousness of Zn^{2+} entry through Ca^{2+} permeable AMPA/kainate channels than through VSCC, these exposures selectively destroyed the Co^{2+}(+) neurons.

We previously found that basal forebrain cholinergic (BFC) neurons are highly kainate sensitive, and are usually Co^{2+}(+). We now report that cortical somatostatin and parvalbumin imunoreactive neurons, which are also exceptionally kainate sensitive, are also generally (over 90%) Co^{2+}(+). Thus, direct permeation of Zn^{2+} through Ca^{2+} permeable AMPA/kainate channels could contribute to selective degeneration of neurons such as these in diseases like Alzheimer's, as well as subserving physiologic signaling functions.

530.7

NEUROPROTECTION IN A RAT MODEL OF EXCITOTOXICITY: NMDA ANTAGONISTS. K.L. Brunson[1], A. Khanna[1], H.C. Cromwell[2] and R.W. Cohen[1]*. [1]Department of Biology, California State University, Northridge, CA. 91330. [2]Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA. 90024.

We are studying mutant Han-Wistar (HW) rats which develop neurodegeneration in two brain regions (hippocampus and cerebellum). Previous data suggest that the observed neuronal degeneration is the result of an alteration in glutamate receptor expression. Here, we analyze the neuroprotective effects of two NMDA antagonists, Ketamine and MK-801 using chronic and acute protocols. In the chronic study, mutants were injected weekly (in a two week interval) with either Ketamine (10 mg/Kg) or MK-801 (1 mg/Kg) starting at 30-32 days of age. Chronic MK-801-injected mutants (n=3) exhibited a longer life span (16% increase) and prolonged weight gain (14% heavier after 45 days of age) compared to saline-injected mutants (n=3). Motor skill deterioration was monitored by an open-field test for two minute sessions. After 45 days of age the MK-801-injected mutants displayed 29.5% greater motor skill activity in the open field test than the saline-injected mutants. Ketamine-injected mutants (n=8) showed minor differences compared with the saline-injected mutants (n=7). In the acute study, mutants were injected with a single, anesthetic dose of Ketamine (50 mg/Kg) at 30 days of age. Acute Ketamine-injected mutants (n=5) lived 76 days (30% increase) while acute saline-injected mutants (n=2) lived 53 days. The data show that the mutant rats derive protective effects from the NMDA antagonists, suggesting a role of glutamate-induced excitotoxicity in neuronal degeneration of our model.

530.8

N-METHYL-D-ASPARTATE-MEDIATED TOXICITY IN CULTURED RAT CORTICAL NEURONS IS ANTAGONIZED BY FPL 15896AR. M. A. Black, R. Tremblay, G.A.R. Mealing, R. Ray, J. P. Durkin, J. F. Whitfield, J. Blosser* and P. Morley National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6 and Fisons Pharmaceuticals, P.O. Box 1710, Rochester, New York, 14603.

Overstimulation of glutamate receptors is believed to initiate cellular processes resulting in neuronal cell death, due in large part to the influx of Ca^{2+} through N-methyl-D-aspartate (NMDA) receptors. While NMDA-antagonists have been shown to be neuroprotective *in vivo* and *in vitro*, their mechanism of action is not well understood. In primary cultures of rat cortical neurons, we have examined the action of a novel NMDA receptor antagonist, (S)-α-phenyl-2-pyridine-ethanamine dihydrochloride (FPL 15896AR), on glutamate- and NMDA-induced neurotoxicity as well as on two early NMDA-triggered events, namely the rise in intracellular Ca^{2+} levels ($[Ca^{2+}]_i$) and the loss of membrane-associated protein kinase C (PKC) activity.

Ligand-binding assays, using a membrane fraction from rat cortical tissue, indicated that FPL 15896AR is an uncompetitive NMDA receptor antagonist (K_d = 1-2 μM). To test its neuroprotective effects, 12-15 day-old cortical cultures, prepared from day 18 fetuses, were exposed to NMDA (50 μM) or glutamate (50 μM) in the presence or absence of FPL 15896AR (50 μM) for 15 minutes and viability assessed 24 h later. Exposure to NMDA or glutamate resulted in the death of ~ 90 % of the neurons. This neurotoxicity was completely blocked by FPL 15896AR. Furthermore, FPL 15896AR (50 μM) prevented the loss of membrane-associated protein kinase C that developed 4 h following transient glutamate or NMDA exposure. The effect of FPL 15896AR on the NMDA-induced rise in $[Ca^{2+}]_i$ was determined by microfluorometry using the Ca^{2+}-sensitive dye, fura-2. FPL 15896AR (25 and 50 μM) significantly ($p<0.01$) reduced the NMDA-triggered $[Ca^{2+}]_i$ response by 33% and 45%, respectively.

The results show that FPL 15896AR protects against glutamate- and NMDA-mediated toxicity in primary cultures of rat cortical neurons. Whether it protects by reducing the NMDA-induced $[Ca^{2+}]_i$ surge and/or by preventing the loss of PKC activity is not known.

530.9

CYTOTOXIC EFFECTS MEDIATED THROUGH KAINATE RECEPTOR ACTIVATION. P.-E. Mansson, J.M. Carver, J. Shu, L. Cortes-Burgos, L.-M. Zhou, J.R. Howe[1], and T. Giordano*. Symphony Pharmaceuticals, Inc., Malvern, PA 19355, [1] Yale University School of Medicine, New Haven, CT 06520.

Exposure of neurons for prolonged periods of time or to high concentrations of excitatory amino acids (EAA), such as glutamate, results in neuronal death. The activation of glutamate receptors by EAA leads to a large influx of calcium which is thought to be responsible for the neurotoxicity. This has been well documented for two classes of glutamate receptors: the NMDA and AMPA families. However, due to the rapid desensitization of the kainate receptor family, it is not believed that EAA will elicit toxicity through this class of glutamate receptors. Exposure of cerebellar granule cells for 24 hours to 50 μM kainate results in an ~80% decrease in viability. To determine whether any of this toxicity is mediated through kainate receptors, the kainate specific antagonist, SYM2081, was added at concentrations ranging from 0.1 μM to 10 μM. No neuroprotection was observed at any concentration tested. Because of the difficulty of observing glutamate subtype specific responses in a heterogeneous population of cells expressing multiple receptor subtypes, cloned recombinant cell lines expressing either GluR6 or GluR6 and KA2 were studied for their susceptibility to toxicity through the kainate receptor. Preliminary experiments suggests that both glutamate and kainate at high concentrations (≥100 μM) are toxic to the HEK 293 cells stably transfected with either GluR6 or GluR6-KA2 subunits but not the parental cell line. Future experiments will focus on whether this toxicity can be blocked by kainate antagonists, such as SYM2081.

530.10

VISUALIZATION OF NMDA-INDUCED MITOCHONDRIAL CALCIUM UPTAKE IN STRIATAL NEURONS USING CONFOCAL MICROSCOPY T-I Peng*, S-S Sheu, JT Greenamyre Depts of Neurology and Pharmacology, University of Rochester, Rochester, NY 14642

Impaired mitochondrial energy production increases neuronal vulnerability to NMDA excitotoxicity, in part, because ATP deficiency depresses Na^+/K^+ ATPase function and leads to membrane depolarization. Membrane depolarization reduces the voltage dependent Mg^{++} blockade of NMDA channels and results in excessive activation of NMDA receptors. Mitochondria also serve as a Ca^{++} buffering system. Ca^{++} overloading may impair mitochondrial ATP synthesis and also cause free radical formation and lipid peroxidation. We examined whether NMDA receptor stimulation causes Ca^{++} sequestration in mitochondria of intact cultured striatal neurons. Mitochondrial Ca^{++} was monitored with the fluorescent probe, Rhod-2, and laser scanning confocal microscopy. A rapid increase of mitochondrial Ca^{++} was observed when the neurons were treated with 200 μM NMDA. Increased mitochondrial Ca^{++} was not seen in the presence of ruthenium red, an inhibitor of the mitochondrial Ca^{++} uniporter, or CCCP, a protonophore that breaks down the mitochondrial membrane potential necessary for Ca^{++} uptake. Mitochondrial Ca^{++} returned quickly to baseline if neurons were treated with NMDA for < 1 min; if treated for > 1 min, mitochondrial Ca^{++} remained somewhat elevated even after NMDA had been washed away for 10 min. The longer neurons were exposed to NMDA, the higher the residual mitochondrial Ca^{++} level observed. These data show directly that *(i)* Ca^{++} accumulates in mitochondria of intact neurons in response to NMDA receptor activation, and *(ii)* that the ability to normalize mitochondrial Ca^{++} decreases with the duration of receptor activation. Ca^{++} accumulation may play a role in mitochondrial dysfunction and render neurons more vulnerable to NMDA excitotoxicity. *(Supported by a Mallinckrodt Scholar Award, a Markey Foundation Pilot Project Grant and the NPF Center of Excellence at the University of Rochester.)*

530.11

L-CYSTEINE AND DITHIOTHREITOL ENHANCE CYANIDE-INDUCED POTENTIATION OF NMDA RECEPTOR-ACTIVATED Ca^{2+} INFLUX. P. Sun*[1], S.G. Rane[2], P.G. Gunasekar[1] and G.E. Isom[1]. Depts. of [1]Pharmacol. & Toxicol., and [2]Biol. Sci., Purdue Univ., West Lafayette, IN 47907

In rat cerebellar granule cells, NMDA (50 μM with 10 μM glycine) stimulated a 4 fold increase of cytosolic free calcium ($[Ca^{2+}]_i$). The NMDA response was potentiated 15-60% by NaCN at doses of 20-100 μM, which alone had no effect on basal $[Ca^{2+}]_i$. The present study characterized in more detail the potentiation produced by cyanide. In whole cell patch clamp recordings, cyanide (100 μM) increased the amplitude and duration of NMDA-stimulated inward current (75% and 164%, respectively), suggesting a direct interaction of cyanide with the NMDA receptor. The involvement of sulfhydryl groups in cyanide's action was studied using microfluorescence to measure $[Ca^{2+}]_i$ in fura-2 loaded neurons. Addition of the thio compound L-cysteine (100 μM) further enhanced the cyanide-induced potentiation from 27% to 66% above the NMDA-stimulated $[Ca^{2+}]_i$ levels. In separate studies, the disulfide reducing agent dithiothreitol (DTT) at 2 mM slightly increased the NMDA response after 2 min incubation followed by washing. The DTT pre-treatment increased cyanide potentiation from 25% to 43% above the NMDA-stimulated $[Ca^{2+}]_i$ levels. Pre-incubation with the sulfhydryl alkylating agent N-ethylmaleimide (NEM) (100 μM) for 2 min followed by washing resulted in attenuation of the cyanide potentiation from 25% to 9% above NMDA-stimulated $[Ca^{2+}]_i$ levels. These results indicate that cyanide-induced potentiation of NMDA response can be modulated by sulfhydryl redox agents, suggesting the NMDA receptor sulfhydryl groups may be involved in cyanide's action. (Supported by NIEHS Grant No. 04140)

530.12

CHANGES IN INTRACELLULAR FREE CALCIUM LEVELS DURING DELAYED EXCITOTOXICITY AND RESCUE IN NT2-N NEURONS. **M. Munir*, M.C. LaPlaca, L.E.Thibault and P. McGonigle Depts. of Pharmacology and Bioengineering, University of Pennsylvania, Philadelphia, PA 19104.**

Using digitized videomicroscopy, Ca^{2+}-sensitive dye fura-2 and release of lactate dehydrogenase (LDH), mechanisms of Ca^{2+} regulation during the delayed phase of excitotoxicity and their relationship to cell death were examined in the NT2-N clonal line of human neurons. Exposure to 1 mM glutamate caused a rapid elevation in the $[Ca^{2+}]_i$ which was sustained as long as glutamate was present in the media. After termination of a 15 min exposure to 1 mM glutamate $[Ca^{2+}]_i$ declined gradually and reached near basal levels in 40-60 min. Removal of extracellular Ca^{2+} after termination of a 15 min exposure to 1 mM glutamate caused a sharp decrease in $[Ca^{2+}]_i$ which recovered to resting levels within 10 min. Removal of extracellular Ca^{2+} for 3 hr following glutamate exposure also prevented glutamate-mediated toxicity. Blockade of NMDA receptors with dizocilpine (MK-801) or by lowering the extracellular pH, produced a faster recovery of $[Ca^{2+}]_i$ to near basal levels similar to the one produced by removal of extracellular Ca^{2+} and also rescued the cells from excitotoxic cell death. Blockade of non-NMDA channels with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) or voltage-gated channels with nimodipine did not alter the time course of recovery of $[Ca^{2+}]_i$ to basal levels and did not rescue the cells from glutamate toxicity. These observations indicate that Ca^{2+} influx occurring through the NMDA receptors during the delayed phase of rapidly triggered excitotoxicity plays a critical role in determining the final outcome of the initial glutamate insult. Influx of Ca^{2+} through non-NMDA and voltage-gated Ca^{2+} channels do not play a critical role during the delayed phase of toxicity. (Supported by NS-08803 and GM-34781)

530.13

ROLE OF INTRACELLULAR CALCIUM IN NEURONAL SURVIVAL TO EXCITATORY AMINOACID STIMULATION. A. Novelli*, A. Torreblanca, P. Losa-Uría, and M.T. Fernández-Sánchez. Dept. Funct. Biol., Biochem. and Mol. Biol... Sch. of Med. and Dept. Phylosophy and Psychol., Psychobiol., Univ. Oviedo. 33071 Oviedo. Spain.

We have investigated the effects of intracellular calcium concentration [Ca++]i on cultured cerebellar neuron survival following exposure to excitatory aminoacids (EAA)s. Glutamate (GLU)(40μM), N-methyl-D-aspartate (NMDA)(100μM) and domoate (DOM)(15μM) but not α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA)(100μM), powerfully elicited [Ca++]i increase, cGMP formation and neurotoxicity. AMPA antagonized DOM-mediated [Ca++]i increase, cGMP formation and neurotoxicity. Reduction of extracellular Ca++ concentration reduced both [Ca++]i and cGMP increase by EAAs but not neurotoxicity. Moreover, the nitric oxide synthase inhibitor N^G-monomethyl-L-Arginine (L-NMMA)(1mM) prevented EAAs-mediated cGMP increase but was ineffective in reducing excitotoxicity. Most of DOM-mediated [Ca++]i increase was reduced by nifedipine (NIF)(1μM) which did not reduce DOM toxicity. Culture exposure to high KCl (50mM) significantly increased [Ca++]i and cGMP levels but did not produce neurotoxicity when MK-801(1μM) was present. Similar results were obtained following addition of the L-type voltage sensitive calcium channel agonist S(-)BAY-K8644 (1μM). Long-term (15 days) culture exposure to either high KCl or S(-)BAY-K8644 did significantly increase glucose consumption. Such increase was abolished by reducing extracellular Ca++ concentration. Long-term exposure to either high KCl or S(-)BAY-K6844 did not significantly reduce [Ca++]i increase in response to either GLU or DOM but significantly reduced excitotoxicity. Neuroprotection was abolished by reducing either extracellular or intracellular Ca++ concentration, or by treatment with protein synthesis inhibitors. We conclude that in cultured cerebellar neurons excitotoxicity is independent of [Ca++]i increase, and elevated [Ca++]i may be important to the development of a protein synthesis-dependent excitoprotective mechanism. Such mechanism does not appear to involve EAA receptor desensitization, nor nitric oxide production. This work was supported by CICYT, grant SAL91-0613 & SAF94-0394

530.14

CATION DEPENDENCE OF EXCITOTOXIC GLUTAMATE-INDUCED INHIBITION OF CaM KINASE II ACTIVITY. S.B. Churn* and R.J.DeLorenzo. Department of Neurology, Medical College of Virginia, Richmond, VA 23298

Excitotoxic glutamate exposure results in an immediate osmotic lysis and a delayed, Ca^{2+}-dependent neuronal cell death. The Ca^{2+} dependence of delayed neuronal cell death prompted the investigation of Ca^{2+} regulated neuronal enzyme systems that may be involved in mediating delayed neuronal cell death. Ca^{2+}/calmodulin-dependent kinase II (CaM kinase II) is a neuronally enriched, Ca^{2+}-regulated effector system. CaM kinase II activity is significantly inhibited following excitotoxic glutamate exposure. The excitotoxic glutamate-induced inhibition of CaM kinase II activity has been shown to be dependent upon NMDA receptor stimulation and influx of Ca^{2+}. The present study was designed to determine whether stimulation of CaM kinase II was necessary for glutamate-induced inhibition of kinase activity. Excitotoxic glutamate exposure (500 μM, 10 min) was performed under standard conditions (2 mm Ca^{2+}) or with Ca^{2+} replaced by other cations. The cations were chosen for ability to enter neurons and ability to replace Ca^{2+} in calmodulin stimulation (Chao et al. 1984 Mol. Pharm. 26:75). Cations that supported calmodulin-dependent activity also supported glutamate-induced inhibition of kinase activity (e.g Sr^{2+} 69.4% control activity, n = 6, and Mn^{2} 63.9% control, n = 4). However, replacing Ca^{2+} with cations that do not support calmodulin-dependent activity such as Ba^{2+} (94.1% control, n = 6) or Co^{2+} (84.6%control, n = 4), or omitting Ca^{2+} alone (99.8% control, n = 6) protected kinase activity following glutamate exposure. A positive correlation was observed between ability to support calmodulin-dependent activity and ability to support glutamate-induced inhibition of kinase activity. The data support the conclusion that glutamate-induced inhibition of kinase activity occurs via a calmodulin-dependent process.

GABA RECEPTORS III

531.1

ALLOSTERIC MODULATORS OF THE $GABA_A$ RECEPTOR: DIFFERENTIAL INTERACTION OF BENZODIAZEPINES AND NEUROACTIVE STEROIDS WITH ETHANOL. M. Suruki[1], S. Robledo[1], S. Wieland*[1], N.C. Lan[1], K.W. Gee[2], P.L. Wood[1] and R.B. Carter[1], Dept. Pharmacology, [1]CoCensys and [2]Univ. of California, Irvine, CA 92718.

Pregnane steroids allosterically modulate $GABA_A$ receptor function in a manner similar to that of benzodiazepines, though acting at a distinct recognition site. Accordingly, neuroactive steroids exhibit pharmacologic properties similar to those of benzodiazepines, such as anticonvulsant, anxiolytic, and sedative-hypnotic activity. Inasmuch as positive allosteric modulators of $GABA_A$ receptor function exhibit profound interactions with ethanol, the effects of two endogenous neuroactive steroids, 3α-OH, 5α-pregnan-20-one (3α,5α-P) and 3α-OH, 5β-pregnan-20-one (3α,5β-P), were compared to those of two benzodiazepines, alprazolam and diazepam, on the motor function of rats when administered either alone or in combination with ethanol. Dose-dependent decreases in rotorod performance were obtained for all compounds (i.p.). Benzodiazepines (alprazolam, TD_{50}=3.2; diazepam TD_{50}=7.4) were more potent than neuroactive steroids (3α,5α-P, TD_{50}=21.2; 3α,5β-P, TD_{50}=22.8) in this regard. When administered in combination with a subhypnotic, subataxic dose of ethanol (1.0 g/kg, i.p.), dose-response curves for the effect of the benzodiazepines on rotorod performance were shifted to the left by a factor of 10 (alprazolam, TD_{50}=0.3; diazepam TD_{50}=0.8). In contrast, the ataxic properties of the neuroactive steroids were unaffected by concomitant ethanol administration (3α,5α-P, TD_{50}=29.3; 3α,5β-P, TD_{50}=18.2). These data indicate that positive allosteric modulators of the $GABA_A$ receptor differ with respect to their interaction with ethanol.

531.2

Modulation of $GABA_A$ Receptors by Neurosteroids, Benz[*e*]indenes, and Their Enantiomers L.L. Wittmer, C.F. Zorumski*, M. Kalkbrenner, A.S. Evers, Y. Hu, D.F. Covey. Depts. of Psychiatry, Anesthesiology, and Mol. Bio. & Pharmacology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Modulation of the $GABA_A$ receptor complex is thought to play a role in steroid anesthesia. We have used a series of compounds including (+) and (-) enantiomers of neurosteroids and benz[*e*]indenes as molecular probes for a putative steroid binding site on $GABA_A$ receptors. Compounds were assayed for their effects on GABA-mediated Cl^- currents in cultured rat hippocampal neurons and for their potency as anesthetics in a tadpole righting-reflex assay. Results indicate that (-) enantiomers are markedly less effective at potentiating the effects of GABA on $GABA_A$ Cl^- currents than the (+) enantiomers, in the most extreme case showing a 10-fold difference. Unlike the (+) enantiomers, the (-) enantiomers do not gate $GABA_A$ channels at concentrations up to 200 μM. Both the (+) and (-) enantiomers are anesthetics in the tadpole assay; however the (-) enantiomers are strikingly less potent than the (+) enantiomers. These data provide strong evidence for the existence of an enantioselective neurosteroid binding site on $GABA_A$ receptors and suggest that direct gating of Cl^- currents by the compounds studied is not required for anesthetic effects.

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531.3

ANXIOLYTIC PROPERTIES OF THE NOVEL ORALLY-ACTIVE NEUROSTEROID CO 6-0549. R.B. Carter*[1], S. Wieland[1], N.C. Lan[1], H. Xia[2], J.D. Belluzi[3], L. Stein[3], K.W. Gee[3], and P.L. Wood[1], Depts. [1]Pharmacol. and [2]Chem., CoCensys and [3]Dept. Pharmacol., Univ. of Calif., Irvine, CA 92718.

Neuroactive steroids are allosteric modulators of the $GABA_A$ receptor that act at a novel recognition site. Co 6-0549 is a water-soluble, orally-bioavailable neuroactive steroid prodrug, the parent of which has been demonstrated by binding and electrophysiology to be a potent modulator of the $GABA_A$ receptor. The anxiolytic-like properties of Co 6-0549 and alprazolam were characterized in the rat Geller-Seifter test. Co 6-0549 (2.0-64.0 mg/kg, p.o.) produced dose-related increases in responding under punished and unpunished components. The minimum effective dose (MED) for activity on punished responding (20-shock increase over control) was 4.8 mg/kg. Unpunished responding was suppressed to 25% of control levels at 40.1 mg/kg, yielding a therapeutic index (TI) of 8.4. In comparison alprazolam (2.0-64.0 mg/kg, p.o.) exhibited an MED of 4.1 mg/kg and a TI of 11. Neither Co 6-0549 nor alprazolam exhibited tolerance upon chronic administration (2X MED, daily, 14 days); however, whereas the maximum effects of alprazolam on punished responding required 2-3 days to develop, Co 6-0549 produced full anxiolytic-like effects following a single administration. The ataxic effects of Co 6-0549 (40.0-100.0 mg/kg, p.o.) and alprazolam (1.0-20.0 mg/kg, p.o.) were compared using the rat rotorod procedure. Whereas alprazolam impaired rotorod performance at a dose 3.2 times its MED in the Geller-Seifter (TD_{50}=13.3), Co 6-0549 did so at a dose 16 times its MED (TD_{50}=76.9). Moreover, inasmuch as a subhypnotic dose of ethanol (1.0 g/kg, i.p.) administered in combination, markedly potentiated alprazolam's ataxic effects (TD_{50}=4.4), Co 6-0549 was less affected and retained a good therapeutic index (TD_{50}=55.0; TI=11.5). These data suggest that Co 6-0549 may offer clinically-relevant advantages over current drugs.

531.4

DIFFERENTIAL SENSITIVITY OF SYNAPTIC GABAERGIC CURRENTS TO A NEUROACTIVE STEROID IN BRAIN SLICES FROM MALE RATS

E.J. Cooper, G.A.R. Johnston*, F.A. Edwards Dept. Pharmacology, University of Sydney, N.S.W., 2006, AUSTRALIA

The concentration of the neuroactive steroid tetrahydrodeoxycorticosterone (THDOC, 5α-pregnane-3α,21-diol-20-one), in the rat brain is increased in response to conditions of mild stress. Micromolar concentrations of this steroid and other similar steroids have been reported to potentiate responses to the inhibitory neurotransmitter GABA, in isolated neurons. We have investigated the effects of more physiological levels of THDOC on synaptic GABA currents (in CNQX, 10μM and TTX, 1μM). Whole cell patch-clamp recordings were made from hippocampal dentate granule cells and cerebellar Purkinje cells in slices taken from the same animal for comparison. In all cells 1 - 2μM THDOC caused a rapid reversible increase in the decay time of GABA currents, with no effect on amplitude or rise time. The control currents in Purkinje cells showed a much more rapid decay (decay to half amplitude, T50% = 9.4± 1.1ms, n=14) than hippocampal granule cells (T50% = 21.0 ±0.7ms, n=16). After potentiation the decay times from the two cell types were not significantly different (T50% ~ 80ms, 2μM THDOC, all groups). Thus the percentage potentiation was considerably greater in Purkinje cells (PC) than in hippocampal granule cells (HG) at these high concentrations (PC 900% vs HG 400%, 10-13 day old rats). Comparison was made between different age groups in both cell types (10-13days vs 18-21days). A significantly higher sensitivity to THDOC was found in hippocampal granule cells from the younger group than any of the other of the three groups with an increase of 29±1.3% (n=6) in decay time at 50nM. This was the only group significantly affected by this low, more physiological level, of THDOC, although GABA synaptic currents in Purkinje cells from younger rats were also more sensitive to neuroactive steroids than the older group. It is suggested that the differences seen may be due to different regional and age specific distributions of GABAA-receptor subunits.

531.5

BRAIN ALLOPREGNANOLONE (AP) CONCENTRATIONS AND $GABA_A$ RECEPTOR FUNCTION IN STRESSED RATS. M.L.Barbaccia*, G.Roscetti, M.Trabucchi, A.Concas#, L. Dazzi#, R.H.Purdy* and G. Biggio#.Dept. Exp. Med. Univ. of Rome "Tor Vergata"-00133 Rome-Italy,*Dept. Psych. UCSD- San Diego,CA 92161-USA and# Dept. Exp. Biol. Univ. of Cagliari-09123 Cagliari-Italy.

Acute stress (CO_2 inhalation,foot shock) increased rat brain cortical steroid concentrations. Two temporal patterns were observed after CO_2: a) progesterone and deoxycorticosterone were maximally increased (9 and 4 folds, respectively) 10 min following stress and decreased thereafter; b) pregnenolone and AP levels peaked (+170% and +200%, respectively) 30 min post-stress, were still high at time 60 and returned to control values by 120 min. The same stress paradigm elicited an alteration in CNS $GABA_A$ receptor function that was specular, with respect to the time course, to the changes in brain AP content. ^{35}S-TBPS binding was maximally increased, +51%, and punished responding score in the Vogel's test was decreased, -40%, 10 min after stress. The behavioral response declined more rapidly, reaching control values by 60 min, while ^{35}S-TBPS binding returned to control values by 120 min. Moreover, the immediate increase in brain cortical dopamine release evoked by foot shock (that also increased brain neurosteroids) was blunted by AP (5 ug,i.c.v.). These results suggest a physiological role for AP in the modulation of the CNS response to acute stress.

531.6

NEUROSTEROID POTENTIATION OF A GABA-WITHDRAWAL SYNDROME. **E. Calixto, T. Montiel and S. Brailowsky*. Instituto de Fisiología Celular, U.N.A.M., 04510 México D.F., MEXICO**

The GABA-withdrawal syndrome (GWS) is a model of partial epilepsy which depends, for its induction, of GABA receptor activation. Paroxysmal activity appears at the intracerebral infusion site a short time after aminoacid discontinuation (min to hrs) and lasts for days. We have studied the neuromodulatory effect of the neurosteroid (NE) 3α-hydroxi-5α-pregnan-20-one (allopregnanollone) in this paradigm. In rats prepared for chronic EEG recording and intracortical microinfusions, the instillation of the NE alone or of its antagonist (a sulfate derivative), had no effects; however, when the NE was administered before or concurrently with GABA, we observed a potentiation of the GWS: shorter latency for paroxysmal discharges to appear (from 91.2±2.28 min in the GABA-only group to 56.2±6.1 min in the GABA+NE group) and prolonged duration (from 7.4±0.2 days in the GABA-only group to 9.7±0.4 days in the GABA+NE group).

These results indicate a neuromodulatory effect of allopregnanollone on GWS, which is dependent on the presence of GABA at the receptor site.

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531.7

BINDING CHARACTERISTICS OF [^{3}H]ABECARNIL IN THE RAT CEREBRAL CORTEX. A. K. Mehta* and R. P. Shank. Drug Discovery, The R. W. Johnson Pharmaceutical Research Institute, Spring House PA 19477-0776, USA.

Abecarnil is a GABA-based anxiolytic, and is less sedating and less prone to cause motor incoordination than traditional benzodiazepine (BZ) receptor agonists. We have previously reported that abecarnil inhibits the binding of [^{3}H]Ro 15-4513, [^{3}H]Ro 15-1788, and [^{3}H]flunitrazepam with a slope factor >1 in the rat cerebellum and cerebral cortex. In this study, we have characterized the binding of [^{3}H]abecarnil (0.1-300 nM) to membranes prepared from the rat cerebral cortex. Binding reached equilibrium within 20 min at 22°C. Nonspecific binding to the biological and non-biological material accounted for ≈50% of total binding. Several procedures; e.g., presoaking the filtermats with polyethylenimine, increasing the filtration washes and employing different filtration temperatures (2°C, 22°C, 37°C), were used in an attempt to reduce the nonspecific binding but there was no substantial improvement. A curve-fit analysis of saturation binding data revealed an apparent K_d of 0.60 ± 0.21 nM and B_{max} of 2.8 ± 0.7 pmol/mg protein (n = 9) at 22°C. A good fit between actual and predicted specific binding was obtained only when the equation to which the data were fitted contained a saturable site with a slope factor >1. Whether this reflects positive cooperativity in the binding of abecarnil to $GABA_A$ receptors, or an artifact arising from the high degree of nonspecific binding is unclear. The calculated K_d value for abecarnil is similar to the IC_{50} value obtained for the inhibition of [^{3}H]Ro 15-4513 binding by unlabeled abecarnil (0.60 ± 0.21 nM vs 0.85 ± 0.24 nM). The B_{max} value is also similar to those obtained for [^{3}H]Ro 15-4513, [^{3}H]Ro 15-1788 and [^{3}H]flunitrazepam in the rat cerebral cortex (2.8 ± 0.7 vs 2.3 ± 0.6, 2.3 ± 0.5 and 2.7 ± 0.6 pmol/mg protein, respectively).

531.8

ANDROGENIC:ANABOLIC STEROID INTERACTIONS WITH THE $GABA_A$-BZ RECEPTOR COMPLEX A.E.T. Masonis and M.P. McCarthy*. CABM/RWJMS-UMDNJ, Piscataway, N.J., 08854.

We describe the complex interactions of two androgenic-anabolic steroids (AAS), 17α-methyltestosterone (17α-MT) and stanozolol, with at least three major binding sites on the γ-aminobutyric $acid_A$-benzodiazepine receptor ($GABA_A$-BZ receptor) complex. Interestingly, the effects of the AAS on BZ binding to synaptoneurosomes from male and female rat cortex, hippocampus, and olfactory bulb were differentially sensitive to the binding of endogenous GABA. Additionally, the magnitude of the GABA-insensitive component of the AAS effects differed between brain regions. The AAS 17α-MT had both enhancing and inhibitory effects on BZ binding, depending upon the selectivity of the BZ ligand and the brain region tested. Conversely, the AAS stanozolol acted as a potent, strict inhibitor of high affinity BZ binding. The effects of stanozolol on the binding of the $GABA_A$ receptor ligands [^{3}H]muscimol and [^{3}H]TBPS were also determined. Stanozolol enhanced both 25nM [^{3}H]muscimol binding to cortical synaptoneurosomes and 5nM [^{35}S]TBPS binding to purified cerebrocortical membranes. Stanozolol-mediated enhancement of 5nM [^{35}S]TBPS binding was lost upon the addition of exogenous GABA. Our results are consistent with the interaction of stanozolol with a novel, GABA-sensitive stabilization site on the $GABA_A$-BZ receptor complex.

531.9

ISOLATION OF AN ENDOGENOUS INHIBITOR WITH AGONISTIC ACTION ON BENZODIAZEPINE RECEPTORS. S. Fiszer de Plazas*, M. C. Gravielle, C. Peña and L. Pignataro. Instituto de Biologia Celular, Facultad de Medicina and IQUIFIB, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Paraguay 2155 (1121) Buenos Aires, Argentina.

In a previous work we have described the time-course of appearance of an endogenous material capable of displacing [^{3}H]Flunitrazepam binding from benzodiazepine (BZD) receptor sites present in the developing chick optic lobe (*NeuroReport*, 5: 1957, 1994). The aim of the present study was to purify and determine the pharmacological action of this endogenous inhibitor. The endogenous factor was isolated from synaptic membranes of chick optic lobes. The purification was performed by means of several steps of high performance liquid cromatography with C18 and C4 phase reverse columns. The effect of GABA on the inhibition of [^{3}H]Ro 15-1788 binding by compounds which bind to BZD receptors could predict whether their pharmacological actions would be agonistic or antagonistic to the actions of benzodiazepines (GABA "shift" test). In this work we observed that 10^{-4}M GABA potentiated the inhibition of [^{3}H]Ro 15-1788 binding by the endogenous compound. These results demonstrated that the endogenous inhibitor possess the properties of an agonist of the BZD receptor.

531.10

3α,21-DIHYDROXY-5β-PREGNAN-20-ONE (5β-THDOC) SHARES THE SAME SITE AS 3α-HYDROXY-5α-PREGNAN-20-ONE (3α,5α-P) IN THE MODULATION OF GABA ACTION AT THE GABA$_A$ RECEPTOR COMPLEX. **B.G. Xue, R. Woodward[1], C.H. -Park, N.C. Lan and K.W. Gee*, Department of Pharmacology, College of Medicine, University of California, Irvine and [1]Acea Pharmaceuticals, a subsidiary of CoCensys Inc., Irvine, CA 92718.**

The allosteric modulation of [^{35}S]TBPS binding by the endogenous neuroactive steroids 3α,5α-P and 5β-THDOC was studied in the rat cortex. Relative to 3α,5α-P, 5β-THDOC has been observed to have limited efficacy as an allosteric modulator of [^{35}S]TBPS binding. Interactions between 3α,5α-P and 5β-THDOC were examined to determine whether both neurosteroids share a common site in the modulation of [^{35}S]TBPS binding. Increasing concentrations of 5β-THDOC antagonized the action of 3α,5α-P by shifting the concentration-response curves of 3α,5α-P modulation of [^{35}S]TBPS binding to the right. Schild analysis of the data reveals that 5β-THDOC competitively antagonized the effect of 3α,5α-P on [^{35}S]TBPS binding. 5β-THDOC also antagonized the potentiation of GABA-evoked chloride currents by maximally stimulating concentrations of 3α,5α-P. Behavioral studies with 3α,5α-P reveal the ability of 5β-THDOC to act as an antagonist of 3α,5α-P under certain conditions. Collectively these findings support the hypothesis that 5β-THDOC may act as a partial agonist at the same site as 3α,5α-P in the modulation of the GABA$_A$ receptor complex. (Supported by a grant from CoCensys Inc.)

531.11

KOJIC AMINE: A PARTIAL AGONIST OF THE GABA$_A$ RECEPTOR Y. T. Lee, T.T. Gibbs* and D.H. Farb, Laboratory of Molecular Neurobiology, Department of Pharmacology, Boston University School of Medicine, Boston, MA 02118.

Although benzodiazepine, barbiturate, and neurosteroid modulators of the γ-aminobutyric acid$_A$ receptor (GABA$_A$R) have been characterized in detail, GABA$_A$R agonists have been much less studied, with the exception of GABA and muscimol, both of which appear to be full agonists. Partial agonists may be useful as probes of GABA$_A$R diversity, and may have clinical value if they produce less desensitization than full agonists. A number of drugs have been suggested to be partial GABA$_A$R agonists, but few of these have been extensively studied electrophysiologically. Using whole cell voltage clamp recording of chick spinal cord neurons in primary tissue culture, we measured the potency and efficacy of a series of GABA$_A$R agonists. Of these, THIP, 3-aminopropane sulfonic acid, and isoguvacine produced maximal responses smaller than that of GABA, but these drugs elicited an "off-response" upon washout. This suggests that the lower maximum response to these agonists is due to an action at a secondary inhibitory site, from which the drug dissociates more rapidly than from the agonist recognition site. This may also explain why the responses to these agonists appear to desensitize rapidly, in spite of a smaller peak amplitude. In contrast, kojic amine has characteristics expected of a partial agonist at the GABA site. The kojic amine response is inhibited by the competitive GABA antagonist SR-95531, and the maximum response to kojic amine is about half that of GABA. When applied in combination with GABA, kojic amine inhibits the GABA response, and this inhibition may be surmounted by increasing the concentration of GABA, consistent with a competitive mechanism. We conclude that kojic amine is a partial agonist of the GABA$_A$R of chick spinal cord neurons.

531.12

MULTIPHASIC EFFECTS OF ALLOSTERIC MODULATORS ON GABA WHOLE-CELL CURRENTS IN ACUTELY DISSOCIATED HIPPOCAMPAL CA1 PYRAMIDAL CELLS. E.I.Tietz[1,2], J. Kapur[1], N. Esmaeil[1] and R.L. Macdonald[1,3]*. Departments of Neurology[1] and Physiology[2], University of Michigan, Ann Arbor, MI, 48105 and Department of Pharmacology[3], Medical College of Ohio, Toledo, OH 43699 .

The hippocampus contains multiple GABAA receptor (GABAR) subunit mRNAs. α1, α2, α5, β1, β3 and γ2 subunit mRNAs are in high abundance. α3, α4 and β2 mRNAs are moderately expressed, whereas α6 and δ mRNAs are absent. The effects of GABA and several allosteric modulators [diazepam (DZP); zolpidem (ZOL); DMCM and loreclezole (LOR)] on native GABARs in hippocampus were studied. Whole-cell recordings were obtained from acutely dissociated CA1 pyramidal cells using standard whole-cell patch clamp techniques (V_h=-50 mV). Patch pipettes contained 130 mM KCl (E_{Cl}=0 mV) and an ATP regenerating system. GABA (1 μM-3 mM) evoked inward currents (EC50=10.2 μM; Peak current=750 pA, n=12). In 20% of cells, GABA responses suggested the presence of GABARs with two GABA affinities. Modulator effects on 10 μM GABA currents were multiphasic. DZP potentiated GABA responses at two high affinity sites (EC50=2.3 and 236 nM, 8 cells) by 25% and 60%. ZOL (0.3 nM-10 μM) inhibited GABA currents at low nM concentrations and potentiated GABAR currents at higher concentrations. The inhibitory effects of ZOL, 1-100 nM, but not 1 μM, were reversed by 1 μM flumazenil, suggesting inverse agonist-like activity. DMCM (0.3 nM-30 μM) inhibited GABA currents in 4/8 cells up to 55%. DMCM (0.3-100 nM & 20 μM) potentiated GABA currents by 10-45% in 4/8 cells. LOR, which preferentially acts at recombinant GABARs with β2/3 subunits, potentiated GABA currents in 5 of 6 cells. The heterogeneous responses of CA1 neurons to GABA and to allosteric modulators suggests that functional GABARs are assembled from those subunits with highly expressed mRNAs and that individual CA1 pyramidal cells contain GABARs with α subunit heteromers and/or that multiple subtypes of GABARs exist in a single neuron.

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531.13

SELECTIVE MODULATION OF FAST AND SLOW GABA$_A$ RESPONSES IN THE RAT HIPPOCAMPUS BY VOLATILE ANESTHETICS. R.A.Pearce*, Depts. of Anesthesiology and Anatomy, Univ. Wisconsin, Madison.

Paired-pulse depression (PPD) of the evoked population spike *in vivo* is prolonged by many anesthetics, including volatile agents such as halothane. This action is thought to be due to enhancement of GABA$_A$-mediated feedback inhibition. Spontaneous IPSCs are prolonged by halothane, but their duration (7 ms prolonged to 20 ms) is an order of magnitude faster than PPD (70 ms prolonged to 200 ms). The present study tested the effect of two volatile anesthetics, halothane and enflurane, on fast and slow components of the evoked GABA$_A$ current, to determine if their effect on PPD could be due to prolongation of the slow component.

Evoked monosynaptic IPSCs were obtained by stimulating in the pyramidal (SP) and molecular (SL-M) layers to elicit GABA$_{A,fast}$ and GABA$_{A,slow}$. Glutamate antagonists APV (40 μM) and CNQX (20 μM) were present in the perfusate, and recordings were made with 3M CsAc and 50 mM QX-314 in the electrodes. Volatile anesthetics at a concentration of 2 MAC were applied in the interface configuration.

Halothane and enflurane prolonged the decay of the slow dendritic IPSC, to approximately twice control, but did not alter the response amplitude. Halothane also prolonged the duration of the fast somatic IPSC, to over three times control, without affecting its amplitude. However, enflurane markedly reduced the amplitude of the fast somatic response, in some cases apparently eliminating the response completely, so that only a small slow component remained following SP stimulation.

It is concluded that anesthetics enhance PPD by prolonging the decay of the slow dendritic GABA$_A$ response. Furthermore, it is speculated that the proconvulsant property of enflurane is related to its reduction of the fast somatic component of GABA$_A$ inhibition.

This work was supported by NS-01548 from NINDS.

531.14

PHARMACOLOGICALLY-DISTINCT GABA$_B$ RECEPTORS MODULATE cAMP ACCUMULATION IN A MODEL OF COINCIDENT SIGNALLING Cunningham, M.D.* and Enna, S.J. Department of Pharmacology, Toxicology and Therapeutics, Kansas University Medical Center, Kansas City, Kansas 66160.

GABA$_B$ receptors, coupled through pertussis toxin-sensitive G proteins, modulate presynaptic neurotransmitter release, Ca^{+2} and K^+ conductance, and cAMP production. GABA$_B$ receptor activation by baclofen attenuates forskolin-induced cAMP production and augments the effect of various neurotransmitters or drugs that stimulate production of cAMP through interaction with membrane-bound receptors coupled to G proteins. The dual effects of baclofen on cAMP accumulation has led to the suggestion that there exists multiple GABA$_B$ receptor subtypes. While biochemical and electrophysiological studies are generally supportive, pharmacological evidence for two GABA$_B$ receptors associated with cAMP production is lacking.

In the present study the influence of three GABA$_B$ receptor agonists and five antagonists on rat brain cAMP production was examined. While all of the agonists are equipotent with respect to inhibiting the cAMP response to forskolin and augmenting the isoproterenol-stimulated second messenger production, there is a 30-fold difference ($P < 0.05$) in the potency of the antagonist CGP 52432 to inhibit the effects of baclofen in the two systems. Two other recently-synthesized GABA$_B$ antagonists, SCH 50911 and CGP 54626a, exhibited over a ten-fold difference in potency. These data support the notion that distinct GABA$_B$ receptor subtypes mediate these effects in brain.

(Supported in part by a grant from Marion Merrell Dow).

531.15

ANTAGONISM OF PRESYNAPTIC $GABA_B$ RECEPTORS BY CGP35348 AND CGP52432. K.M. Syed*, J.A. Falchetto, and A.L. Padjen, Department of Pharmacology, McGill University, Montréal, Quebec, H3G1Y6, CANADA

Previous work in this laboratory has identified $GABA_B$ receptor-mediated responses on primary afferent (PA) terminals in amphibian spinal cord [agonist rank order: L-baclofen, kojic amine, and GABA; hyperpolarizing - mediated by a K^+ permeability increase; bicuculline insensitive; poorly antagonized by phaclofen; not recorded from large myelinated axons]. Other evidence suggests that subtypes of $GABA_B$ exist in CNS both pre- and postsynaptically. The objective of this study was to examine the pharmacology of the $GABA_B$ receptors on PA using putative $GABA_B$ antagonists with presumably selective sites of action.

Experiments were done on isolated amphibian spinal cord, using sucrose gap (membrane and evoked potentials from dorsal or ventral roots, DR/VR) and intra-axonal (from single large myelinated axons) recordings in the presence of .2 μM TTX. Concentration dependant reversible responses to 5μM L-baclofen were blocked 100% by CGP35348 at 30 μM (selective for somatic $GABA_B$ receptors); and 50% by CGP52432 at 0.1μM (100x more potent than CGP35348, selective for autoreceptors).

The effects of the antagonists on synaptic activity were variable. CGP52432 had no effect on amplitude or duration of DR-DRP, DR-VRP, or VR-DRPs at concentrations up to 5μM, though CGP35348 showed a 15% increase in DR-DRP and DR-VRP amplitude at 60 μM. The antagonists also increased the frequency of spontaneous excitatory synaptic activity.

The results demonstrate that CGP35348 and CGP52432 have different blocking properties on presynaptic $GABA_B$ receptors. The variable proconvulsant effects of the antagonists (on evoked and spontaneous synaptic activity) suggest a $GABA_B$-mediated inhibitory pathway activated by DR stimulation. *(Supported in part by MRC)*

531.16

CHANGES IN $[K^+]_o$ EVOKED BY BACLOFEN IN GUINEA PIG HIPPOCAMPUS. G.V. Obrocea* and M.E. Morris, Department of Pharmacology, University of Ottawa, Ottawa, Canada K1H 8M5.

Ion-selective microelectrodes have been used to measure changes in $[K^+]_o$ and field potentials in stratum pyramidale (SP) and stratum radiatum (SR) in the CA1 region of guinea pig hippocampal slices during applications of the $GABA_B$ agonist, (±) baclofen by bath perfusion (1-3000 μM for 5 min) and pressure ejection (100 μM, 100-900 ms). Bath application evoked dose-dependent increases in $[K^+]_o$ (above resting level of 3.6 ± SE 0.23 mM) of ≤ 0.59 ± 0.10 mM in SP (EC_{50} = 39.7 μM) and ≤ 0.65 ± 0.09 mM in SR (EC_{50} = 58.6 μM), which were antagonized by 50 μM CGP35348. At threshold 10 μM baclofen produced variable changes -- either no effect or a small increase (n = 3/6) or a decrease of -0.16 ± 0.40 mM in SP and -0.18 ± 0.0 mM in SR (n = 3/6). With agonist concentrations ≤ 20 μM the $[K^+]_o$ increases usually attained an early peak, with rapid decline to a lower plateau or the resting level; higher concentrations caused changes which were sustained throughout the application.

In some experiments both bath and pressure applications of baclofen also evoked repetitive transients of $[K^+]_o$ increase (2.7 ± 0.03 mM, duration 5-8 s, frequency 1/12 s) which persisted throughout and after recovery, were accompanied by long-lasting field potentials and briefer, more frequent inter-ictal discharges and closely resembled the potentials observed with 4-aminopyridine (4-AP) *(Morris, Obrocea & Avoli, 1993, Soc for Neurosci Abs 19: 1817)*.

The dose-dependent $[K^+]_o$ increases evoked by baclofen are presumed to reflect a K^+ conductance activated by $GABA_B$ receptors. Such changes may be expected to play a role in the $[K^+]_o$ accumulation evoked by GABA (especially when it is present at a high concentration) and add to that previously demonstrated for $GABA_A$ receptors *(Barolet & Morris, 1991, Expl Br Res 84: 591-598)*.

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531.17

THE POSTSYNAPTIC CONDUCTANCE LINKED TO $GABA_B$ RECEPTORS ACTIVATED BY ENDOGENOUSLY RELEASED TRANSMITTER. L. Mody* and Y. De Koninck. Dept. of Neurology, UCLA Sch. of Medicine, Los Angeles, CA, and Dept. of Anesthes./Pain Mgmt.UT Southwestern Med. Ctr., Dallas, TX.

In the hippocampus, activation of postsynaptic $GABA_B$ receptors generally requires stimulation of the presynaptic terminals with a stimulus intensity greater than that necessary to elicit $GABA_A$ responses. The large stimulus intensities may be required to activate extrasynaptic $GABA_B$ receptors or to release another neurotransmitter necessary for the activation of these receptors. To establish the relative contribution of endogenously released GABA to the activation of $GABA_A$ and $GABA_B$ receptor-mediated IPSCs we depleted GABA levels through inhibition of its synthesizing enzyme, glutamic acid decarboxylase (GAD).

Isonicotinic hydrazide (isoniazid, INH; 10 mM), an inhibitor of the pyridoxine cofactor of GAD, and nipecotic acid (NIP; 2 mM), a GABA uptake blocker were perfused onto the slices. Within an hour, multiple population spikes characteristic of a diminished GABAergic inhibition appeared. The epileptic field potentials were enhanced by only <10% upon blocking postsynaptic $GABA_A$ receptors with bicuculline (100 μM) or picrotoxin (100 μM). Thus, reduced GAD activity and GABA uptake effectively deplete over 90% of the releasable GABA pool. We then investigated $GABA_A$ and $GABA_B$ receptor-mediated monosynaptic IPSCs in whole-cell recordings. Consistent with the above findings, INH+NIP produced a parallel reduction in the size of both types of IPSCs by 90%. This demonstrates that GABA is the endogenous transmitter responsible for the activation of both $GABA_A$ and $GABA_B$ receptor-mediated IPSCs.

The properties and the relative number of postsynaptic K^+ channels linked to $GABA_B$ receptors and activated by synaptically released GABA were then studied using non-stationary fluctuation analysis. We have determined that $GABA_B$ receptors are linked to outwardly rectifying small conductance (5-12 pS) K^+ channels. A large number (157 ± 51) of these channels are activated during an average size stimulus-evoked $GABA_B$ IPSC.

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531.18

$GABA_B$ ANTAGONISTS BLOCK γ-BUTYROLACTONE-INDUCED ABSENCE SEIZURES AND COORDINATED INDUCTION OF CRE- AND AP-1 DNA-BINDING IN MOUSE BRAIN Y. Ito*, K. Ishige, M. Aizawa and H. Fukuda, Dept. of Pharmacology, College of Pharmacy, Nihon University, Funabashi, Chiba 274, Japan.

We examined the effects of $GABA_B$ receptor blockade on γ-butyrolactone (GBL)-induced absence-like seizures and coordinated induction of nuclear cyclic AMP-responsive element (CRE)- and activator protein 1 (AP-1) DNA-binding activities. Administration of GBL induced absence-like seizures and concomitant increases in nuclear CRE- and AP-1 DNA-binding activities in whole mouse brain. $GABA_B$ antagonists such as CGP 35348 and CGP 46381 suppressed both the GBL-induced absence-like seizures and the increased nuclear CRE- and AP-1 DNA-binding activities. Gel-shift assay in various brain regions revealed that administration of GBL increased nuclear CRE- and AP-1 DNA-binding activities significantly in the midbrain and cerebral cortex but not in the cerebellum, hippocampus or pons-medulla. The region-specific increases in nuclear DNA-binding activities were also suppressed by CGP 35348. In the cerebral cortex, the increased CRE-binding activity was completely supershifted by CRE-binding protein (CREB) antibody, but not affected by c-Jun or c-Fos antibody. In addition, the AP-1 DNA-binding activity was blocked by c-Jun or c-Fos antibody and partially supershifted by CREB antibody. These results suggest that $GABA_B$ receptor-mediated synaptic responses are involved in GBL-induced absence-like seizures and that the increases in nuclear CRE- and AP-1 DNA-binding activities are correlated with the seizures. It is also suggested that CREB is involved in the CRE-binding complexes and c-Jun, c-Fos and CREB are involved in the AP-1 DNA-binding complexes.

531.19

GABA-B AGONIST BACLOFEN ENHANCES THE Gs-COUPLED RECEPTOR-INDUCED cAMP PRODUCTION VIA G-PROTEIN βγ SUBUNITS IN *XENOPUS* OOCYTES EXPRESSING RAT BRAIN POLY $(A)^+$ RNA. Y. Uezono*, Y. Ueda, S. Ueno, N. Yanagihara, Y. Toyohira, T. Nagatomo', H. Yamashita' and F. Izumi. Depts of Pharmacol and 'Ist Physiol, Univ. of Occup. & Environ. Health, School of Medicine, Kitakyushu 807, Japan.

In the CNS, GABA-B receptor is negatively coupled to adenylyl cyclase (AC) via Gi/o protein followed by an inhibition of cAMP production while in some CNS region, baclofen enhances the isoproterenol- and vasoactive intestinal peptide (VIP)-induced cAMP production. Little is known about the mechanisms by which GABA-B receptor mediates an enhancement of cAMP production. We previously reported that the protein encoded by the cystic fibrosis transmembrane conductance regulator gene (CFTR), that is the Cl^- channel activated by A-kinase, can be used as an electrophysiological sensor for cAMP changes in *Xenopus* oocytes. In the present study, we injected cRNA for the CFTR along with rat brain poly $(A)^+$ RNA into oocytes and investigated the effects of baclofen on the isoproterenol-, VIP-, and pituitary adenylyl cyclase activating polypeptide (PACAP)-induced CFTR currents. Baclofen by itself had no effect on CFTR current, however, enhanced CFTR currents caused by isoproterenol, VIP and PACAP 2 - 3 fold, which were inhibited by GABA-B antagonist 2-OH saclofen. Further, the effect of baclofen is augmented by two fold after coinjection of cRNA for AC type II which is known to be activated by Gβγ subunits together with activated forms of Gαs (Gαs*); AC type III that is not activated by Gβγ had no augmentation. These results suggest that the Gβγ subunits released by GABA-B receptor stimulation activate the isotypes of AC together with Gαs* in the oocytes. In the CNS baclofen may enhance the Gs-coupled receptor-induced cAMP production by an activation of the AC through Gβγ subunits and Gαs*.

531.20

D-TRYPTOPHAN-6-LUTEINIZING HORMONE-RELEASING HORMONE ANALOG (DECAPEPTYL) ADMINISTRATION DOWN-REGULATES OVARIAN PERIPHERAL-TYPE BENZODIAZEPINE RECEPTOR EXPRESSION. A. Weizman*[1], I. Bachman[2], S. Leschiner[2] and M. Gavish[2,3]. [1]Sackler Fac. of Med., Tel Aviv Univ. 67197 Tel Aviv, and [2]B. Rappaport Fac. of Med. and [3]Rappaport Family Inst. for Res. in Med. Sci., Technion-I.I.T., 31096 Haifa, Israel.

Peripheral-type benzodiazepine receptors (PBR) are involved in the regulation of steroidogenesis, via activation of mitochondrial cholesterol transport. We previously found that hypophysectomy reduces PBR density in the adrenals and gonads. We were interested in evaluating the effect of "pharmacological" castration on PBR binding and mRNA expression in uterus, ovaries, and adrenal. In the present study, groups of female rats were "chemically" castrated by administration of decapeptyl, 50 μg/rat/day. Half of these rats were treated with estradiol (E_2), 50 μg/rat/day. PBR density and RNA levels were compared with each other and with control rats. Decapeptyl castration resulted in reduction of $[^3H]$PK 11195 binding in the ovaries; this effect was prevented by coadministration of E_2. We are currently studying the impact of decapeptyl treatment on PBR density in other tissues, as well as measuring any difference in PBR RNA expression. Our data indicate that deprivation of pituitary gonadotropins leads to depletion of ovarian $[^3H]$PK 11195 binding sites. This implicates E_2 as a necessary maintenance hormone for PBR expression in female sex organs.

531.21

IMPACT OF SURGICAL CASTRATION ON THE EXPRESSION OF THE PERIPHERAL-TYPE BENZODIAZEPINE RECEPTOR. M. Gavish*[1,2], C. Mazurik[1], G. Weisinger[1] and R. Weizman[3]. [1]Bruce Rappaport Fac. of Med. and [2]Rappaport Family Inst. for Res. in the Med. Sciences, Technion-I.I.T., 31096 Haifa, and Sackler Fac. of Med., Tel Aviv Univ., 67197 Tel Aviv, Israel

Peripheral-type benzodiazepine receptors (PBR) have been found to play a role in steroidogenesis. Since the expression of PBR is under the control of gonadal and pituitary hormones, we undertook to study the regulation of PBR expression in the female rat genital tract axis. In the current study, groups of female rats were either castrated and treated with estradiol (E_2; 3 mg/kg) or just castrated. [^{3}H]PK 11195 binding and PBR RNA levels were measured in both groups and further compared to sham-operated rats. Castration induced a significant reduction in uterine PBR density, while E_2 treatment prevented this reduction. Adrenal and renal PBR binding were not affected by castration or by E_2 treatment. In contrast to the decrease in uterine [^{3}H]PK 11195 binding following castration, PBR steady-state mRNA levels were found to increase. This increase was further enhanced by E_2 treatment. Run-on transcription analysis indicated that these steady-state changes were not due to changes in transcription. These data indicate that hormonal regulation in the uterus affects at least two putative levels of PBR expression. These levels of control seem to be on the post-transcriptional and translational levels of PBR expression.

531.22

CHRONIC DENERVATION OF PARASYMPATHETIC FIBERS BLOCK THE EFFECTS OF $GABA_A$-AGONISTS ON NEUROGENIC INFLAMMATION WITHIN THE RAT MENINGES.

V. Limmroth*, K.I. Maynard, C. Ayata, M.A.Moskowitz, Stroke and Neurovascular Regulation, Mass. Gen. Hospital, Harvard Medical School, Charlestown, MA. 02129

We recently reported, that agents, which activate $GABA_A$-receptors, such as valproate, muscimol or neurosteroids suppress neurogenic inflammation (NI) and substance P-induced plasma-extravasation (PE) within the meninges of rats. In this study we investigated whether the relevant $GABA_A$-receptors were localized on sensory or parasympathetic fibers projecting to the meninges.

50 Sprague Dawley rat pups were treated neonatally with capsaicin (50mgkg^{-1}, s.c.) or vehicle in order to deplete small unmyelinated sensory fibers. Seven weeks later, PE (^{125}I-albumin leakage, 50µCi kg^{-1}) was induced within the meninges by substance P (1nmol kg^{-1},i.v.). In each group, 10 animals received valproate (30 and 100 mg kg^{-1}) and 5 animals valproate (100 mg kg^{-1}) plus bicuculline (10µg kg^{-1}). Valproate dose-dependently reduced PE, which was reversed by bicuculline, suggesting that the involved $GABA_A$-receptors are not expressed on capsaicin sensitive small unmyelinated fibers.

To further clarify the origin of the fibers expressing $GABA_A$-receptors, 48 male SD rats underwent unilateral spheno-palatine ganglionectomy. Ten days later NI was induced by electrical stimulation of the trigeminal ganglion (0.6mA, 5Hz, 5min). Animals were pretreated with valproate (100 mg kg-1, n=10), muscimol (100 µg kg^{-1}, n=9), allopregnanolone (100 µg kg^{-1}, n=5) or sumatriptan (100 µg kg^{-1}, n=7). Only the 5HT1D-agonist sumatriptan suppressed NI in these animals.

These results suggest that a) $GABA_A$- and 5-HT-receptors are located on different populations of nerve fibers projecting to the meninges and b) $GABA_A$- receptors might be expressed on parasympathetic fibers projecting from the sphenopalatine ganglion to meningeal vessels.

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531.23

CONTROL OF PROOPIOMELANOCORTIN (POMC) mRNA LEVELS IN THE RAT ARCUATE NUCLEUS BY THE POTENTIAL ENDOGENOUS BENZODIAZEPINE (BDZ) RECEPTOR LIGAND OCTADECANEUROPEPTIDE (ODN). E. Garcia de Yebenes and G. Pelletier,* MRC Group in Molecular Endocrinology, CHUL Research Center, Laval University, Quebec, G1V 4G2 CANADA.

The neurotransmitter GABA exerts a tonic inhibitory influence on POMC neurons in the hypothalamus and melanotropic cells in the pituitary intermediate lobe. Moreover, activation of the $GABA_A$-BDZ receptors inhibits the expression of the POMC gene. An endogenous 86 amino acid polypeptide with high affinity for the diazepam-binding sites, termed diazepam-binding-inhibitor (DBI), has been found in non-neuronal elements of the rat brain. ODN, generated from a moderate tryptic digestion of DBI (DBI[33-50]), has been shown to have the same affinity as DBI itself for the diazepam binding sites at the $GABA_A$ receptor complex. In order to investigate the influence of these endogenous peptides on the POMC gene expression at the hypothalamic level, we have studied the effects of the acute administration of ODN alone, or in combination with the $GABA_A$ antagonist picrotoxin on POMC mRNA levels as measured by in situ hybridization. Treatment with ODN i.v. at doses of 100, 200 and 400 µg/Kg produced a decrease in the hybridization signal of 11.2%, 33% and 48%, respectively. The concomitant intraperitoneal administration of picrotoxin (5 mg/Kg), completely prevented the effect of ODN. Intraventricular injection of ODN (40 µg/Kg) produced a 9.5% decrease in the POMC mRNA levels. The concomitant administration of picrotoxin not only reversed the inhibitory effect of ODN, but induced a 40% increase in the hybridization signal. In conclusion, these experiments show a negative regulation of the POMC gene expression by the endogenous peptide ODN, an effect that is reversed by the $GABA_A$ antagonist picrotoxin. We them propose that endogenous peptides with BDZ-like actions can exert an additional fine control upon the expression of neuropeptides at the hypothalamic level.

531.24

IN VIVO RELEASE OF GABA WITHIN THE SPINAL CORD OF MONOARTHRITIC RATS USING MICRODIALYSIS. N.G. Bowery* and M. Malcangio. Dept. of Pharmacology, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX, U.K.

We have previously suggested that during the development of monoarthritis activation of the GABAergic system occurs to provide as an endogenous antinociceptive mechanism (Br. J. Pharmacol. 113, 1561-66, 1994). We have now evaluated *in vivo* whether the level of GABA within the spinal cord changes during chronic inflammation. For this purpose 12 Lewis rats were injected with complete Freund's adjuvant (CFA) and 10 with incomplete adjuvant (IFA). 7, 14, 21 and 28 days after innoculation, rats were anaesthetized with halothane and a microdialysis probe (200µm) was inserted into the dorsal horn of the spinal cord. Dialysates were collected at 0.5µl/min for 30 min and amino acid outflow determined by HPLC. The basal outflow of GABA was 3.3 ± 2.1 pmol/15µl in IFA rats and 1.6 ± 0.9 pmol/15µl in CFA rats. During perfusion with KCL (100mM in the probe) the levels of GABA in the dialysates of IFA rats were 101 ± 20%; 166 ± 37%; 103 ± 32%; 229 ± 29% of basal outflow at 7, 14, 21 and 28 days respectively. By contrast the levels of GABA in dialysates of CFA rats were 580 ± 247%; 543 ± 290%; 986 ± 517% and 402 ± 173% of basal outflow at 7, 14, 21 and 28 days. These preliminary results would suggest that a larger amount of GABA may be available for release within the spinal cord of monoarthritis rats during development of chronic inflammation which supports previous *in vitro* data.

PEPTIDE RECEPTOR STRUCTURE AND FUNCTION II

532.1

CLONING AND CHARACTERIZATION OF TWO ALTERNATIVE SPLICE FORMS ($CRF_{2\alpha}$ AND $CRF_{2\beta}$) OF A NOVEL RAT CORTICOTROPIN-RELEASING FACTOR RECEPTOR cDNA. Timothy W. Lovenberg*, Chen Liaw, Dimitri E. Grigoriadis, Derek T. Chalmers, Changlu Liu, Errol B. De Souza, Depts. of Molecular Neurobiology and Neuroscience, Neurocrine Biosciences Inc., San Diego, CA 92121

Corticotropin releasing factor (CRF), a 41 amino acid peptide, regulates the anterior pituitary secretion of adrenocorticotropin and other proopiomelanocortin products as well as coordinates the endocrine, behavioral and autonomic responses to stress. A cDNA encoding the pituitary CRF receptor has recently been cloned (CRF_1 receptor). In this study, we have isolated cDNA clones for two splice forms of a putative second member of the CRF receptor family (CRF_2 receptor). These cDNAs were first identified using degenerate-PCR. The $CRF_{2\alpha}$ form was subsequently isolated from a rat hypothalamus cDNA library, whereas the $CRF_{2\beta}$ form was isolated from rat heart. The putative proteins encoded by these cDNAs are 411 and 431 amino acids, respectively, and have approximately 70% overall identity with the known CRF_1 receptor. When expressed transiently in mouse Ltk$^-$ cells, the $CRF_{2\alpha}$ and $CRF_{2\beta}$ receptors stimulate cAMP accumulation in response to known CRF-like agonists CRF, sauvagine, and urotensin I. $CRF_{2\beta}$ and CRF_1 receptors have nearly identical rank orders of potency for these ligands, whereas the $CRF_{2\alpha}$ receptor displays a unique profile of activation. Tissue distribution of the mRNAs by RNase protection shows expression of $CRF_{2\alpha}$ in the hypothalamus and olfactory bulb, whereas $CRF_{2\beta}$ is expressed primarily in heart and skeletal muscle, with lower levels in lung and intestine. These expression patterns are quite different from that of the CRF_1 receptor. *In situ* hybridization studies confirm these non-overlapping sites of expression for $CRF_{2\alpha}$ and $CRF_{2\beta}$ receptors in the CNS and periphery. Together, these results demonstate the existence of two forms of a second CRF receptor, and suggest several directions for exploring CRF pathways as well as searching for alternate CRF-like ligands in mammals.

532.2

CLONING AND CHARACTERIZATION OF THE HUMAN CRF_2 RECEPTOR GENE AND cDNA. Chen W. Liaw, Timothy W. Lovenberg, Guy Barry, Tilman Oltersdorf*, Dimitri E. Grigoriadis, Errol B. De Souza. Dept of Molecular Neurobiology, Neurocrine Biosciences Inc., San Diego, CA 92121

Two CRF receptor subtypes (CRF_1 and CRF_2 receptor) with distinct brain localization and pharmacological profile have recently been cloned and characterized. For the CRF_2 receptor subtype, at least two splice forms, $CRF_{2\alpha}$ and $CRF_{2\beta}$ with different 5' sequences have been identified in rat. In this article, we report the genomic structure, and the corresponding cDNA sequence of the human CRF_2 receptor. The gene coding for human CRF_2 receptor consists of at least 12 exons and spans approximately 30 kb. The cDNA sequence in the protein coding region is 94% identical to that of the reported rat $CRF_{2\alpha}$ receptor. So far there is no evidence for the existence of a rat $CRF_{2\beta}$ receptor homologue in human. The encoded receptor is 411 amino acid in length and is 70% identical to the human CRF_1 receptor with least sequence homology in the N-terminal extracellular domain (47% identical). Cells transfected with the full length human CRF_2 receptor cDNA responded to rat/human CRF and sauvagine by increasing intracellular cAMP level with EC_{50} values of ~20 nM and ~1 nM, respectively. The abundance of the CRF_2 receptor mRNA appears to be much lower in human than in rat in the tissues studied thus far. Therefore, CRF_2 receptor message may have a different regulation and/or distribution in human.

532.3

PHARMACOLOGICAL CHARACTERIZATION OF TWO NOVEL CORTICOTROPIN-RELEASING FACTOR RECEPTORS ($CRF_{2\alpha}$ AND $CRF_{2\beta}$) EXPRESSED IN STABLE CELL LINES.

Dimitri E. Grigoriadis*, Timothy W. Lovenberg, Chen Liaw, Doug McGrath and Errol B. De Souza. Neurocrine Biosciences Inc., San Diego, CA 92121

Corticotropin-releasing factor (CRF) secreted by the hypothalamus is the primary regulator of the release of ACTH and other proopiomelanocortin derivatives from the anterior pituitary gland. In addition to its endocrine role, CRF has a broad extrahypothalamic distribution in brain and fulfills all of the criteria for a *bona fide* neurotransmitter. CRF_1 receptors and more recently two splice forms of a second member of the CRF receptor family, designated $CRF_{2\alpha}$ and $CRF_{2\beta}$, have been cloned and demonstrated to have clear and distinct patterns of distribution in rat brain and periphery. We have expressed both splice forms of the CRF_2 receptor subtype in stable cell lines and characterized their pharmacological and functional profiles in comparison with the CRF_1 receptor subtype. $CRF_{2\alpha}$ and $CRF_{2\beta}$ receptors are both positively coupled to adenylate cyclase through a G-protein, however, they exhibit a different pharmacological profile between each other and CRF_1 receptors. The CRF-related peptides, r/hCRF, ovine CRF, bovine CRF sauvagine and Urotensin I all bind and potently stimulate cAMP production in stable cell lines for CRF_1 and $CRF_{2\beta}$ receptors with approximately equal affinities. While sauvagine and Urotensin I exhibit the same affinity for $CRF_{2\alpha}$ receptors as CRF_1 and $CRF_{2\beta}$, r/hCRF, oCRF and bCRF, are all weaker by 10 to 100 fold for this subtype. The peptide antagonists, α-helCRF(9-41) and d-PheCRF(12-41), both inhibit CRF- or sauvagine-stimulated cAMP production with the same potency on all receptor subtypes. The differential pharmacological profile of CRF_1, $CRF_{2\alpha}$ and $CRF_{2\beta}$ receptors suggests distinctive and selective functional roles for each receptor subtype. Furthermore, the stable expression of CRF receptor subtypes represents an unique opportunity for the discovery of subtype-selective non-peptide ligands which could target different aspects of CRF-mediated disorders.

532.4

LOCALIZATION OF NOVEL CORTICOTROPIN-RELEASING FACTOR RECEPTOR (CRF_2) mRNA EXPRESSION IN RAT BRAIN. Derek T. Chalmers*, Timothy W. Lovenberg, Errol B. De Souza. Dept. of Molecular Neurobiology and Neuroscience, Neurocrine Biosciences Inc., San Diego, CA 92121

Corticotropin-releasing factor (CRF) is the primary factor involved in controlling the release of ACTH from the anterior pituitary and also acts as a neurotransmitter in a variety of brain systems. The actions of CRF are mediated by G-protein coupled membrane bound receptors and a high affinity CRF receptor, CRF_1, has been previously cloned and functionally characterized. We have recently isolated cDNAs encoding two splice forms of a second member of the CRF receptor family, designated $CRF_{2\alpha}$ and CRF_2 β, which display approximately 70% homology at the nucleotide level to the CRF_1 receptor and exhibit distinctive pharmacological profiles. The present study utilized *in situ* hybridization histochemistry to localize the distribution of $CRF_{2\alpha}$ and $CRF_{2\beta}$ receptor mRNA in rat brain and pituitary gland and compared this with the distribution of CRF_1 receptor expression. While CRF_1 receptor expression was very high in neocortical, cerebellar and sensory relay structures, $CRF_{2\alpha}$ receptor expression was generally confined to sub-cortical structures. The highest levels of $CRF_{2\alpha}$ receptor mRNA in brain were evident within the lateral septal nucleus and the ventromedial hypothalamic nucleus. Moderate levels of $CRF_{2\alpha}$ receptor expression were evident in the olfactory bulb, amygdaloid nuclei, the paraventricular and suraoptic nuclei of the hypothalamus, the inferior colliculus and serotonin-associated raphe nuclei of the midbrain. $CRF_{2\beta}$ receptor mRNA was generally confined to non-neuronal structures, the choroid plexus and cerebral arterioles. Within the pituitary gland, CRF_2 receptor mRNA was detectable only at low to negligable levels in scattered cells while CRF_1 receptor mRNA was readily detectable in anterior and intermediate lobes. This heterogeneous distribution of CRF_1, $CRF_{2\alpha}$, and $CRF_{2\beta}$ receptor mRNAs suggests distinctive functional roles for each receptor in CRF-related systems.

532.5

DIRECT MEASUREMENT OF CORTICOTROPIN RELEASING FACTOR-BINDING PROTEIN AND CRF-BP/CRF COMPLEX IN ALZHEIMER'S DISEASE USING TWO-SITE ELISA ASSAYS.

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Two-site ELISAs were developed for direct measurement of corticotropin releasing factor (CRF), CRF binding protein (CRF-BP) and the CRF-BP/CRF complex in human plasma and brain extracts. The CRF-BP ELISA had a sensitivity of 2.7 fmol/well and a broad range of detection from 0.008-8 pmol/well. Both the CRF and CRF-BP/CRF assays had a sensitivity of 0.4 fmol/well with a useful range of detection from 0.4-40 fmol/well . Plasma CRF-BP levels were 7.1 ± 0.64 nmol/L in Alzheimer's patients (n=21) and 7.5 ± 0.35 nmol/L in age-matched controls (n=19) with no significant differences between groups. CRF-BP levels decreased to 60% of resting levels 15 min after a bolus injection of synthetic human CRF (1 μg/kg) and gradually returned to pre-injection levels after 120 min. Conversely, bound CRF levels reciprocally increased at 15 min to 0.58 ± 0.03 nmol/L in control subjects and 0.49 ± 0.04 nmol/L in Alzheimer's subjects and then rapidly decreased to undetectable levels at 120 min. CRF-BP levels ranged from 5.4 - 13.5 fmol/mg of wet tissue weight in human cerebrocortical extracts with no significant differences found between controls and Alzheimer's patients. The majority (>80%) of the CRF-BP was membrane associated. Bound CRF levels were 0.31 ± 0.018 and 0.24 ± 0.007 fmol/mg wet tissue weight in control and Alzheimer's cerebrocortical extracts, respectively. Furthermore, bound CRF could be displaced from the binding protein using high affinity CRF-BP ligands to replenish free CRF levels to normal in the Alzheimer's extracts. These data demonstrate that CRF-BP, CRF and the CRF-BP/CRF complex can be measured by two-site ELISAs in unextracted plasma samples and brain extracts and suggest that ligand inhibitors of CRF-BP may have utility in elevating free CRF levels in disease states associated with decreased CRF.

532.6

CRF BINDING PROTEIN (CRF-BP) IS EXPRESSED IN CULTURED ASTROCYTES AND NEURONS IN RESPONSE TO cAMP AND PROTEIN KINASE C STIMULATION.

Dominique Maciejewski*, Dominic P. Behan, Derek Chalmers and Errol B. De Souza. Neurocrine Biosciences Inc., San Diego, 92121.

CRF-BP was measured in media and cell lysates of primary rat astrocytes, microglia and neurons with the use of a ligand immunoradiometric assay (LIRMA). A low basal level of CRF-BP was detected in the media but not cell lysates from primary neuronal and astrocyte cells after 48h in culture. No basal expression of CRF-BP was detected in cell lysates or media from primary microglial cultures. After forskolin, IBMX or forskolin/IBMX treatment, a robust increase in secreted CRF-BP levels in the media from astrocytes and neurons, but not microglia, was observed. Also, forskolin, IBMX and dibutyryl cAMP treatment resulted in a dose dependent increase in CRF-BP levels detected in the media from astrocytes and neurons. *In situ* hybridization analysis revealed that CRF-BP mRNA was increased in primary cultured astrocytes after IBMX/forskolin stimulation suggesting that regulation was at the level of gene transcription. Furthermore, treatment of neurons and astrocytes with specific type 3 and type 4 phosphodiesterase inhibitors also resulted in increased secretion of CRF-BP into the media. The increase in CRF-BP expression was not due to increased cell proliferation as measured by radiolabeled ^{3}H-thymidine incorporation. Treatment of astrocytes with PMA also resulted in increased CRF-BP levels in the media suggesting that the AP1 site in the human promoter may be important in CRF-BP gene regulation. Dexamethasone, hydrocortisone, dihydroepiandosterone, progesterone and pregnenolone inhibited Forskolin/IBMX stimulated release of CRF-BP from astrocytes. These data provide evidence for the differential localization and regulation of CRF-BP in different cell types in brain and suggest that CRF-BP expression may be locally increased in disease states associated with astrocytosis.

532.7

CORTICOTROPIN-RELEASING FACTOR BINDING PROTEIN LIGAND INHIBITION: A NOVEL MECHANISM FOR COGNITIVE ENHANCEMENT S.C. Heinrichs*, D.P. Behan, N. Ling and E.B. De Souza. Neurocrine Biosciences, Inc., 3050 Science Park Road, San Diego CA 92121.

Data from postmortem studies suggest that corticotropin-releasing factor (CRF) is a major neurotransmitter affected in Alzheimer's disease (AD). Observations that greater cognitive impairment in AD is associated with lower cerebrospinal fluid concentrations of CRF and rodent studies demonstrating CRF-induced improvment in learning and memory tasks suggest that decreases in brain CRF may contribute to cognitive impairment. The identification in brain of a high-affinity binding protein with the ability to sequester and inactivate corticotropin-releasing factor (CRF-BP) provides a novel target to modulate endogenous levels of CRF *in vivo* (see Behan et al.). Thus, central administration of selective CRF-BP ligands which dissociate CRF from CRF-BP could increase brain levels of unbound CRF. The present studies employed two behavioral measures of arousal in rats, acquisition of a learning task and exploration of an unfamiliar environment, to examine the impact on the CRF system of three distinct pharmacological tools: 1) a high affinity CRF receptor agonist, rat/human CRF (1-41), 2) selective CRF-BP ligand inhibitors, rat/human CRF (6-33) or (9-33) and 3) homologous control peptides, ovine CRF (6-33) or (9-33) which are inactive at both CRF receptors and CRF-BP. Acquisition of the Morris water maze test of spatial learning was facilitated by pre-test intracerebroventricular (icv) administration of r/hCRF (1-41), or either CRF-BP ligand inhibitor but not by either control peptide. Administration of the CRF-BP ligand inhibitor, r/hCRF (6-33) also improved performance in a visual discrimination learning paradigm. Doses of r/hCRF (1-41) which improved performance of the learning task also produced anxiogenic-like behavior in the Elevated Plus-Maze test while doses of r/hCRF (6-33) up to five fold higher than the behaviorally active dose did not modify exploration of the Plus-Maze. These data demonstrate a functional dissociation of the learning enhancement effects from emotionality-related side-effects of CRF-BP ligand inhibitors and suggest that these compounds may have therapeutic potential in treating disorders such as dementia associated with low levels of CRF in brain.

532.8

ASSESSMENT OF COMPOUND AFFINITIES AT RAT BRAIN CORTICOTROPIN RELEASING FACTOR (CRF) RECEPTORS USING A FILTRATION-BASED LIGAND BINDING ASSAY. H. Herdon*, J. Jerman, A. Jennings' and M. Duckworth'. Departments of Psychiatry Research and 'Medicinal Chemistry, SmithKline Beecham Pharmaceuticals, Harlow, Essex, UK.

Previous studies on [^{125}I]-CRF binding to brain CRF receptors have generally used centrifugation-based binding assays. We have developed a simple high-throughput filtration-based binding assay and used it to assess the affinities of compounds at cortical CRF receptors. The assay measures [^{125}I]-oCRF binding to washed homogenates of rat cerebral cortex, based on the method of De Souza (J. Neurosci. 7:88-100, 1987). Termination is by filtration through PEI-soaked GF/C filters, followed by washing with PBS containing 0.1% BSA and 0.01% Triton X-100.

Specific binding of [^{125}I]-oCRF (defined with 1μM r/hCRF) represented 70% of total binding. Saturation analysis gave a Bmax value of 180 fmoles/mg protein and a Kd of 5nM. Competition studies showed Ki values of 6nM for r/h CRF and 9nM for oCRF. The antagonists α-helical CRF-(9-41) and D-PheCRF-(12-41) gave Ki values of 60nM and 80nM respectively. The relative potencies of these four peptides are similar to those reported previously (Curtis et al, J. Pharm. Exp. Ther. 268:359-65, 1994; De Souza, J. Neurosci. 7:88-100, 1987), thus validating the assay. Two novel non-peptide compounds claimed as CRF receptor antagonists in recent patents from Pfizer Pharmaceuticals (Example 1 of WO 94/13643 and Example 5i of WO 94/13677) were also tested; both compounds gave Ki values of 70nM. These results show that our assay can be used for the assessment of CRF agonists and both peptide and non-peptide CRF antagonists.

532.9

AN ANTAGONIST AT THE SOMATOSTATIN RECEPTOR SSTR5 [1]T. Reisine*, [1]M. Tallent, [1]G. Liapakis, [3]A-M. O'Carroll and [2]M. Dichter. Depts. [1]Pharmacology and[2] Neurology, Univ of Penn. Sch. Med. and the Graduate Hospital, Philadphia, PA 19104 and [3]Lab. Cell Biol. NIMH, Bethesda MD.

Somatostatin (SRIF) is a major physiological regulator of growth hormone, glucagon and insulin release. It causes biological actions by interacting with five different receptors referred to as SSTR1-5. Subtype selective agonists have been used to reveal distinct functions of these receptor subtypes. Development of antagonists would be useful in further identifying the selective biological roles of these receptors. The peptide L362,855 binds with high affinity to the cloned receptor SSTR5. At high concentrations it inhibits cAMP accumulation in cells expressing the cloned rat SSTR5. At lower concentrations it blocks SRIF inhibition of cAMP accumulation via this receptor, suggesting the peptide is a partial agonist/antagonist. The SSTR5 selective agonist BIM23052 inhibits an L-type Ca^{++} current in the cell line AtT-20, which expresses SSTR5 mRNA. L362,855 has mininal actions on this current at low concentrations but completely blocks the electrophysiological effects of BIM23052. L362,855 does not prevent the SSTR2 selective agonist MK 678 from inhibiting Ca^{++} currents in AtT-20 cells, suggesting that L362,855 is a selective antagonist at SSTR5. L362,855 has two phenylalanines in its structure. The analogs L363,588 and L363,587 have each individual phenylalanine converted to a tyrosine. L363,588 is a pure agonist at SSTR5 whereas L363,587 is an antagonist. Therefore, the addition of one hydroxyl group to L362,855 converts it from an antagonist to an agonist revealing an important structural determinant for the activity of this compound. L362,855 analogs may be useful in further identifying functions of SSTR5 and could serve as a foundation for the development of new SRIF antagonists with clinical uses. Supported by MH45533 and MH48518.

532.10

LIGAND BINDING DOMAINS OF THE SOMATOSTATIN RECEPTOR SSTR2 G. Liapakis*, D. Fitzpatrick, C. Codispoti, R. Vandlen and T. Reisine. Dept. Pharm., Univ. Penn. Sch. Med. Philadelphia, PA 19104 and Protein Chem. Dept., Genentech, 460 Pt St. Bruno Blvd., S. San Francisco, CA 94080

Somatostatin (SRIF) induces its biological actions by interacting with five different receptors. The subtype SSTR2 may mediate SRIFs actions on growth hormone release. Development of SSTR2 selective drugs would be facilitated by the identification of the ligand binding domains of this receptor. To identify the ligand binding domain of SSTR2, chimeric receptors consisting of fragments of SSTR1 and SSTR2 were generated and tested for their ability to bind selective SSTR2 ligands. Chimera 282 consists of SSTR2 with the second and third extracellular loops of SSTR1. It bound native SRIFs with high affinity. The SSTR2 selective agonists NC4-28B and NC8-12 and the peptides BIM23003, MK 678 and SMS 201-995 which bind preferentially to SSTR2, bound with low affinity to 282. In contrast, they bound to the chimera 258, consisting of SSTR1 with the region spanning extracellular loops 2 and 3 of SSTR2, with high affinity indicating that ligand binding domains of SSTR2 are contained within the second and third extracellular loops. To further define determinants for ligand binding to SSTR2, the chimera 394 was generated. It consists of SSTR2 with four amino acids in the third extracellular loop exchanged for the corresponding region of SSTR1. 394 had greatly reduced affinity for the SSTR2-selective agonists NC8-12 and NC4-28B as well as the SSTR2 preferring agonist SMS 201-995 and BIM23003. These studies define a highly selective, small region of extracellular loop three as being required for the binding of constrained octa- and decapeptides to SSTR2. In contrast, the native SRIF peptides do not require this region for binding. Such information may facilitate the rational design of SSTR2 antagonists and drugs. Supported by MH45533 and MH48518.

532.11

COUPLING OF THE CLONED KAPPA AND MU OPIOID RECEPTOR TO THE INWARD RECTIFIER POTASSIUM CURRENT IS DIFFERENTIALLY REGULATED. [1]M. Tallent*, [1,2]M.A. Dichter, and [1]T. Reisine, [1]Depts. of Pharmacology and [2]Neurology, University of Pennsylvania School of Medicine and the Graduate Hospital, Philadelphia, PA 19104.

Three opioid receptors have recently been cloned, corresponding to the kappa, delta, and mu subtypes which had been previously characterized in endogenous tissue. These three receptor subtypes have all been shown to interact with inward rectifying potassium currents in neurons, and this appears to be an important mechanism by which the opioids mediate many of their inhibitory effects in the nervous system.

We show here that the cloned mouse kappa and rat mu opioid receptors are able to couple to an endogenously expressed inward rectifier K^+ current (IR) when expressed in the mouse anterior pituitary cell line AtT-20. The coupling of the kappa opioid receptor to the IR is desensitized upon pretreatment with kappa agonist. Kappa receptor desensitization appears to be homologous, as the ability of the endogenously expressed somatostatin receptor to couple to the IR is not affected by kappa receptor desensitization. The mu receptor coupling to the IR is also desensitized by pretreatment with mu agonist. However, mu receptor desensitization also causes heterologous desensitization of the endogenous somatostatin receptor. Likewise, desensitization of the somatostatin receptor causes heterologous desensitization of the mu receptor but not the kappa receptor. These results show that the cloned kappa and mu opioid receptor coupling to the IR is differentially desensitized in AtT-20 cells, with kappa receptor desensitization appearing to be homologous and mu receptor desensitization involving heterologous mechanisms. Supported by DA 08951.

532.12

AGONIST INDUCED REGULATION OF MU AND DELTA OPIOID RECEPTORS A. Blake*, M. vonZastrow, J. Freeman and T. Reisine. Dept. Pharm., Univ. Pennsylvania, Philadelphia PA 19104 and Dept. Psych., Univ. Cal. San Francisco, San Francisco, CA 94143.

Opiates are potent analgesics used clinically to treat pain. A major side effect of chronic use of opiates is the development of tolerance. To investigate cellular mechanisms of tolerance development to opiates, the cloned mu and delta opioid receptors were epitope tagged, stably expressed in HEK 293 cells and the effects of chronic treatment of these cells with opiates on the characteristics of the opioid receptors were examined. The pharmacological characteristics of the epitope tagged mu and delta receptors are similar to those reported for mu and delta receptor expressed in the nervous system. The mu receptor selectively bound the agonist ^{3}H-DAMGO and this binding was diminished by GTP analogs and pertussis toxin treatment, indicating the receptor coupled to G proteins. Overnight treatment of mu receptor expressing 293 cells with levorphanol or morphine decreased ^{3}H-DAMGO binding and the binding of the antagonist ^{3}H-diprenorphine. These results indicate that prolonged agonist treatment caused a loss in high affinity agonist binding and a down-regulation of mu receptor. The epitope tagged delta receptor selectively bound the delta agonist ^{3}H-DSLET and this binding was sensitive to GTP analogs and pertussis toxin. Overnight treatment of delta receptor expressing 293 cells with levorphanol caused both a loss of high affinity agonist and antagonist binding indicating that the receptor was downregulated. Thus, prolonged, overnight agonist treatment of both the mu and delta receptors with agonists causes receptor downregulation, which may contribute to tolerance development to opiates. Supported by DA08951 and DA05636.

532.13

ASSOCIATION OF THE CLONED DELTA, KAPPA AND MU OPIOID RECEPTORS WITH G PROTEINS S.F. Law* and T. Reisine. Dept. Pharmacol., Univ. Pennsylvania Sch. Med. Philadelphia, Pa 19104

Opiates are potent analgesics and are used clinically to treat pain. They induce their biological effects by interacting with delta, kappa and mu receptors. These receptors couple to cellular effector systems via G proteins. To identify the G proteins that associate with the opiate receptors, the cloned opiate receptors were expressed in CHO cells, the receptors were solubilized with CHAPS and the receptor/G protein complexes were either uncoupled or immunoprecipitated with antisera directed against the different alpha subunits of G proteins. The mouse delta receptor predominantly associates with G_{ia1}, G_{ia3} and G_{oa}. Upon agonist binding to the receptor, G_{ia1} dissociates and G_{ia2} associates with the receptor whereas G_{ia3} and G_{oa} remain complexed with the receptor. The switch in G_{ia} subtypes may be involved in the activation of the delta receptor following agonist binding. The mouse kappa receptor also associates with G_{ia1}, G_{ia3} and G_{oa}. Following agonist binding, G_{ia2} associates with the receptor and both G_{ia1} and G_{ia3} remained coupled to the receptor. Therefore, agonist binding promotes the association of G_{ia2} with the kappa receptor. Both the delta and kappa receptor can also interact with G_{za} and G_{qa}, which may mediate pertussis toxin insensitive actions of delta and kappa selective agonists. In contrast to the delta and kappa receptors, associations of G proteins with the cloned rat mu receptor could only be detected when agonist was bound to the receptor. Under these conditions, the mu receptor predominantly associates with G_{ia2}, G_{ia3} and G_{oa}. These studies reveal that specificity exists in the coupling of G proteins with the oplate receptors and that changes in G protein coupling occur following agonist binding which may contribute to the molecular basis of signalling via this receptors. Supported by DA08951.

532.14

CLONED HUMAN SOMATOSTATIN SSTR5 RECEPTORS COUPLE TO INHIBITION OF cAMP FORMATION, STIMULATION OF PHOSPHOINOSITIDE TURNOVER, CALCIUM MOBILIZATION, ARACHIDONIC ACID RELEASE, AND CELLULAR METABOLIC RATE. J. E. Taylor*, Biomeasure Inc., 27 Maple St., Milford, MA 01757

Somatostatin (SRIF) exhibits diverse biological effects mediated through a family of five G-protein coupled receptors (SSTR1-5). SSTR (1-5) activation has variably been shown in most native and cloned assay systems to be coupled to an inhibition of stimulated-cAMP formation, and stimulation of phosphoinositide (PI) turnover. In addition, stimulation of tyrosine phosphatase activity and inhibition of voltage-dependent Ca^{2+} channels have been reported for the SSTR2 type. Activation of arachidonic acid (AA) release has also been reported for the SSTR4 subtype. In the present study with CHO-K1 cells stably expressing the human SSTR5 receptor, SRIF-14, SRIF-28 and SSTR5-selective ligands (BIM-23052, L-363,855) inhibited [^{125}I-Tyr^{11}]SRIF-14 binding with Ki values of 0.38, 0.88, 0.76, and 1.2 nM, respectively. Functional analysis, showed for the first time that human SSTR5 receptors were coupled to the stimulation of AA release, in addition to cAMP inhibition and PI/Ca^{2+} stimulation, with a rank order of potency of SRIF-28 > SRIF-14. Furthermore, employing the technique of microphysiometry to assess real-time cellular metabolism, SSTR5 specific peptides stimulated the short-term metabolism of CHO-K1 cells. These results demonstrate that human SSTR5 receptors are functionally coupled to multiple second messenger systems.

532.15

PHARMACOLOGICAL CHARACTERIZATION OF LY303870: A NOVEL, POTENT, AND SELECTIVE NON-PEPTIDE SUBSTANCE P RECEPTOR ANTAGONIST. B. D. Gitter* [a], R. F. Bruns[a], J. J. Howbert[a], P. W. Stengel[b], D. R. Gehlert[a], S. Iyengar[a], D. O. Calligaro[c], D. Regoli[d], and P. A. Hipskind[a]. [a]Central Nervous System, [b]Cardiovascular, and [c]Biochemical Toxicology Research Divisions, Lilly Research Labs., Lilly Corp. Ctr., Indianapolis, IN 46285 and [d]Dept. of Pharmacol., Medical School, Univ. of Sherbrooke, Sherbrooke, Que., Canada J1H5N4

(R)-1-[N-(2-methoxybenzyl)acetylamino]-3-(1H-indol-3-yl)-2-[N-(2-(4-(piperidin-1-yl)piperidin-1-yl)acetyl)amino]propane (LY303870) is a new, potent, and selective non-peptide neurokinin-1 (NK-1) receptor antagonist. LY303870 bound selectively and with high affinity to human peripheral (K_i = 0.15 nM) and central (K_i = 0.10 nM) NK-1 receptors. LY303870 inhibited [^{125}I]SP binding to guinea pig brain homogenates with similar affinity, however, it had approximately 50 fold lesser affinity for rat NK-1 sites. The less active enantiomer, (S)-1-[N-(2-methoxybenzyl)acetylamino]-3-(1H-indol-3-yl)-2-[N-(2-(4-(piperidin-1-yl)piperidin-1-yl)acetyl)amino]propane (LY306155), was 1,000-15,000 fold less potent in all the species examined. LY303870 antagonized *in vitro* NK-1 receptor effects as demonstrated by blockade of substance P (SP) stimulated phosphoinositide (PI) turnover in UC-11 MG human astrocytoma cells (K_i = 1.5 nM) and interleukin-6 (IL-6) secretion from U-373 MG human astrocytoma cells (K_i = 5 nM). In addition, LY303870 inhibited SP induced rabbit vena cava contractions (pA_2 = 9.4) with high (50,000 fold) selectivity vs. NK-2 or NK-3 receptor-mediated responses. In vivo, LY303870 inhibited [Sar^9,Met(O$_2$)^{11}]-SP induced guinea pig bronchoconstriction (ED_{50} = 75 µg/kg i.v.) and pulmonary microvascular leakage in the bronchi (ED_{50} = 12.8 µg/kg i.v.) and trachea (ED_{50} = 18.5 µg/kg i.v.). Therefore, LY303870 is a potent and selective NK-1 receptor antagonist *in vitro* and *in vivo*. The use of LY303870 will facilitate a better understanding of NK-1 receptors in physiological processes.

532.16

CHARACTERIZATION OF GUINEA PIG NK-1 RECEPTORS USING THE POTENT ANTAGONIST LIGAND, [^{3}H]-LY303870. D.R. Gehlert*, D.A. Schober P.A. Hipskind, J. Nixon, B.D. Gitter and J.J. Howbert Central Nervous System Research, Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285 U.S.A.

The neuropeptide, substance P (SP), produces its biological responses by binding to NK-1 receptors found in the brain and periphery. In general, the binding properties of the NK-1 receptor have been characterized using radiolabeled SP or SP analogs. Until recently, no useful antagonist radioligands were available for the study of these receptors. [^{3}H]-CP96,345 and the related ligand, [^{125}I]-L-703606, have been introduced, but may interact with non-NK-1 sites. Another radioligand, [^{3}H]-99,994 appears to bind to a single site in guinea pig striatum. Recently, another class of nonpeptide NK-1 antagonists have been discovered. In the present study, we used a radiolabelled form of the NK-1 antagonist, LY303870, for use in homogenate and autoradiographic binding studies.

[^{3}H]-LY303870 rapidly associated with guinea pig striatal membranes and readily dissociated upon addition of unlabeled LY303870. Under equilibrium conditions, the ligand bound to a single site with a K_d of 0.22 nM and a B_{max} of 22 fmoles/mg protein. (±)CP96,345, LY303870 and the related antagonist LY306740 were the most potent inhibitors of binding tested. While the nonpeptide antagonists had Hill coefficients near unity, peptide agonists were generally less potent and exhibited biphasic displacement curves. Consistent with binding to a G-protein coupled receptor, 10 µM Gpp(NH)p shifted inhibition by SP to a single, lower affinity site. The dissociation of specific [^{3}H]-LY303870 binding was unaffected by the presence of 10 µM Gpp(NH)p.

Autoradiographic studies were conducted using sections from guinea pig forebrain and brainstem. Adjacent sections were labelled with either [^{3}H]-LY303870 or [^{3}H]-SP. The distribution of binding sites for these ligands was similar. The highest levels of specific binding were seen in noradrenergic areas such as the locus coeruleus and bed nucleus of the stria terminalis while lower levels were seen in areas such as the striatum and dorsal horn of the spinal cord.

In conclusion, [^{3}H]-LY303870 binds with high affinity and selectivity to NK-1 receptors in guinea pig brain. This radioligand should provide a useful tool for the further study of mammalian NK-1 receptors.

532.17

LY303870: A CENTRALLY ACTIVE NK-1 ANTAGONIST WITH A LONG DURATION OF ACTION. R.M.A. Simmons*, B.D. Gitter, P.A. Hipskind, D.R. Gehlert, C. McMillian, R. Couture, D. Li and S. Iyengar. CNS Research, Lilly Corp. Ctr., Eli Lilly and Company, Indianapolis, IN 46285.

The selective NK-1 antagonist, LY303870, has high affinity and specificity for human and guinea pig NK-1 receptors labelled with [^{125}I]-SP (K_i of 0.10nM and 0.31nM respectively) and approximately 15- to 30-fold lower affinity for rat and mouse brain NK-1 receptors (K_i of 8.7nM and 7.5nM), consistent with previously reported species differences in the affinities of non-peptide antagonists for NK-1 receptors. *Ex-vivo* binding studies revealed that LY303870 potently bound to central NK-1 receptors labelled by [^{125}I]-SP over a long duration of time. *In vivo*, LY303870 blocked the characteristic noci-ceptive behavioral response elicited by intrathecal administration of the selective NK-1 agonist Ac-[Arg^6, Sar^9, Met(O$_2$)^{11}]-substance P $_{6-11}$ in conscious mice [est. ED_{50} 0.2nmoles (i.t.) and 2.5 mg/kg (i.p.) respectively]. LY303870 also selectively blocked the potentiation of the tail-flick response in the rat, elicited by intrathecally administered NK-1 agonist, [Sar^9, Met(O$_2$)^{11}]-substance P [est. ED_{50} 4 mg/kg (i.v.) and 6 mg/kg (p.o.) respectively]. LY303870 was tested in a model of persistent nociceptive activation induced by tissue injury (the formalin test). LY303870 blocked pain-related behavior in the second phase of the formalin test in a dose-dependent manner [est. ED_{50} 4 mg/kg(i.p.) and 10 mg/kg (p.o.)]. Following oral administration of 10 mg/kg, the blockade of the second phase pain-related behavior was evident for at least 24 hours, consistent with *ex-vivo* binding studies to the NK-1 receptor in the brain and pharmacokinetic studies of plasma levels of LY303870. In conclusion, LY303870 blocked central NK-1 receptor-mediated nociceptive behavioral responses in both rats and mice and showed analgesic activity in the formalin test at doses that were consistent with its affinity for rodent NK-1 receptors. LY303870 is a potent, centrally active, NK-1 antagonistin vivo, with long lasting oral activity.

532.18

NK-1 RECEPTORS MEDIATE A SPINAL NOCICEPTIVE REFLEX. D. Li, R.M.A. Simmons, R.F. Bruns, P.A. Hipskind and S. Iyengar*. CNS Research, Lilly Corp. Ctr., Eli Lilly and Company, Indianapolis, IN 46285.

Substance P (SP) is found in the primary afferent neurons that carry nociceptive signals to the brain. NK-1 receptors are widely distributed at spinal and supraspinal sites. The involvement of central NK-1 receptors in nociceptive behavior was tested. (1) SP, when administered intrathecally, induces a characteristic nociceptive behavioral response. Using selective agonists and antagonists, this response was further characterized in our laboratory and shown to be selectively mediated via NK-1 receptors. The selective NK-1 agonist Ac-[Arg^{6}Sar^9 Met (O$_2$)^{11}]SP$_{6-11}$, (Ac-Sar^9), when administered intrathecally, mimicked the SP-elicited response described in the early studies. Selective NK-2 or NK-3 agonists did not elicit the same response. Selected Lilly NK-1 antagonists, including LY303870 and LY309809, when co-injected intrathecally (i.t.) with the agonist, blocked the Ac-Sar^9-mediated nociceptive response in a dose-dependent and stereoselective manner. This response showed a structure activity relationship (SAR) that was consistent with a NK-1 receptor mediated event. Structurally unrelated NK-1 antagonists, RP67,580 and GR83224 also blocked this response, further confirming that this response was NK-1 receptor mediated. (2) CGRP, another neuropeptide found in the primary afferents, can potentiate this NK-1 receptor mediated nociceptive response when coinjected with subthreshold doses of the NK-1 agonist, while having no effect by itself. Interestingly, this potentiation was blocked by the NK-1 antagonists at doses that blocked Ac-Sar^9 mediated behavior alone. NK-1 antagonists (i.t.) were less potent at blocking acetic acid induced writhing. In contrast, morphine (i.t.) blocked acetic acid-induced writhing very potently, was less potent at blocking Ac-Sar^9- induced behavior and did not block CGRP-induced potentiation of NK-1 receptor mediated nociceptive behavior. These data suggest that NK-1 receptors may play a unique role in mediating spinal nociceptive reflexes.

532.19

INTRAVENOUS SUBSTANCE P ADMINISTRATION TRANSIENTLY INCREASES RAT EAR BLOOD FLOW VIA NK-1 RECEPTOR ACTIVATION: AN IN VIVO NK-1 ASSAY. L.A. Phebus*, M. E. Roush, J.H. Krushinski, K.L. Lobb, I.A. Nixon and P.A. Hipskind. The Lilly Research Laboratories, Eli Lilly & Co. Indianapolis, IN 46285.

Substance P (SP), an eleven amino acid neuropeptide, is a member of the neurokinin family which exert their effects via neurokinin (NK) receptors. SP is somewhat selective for the NK-1 receptor. Stimulation of NK-1 receptors by released neuronal SP is thought to be involved in various physiological processes including pain transmission, inflammation and vascular tone. Non-peptide NK-1 antagonists might be effective treatments for various pathophysiological conditions including persistent pain, migraine and asthma. In our search for such agents, we employed a rapid, in vivo test of NK-1 antagonism described here.

Male Wistar rats were anesthetized with isoflurane and placed in stereotaxic apparatus. One of the rat's ears was then gently taped, using double-sided tape, to the stereotaxic ear bar. A laser Doppler blood flow probe was positioned to measure capillary blood flow in the ear. Intravenous, bolus injections of SP (1-1000 pmols/kg) produced transient increases in ear blood flow. Using selective agonists and antagonists, this increase was documented to be mediated by NK-1 receptors. Non-peptide NK-1 antagonists were tested for their ability to shift the SP dose-response curve. In other tests, repeated low iv doses (4 pmols/kg) of SP were injected at 5 minute intervals for hours without desensitization. After a baseline was established, non-peptide NK-1 antagonists were administered and their onset, duration of action and availability by various routes of administration were examined. This method proved to be a reliable and relatively rapid in vivo assay of NK-1 antagonism.

532.20

INHIBITION OF NEUROGENIC DURAL INFLAMMATION BY LY303870, A NOVEL, NON-PEPTIDE NK-1 RECEPTOR ANTAGONIST. K. W. Johnson*, P. A. Hipskind, K. L. Lobb, J. A. Nixon, P. W. Stengel and L. A. Phebus Central Nervous System Research, Lilly Research Laboratories, Indianapolis, IN 46285

Neurogenic dural inflammation has been proposed as a source of pain during migraine. This inflammation is produced by the release of substance P and other inflammatory neuropeptides from sensory afferents in dural tissue. Substance P is thought to induce protein extravasation from dural postcapillary venuoles by activation of NK-1 receptors on the endothelium of the venuoles. Unilateral electrical stimulation of the trigeminal ganglion is known to induce ipsilateral release of inflammatory neuropeptides, particularly Substance P. The resulting ipsilateral dural protein extravasation from post-capillary venuoles and increased dural blood flow are both components of neurogenic dural inflammation. We have previously shown (Soc. for Neuroscience, 1994) that this model successfully discriminates several clinically effective and ineffective compounds for the alleviation of migraine pain. Several other groups have shown dural protein extravasation can be blocked in this model by NK-1 receptor antagonists.

We evaluated the ability of a novel, non-peptide NK-1 antagonist (LY303870) to block dural protein extravasation in guinea pigs induced by ipsilateral stimulation of the trigeminal ganglion. The compound inhibited dural extravasation in a dose dependent manner when administered by several routes. The compound was equipotent compared to sumatriptan when administered intravenously 10 minutes prior to stimulation. LY303870 was also found to be approximately 50 times more potent than sumatriptan when orally administered approximately one hour prior to stimulation. Oral doses of 1 and 100 µg/kg were found to be totally effective in blocking dural extravasation for at least 8 and 24 hours post-dose respectively. The compound was also delivered successfully as an aerosol. These effects were enantiospecific; the enantiomer, LY306155, was approximately 100 times less potent when administered intravenously. These data suggest that the inhibition of extravasation produced by LY303870 is NK-1 receptor mediated.

533.1

SUPPRESSION OF CELLULAR IMMUNE FUNCTION BY DELTA OPIOID RECEPTOR ANTAGONISTS. R. V. House*, H. N. Bhargava, J. T. Kozak, A. S. Guy and P. T. Thomas. Life Sciences Dept., IIT Res. Inst., Chicago, IL 60616 and Dept. Pharmaceutics and Pharmacodynamics, Univ. of Illinois at Chicago, Chicago, IL 60612.

Previous studies in our laboratory have demonstrated that in vitro exposure to compounds acting as δ-opioid receptor agonists results in a significant stimulation of cellular immune function. The purpose of the present studies was to assess potential immunomodulation by the peptide receptor antagonists TIPP, d-TIPP, and ICI 174864 as well as the non-peptide receptor antagonists benzylidinenaltrexone (BNTX), naltrindole (NTI) and naltriben (NTB) by using a panel of in vitro cellular immune function assays. Murine B6C3F1 splenocytes were cultured with the compounds at concentrations between 0.00001-10 μM. B-cell proliferation was quantitated following cellular activation, T-cell function was assessed by cytokine production following stimulation with anti-CD3 monoclonal antibody, and natural immunity was assessed by quantitating natural killer (NK) cell activity following a 24-h exposure to δ-opioid receptor antagonists. The peptide antagonists exhibited limited activity against B-cells, exposure to BNTX and NTI suppressed B-cell proliferation, and NTB had no effect. Cytokine production by T cells was enhanced by exposure to the peptide antagonists, exposure to either BNTX or NTI resulted in suppressed cytokine production, while exposure to NTB had no significant effect. NK cell function exhibited the greatest sensitivity, with suppression observed following exposure to either peptide or non-peptide antagonists. These data suggest that δ-opioid receptor antagonists are broadly immunosuppressive at physiological concentrations (Supported by contract No. 271-91-9201 and grant No. DA-08867 from the National Institute on Drug Abuse).

533.2

ANTISENSE CONFIRMATION OF μ- AND κ-OPIOID RECEPTOR MEDIATION OF MORPHINE'S EFFECTS ON BODY TEMPERATURE IN RATS. Xiao-Hong Chen, Ellen B. Geller*, Lee-Yuan Liu-Chen and Martin W. Adler. Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140

Low doses of morphine (4-16 mg/kg, s.c.) can markedly increase body temperature in the rat, but a high dose of morphine (>30 mg/kg, s.c.) can cause a profound decrease in body temperature. Based on the use of selective opioid agonists and antagonists, we postulated that these effects were due to morphine's actions on μ and κ receptors, respectively. In the present study, we examined whether an antisense (AS) oligodeoxynucleotide (oligo) against cloned μ or κ opioid receptors could affect morphine-induced body temperature changes. AS oligos were directed against nucleotides 1-18 of the μ receptors and 4-21 of the κ receptors, respectively. Male SD rats were surgically implanted with icv cannulae. Rats received icv injections of vehicle or oligo in the animal colony room on days 1, 3 and 5. Either AS oligo or missense (MS) oligo was infused in a volume of 5 μl over 30 sec to freely moving animals. On day 6, rats were tested. The results showed that treatment with an AS oligo against μ opioid receptors, but not MS oligo, shifted the hyperthermia curve to the right. In addition, treatment with an AS oligo against κ receptors, but not MS oligo, almost completely blocked the hypothermia induced by a high dose morphine. This study confirms our earlier postulate that low doses of morphine induce an increase in body temperature primarily via μ opioid receptors and high doses of morphine produce a decrease through κ opioid receptors. (NIDA Grants DA 00376 and DA 04745).

533.3

SINGLE CELL RT-PCR DEMONSTRATES DIFFERENTIAL EXPRESSION OF OPIOID RECEPTOR mRNA AMONG IDENTIFIED PRIMARY AFFERENT NOCICEPTIVE NEURONS

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Opioids inhibit "2nd pain", the dull ache that persists after a noxious stimulus, while having little effect on "1st pain", the sharp initial sensation (Cooper *et al.* Pain 24:93, 1986). The lab has previously shown significant inhibition of *in vitro* Ca^{2+} currents by μ-opioid agonists applied to small, but not large, rat dental pulp nociceptors. We propose that selective inhibition of presynaptic Ca^{2+} entry in small nociceptors with unmyelinated axons enables opioid analgesics to specifically interrupt 2nd pain sensations.

In the current study we apply reverse transcription, followed by competitive PCR analysis, of single neurons to address whether differential expression of opioid receptor mRNA in nociceptors underlies these selective opioid actions. Key to this approach, as an internal control against tube-to-tube variation in the efficiency of the PCR reaction, we include in each PCR sample tube a known number of competitor target molecules containing a small insertion in the native cDNA sequence. With each PCR reaction, a series of tubes containing known quantities of both the mutant and native sequence establish a standard quantitative scale against which we measure ratios of mutant and wild-type PCR product bands derived from individual nociceptor cDNA. Using this approach we have identifed cells that, following reverse transcription, contain fewer than 5 μ-opioid receptor cDNA molecules, compared to others containing at least 50-150 μ-receptor cDNA molecules. We can therefore test whether those cells which lack a pharmacologic response to opioids do so because they fail to transcribe mRNA specific for this receptor.

533.4

Biosynthesis and regional distribution of a novel neuropeptide acting at an opioid-like G protein-coupled receptor

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We have identified a natural ligand of an "orphan" G protein-coupled receptor which bears high homology to the known opioid receptors. This ligand consists of a 17 amino acid peptide which also shows some sequence similarities with the opioid peptides. Consequently, we have begun to analyze the mode of biosynthesis of this novel neuropeptide. We have carried out preliminary experiments to determine the structure of the polypeptide precursor encoding this novel peptide by using molecular biological techniques. In addition, we have developed polyclonal antisera to the peptide and started to analyze the regional distribution of peptide expression by immunocytochemical methods. These analysis will be correlated with receptor autoradiography experiments and will allow us to draw a map of this novel peptidergic neuronal pathway.

533.5

Behavioral profile induced by a novel neuropeptide acting at an opioid-like G protein-coupled receptor

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We have isolated and characterized an endogenous peptidergic ligand to a formerly "orphan" G protein-coupled receptor which has significant homology to the cloned opioid receptors. Although this peptide shares some structural similarity with the opioid peptides it does not exhibit pharmacological activity at the opioid receptors. When injected into the cerebral ventricle of rats, this peptide induced a strong decrease in locomotor activity which was dose-dependent and lasted for up to one hour. This inactivity state was accompanied by a significant loss in muscle tonus but the animals were not cataleptic. No changes were seen in body temperature, heart rate, blood pressure and food intake. Our preliminary results indicate, that this novel peptide is biologically active and can induce behavioral responses different from those commonly associated with the opioid peptide system. Experiments to determine the involvement of this novel neuropeptide in analgesic responses are currently being carried out in our laboratory.

533.6

VARIANTS OF THE HUMAN MU OPIOID RECEPTORS: EVIDENCE FOR HETERONUCLEAR RNA FORMS. A. Mansour*, L.A. Bare, E. Mansson, H. Akil, and S. J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI USA and Ohmeda Pharmaceuticals, NJ USA.

The recent cloning of the opioid receptors, μ, δ and κ, suggests that they are members of the G-protein family of seven transmembrane receptors. While several lines of evidence suggest the presence of opioid receptor subtypes, there is little molecular biological data to support these findings. Recently, a variant of the μ opioid receptor has been cloned from both the rat and human (MORIA) that differs at the '3 end. Given the variation is at an exon-intron border, it may represent an alternatively spliced form of the μ receptor or the result of incomplete hetereonuclear RNA splicing. If the μ receptor variant is an alternatively spliced form, it should be distributed over both cytoplasmic and nuclear cellular compartments. If, on the other hand, it represents an incompletely spliced hetereonuclear RNA, then it should be localized primarily in cell nuclei. *In situ* hybridization studies were performed on formaldehyde post-fixed human brain sections with cRNA probes directed either to a EcoR1-Bam H1 fragment of exon 2, a Pst 1-Xba 1 fragment of intron 1, or a Xba 1 -Nde 2 fragment of intron 3 of the human μ receptor gene. The Eco1 - BAM H1 and Pst1 - Xba 1 fragments are "prototypical" exonic and intronic sequences, respectively, to which the Xba1-Nde 2 fragment (MORIA) may be compared. Results from emulsion-dipped sections suggest that cRNA probes directed at exon 2 of the μ receptor produced silver grains that were distributed over cell nuclei and cytosol. In contrast, the cRNA probe to the MORIA variant showed a localization primarily in cell nuclei, similar to that seen with the intronic Pst 1-Xba 1 cRNA probe. The results suggest that the MORIA variant is an incompletely spliced hetereonuclear RNA form.

533.7

CHARACTERIZATION OF CLONED μ AND κ OPIOID RECEPTORS IN HETEROLOGOUS SYSTEMS. A. Kouvelas[1]*, G. YK. Ng[1], M. Scheidler[2], M. Caron[3], M. Dennis[3], B.F.O'Dowd[1], and S.R. George[1] Depart. of Pharmacol., University of Toronto and Addiction Research Foundation, Toronto, ON, [2]Novo Nordisk A/S, Denmark, [3]BioSignal, Montreal, QC.

Cloned μ and κ opioid receptors have been expressed in vitro for elucidating receptor subtype-specific processes. μ receptor expressing BHK cells showed high affinity binding for antagonists and agonists. Agonist (DAMGO) detected high affinity binding (Ki ~1nM) was sensitive to GTP and analogues, and mediated inhibition of adenylyl cyclase. Pertussis toxin treatment resulted in the loss of agonist detected high affinity binding and the abolishment of DAMGO-mediated inhibition of adenylyl cyclase activity, suggesting μ receptor coupling to pertussis toxin-sensitive signalling pathways in the BHK cells. An alternate model was used to express cloned κ receptors in Sf9 cells which have been used successfully to express a number of other G protein coupled receptors for biochemical and functional studies. Expressed κ receptors showed saturable binding for the antagonist [^{3}H]naloxone with a K_d of ~2nM in good agreement with endogenous receptors. U50488 agonist/[^{3}H]naloxone competition binding revealed the presence of guanine nucleotide-sensitive high affinity binding sites suggesting functional coupling of expressed receptors, and is consistent with preliminary studies showing U50488- mediated inhibition of adenylyl cyclase activity in the Sf9 cells. A monoclonal κ receptor antibody revealed the presence of a discrete ~65 kDa immunoreactive species in membranes prepared from κ receptor expressing Sf9 cells not present in wild-type Sf9 membranes. Further, this antibody showed no immunoreactivity in membranes prepared from μ receptor expressing BHK cells demonstrating the specificity of this antibody for the specific identification and purification of κ receptors for further biochemical studies.

533.8

TRANSCRIPTION OF MU, DELTA, AND KAPPA OPIOID RECEPTORS IN PERIPHERAL BLOOD CELLS. R. E. Rodríguez*, A. Barrallo, M. V. Santos and R. González Sarmiento. Department of Biochemistry and Molecular Biology and Department of Medicine, Faculty of Medicine, University of Samanca, Spain.

Several authors have observed by Northern blot analysis the lack of opioid receptor expression in mouse or rat heart, spleen, lung, liver and *vas deferens*. We have studied the transcription of mu, delta and kappa opioid receptors in adult male CFLP mice by extraction of RNA from different central and peripheral tissues using the acid guanidium thiocyanate-phenol-chloroform method. Opioid receptor fragments were generated using specific oligonucleotides for mu, delta and kappa opioid receptors by RT-PCR. Amplified cDNA from the different tissues were studied by Southern blot and hybridized with specific probes. Northern blot analysis was also done to compare results obtained by PCR and by this method. We observe opioid receptor (mu, delta, and kappa) transcription in all tissues studied by RT-PCR (brain stem, anterior brain, cerebellum, spinal cord, ileum, liver, spleen, heart, lung and kidney). These results suggest that RT-PCR is a more sensitive technique than Northern blot analysis for the determination of the transcription of these receptors. Nevertheless, RT-PCR done in peripheral blood as well as in isolated T-lymphocytes, B-lymphocytes and monocytes also show opioid receptor transcription. As a consequence we conclude that RT-PCR done on fresh tissue is not a suitable technique for the determination of opioid receptor transcription since these receptors are also expressed in peripheral blood and we can not ignore that the tissue used for the determinations is not free from blood.

533.9

RAPID ENDOCYTOSIS OF OPIOID RECEPTORS: DIFFERENTIAL REGULATION BY OPIOID PEPTIDE AND MORPHINE. D. Keith, S. Murray, P. Zaki, P. Chu, D. Lissin, L. Kang, J. Aimi*, C. Evans, and M. vonZastrow. Dept. Psych., UCLA, Los Angeles CA 90024, Dept Psych., Stanford Univ., Stanford CA 94305, and Dept Psych., UCSF, San Francisco CA 94143

Morphine, an important therapeutic and abused drug, activates the same receptors which are the physiological targets of endogenous opioid peptides. It is not known to what extent this molecular mimickry extends to specific mechanisms of receptor regulation. We have utilized epitope tagging and immunochemical techniques to study the intracellular trafficking of cloned delta and mu -subtype opioid receptors expressed in human 293 fibroblastic and mouse Neuro2 neuroblastoma cells. In the absence of receptor agonist, both subtypes of opioid receptor are localized predominantly in the plasma membrane. In the presence of enkephalin (an endogenous peptide agonist) or etorphine (an alkaloid agonist drug), both receptor subtypes undergo rapid internalization ($t_{1/2}$ ~ 15 minutes). Enkephalin and etorphine cause opioid receptors to be sorted into a rapid endocytic pathway similar to that which mediates the constitutive endocytosis of transferrin. Morphine fails to stimulate detectable rapid endocytosis of either receptor subtype, even when used at high doses which cause maximal receptor-mediated inhibition of adenylyl cyclase. Furthermore, morphine inhibits the rapid endocytosis of opioid receptors when added to cells in combination with enkephalin or etorphine. These observations indicate that morphine and opioid peptide have opposing effects on a fundamental mechanism of receptor regulation.

533.10

EXPRESSION OF A KAPPA$_3$-RELATED (KOR-3) OPIOID RECEPTOR IN A MOUSE OSTEOSARCOMA CELL LINE, MC3T3-E1

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The MC3T3-E1 mouse osteosarcoma cell line naturally expresses a kappa$_3$-related receptor encoded by KOR-3 cDNA. The presence of this receptor has been demonstrated by Western blots and immunocytochemical staining with a monoclonal antibody (mAb 8D8). The molecular size of the band recognized on immunoblot and immunocytochemical staining patterns are similar to BE(2)-C human neuroblastoma cells. The functionality of this kappa$_3$-related receptor has been investigated through cAMP inhibition assays. Cyclazocine and nalorphine produced inhibitory effects with EC_{50} values of 6600 ± 800 nM and 5500 ± 2000 nM, respectively. Morphine, DAMGO and DPDPE did not inhibit cAMP accumulation greater than 10 %.

533.11

EXPRESSION OF N-METHYL-D-ASPARTATE RECEPTOR (NMDAR1), DELTA OPIOID RECEPTOR (DOR) AND MU OPIOID RECEPTOR (MOR) mRNAs IN TERMINALLY DIFFERENTIATED NT2-N CELLS. I.W. Beczkowska*, N. Gracey, V.M. Pickel, C.E. Inturrisi. Depts. of Pharmacology and Neurology and Neuroscience. Cornell U. Med. Coll., New York, NY 10021.

Retinoic acid (RA) treatment of NTera2/cl.D1 (NT2) cells, a human teratocarcinoma cell line, yields 95% pure cultures of terminally differentiated neuronal cells (NT2-N; Pleasure et al., J. Neurosci. 12:1802, 1992). Following RA-induced differentiation, NT2-N cells express functional NMDA receptors (Younkin et al., PNAS 90:2174, 1993). We compared the levels of NMDAR1, delta opioid receptor (DOR) and mu opioid receptor (MOR) mRNAs in NT2 cells, before and after treatment with RA, using a quantitative solution hybridization method (Franklin et al., Mol. Brain Res. 19:93, 1993). The level of NMDAR1 mRNA in NT2-N cells increased 10 fold, DOR mRNA increased 3 fold, and MOR mRNA increased 4 fold, as compared to NT2 cells. Northern blot analysis revealed two transcripts for NMDAR1 mRNA (4.4 and 4.2 kb) and two transcripts for DOR mRNA (7.0 and 11.0 kb). Cells were also processed for immunoperoxidase localization of NMDA and DOR antisera. In NT2-N cells the labeling for NMDA was seen within the cell bodies as well as on the processes, while it was undetectable in NT2 cells. In contrast, DOR-like immunolabeling was detected in both, NT-2 and NT2-N cells. Thus, RA differentiated NT2-N cells express increased levels for NMDAR1, DOR and MOR mRNA providing a model to study interaction between these receptor systems in human terminally differentiated neuronal cell culture. Supported by NIDA Grants DA01457, DA07274, DA00198 and DA04600.

533.12

INTERNALIZATION OF FLUORESCENT OPIOID AGONISTS IN COS CELLS TRANSFECTED WITH MU AND DELTA OPIOID RECEPTORS: A CONFOCAL MICROSCOPIC STUDY. G. Gaudriault, D. Nouel[1]*, J.P. Vincent and A. Beaudet[1]. IPMC - CNRS UPR 411, Sophia-Antipolis, 06560 Valbonne, France, [1] Montreal Neurol. Inst., Montreal, Canada H3A 2B4.

In order to visualize mu- and delta-opioid receptor internalization kinetics by confocal microscopy, we synthesized fluorescent derivatives of dermorphin (Bodipy-dermorphin, BDM) and deltorphin (Bodipy-deltorphin, BDLT) with high affinity (nanomolar range) and nearly complete specificity (selectivity factor of 100-1000) for mu-and delta-opioid receptors, respectively. Confocal scanning of mu- or delta-transfected COS cells incubated with 5nM BDM or BDLT for various times revealed the following: (i) dense clustering of each fluorescent ligand was initially observed on the surface of cells transfected with the appropriate receptor. This labeling was not present in non-transfected cells and was abolished by a thousand fold excess of naloxone, indicating that it was receptor dependent ; it was also totally washed out by a hypertonic acid wash, confirming that it was confined to the cell surface. (ii) after longer incubation times, transfected cells incubated with the appropriate fluorescent peptides exhibited numerous, small, intensely fluorescent endosome-like particules. These were acid wash-resistant and confirmed by serial optical sectionning to be mainly intracellular. (iii) kinetic data indicated that the delta ligand was internalized considerably more efficiently than the mu one. (iv) Experiments involving COS cells cotransfected with both the mu and delta receptors showed no overlap of the two different fluorophores (green for BDM and red for BDLT) within the same endosomal compartments. These observations, combined with the kinetics data, suggest that mu- and delta-opioid receptors are internalized along two different pathways.

533.13

ROLE OF THE C-TERMINAL TAIL IN CELLULAR LOCALIZATION OF THE μ OPIOID RECEPTOR MUTANTS.

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In Human Embryonic Kidney Cells (H.E.K.) stably transfected with the μ opioid receptor, acute morphine treatment inhibits adenylyl cyclase, indicating that the receptor is functionally expressed. The C-terminal tail of the μ opioid receptor is rich in Ser and Thr residues which are putative sites for receptor phosphorylation. In order to assess the role of phosphorylation in agonist-induced internalization and downregulation of the μ opioid receptor, we constructed two deletion mutants, one lacking any Ser or Thr residues in the C-terminal tail (TRUNC354), and the other maintaining the TSST region (TRUNC358). An epitope tag was added to the N-terminus of the wild type and both mutant receptors to allow immunocytochemistry with a monoclonal antibody. We found that all constructs are functional, as they inhibit adenylyl cyclase. The epitope tagged wild-type μ receptors and TRUNC358 stably expressed in H.E.K. cells, were located at the cell surface as visualized by confocal microscopy. DAMGO, a peptide agonist, induced μ receptor redistribution into intracellular vesicles whereas morphine treatment did not change μ receptor localization. The TRUNC354 mutant however, was mostly localized in intracellular vesicles before any treatment. Our results suggest that the TSST region may be involved in cellular localization of the μ opioid receptor.

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533.14

Delta and Mu Opioid Receptor Targeting in Madin-Darby Canine Kidney Cells.

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Investigation of opioid peptidergic neurotranmission has been greatly facilitated with the cloning of the opioid receptors. We have been able to raise rabbit antisera to the delta and mu opioid receptors (DOR1 and MOR1, respectively). During our anatomical survey of the rat CNS using immunolocalization methods, DOR1 was found predominantly in axons, suggesting its function as a presynaptic receptor (Dado et al., Neuroreport 5(3): 341-4, 1993; Arvidsson et al., J Neurosci 15(2): 1215-1235, 1995). In contrast, our study of the MOR1 has shown predominant localization to the soma and dendrites, suggesting a postsynaptic receptor (Arvidsson et al., J Neurosci 15(5): 3328-3341, 1995).

Since the initial studies by Dotti and Simon (Cell 62(1): 63-72, 1990), it has been suggested that the axonal and soma-dendritic cell surface domains of the neuron are similar to the apical and basolateral domains of polarized epithelial cells, respectively. We have attempted to take advantage of this by transfecting Madin-Darby canine kidney cells (MDCK), a well characterized polarizable epithelial cell line, with DOR1 and MOR1. Surprisingly, our preliminary results show that both opioid receptor types appear to be targeted predominantly to the basolateral surface of MDCK cells.

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533.15

μ-OPIOIDS ACTIVATE TYROSINE KINASES C-SRC AND FAK.

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We have previously shown that the G-protein μ-opioid receptor is coupled to tyrosine kinase activation. We have shown that 1μM met-enkephalin caused specific increases in protein tyrosine phosphorylation. One of these proteins is pp125^{FAK}, a focal adhesion tyrosine kinase. Both the phosphotyrosine content of FAK and the kinase activity of pp125^{FAK}, as investigated by in vitro kinase assays of immunoprecipitates from control and treated cultures, were increased. In addition, these assays revealed that met-enkephalin activate tyrosine kinases, which form complexes with pp125^{FAK}. We now report that one of them was identified as c-src (pp60^{src}). The time curves of tyrosine phosphorylation we obtained indicate that the initial tyrosine phosphorylation (<2 min) of pp125^{FAK} is not mediated by pp60^{src}. Our data are consistent with a complex regulation scheme of pp125^{FAK}, where the G-protein coupled μ-opioid receptor activates a yet unknown tyrosine kinase, which rapidly activates pp125^{FAK}. This kinase may be the one that initiates the rapid activation of PLD, as we have observed with μ-opioids. The tyrosine phosphorylation of pp125^{FAK} promotes formation of pp125^{FAK}/pp60^{src} complexes, which in turn facilitate tyrosine phosphorylation of pp125^{FAK} by pp60^{src}, detected as a second peak of pp125^{FAK} activation. This last peak indeed coincides with the met-enkephalin-stimulated activation of pp60^{src}. All met-enkephalin-stimulated signaling was inhibited by the antagonist naloxone. These data provide novel evidence that opioid intracellular signaling engages activation of the tyrosine kinases pp125^{FAK} and pp60^{src}. We propose that these protein-protein interactions may be the primary point of regulation of shape and function by opioids during brain morphogenesis (HD 09402 and BRF).

533.16

BINDING PROFILES OF δ-SPECIFIC AGONISTS IN A STABLY TRANSFECTED DOR-1 CHO CELL LINE SUGGEST THE PRESENCE OF A HIGH PROPORTION OF UNCOUPLED RECEPTORS.

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Transfection of cell lines with the opioid receptor clones provides a useful tool in the understanding of receptor-ligand and receptor-G protein interactions. Unfortunately, the binding profiles may differ from those of natively expressed opioid receptors in brain and cultured cell membranes. These discrepancies may be attributable to differences in processing, glycosylation or availability and abundance of G proteins in the cell line used for the transfection. We have transfected a CHO cell line with the mouse DOR-1 and find that a high proportion of the expressed delta receptors appear to be uncoupled from G proteins. Saturation binding studies using readiolabeled naltrindole (NTI), [D-Pen^2, D-Pen^5]-enkephalin (DPDPE), [D-Ala^2,D-Leu^5]-enkephalin (DADLE), and [D-Ala^2, D-Glu^4] deltorphin (deltorphin II) demonstrated that the high affinity agonist binding for each of the tritiated agonists did not account for more than approximately one third of the total ^{3}H-NTI binding. Affinity constants of all ligands in saturation studies were comparable to those seen in NG108-15 membranes, but the K$_i$ values as competitors of ^{3}H-NTI binding are almost 10-fold greater than would be expected. Addition of the non-hydrolyzable guanine nucleotide analogue, Gpp(NH)p, did not shift the agonist competition curves in the stably transfected cells, suggesting that these ligands are competing at uncoupled receptors. These cells will provide a useful tool in the study of receptor-ligand and receptor-G protein interactions.

533.17

TRANSDUCTION SIGNALING AT *DELTA* OPIOID RECEPTORS IN MOUSE CORTICAL NEURONS *IN VITRO*.

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The existence of subtypes of the *delta* opioid receptor is based on *in vivo* pharmacological data. No clear distinction, however, has yet been made at the molecular or cellular level. In the present study, the effects of preferential *delta*1 and *delta*2 opioid receptor agonists (DPDPE and Deltorphin II, respectively) on cAMP formation in mouse cerebral cortical neurons *in vitro* have been studied. DPDPE produced a concentration-dependent inhibition that was maximal (25%) at 1-5 μM. The response to Deltorphin II was different, being biphasic with an initial shallow concentration-effect curve (inhibition of 30% at 0.1-1 μM) and a second steeper phase with maximal (90%) inhibition observed at 100 μM. A biphasic concentration-dependent reduction in cAMP levels was also observed with the novel non-peptidic selective *delta* agonist (-)trans-4a-(3-hydroxyphenyl)-2-methyl-1,2,3,4,4a,5,12,12a-octahydropyrido[3,4-b] acridine (SB213698B), with maximal reduction of 65% at 100 μM.

DPDPE and deltorphin II effects were antagonized by BNTX and NTB (0.1-1 μM), respectively, supporting the suggestion that different *delta* receptor subtypes are responsible for the responses observed.

Acute neuronal pretreatment with Deltorphin II (0.001-1 μM) also reduced cGMP levels, in controls and after stimulation with glutamatergic agonist NMDA (300 μM). This suggests that complex intracellular interactions may occur between opioid and glutamate receptor systems in cerebral cortical neurons.

533.18

EFFECTS OF FULL AND PARTIAL μ-OPIOID AGONISTS ON RECEPTOR-STIMULATED [^{35}S]GTPγS BINDING.

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The ability of full and partial μ-opioid agonists to stimulate [^{35}S]GTPγS binding was examined in rat thalamic membranes. Agonist stimulation of [^{35}S]GTPγS binding required the presence of excess GDP to suppress basal labeling of G-proteins by [^{35}S]GTPγS. In the presence of 30 μM GDP, the μ-selective agonist DAMGO stimulated [^{35}S]GTPγS binding in with a maximal stimulation of 135 % and an ED$_{50}$ value of 214 nM. Stimulation of [^{35}S]GTPγS binding by DAMGO was consistent with a μ opioid receptor-mediated mechanism, since naloxone reversed this effect with a K$_e$ value of 1.8 nM. Comparison of DAMGO with other μ-opioid agonists indicated that it was a full agonist in this system. Morphine was a partial agonist of relatively high efficacy, since it produced approximately 60-70% of the maximal stimulation produced by DAMGO, whereas buprenorphine was a partial agonist of low efficacy (20-30% of DAMGO-stimulation). Similar efficacy relationships among these agonists were observed in membranes from the μ-opioid receptor-containing cell line SK-N-SH, which contained about a 10-fold lower density of [^{35}S]GTPγS binding sites than rat thalamic membranes. These results indicate that the difference in efficacy for G-protein activation among μ agonists is determined at the level of the receptor, since these efficacy differences were conserved between the two systems.

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533.19

ACUTE AND CHRONIC EFFECTS OF OPIOIDS ON [^{35}S]GTPγS BINDING IN NG108-15 AND SK-N-SH CELLS. Christopher S. Breivogel, Dana E. Selley, and Steven R. Childers*. Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157.

The activation of inhibitory GTP-binding proteins (G_i) by opioid agonists was measured in NG108-15 and SK-N-SH cell lines by their ability to stimulate [^{35}S]GTPγS binding in membranes along with excess GDP. In NG108-15 membranes, GDP decreased total [^{35}S]GTPγS binding while increasing the percent stimulation of binding by agonists; 10-20 μM GDP optimized these parameters. In these cells, the δ-selective agonist DSLET stimulated binding approx. two-fold with an ED_{50} of 13 nM, and the δ-selective antagonist naltrindole (NTI) exhibited a K_e of 0.04 nM. In membranes from SK-N-SH cells, which express both μ and δ receptors, GDP had similar effects to decrease total [^{35}S]GTPγS binding and increase agonist stimulation. In these cells, the μ agonist DAMGO stimulated binding approx. two-fold with an ED_{50} of 0.5 μM, while δ agonists were much less potent. Effects of μ and δ antagonists were consistent with the idea that agonist stimulation of [^{35}S]GTPγS binding occurred almost entirely through μ receptors.

Chronic treatment of NG108-15 cells with DSLET (1 μM) from 0.5 to 22 hr resulted in rapid loss of DSLET-stimulated [^{35}S]GTPγS binding in membranes. Maximal agonist stimulation decreased by 80% within 0.5-1 hr, and declined even further after overnight treatment. Treatment of SK-N-SH cells with morphine (10 μM) decreased DAMGO stimulation of binding by 60% after overnight treatment, but had little effect on DAMGO stimulation after 0.5 hr of morphine treatment.

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533.20

OPIOID REGULATION OF INTRACELLULAR FREE CALCIUM IN CULTURED MOUSE DORSAL ROOT GANGLION NEURONS. Tianlai Tang and Brian M. Cox*. Department of Pharmacology, Uniformed Services University of Health Sciences, Bethesda, MD 20814

We have examined opioid effects on intracellular free calcium concentrations ($[Ca^{2+}]i$) in cultured single mouse dorsal root ganglion (DRG) neurons using Fluo-3-based fluocytometry. Opioid agonists induced an increase in resting $[Ca^{2+}]i$ or an inhibition of K^+-stimulated increase in $[Ca^{2+}]i$ in different subsets of DRG neurons. The total neuronal population was grouped into three classes according to their somatic diameter and defined as small- (<16 μm), intermediate- (16-25 μm), or large- (>25 μm) sized neurons. Substance P-like immunoreactivity was mainly detected in the small- and intermediate-sized neurons. The δ, κ, and μ opioid receptor agonists, [D-Ser^2,Leu^5]enkephalin-Thr (DSLET), U69593, and [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin (DAMGO) each induced an transient increase in $[Ca^{2+}]i$ in a small fraction (<30%) of neurons. The increases in $[Ca^{2+}]i$ were blocked by the opioid antagonist, naloxone. The dihydropyridine-sensitive calcium channel blocker, nifedipine, also blocked the increase in $[Ca^{2+}]i$ induced by 1 μM DSLET, indicating that the opioids induced a calcium influx through dihydropyridine-sensitive calcium channels. The rank order of potency (percentage of cells responding to each opioid agonist) was DSLET > U69593 > DAMGO. The opioid-induced increase in $[Ca^{2+}]i$ was mainly observed in large-sized neurons with a low incidence in the small- and intermediate-sized neurons. Opioid agonists also caused inhibition of K^+ (25 mM)-stimulated increases in $[Ca^{2+}]i$, which were blocked by naloxone. Greater inhibition by 1 μM DSLET or U69593 was found in small- and intermediate-sized neurons with a less inhibition in large-sized neurons. *(Supported by a grant from the National Institute on Drug Abuse).*

533.21

ASTROGIAL CELLS FROM THE RAT CEREBRAL CORTEX COEXPRESS DELTA OPIOID AND GLUTAMATE RECEPTORS T.Thorlin[1], P. S. Eriksson[1,2], E. Hansson[1,3] and L. Rönnbäck*[1,2]. Institute of Neurobiology[1] and Department of Neurology[2] University of Göteborg and Department of Cell Biology, Faculty of Health Sciences, University of Linköping[3], Sweden.

Astroglial cultures from the cerebral cortex were compared with tissue from the cerebral cortex concerning the expression of delta opioid receptor mRNA. The presence of δ-receptor mRNA in glial cultures was verified using RT-PCR and Northern blot. The level of expression was shown to be lower in glial cultures compared with brain tissue when the expression was normalized to total RNA. The amount of delta receptor mRNA was quantified using an RNAse protection assay.

Cultured neurons were shown to respond with a substantial decrease in the cytoplasmic free calcium after stimulation of μ, δ and κ receptors. On the other hand, stimulation of cultured astroglial δ receptors resulted in a substantial increase in the cytoplasmic free calcium. Glutamate receptors were shown to be co-localized with opioid receptors on cultured astroglial cells. Corresponding results were obtained in dissociated acute preparations of astroglial cells the cerebral cortex. Astroglial cells in culture and acute dissociated astrocytes were identified by positive immunofluorescent staining for GFAp. The results obtained in acute dissociated preparations support the *in vitro* findings. The presence of glial opioid receptors might represent a novel mechanism contributing to the depressant action of opioids on synaptic transmission via decreasing the availability of calcium necessary for presynaptic transmitter release.

533.22

Functional determination of opioid receptor subtypes in the hypothalamic ventromedial nucleus of the rat. Zhang, C*., Kow, L. M. & Pfaff, D. W. Lab. of Neurobiology and Behavior, The Rockefeller University, 1230 York Ave., New York, NY 10021, USA.

The preproenkephalin gene is expressed in the ventromedial nucleus (VMN) of the hypothalamus and is up-regulated by estrogen in ovariectomized female rats. The major product of preproenkephalin gene, met-enkephalin (mEnk) has been suggested to play a role in reproductive and emotional behaviors, but the nature of possible mEnk effects in VMN itself and the receptor subtypes that could mediate mEnk action have not been functionally characterized. In the present study, thin (400 μm) hypothalamic slices containing VMN were prepared from ovariectomized rats primed with estrogen. Single unit activity was recorded extracellularly with glass microelectrodes from VMN neurons, and neuronal responses to bath-applied mEnk and agonists selective for μ (DAGO), δ (DPDPE) and κ (U50,488), opioid receptors, were examined. mEnk, DAGO and DPDPE inhibited, in a dose-dependent fashion (10 nM -- 10 μM), the spontaneous firing of VMN neurons with similar potencies, while U50,488 produced a much smaller inhibition. No excitatory response was observed. The inhibition produced by mEnk was blocked by naloxone, by the δ-selective antagonist naltrindole, and to a lesser extent by the μ-selective antagonist β-funaltrexamine, while the κ-selective antagonist Nor-BNI had no effect on the inhibition. The antagonists had no effect of their own on the spontaneous firing of VMN neurons under the present *in vitro* conditions. These results suggest that the major opioid receptors in the rat VMN are of the μ and δ subtypes, and that mEnk, a potential endogenous opioid transmitter in the VMN, can exert inhibitory effects by acting on δ and μ receptors. Supported by: grant # HD05751.

OPIOID RECEPTORS: DEVELOPMENT AND REGULATION

534.1

ATTENUATION OF MORPHINE TOLERANCE AND ACUTE DEPENDENCE BY DELTA OPIOID RECEPTOR (DOR) ANTISENSE OLIGODEOXYNUCLEOTIDE TREATMENT. B. KEST*, C.E. Lee, G. McLemore and C.E. Inturrisi. Dept. of Pharmacology, Cornell U. Medical College, New York, NY 10021.

Studies from several laboratories indicate a modulatory role for the DOR in morphine (MOR), tolerance (TOL), and dependence (DEP). We evaluated the role of the DOR in MOR TOL and acute DEP using an in vivo antisense strategy in mice. Intracerebroventricular (icv) saline, antisense or mismatch (different at 4 bases) oligos (ODNs; 12.5 ug in 5 ul) targeting nucleotides 7-26 of the DOR-1 mRNA for 3 days did not affect MOR tail-flick analgesia. When morphine was administered along with mismatch ODN or saline on days 4, 5, and 6 the MOR ED_{50} increased 4-fold on day 7, indicating the development of MOR tolerance. In antisense treated mice, however, the MOR ED_{50} was increased only 2-fold, representing an attenuation of MOR TOL relative to saline and mismatch treated mice. Naloxone (50 mg/kg, sc) produced vertical jumping, (acute DEP), 3 h after a single dose of MOR (50 mg/kg, sc). Three days of antisense, but not mismatch, ODN reduced the incidence and magnitude of jumping. These results suggest that downregulation of the DOR affects the development of MOR TOL and acute DEP and provides new evidence for a modulatory role of the DOR in morphine pharmacodynamics. Supported by NIDA Grants DA07274, DA01457, DA00198 and Grants from the Aaron Diamond Foundation.

534.2

PROOPIOMELANOCORTIN AND MU OPIOID RECEPTOR mRNA EXPRESSION IN THE FEMALE GUINEA PIG HYPOTHALAMUS: EFFECTS OF CHRONIC MORPHINE TREATMENT. Y. Fang, M.J. Kelly, D.K. Grandy, O.K. Ronnekleiv*. Dept. of Physiology, Oregon Health Sciences Univ., and ORPRC, Portland, OR 97201.

Our previous electrophysiological studies have shown that hypothalamic beta-endorphin (BE) neurons express μ-opioid receptors, which exhibit tolerance to chronic morphine in the ovariectomized (OVX) female guinea pig (GP). To further identify the response of BE to chronic morphine treatment, female GPs were OVX and implanted (s.c.) with 4x75 mg pellets for 2 days plus 6 more pellets of either morphine or placebo for 5 days. Animals were killed between 1000 and 1100 h on day 7, the brains quickly dissected and frozen. The expression of POMC and μ-receptor mRNAs were investigated using *in situ* hybridization with GP-specific ^{35}S-labeled cRNA probes. The results from X-ray film showed that the level of POMC mRNA in the arcuate nucleus (ARC) of morphine-treated GPs (n=6) was decreased by 22 % compared with the placebo controls (n=6; $P<0.05$; paired two-tailed t-test), suggesting that the activity of POMC is down-regulated due to the exposure to chronic morphine. The expression of μ-opioid receptor mRNA was determined from emulsion-coated tissue sections. This analysis revealed that the μ-opioid receptor mRNA was distributed in many areas of the hypothalamus including the preoptic, anterior, mediobasal (MBH), dorsomedial, posterior and mammillary regions of both placebo and morphine treated GPs. Within the MBH μ-opioid receptor mRNA was found in the ARC. Experiments are in progress to elucidate the effects of chronic morphine on the expression of μ-opioid receptor mRNA in the female GP. Supported by NIH grants DA07165, DA05158, and DA08562.

534.3

EFFECTS OF CHRONIC MORPHINE ON μ-OPIOID RECEPTOR BINDING AND mRNA IN THE GUINEA PIG CNS. M.A. Bosch*, M.J. Cunningham, G. Zhang, E.J. Wagner, M.J. Kelly, D.K. Grandy, O.K. Ronnekleiv. Department of Physiology, the Oregon Health Sciences University, the Vollum Institute and ORPRC, Portland, OR, 97201

To elucidate the effects of chronic morphine on the expression of μ-opioid receptors in different regions of the guinea pig (GP) CNS, females were ovariectomized and implanted (s.c.) with 4 × 75 mg pellets, followed two days later with 6 more pellets of either morphine or placebo (n=8, each). This treatment produced plasma morphine levels of 559 ± 180 ng/ml as measured by HPLC. Animals were killed between 1000-1100 h on day 7 and the preoptic area (POA), mediobasal hypothalamus (MBH) and the thalamus (THAL) were microdissected. Total RNA was isolated and μ-receptor mRNA quantified using a ribonuclease protection assay with a GP-specific cRNA probe. Morphine, compared to placebo, significantly reduced the expression of μ-receptor mRNA in the MBH ($p<0.035$; n=8, each) but not in the POA or the THAL. To evaluate morphine's effect on μ-opioid receptor density, saturation binding studies were performed in P_2 membrane fractions using antagonist radioligand [^{3}H] diprenorphine. DPDPE and U50488 were included in the assays to occupy δ- and κ-receptors, respectively. The μ-receptor density was significantly decreased in the MBH of morphine-treated GP (B_{max} = 217 ± 9 fmol/mg protein) vs. controls (B_{max} 276 ± 16 fmol/mg protein), with no change in the K_d (0.38 ± 0.03 nM in morph vs. 0.37±0.05 nM in placebo). These data suggest that chronic morphine causes a down regulation of μ-opioid receptor binding and receptor mRNA in the MBH of the GP. Supported by NIH grants DA07165, DA05158, and DA08562.

534.4

PRENATAL MORPHINE EXPOSURE DIFFERENTIALLY ALTERS EXPRESSION OF OPIOID PEPTIDES IN STRIATUM OF NEWBORNS

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Chronic morphine treatment in the adult central nervous system (CNS) has produced contradictory results, ranging from no change, to an upregulation as well as a downregulation of opioid receptors. In addition, decreases in proenkephalin (PPE) and dynorphin levels in striatum and hypothalamus have been reported while peptide levels are increased following morphine treatment. In the neonate, we have demonstrated a downregulation of μ-opioid receptors in striatum of morphine-addicted newborns. The present study was carried out to investigate the effects of prenatal morphine on the levels and expression of endogenous opioid peptides in brain regions of newborns. Dams were implanted with one morphine pellet (75mg each) or placebo one week prior to the birth of pups. Changes in mRNA levels for the opioid peptides were determined by Northern blot analysis. Alterations in opioid peptide levels were determined by radioimmunoassays (RIAs). Prenatal morphine treatment significantly increased (38%; $p<0.03$) PPE mRNA levels and decreased met-enkephalin levels (40%; $p<0.01$) in striatum of newborns. No significant alterations in dynorphin levels were observed. This data is in contrast to what is observed in the adult CNS. These data indicate that prenatal morphine treatment may increase met-enkephalin release and/or cause inhibition at the level of translation. In addition, increased transcription may be necessary to maintain equilibrium in the system when there is an increase in met-enkephalin release (supported by LIJMC Faculty Research Award).

534.5

OPIOID RECEPTORS ARE BASALLY PHOSPHORYLATED IN GUINEA PIG BRAIN SLICES BY PKC AND PKA T.A. Patterson*, S.M. Appleyard and C. Chavkin, Univ. of WA. Dept of Pharmacology, Box 357280, Seattle WA 98195

Antipeptide antibodies were generated against the guinea pig kappa and rat mu opioid receptor subtypes. Specificity of the antibodies was shown by western blotting analysis, peptide block and immunoprecipitation studies. The kappa and mu antibodies precipitated proteins of approximate molecular weights 54 kD and 73 kD, respectively.

Studies were performed to determine the phosphorylation state of opioid receptors in intact neurons. Slices were made from guinea pig whole brain or forebrain for kappa and mu studies respectively. Slices were metabolically labeled with ^{32}P orthophosphoric acid prior to the addition of staurosporine, H-7, RpCAMPS or R24571. Receptors were immunoprecipitated and incorporation of ^{32}P was determined with scintillation counting or phosphoimaging and normalized for relative receptor concentration based on western blotting.

The kappa receptor was basally phosphorylated under these conditions. Phosphorylation of the kappa receptor was significantly decreased ($p<0.05$) in the presence of the PKC and PKA inhibitors staurosporine and H-7 and not changed in the presence of the PKA inhibitor RpCAMPS or the CAM kinase inhibitor R24571. *In vitro* back-phosphorylation studies of the solubilized kappa receptor showed it to be an excellent PKC substrate and a weaker PKA substrate, in agreement with the metabolic labeling studies. Supported by DA04123.

534.6

NOVEL EFFECTS OF FENTANYL OPIATES WITH μ-OPIATE RECEPTORS IN A RECOMBINANT CELL LINE. C. CASEY* AND M. TEITLER. Dept. of Pharmacology & Neuroscience, Albany Medical College, Albany, NY 12208

Previous reports have indicated that, in general, fentanyl and fentanyl-like compounds such as carfentanil are selective μ-opiate receptor agonists (1,2). In order to further explore the nature of novel direct molecular interactions of fentanyl derivatives with μ-opiate receptors, and to explore possible differences in the effects of chronic fentanyl stimulation on μ-opiate receptor function, studies were undertaken taking advantage of the recent development of recombinant cells selectively expressing the μ-opiate receptor (3). Initial characterization indicated that there was a striking difference in the effect of GppNHp on the levels of binding of ^{3}H-carfentanil-labelled μ-opiate receptors, versus ^{3}H-DAMGO-labelled receptors. GppNHp produced a far more dramatic inhibition of the levels of ^{3}H-carfentanil-labelled receptors (~90% inhibition) than of ^{3}H-DAMGO-labelled receptors (~40%). These results indicate a difference in the molecular interaction of a fentanyl-stimulated μ-opiate receptor with a G-protein, versus a DAMGO-stimulated μ-opiate receptor. Studies involving chronic treatments with fentanyl derivatives will be presented along with data on the initial characterization of ^{3}H-carfentanil binding in recombinant cells expressing μ-opiate receptors. Mechanisms responsible for the dramatic desensitization characteristic of chronic opiate adinistration should be revealed by these studies.

1. Teitler et al, Eur. J. Pharmacol., 167, 221-228 (1989)
2. L.W. Fitzgerald and M. Teitler, Synapse, 14, 154-159 (1993).
3. Chen et al, Mol. Pharmacol., 44, 8-12 (1993)

534.7

PROTEIN KINASE A (PKA) REGULATES DELTA OPIOID RECEPTOR (DOR) mRNA IN NG108-15 CELLS. S. Jenab and C. E. Inturrisi*, Dept. of Pharmacology, Cornell U. Med. Coll., New York, NY 10021.

In NG108-15 cells forskolin (FOR) increases intracellular cAMP and cAMP-dependent protein kinases (PKA) while it decreases DOR binding (Bergsbaken et al., 1993). In contrast ethanol up-regulates DOR binding and mRNA (Charness et al., 1993; Jenab and Inturrisi, 1994). We now report that the ethanol-induced increase in DOR mRNA is blocked by FOR (10 uM). DOR mRNA was measured using solution hybridization and Northern blot analysis. FOR alone, but not the inactive dideoxy FOR, reduced DOR mRNA by 50%. Incubation of NG108-15 cells with 200 mM ethanol increased DOR mRNA transcript levels 2 fold and FOR blocked this induction. Northern blot analysis of RNA extracts indicated that these treatments altered each of the multiple DOR transcripts proportionately so that no difference was observed in the fraction of the total hybridization signal produced by each band. Inhibition of PKA by an analogue of cAMP (cAMPS, Rp-diastereomer 50 uM) resulted in a 2 fold increase in DOR mRNA but no further increase when combined with ethanol. These results suggest that the induction of DOR mRNA by ethanol in NG108-15 cells is related to ethanol's ability to reduce cAMP-dependent PKA activities. Furthermore, DOR gene expression can be regulated by second messengers via PKA. Supported by NIDA Grants DA01457, DA05130, DA07274 and DA00198.

534.8

DOWN REGULATION OF DELTA-OPIOID mRNA LEVELS BY INCREASED cAMP LEVELS IN DIFFERENTIATED NG108-15 CELLS. **K.H. Gylys*, D. E. Keith, and C.J. Evans.** *Dept. of Psychiatry & Biobehavioral Sciences, University of California, Los Angeles CA 90024.*

The second messenger cAMP has been shown to regulate adrenergic receptor mRNA (Collins et al., 1991), and the μ-opioid promoter contains the consensus sequence for a cAMP regulatory element (Min et al., 1994). We have examined cAMP regulation of δ-opioid mRNA in differentiated NG108-15 cells. Because the IBMX/forskolin combination is nonselective in its actions we have also used the selective PDE inhibitor rolipram and prostaglandin E_2 to look at cAMP regulation in NG108-15 cells differentiated with retinoic acid. Cultures were differentiated for 3 days with retinoic acid before exposure to drugs for varying lengths of time. Cells were then harvested, total RNA was isolated, and quantitative Northern analysis performed. The three agents examined: rolipram (100 μM), IBMX/forskolin (250/5μM), and PGE_2 (10μM), all caused a down-regulation of δ-opioid message to less than 10% of control levels. Maximum inhibition occurred after 8 or 24 hours of treatment. These results suggest a possible mechanism for heterologous regulation of δ-opioid receptors.
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534.9

PHOSPHORYLATION OF EXPRESSEDHUMAN MU OPIATE RECEPTORS J. B.Wang*, G. Pei[1] and G. R.Uhl[2] Dept. of Pharmaceutical Sciences, School of Pharmacy, University of Maryland at Baltimore, MD 21201, [1]Shanghai Institute of Cell Biology, People's Republic of China and [2] Molecular Neurobiology Branch, NIDA/IRP, NIH, Baltimore MD 21224

Mu opiate receptors can desensitize when agonist-induced K^+ channel responses are examined in expression systems including Xenopus oocytes. Mechanisms underlying such processes could include phosphorylation events involved in desensitizing other G-linked receptors. To explore the possible roles of phosphorylation in mu opiate receptor desensitization, we constructed mu receptors fused at N- and C- terminals to HA12CA5 epitope tags and examined their expression in COS cells. These constructions each appear to express nearly-normally affinities and Bmax values for [3H] DAMGO binding. These constructions expressed in COS cells were exposed to various drug treatments in the presence of ^{32}Pi, tagged proteins were immunoprecipiatated with anti-HA12CA5 antibodies, resolved on SDS-PAGE gels and radiolabelled mu receptor ca. 60 kd band identified by autoradiography. mu receptor-expressing COS cells free from opiate agonists revealed no ^{32}Pi radiolabelling of the ca. 60 kd band. Cells exposed to 1 uM DAMGO for 3 min reveal apparent agonist-dependent mu receptor phosphorylation. Phorbol ester treatment for 3 min also enhanced receptor phosphorylation while forskolin failed to stimulate phosphorylation. These observations are conistent with previous suggstions of protein kinase C involvement in desensitization. Candidate sites for receptor phosphorylation are being tested by assessment of mu receptor mutants. Both mu and other G-linked receptors can alter PKC function; these mechanisms could thus be implicated in both homologous and heterologous desensitization.

534.10

DIFFERENT RATE OF DESENSITIZATION IN THE MULTIPLE SIGNALING PATHWAYS OF OPIOID RECEPTOR. Shuji Kaneko*,[1] Nobumichi Yada[1], Kei Adachi[1], Akinori Akaike[1] and Masamichi Satoh[2]. Depts. of [1]Pharmacol. and [2]Mol. Pharmacol., Faculty of Pharm. Sci., Kyoto Univ., Kyoto 606-01, Japan.

RNA-injected *Xenopus* oocytes were used for studying homologous desensitization of opioid receptors to G-protein-coupled intracellular signaling pathways. In the oocytes expressing κ opioid receptor, voltage-dependent Ca^{2+} channel α_1 and β subunits, the κ agonist U50488H (1 μM) inhibited Ca^{2+} channel current in a reversible manner, and the inhibition was diminished by sustained agonist exposure (homologous desensitization). More than 30 min was required to halve the inhibition of BI- or Q-type Ca^{2+} channels by the κ agonist, however, the $t_{1/2}$ for U50488H-induced inhibition of BIII- or N-type channels was only 7 ± 1 min. Moreover, in the oocytes expressing κ opioid receptor, EP_4 prostaglandin receptor and CFTR channel, no apparent desensitization was observed in the κ receptor-mediated potentiation of the cyclic AMP production by prostaglandin E_2 even when the oocytes were treated with U50488H for 2 hours or more. The time-course of desensitization of μ opioid receptor was also different in the effector molecules. These observations suggest that rate of homologous desensitization of opioid receptor is not only dependent on the receptor molecule itself but also on its intracellular signaling systems.

534.11

DEVELOPMENTAL EXPRESSION OF OPIOID RECEPTOR mRNAS. **R.C. Thompson*, J. Wong, H. Akil, and S. J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan, 48109-0720.**

Endogenous opioid peptide systems have been implicated in neuronal development including putative roles as trophic factors. Binding studies of the three major classes of opioid receptors (mu (μ), delta (δ), and kappa (κ)) have demonstrated that mu and kappa receptors are detected relative early in development in the rodent (approximately E12 in rat) whereas delta receptors are not detected until postnatal time periods. Based in part on this work, much focus has been placed upon the roles of β-endorphin and dynorphin during development. However, little is known about the precise anatomical expression of the opioid receptors in the rat. In order to evaluate the potential roles of endogenous opioid peptide systems during development, we felt that it was very important to determine which opioid receptor mRNAs were expressed during specific developmental time points. The expression of mu, delta, and kappa opioid receptor mRNA was examined in rat embryos and pups by in situ hybridization with cRNA probes derived from the three cloned opioid receptors. Autoradiograms from these studies document several interesting findings. Mu receptor mRNA is detected as early as E12 within the developing central nervous system and in components of the peripheral nervous system at multiple developmental time points. Kappa RNA is also expressed at E12 stages. Preliminary data on delta mRNA expression suggests that it is also expressed during development but not at the same levels as kappa or mu mRNAs. Complete anatomical comparisons will be presented and discussed. This work is supported by grants DA02265 and DA 08920.

534.12

DEVELOPMENTAL EXPRESSION OF THE MU OPIOID RECEPTOR IN THE MOUSE Y. Zhu*, B. Anton, M. Hsu, C. J. Evans, J. E. Pintar. Dept. Neurosci. & Cell Biol., UMDNJ Robert Wood Johnson Med. Sch., Piscataway, NJ 08854 and Dept. Psychiatry, UCLA Sch. of Med., Los Angeles, CA 90024.

To characterize the establishment of opioid systems during development, we have begun to examine the distribution of μ receptor mRNA at both prenatal and postnatal stages of mouse development using in situ hybridization. Restricted μ receptor gene expression has been detected as early as e10.5, in the VII-VIII preganglion complex. By e12.5, cells expressing μ-receptor mRNA are found in the facial (VII) and vestibulocochlear (VIII) ganglia. In the central nervous system at this age, μ-receptor mRNA expression is detected in the basal telencephalon and ventral spinal cord. At e13.5, cells expressing μ-receptor are found in the thalamus, hypothalamus and dorsal medulla with relatively high levels of μ receptor mRNA observed in caudate-putamen. At e15.5, the expression pattern of the μ receptor is similar to that at e13.5, though the intensity of the hybridization signal has increased. The mRNA expression at e15.5 has expanded to cover most of the dorsal spinal cord in addition to the ventral regions of the spinal cord seen earlier. By e17.5, μ receptor mRNA starts to appear in the mitral cell layer of olfatory bulb and in the subplate of cortex. Therefore, μ receptor expression develops earlier in many other brain regions than in the cortex. Besides caudate-putamen, strong signals are also found in the medial habenula. In the trunk, μ receptor can still only be detected in the spinal cord and the dorsal root ganglion. Our results confirm and extend results from previous ligand binding studies using whole mouse brain that showed that μ receptor binding activity can be detected as early as e12.5, and in addition, provide direct evidence for opioid receptor gene expression in the earliest stages of peripheral gangliogenesis. Supported by DA-09040 to JP.

534.13

ONTOGENY OF THE EXPRESSION OF THE MU OPIOID RECEPTOR GENE IN RAT BRAIN: AN *IN SITU* HYBRIDIZATION STUDY. J.-G. Chabot[1*], Y. Tong[1,2], S.-H. Shen[2], B.F. O'Dowd[3], S.R. George[3] and R. Quirion[1]. [1] Douglas Hosp. Res. Ctr., Dept. of Psychiatry, McGill University, Montreal, H4H 1R3. [2] Biotechnology Research Institute, Montreal, H4P 2R2. [3] Dept. of Pharmacology, Medical Sciences Building, University of Toronto, Toronto, M5S 1A8, CANADA.

The recent cloning of the μ opioid receptor has allowed the comparative localization of the receptor mRNA and its translated protein using *in situ* hybridization and quantitative receptor autoradiography (e.g., Mansour et al., *TINS*, 18, 22, 1995). The aim of the present study was to examine the developmental profile of the expression of the μ receptor mRNA in the rat brain in order to provide new insights as to the putative role of this receptor in normal brain ontogeny. The anatomical distribution of μ receptor gene expression in the developing (ED11 - PD40) and adult (PD60) rat brain was investigated using quantitative *in situ* hybridization. Eighteen μm-thick brain sections were hybridized with [^{35}S]UTP-labeled riboprobes generated to the rat μ receptor (George et al., *BBRC* 205, 1438, 1994). μ receptor mRNA was observed to be first detectable in the neuroepithelium at ED12. In the developing CNS (ED14 - P40), transcripts were seen over numerous telencephalic, diencephalic and mesencephalic areas. For example, in the developing striatum, transcripts were detected first in the striatal anlage at ED14, increasing rapidly to reach maximal levels during the first postnatal week to then decrease to reach adult levels during the next two postnatal weeks. Hybridization signal remained diffuse in the striatum until PD6 to then began to assume the adult patchy distribution. Similar results were previously reported for μ receptor binding sites (Kent et al., *Dev. Brain Res.* 2, 487, 1982; Kornblum et al., *Brain Res.* 465, 21, 1987). In summary, endogenous opioids, μ receptor mRNA and its translated protein are present very early on in the embryonic brain and demonstrate transient increases during the critical period of neurogenesis, neuronal migration and synaptogenesis. Supported by the MRCC.

534.14

THE EFFECTS OF AGING ON THE BINDING OF ^{3}H-NALTRINDOLE IN THE BRAIN AND SPINAL CORD OF F-344 RATS. D. L. Hoskins* and T. Crisp. Department of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

Previous studies have shown a dramatic decline in the antinociceptive efficacy of δ opioid receptor agonists with advancing age. This study was performed to ascertain whether this may be due to either a decline in the total number of receptors (B_{max}), or a general decline in the affinity (K_d) of the δ opioid receptor for its substrate. Saturation studies were performed on P2 fractions from cortex, striatum and hippocampus; while S2 fractions were used for the brainstem and spinal cord. Membranes from young (5-6 months) mature (15-16 months) and aged (25-26 months)rats were incubated with increasing concentrations of ^{3}H-naltrindole (a δ- specific opioid antagonist) for 5 hours at 25°C. Preliminary Scatchard analysis reveals no age-related decrease in either affinity or B_{max}.

534.15

THE REGULATION OF ASTROGLIAL PROENKEPHALIN AND OPIOID RECEPTOR mRNA EXPRESSION BY INTERLEUKIN-1ß. B.B. Ruzicka*, R.C. Thompson, S.J. Watson and H. Akil, Mntl Hlth Res Inst, University of Michigan, Ann Arbor, Michigan, 48109-0720.

Opioids have been found to modulate the immune system by regulating the function of immunocompetent cells via both classical and non-classical opioid receptors. Several studies suggest that the interaction between the opioid and immune systems is not unidirectional, but rather, reciprocal, in nature. In the central nervous system (CNS), one cellular target of immune system activation is the astrocytes, glial cells which have been shown to synthesize the opioid peptide, pro-enkephalin (ENK), and, very recently, to express the μ, δ and κ opioid receptors.

To study the potential interaction between immune factors and the astroglial opioid "system", we examined the effect of interleukin-1ß (IL1ß, 1 ng/ml, 24 h treatment) on the levels of ENK and μ, δ and κ receptor mRNA expression in primary cultures of astrocytes derived from rat (postnatal day 1-2) cortex, striatum, cerebellum, hippocampus and hypothalamus. Preliminary data show that IL1ß regulated astroglial ENK mRNA levels in a brain region-dependent manner. IL1ß dramatically reduced (>50%) ENK mRNA levels in the hippocampal, but not in the other four astroglial cultures. The effect of IL1ß on the opioid receptor mRNA varied both with respect to the receptor type and brain region. IL produced a significant increase (70-80%) in the μ receptor mRNA levels in the striatal and cerebellar cultures, but had little effect on these levels in the cortical, hippocampal or hypothalamic cells. In marked contrast, IL1ß profoundly decreased (>70%) the levels of κ receptor mRNA in the cortical and hypothalamic astrocytes, but had little effect on these levels in the cultures derived from other brain regions. The effects of IL1ß on the levels of δ receptor mRNA are currently under investigation, as are the possible intracellular signalling cascades associated with the IL1ß-elicited effects. However, the present findings support an IL1ß-mediated regulation of astroglial ENK and opioid receptors *in vitro*, and suggest that the glial opioid "system" may be important in the IL1ß-initiated, coordinated response to CNS infection, trauma or injury. (Supported by the MRC of Canada and NIDA).

534.16

CHANGES IN δ-OPIOID RECEPTOR mRNA DETECTED IN NG108-15 CELLS BY RIBONUCLEASE PROTECTION ASSAY. E.A. Sehba, B.C. Yoburn, J.M. Carroll* College of Pharmacy and Allied Health Professions, St. John's University, Jamaica, N.Y. 11439

The measurement of δ-opioid receptor (DOR) mRNA provides an important insight into the mechanism of opioid receptor regulation. Both cDNA and riboprobes can be used to assess changes in DOR mRNA. However, using Northern analysis, it was difficult to detect DOR mRNA in total RNA preparations from NG108-15 cells with a cDNA probe. Therefore, we used a ribonuclease protection assay (RPA) which afforded enhanced sensitivity. The RPA was optimized for detection of DOR, glyceraldehyde phosphate dehydrogenase (GAPDH) and actin mRNAs in total RNA from NG108-15 cells. Two riboprobes of 1116 and 450 nt were prepared from the DOR-1 plasmid (Evans et al., *Science,* 258:1952, 1992). Hybridization with either probe generated a single protected band on PAGE. Cells were treated for 24-36hr with 200mM ethanol. Ethanol produced an increase in DOR mRNA compared to controls as detected by the RPA. The change in DOR mRNA was selective since no change in actin mRNA was observed. Currently, we are investigating the temporal relationship between increased mRNA and expression of functional receptor using a binding assay. This system will be used to study the effects of various agents on DOR mRNA and receptor protein expression in NG108-15 cells. (Supported by NIDA DA 04185)

534.17

3'-cDNA REGIONS NEGATIVELY INFLUENCE μ OPIATE RECEPTOR EXPRESSION. *P.S. Johnson*, J.B. Wang#, J.M. Wu#, C.E. Spivak#, G.R. Uhl#!@, C.K. Surratt#.* Molecular Neurobiology Branch#, Office of the Director!, Intramural Research Program/NIDA; Dept. of Neurology and Neuroscience@, Johns Hopkins School of Medicine.

cDNA clones encoding rat and human μ opiate receptors include products of alternative splicing that confer C-terminal receptor variant sequences, as well as lengthy stretches of 3'-untranslated sequence (3'-UTR). COS cells transiently expressing the μOR1 sequence with 30 bp of 3'-UTR sequence cloned into pCDNA-2 displayed [^{3}H]DAMGO and [^{3}H]naloxone binding B_{max} values 3- to 6-fold higher than those displayed by a μOR1 construction with 700 bp of 3'-UTR (μOR1-700). μOR1-700 expressed at levels several-fold higher than those of a hμOR1A splice variant (Bare et al. (1994) *FEBS Lett.* 354, 213) lacking μOR 3'-UTR. Both μOR1 and μOR1A display similar affinities for [^{3}H]DAMGO and both mediate opiate-induced adenylate cyclase inhibition. Conversely, a construction lacking the 33 C-terminal μOR amino acids and its 3'-UTR expressed at ca. 80% of wildtype levels. 3'-Translated and untranslated sequences play significant roles in μOR expression levels in an *in vitro* test system. Conceivably, these sequences could function to alter mRNA stability, protein stability, and/or mRNA translation rates to regulate μOR expression in neurons.

OPIOID RECEPTORS: LOCALIZATION

535.1

LOCALIZATION OF OPIOID RECEPTOR-LIKE IMMUNOREACTIVITY (OR-LI) IN PORCINE SMALL INTESTINE. M.A. Osinski, T.R. Kowalski, M.P. Murtaugh, R.P. Elde, and D.R. Brown*. Depts. of Vet. PathoBiology and Cell Biology & Neuroanatomy, Univ. Minnesota, St. Paul, MN 55108.

The localization of opioid receptors in the central and peripheral nervous systems has been limited to autoradiographic and in situ hybridization studies due to the lack of receptor-specific antisera. One of us (R.P.E.) has recently produced polyclonal antisera against unique peptide sequences of the mu (MOR) and delta (DOR) opioid receptors. An indirect immunofluorescence procedure was used to detect opioid receptor immunoreactivity of 10 μm-thick cryosections of Zamboni-fixed ileum from 8-12 week old pigs of both sexes. DOR-LI was observed in the myenteric and submucosal ganglia as well as in transected axonal profiles in the circular muscle layer of the muscularis externa. Some submucosal ganglia subjacent to ileal lymphoid follicles of Peyer's patch-containing intestinal segments also exhibited DOR-LI. In the mucosa, DOR-LI was displayed by nerve fibers in the lamina propria of the villi. MOR-LI was present in the submucosal and myenteric ganglia. Immunohistochemical staining with antiserum to [Leu]enkephalin revealed a pattern of immunoreactivity similar to DOR-LI. Thus, the structural data we describe agree with previously reported pharmacological data on the role of OR neurotransmission in the control of secretory and motor function of the mammalian small intestine. Additionally, the novel finding of OR-LI near immune structures suggests a possible role for OR in intestinal neuroimmune regulation.

535.2

EXPRESSION OF δ- AND κ-OPIOID RECEPTOR mRNA IN THE HUMAN NEOCORTEX. P. Schmidt[1*], A. Wevers[2], K. Maderspach[3], E. Cserpan[3], I. Cserpan[4], M. Staak[1], H. Schröder[2], [1]Inst. für Rechtsmedizin and [2]Inst. II für Anatomie, Univ. zu Köln, 50823 Köln, FRG, Inst. of [3]Biochemistry and [4]Genetics, Biological Research Center, Hungarian Academy of Sciences, 6701 Szeged, Hungary

The effects of opiates in the human central nervous system are mediated via at least three major classes of opioid receptors (OR): μ, δ and κ. By means of immunohistochemistry we have previously shown the localization of the κ-OR protein in layers II/III, V and partly VI of the human frontal cortex.

Presently the cellular expression of the δ- and κ-OR mRNA in different cortical areas (A4, A10, A17 according to Brodmann) is studied by non-isotopic in situ hybridization. Digoxigenin 3' endlabeled oligonucleotide probes were applied. Hybrids were visualized by alkaline phosphatase coupled anti-digoxigenin antibodies and incubation with NBT/BCIP. The mRNAs for the δ- and κ-OR were expressed in all cortical areas, especially in the pyramidal cells of layer III and V as well as in the multiform neurons of layer VI. In A4 the giant pyramidal cells and in A17 the stellate neurons stood out prominently labelled. In comparison, the transcripts of the δ-OR seemed to be more abundant than those for the κ-OR. For the latter, thus, a good correlation of mRNA and protein expression sites was encountered.

The combined cellular assessment of the mRNA is intended to be compared with the expression under pathological conditions, i.e. in drug-related fatalities of heroin/morphine addicts.

Supported by DFG, grant Schr 283/13-1, and by OTKA T6374, Hungary

535.3

LOCALIZATION OF DOR1 AND MOR1 IMMUNOREACTIVITY IN THE MONKEY CENTRAL NERVOUS SYSTEM. M. Riedl*, U. Arvidsson, R.J. Dado, A. Nakano,L. Vulchanova, P.-Y. Law, H.H. Loh, C.N. Honda, M.W. Wessendorf, and R. Elde. Departments of Cell Biology and Neuroanatomy and Pharmacology, University of Minnesota, Minneapolis, MN 55455.

Antisera to the cloned δ- and μ-opioid receptors have been raised in rabbits and extensively characterized(Dado et al., Neuroreport 1993, Arvidsson et al. J. Neurosci 1995 a, b). The distribution of DOR1- and MOR1-immunoreactivity (-ir) is generally consistent with the distribution of the receptors as ascertained by binding as well as in situ hybridization studies in the rodent brain. However, the efficacy of the antisera to recognize the receptor and the distribution of DOR1- and MOR1-ir has not been described in the primate brain. Therefore, three Maccaca mulatta monkeys (1.5 - 4 yrs.) were transcardially perfused with 4% paraformaldehyde, followed by 10% sucrose in PBS. The brain and spinal cord were blocked and 50 μ frozen sections were cut on a freezing microtome. The sections were then processed for immunohistochemistry. Prominent DOR1-ir was seen in the medial septal n., anterior hypothalamic area, globus pallidus, substantia nigra, periaqueductal gray, and spinal cord dorsal horn. Interestingly, most of the DOR1-ir was confined to axons, suggesting a presynaptic receptor. These results are similar to those reported in rat brain. Intense MOR1-ir was seen in the preoptic area of the hypothalamus, paraventricular n. of the thalamus, interpeduncular n., central linear raphe n., and spinal cord dorsal horn. In contrast to DOR1-ir, the major portion of MOR1-ir was seen in dendrites and cell bodies, suggesting a postsynaptic receptor. Supported by grants from NIDA.

535.4

THE MU OPIOID RECEPTOR IS EXPRESSED IN THE RETINA AND RETINAL-RECIPIENT NUCLEI. N. Brecha*, J. Johnson, R. Kui, B. Anton, D. Keith Jr., C. Evans, and C. Sternini. Depts. of Neurobiol., Med. and Psych., UCLA and VAMC-WLA, LA, CA 90073.

The purpose of this study was to determine the cellular localization of the mu opioid receptor in the rat visual system using immunohistochemistry with an affinity purified polyclonal antibody directed to the C-terminus of the mu receptor. Prominently immunostained medium to large cell bodies are found in the ganglion cell layer in all retinal regions. Immunoreactive (IR) processes are confined to the inner plexiform layer. Mu receptor-IR cells are likely to be ganglion cells. In retinal-recipient nuclei, mu receptor-IR is expressed by fibers. The highest level of mu receptor-IR is in the accessory optic nuclei (AON), and in the olivary pretectal nucleus. Moderate levels of IR are present in the superficial layers of the superior colliculus, the nucleus of the optic tract and the lateral geniculate nucleus (LGN) with stronger immunostaining in the ventral LGN and intergeniculate leaflet compared to the dorsal LGN. The lowest level of mu receptor-IR is in the suprachiasmatic nucleus. Retinal removal resulted in the elimination or reduction of mu receptor-IR in several retinal-recipient nuclei, including the LGN and the AON. These observations indicate that retinal ganglion cells give rise to the prominent mu receptor-IR fibers located in several retinal-recipient structures. These findings indicate that endogenous opioids can influence sensory processing in the visual system at mu receptors.

Supported by EY 04067, DA 05010, and VA Medical Research Funds.

535.5

ULTRASTRUCTURAL LOCALIZATION OF MU, DELTA AND KAPPA OPIOID RECEPTORS IN THE NUCLEUS SOLITARIUS. B.E. Maley*[1], M.G. Engle[1] and R.P. Elde[2], [1]Dept. of Anatomy and Neurobiology, Univ. of Kentucky Chandler Med. Ctr., Lexington, KY 40536 and [2]Dept. of Cell Biology and Neuroanatomy, Univ. of Minnesota Med. Ctr., Minneapolis, MN 55455.

Opioid peptides are known to have profound effects on the cardiovascular and respiratory system. One area which is known to be involved in the synaptic circuits regulating the cardiovascular and respiratory systems is the nucleus solitarius which we have reported previously to have significant amounts of enkephalin immunoreactivity. In the present study we report the immunohistochemical demonstration at the ultrastructural level of mu, delta and kappa opioid receptors along the neuronal membrane of nucleus solitarius cells. Male Sprague-Dawley rats were perfused with 4% paraformaldehyde-0.5% glutaraldehyde in Sorenson's phosphate buffer, pH 7.4. The caudal brainstem containing the nucleus solitarius was sectioned in the coronal plane. Sections were immunostained either with the peroxidase, anti-peroxidase method for pre-embedding immunocytochemistry or with colloidal gold for the post-embedding technique. All sections were embedded in LR White resin for ultrastructural analysis. Mu and kappa opioid receptors were associated with the neuronal membrane of presynaptic terminals and postsynaptic structures at both conventional postsynaptic specializations as well as at extra-synaptic sites. In contrast, delta opioid receptors were associated with presynaptic terminals. Results of the present investigation indicate that at least the mu and kappa opioid receptors are associated with many of the same structures which we have reported to be postsynaptic to the endogenous opioid peptide, enkephalin. The presence of mu opioid receptors at extra-synaptic sites suggests that opioid peptides may exert their influence at sites other than synaptic junctions. The association of delta opioid receptors with presynaptic terminals indicates that it acts in the presynaptic regulation of those structures.

535.6

LIGHT AND ELECTRON MICROSCOPIC LOCALIZATION OF μ-OPIOID RECEPTOR IMMUNOREACTIVITY WITHIN THE NUCLEUS OF THE SOLITARY TRACT. TA Milner*[1], PY Cheng[1], LY Liu-Chen[2], C Chen[2], VM Pickel[1], [1]Dept of Neurology and Neuroscience, Cornell Univ Medical College, New York, NY, 10021. [2]Dept of Pharmacology, Temple Univ School of Medicine, Philadelphia, PA, 19140.

Opioids within the nucleus of the solitary tract (NTS) are involved in analgesic, cardiovascular and respiratory responses to stress. To determine the potential cellular sites for the physiological effects of the opioids within the NTS of the rat brainstem, we examined the cellular localization of the μ-opioid receptor (MOR) using a rabbit polyclonal antiserum raised against a 17 amino acid peptide of the carboxyl terminal domain of the cloned receptor. By light microscopy, MOR-like immunoperoxidase labeling was detected throughout the rostrocaudal extent of the solitary tract and medial NTS. Electron microscopy established that varicose processes within the medial and commissural nuclei at the level of the area postrema were axon terminals. In addition, using silver-intensified immunogold, we more discretely demonstrated a preferential localization of MOR along plasma membranes of both axons and dendrites. These particles were not usually associated with synaptic specializations. Our results indicate that visceral responses to stress or pain are modulated by opioids acting non-synaptically on axons, which are most likely to be primary afferents, as well as target neurons within the NTS.
(Supported by an Aaron Diamond Foundation Postdoctoral Fellowship to PYC, NIDA DA04600 to VMP and DA04745 to LYLC).

535.7

ULTRASTRUCTURAL LOCALIZATION OF μ-OPIOID RECEPTOR IMMUNOREACTIVITY AND RELATIONSHIP TO ENKEPHALIN IN CERVICAL DORSAL HORN OF THE RAT SPINAL CORD. PY Cheng*[1], A Moriwaki[2], JB Wang[2], GR Uhl[2], VM Pickel[1]. [1] Dept of Neurology and Neuroscience, Cornell Univ Medical College, New York, NY, 10021. [2]Molecular Neurobiology Branch, Intramural Research Program, NIDA and Dept of Neurology and Neuroscience, Johns Hopkins Univ School of Medicine, Baltimore, MD, 21224.

We used a polyclonal rabbit antiserum against an 18 amino acid sequence from the carboxyl terminus of the μ-opioid receptor (MOR) to determine the potential cellular sites for the analgesic actions of opiates in the rat cervical spinal cord. By light microscopy, MOR-like immunoreactivity (MOR-LI) was most intensely localized, using either peroxidase or gold-silver labeling methods, to processes in lamina I and II of the dorsal horn. Electron microscopic examination of this region revealed prominent localization of immunogold-silver labeling for the MOR specifically along membranes of dendrites, axons and axon terminals. The majority of the gold-silver particles were associated with portions of the dendritic membrane not contacted by afferent terminals. We have also shown by dual labeling that Leu[5]-enkephalin (LE) was sometimes present in axon terminals which contacted the dendrites showing MOR-LI. However, the majority of the LE-labeled axon terminals did not directly appose either dendrites or axons within the plane of sections that contained MOR-LI. These results provide the first direct evidence that the MOR is preferentially localized both to non-synaptic as well as possible synaptic sites along the membrane of dendrites and axons within the superficial layers in the dorsal horn of the rat cervical spinal cord. Activation of the receptors by LE or exogenous opiates thus may produce analgesia through modulation of the postsynaptic receptivity or presynaptic release of other transmitters within superficial laminae of the spinal cord. (Supported in part by an Aaron Diamond Foundation Postdoctoral Fellowship to PYC, NIDA DA 04600 and HL 18974 to VMP and NIDA Intramural Research Program).

535.8

CELLULAR DISTRIBUTION OF DELTA OPIOID RECEPTORS IN THE RAT SPINAL CORD: CONFOCAL AND EM ANALYSIS WITH THREE-DIMENSIONAL RECONSTRUCTION. M. Gastard*, P. Mailly, M. Conrath. Laboratoire de Neurobiologie des Signaux Intercellulaires, Universite P. & M. Curie, 7 quai Saint-Bernard, 75252, Paris Cedex 05, France.

Using a monoclonal anti-idiotypic antibody and immunocytochemistry, we studied the distribution of delta opioid receptors in the spinal cord of rats fixed with 4% paraformaldehyde. Confocal microscopy revealed that delta opioid receptor immunoreactivity was present in many labeled axons of the superficial dorsal horn (notably lamina I) and of lamina X. EM revealed that most of immunoreactivity was associated with the plasma membrane at the interface between adjacent neurons. In lamina I, we also found membrane labeling between two axon terminals confirming the presynaptic localization of delta receptors. We found no immunolabeling at synaptic specializations.

Labeled cells bodies were found in lamina X and in ventral horn. To study the 3-D intracellular distribution of delta receptor immunoreactivity, we collected a confocal Z series of cell bodies and generated a 3-D reconstruction. Approximately 60% of total cell body immunoreactivity was found in the cytoplasm, primarily around the nucleus. EM analysis of the latter revealed immunoreactivity associated with rough ER and Golgi apparatus. About 10% of the labeling was found in the nucleus and EM analysis showd that it was associated with chromatin. This result suggests an involvement of delta receptors in long term adaptation processes as well as in dorsal horn antinociceptive mechanisms.

535.9

EXTRA-SYNAPTIC NEURONAL SITES FOR ENKEPHALIN ACTIVATION OF μ-OPIOID RECEPTORS IN RAT NUCLEUS ACCUMBENS.

A.L. Svingos*[1], A. Moriwaki [2], J.B. Wang [2], G.R. Uhl [2] and V.M. Pickel [1], Cornell Univ. Med. College, Dept. of Neurology and Neuroscience, NY, NY, 10021[1]. Intramural Res. Program, NIDA and Depts. of Neurology and Neuroscience, Johns Hopkins Univ., Baltimore, MD, 21224[2].

The μ-opioid receptor (MOR), and its endogenous ligands, including Leu[5]-enkephalin (LE), are abundantly distributed in the nucleus accumbens, a region implicated in opiate reinforcement. To determine ultrastructural sites for functions ascribed to MOR, we used an anti-MOR rabbit polyclonal antiserum raised against an 18 amino acid sequence from the C-terminal unique to MOR (Surratt *et al.*, 1994) to examine the immunoperoxidase and immunogold-silver localization of the receptor in rat nucleus accumbens. Electron microscopy localized MOR-like immunoreactivity predominantly to extra-synaptic sites in plasma membranes of dendrites. Unmyelinated axons and axon terminals were also immunoreactive for MOR. Cytoplasmic labeling was largely associated with smooth endoplasmic reticulum in dendrites, and small clear vesicles in axons. We subsequently combined immunogold-silver labeling for MOR with immunoperoxidase detection of LE to determine potential sites for MOR activation by the endogenous ligand. LE labeled terminals formed symmetric synapses with dendrites both with and without detectable MOR-like immunoreactivity. The gold-silver MOR labeling was localized chiefly along portions of plasma membrane exclusive of the synaptic specialization. These results suggest that in rat nucleus accumbens, activation of MOR by endogenous opioid peptides or by exogenous opiates results primarily in neuronal modulation at extra-synaptic sites. This modulation most likely includes changes in the receptivity of target dendrites to other transmitters, and presynaptic modulation of transmitter release. (Supported by the Aaron Diamond Foundation to ALS, NIDA DA04600 to VMP and the NIDA Intramural Research Program).

535.10

ULTRASTRUCTURAL IMMUNOCYTOCHEMICAL LOCALIZATION OF MU OPIOID RECEPTORS AND LEU[5]-ENKEPHALIN IN PATCH COMPARTMENT OF RAT CAUDATE-PUTAMEN NUCLEUS.

H. Wang*, A. Moriwaki°, J.B. Wang°, G.R. Uhl° and V.M. Pickel. Dept. of Neurol. and Neurosci., Cornell Univ. Medical College, New York, NY 10021, and °Mol. Neurobiol. Branch, IRP, NIDA, NIH and Johns Hopkins Univ. School of Medicine, Baltimore, MD 21224.

The mu opioid receptor (MOR) is highly enriched in striatal patch zones. To determine cellular sites for MOR activation in the patch compartment, we examined the ultrastructral localization of MOR and one of its endogenous ligands, Leu[5]-enkephalin (LE), in rat caudate-putamen nucleus, using combined immunogold-silver and immunoperoxidase methods. The anti-MOR antibody was raised against the C-terminal 18 amino acids of the rat MOR-1 receptor (Surratt et al. (1994), JBC, 269, 20548), and shown by light microscopy to be specifically localizied in the patch compartment. Electron microscopy of the patch region showed immunogold-silver particles localizing MOR like immunoreactivity (LI) mainly associated with non-synaptic plasma membranes of soma and dendrites. These were typically spiny neurons receiving abundant asymmetrical excitatory-type synapses from unlabeled axon terminals. Other axon terminals in contact with these neurons formed symmetric junctions, and were also either unlabeled or immunolabeled for LE. MORLI was infrequently found in axon terminals, some of which colocalized LE. We conclude that endogenous opioids and morphine act mainly at non-synaptic MOR reactive sites along the plasma membrane of spiny neurons in rat caudate-putamen nucleus.

Supported by NIDA DA 04600 & NIDA Intramural Research Program.

535.11

IMMUNOHISTOCHEMICAL MAPPING OF μ-OPIOID RECEPTOR IN THE CNS OF BOTH RAT & MOUSE

B. Anton*, D.E. Keith Jr, J. Rogowski, C. Yan, X. Li, T. To, and C.J. Evans. Dept. of Psychiatry and Biobehavioral Sciences, UCLA, Los Angeles, California 90024.

We have generated a rabbit peptide antisera (Cμ-12) to the C-terminus of a murine μ-opioid receptor recently cloned in our laboratory (Kaufman et al., *J. Biol. Chem.*, in press). Histochemistry used antigen-affinity purified Cμ-12 and revealed similar but not identical distribution of μ-opioid receptor-like immunoreactivity (MOR-LI) in rat and mouse neuroaxis. Localization of MOR-LI showed a good correlation with the previously reported distribution of μ-opioid receptor binding sites and μ-opioid receptor mRNA in mouse and rat brain. High to very high expression of MOR-LI material was found in multiple areas of diencephalon, mesencephalon, pons-medulla , dorsalmost aspects of spinal cord and deep cerebellar nuclei. Within most of all these structures, staining was predominantly localized in long fibers, short dendritic-like processes and fine puncta. Overall, most of the telencephalic regions showed low labeling. However, notable exceptions were the olfactory areas as well as the caudate-putamen and nucleus accumbens. These two latter subcortical regions showed a characteristic patchy-like distribution of MOR-LI mostly found in neuropil. Staining in perikarya was rarely observed throughout most of the neuroaxis, although moderately labeled scattered cell bodies were observed in specific cellular layers of few brain areas such as the neocortex, ventral aspects of the nucleus accumbens and hippocampus. Our results also revealed fine anatomical differences in MOR-LI between rat and mouse brain specifically in areas such as the olfactory bulb, neocortex, hippocampus and ventral horn in spinal cord. These latter results suggest the possibility of differential circuitry modulation by MOR-specific drugs between these two rodent species.

Supported by NIDA DA05010 and the W.M. Keck Foundation

535.12

KAPPA OPIOID RECEPTOR-LIKE IMMUNOREACTIVITY IS PRESENT IN SUBSTANCE P-CONTAINING SUBCORTICAL PROJECTIONS TO GUINEA PIG DENTATE GYRUS.

C.T. Drake* and T.A. Milner, Dept. of Neurology and Neuroscience, Cornell University Medical College, New York, NY 10021

We have previously used light and electron microscopy to demonstrate kappa opioid receptor-like immunoreactivity (κ-LI) in axons and terminals in the granule cell layer and inner molecular layer of the guinea pig dentate gyrus. Although several populations of neurons project to this area, the distribution and ultrastructural appearance of processes with κ-LI most strongly resembled previous descriptions of the substance P (SP) afferents arising from the supramammillary region of the hypothalamus (SUM). In the present study we used dual labeling electron microscopy to determine whether κ-LI is colocalized with SP-LI in the dentate gyrus, and unilateral fornix lesions to determine whether the processes with κ-LI originate extrinsically. By light microscopy, both κ-LI and SP-LI were present in varicose processes concentrated in the granule cell layer and supragranular region. By electron microscopy, κ-LI was in small unmyelinated axons and axon terminals, where it was predominantly associated with large dense-core vesicles, but was also localized to the plasmalemma and small vesicle membranes. SP-LI was found in axons and terminals with similar morphology, and rimmed small vesicles and a few dense-core vesicles. A number of terminals colocalized both κ-LI and SP-LI. Both single- and double-immunolabeled terminals formed predominantly asymmetric synapses with large dendrites and perikarya of granule cells. In fornix-lesioned animals, by light microscopy processes with κ-LI and processes with SP-LI were dramatically decreased in the supragranular region unilateral to the lesion. These findings suggest a role for κ receptors in presynaptic modulation of an extrinsic SP-containing projection to the dentate gyrus. Supported by DA08259, DA07274.

535.13

REGIONAL AND TEMPORAL DIFFERENCES IN μ, δ, AND κ OPIOID RECEPTOR EXPRESSION BY MURINE TYPE 1 ASTROCYTES FROM THE CEREBRAL CORTEX, HIPPOCAMPUS, CEREBELLUM, AND STRIATUM *IN VITRO*.

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Flat, polyhedral (type 1) astrocytes from mouse forebrain express μ, δ, and κ opioid receptors (ORs) in dissociated culture. To determine whether the heterogeneity of OR types expressed among type 1 astrocytes results from regional or temporal differences in the pattern of expression, primary, mixed-glial cultures were obtained from the cerebral cortex, hippocampus, cerebellum, and striatum of 1-day-old mice and examined at various days *in vitro* (DIV). OR types were identified immunocytochemically using antibodies selective for unique μ and δ OR epitopes. OR types were assessed functionally using fura-2-based measures of opioid-induced mobilization of free intracellular calcium ($[Ca^{+2}]_i$) following activation by selective κ, δ, or μ agonists. At 6 DIV, μ, δ,and/or κ ORs were expressed by subpopulations of type 1 astrocytes in all regions, although the proportion of astrocytes expressing each OR type varied among regions. Based on immunocytochemical evidence, hippocampal astrocytes had the greatest proportion of μORs (~60-75%), while astrocytes from other regions had fewer (25-40%). δORs were most abundant in striatal astrocytes (60-65%), but least abundant in hippocampal astrocytes (~35%). Temporal differences were evident in opioid-induced changes in $[Ca^{2+}]_i$. Hippocampal and cerebral cortical astrocytes expressed ORs at 1-2 DIV, whereas astrocytes from striatum and cerebellum did not express ORs until 6 DIV. Spatio-temporal differences in the expression of particular OR types by astroglia may, in part, underlie region-specific critical periods of sensitivity to opioids during development, and may contribute to regional differences in opioid function in the adult. Supported by NIDA grant 06204.

535.14

IMMUNOHISTOCHEMISTRY FOR ORL-1 IN THE RAT CNS

C.J.Evans*, J. Fein, T. To, J. Rogowski, X. Li, L. Silberstein & B. Anton. Dept. of Psychiatry and Biobehavioral Sciences, UCLA, Los Angeles, CA 90024 & San Jose State University, San Jose, CA.

We have generated a murine IgG1 monoclonal antibody (mN18-ORL-1) to the N-terminus of the mouse ORL-1 protein using a MAPS-based synthesis of the peptide MESLFPAPFWEVLYGSHF. Localization of ORL-1-like immunoreactivity (ORL-1-LI) showed a good correlation with the neuroanatomical distribution of ORL-1 mRNA in rat brain. High to very high labeling to ORL-1 was observed in multiple areas from the diencephalon, mesencephalon, pons/medulla and ventral and dorsal horns in the spinal cord. In the telencephalon, the pattern of expression of ORL-1-LI was more variable being very high in a dense neuropil distributed through cortical layers II-V in the neocortex, in the anterior olfactory nuclear complex, pyriform cortex, in CA1-CA4 fields and dentate gyrus in the hippocampus and in most of the septal and basal forebrain areas. In contrast, low labeling was observed in the olfactory bulb and most of the basal ganglia structures. In the cerebellum ORL-1-LI was highly expressed in the deep nuclei and no specific labeling was observed in any of the cerebellar cortical layers. ORL-1-LI was predominantly localized in long fiber processes and short dendritic-like fibers and fine puncta. Perikaryal staining was rarely observed through-out most of the stained areas, although low to moderately labeled scattered cell bodies were observed in few brain areas such as the hilus dentate in the hippocampus, caudate-putamen and cortical layers III-IV in the neocortex. Currently the endogenous ligand for ORL-1 has not been chemically identified thereby making difficult to hypothesize putative functional circuitry. However, our immunohistochemical findings enable us to anticipate a key role of the ORL-1 system in pain and analgesia, cortical olfactory processing, auditory and visual processing, hypothalamic autonomic functions, motor and sensory control, as well as a major role in learning and memory. Supported by NIDA DA05010 & W.M. Keck Fdn

535.15

CHROMOSOMAL LOCALIZATION AND EXPRESSION PATTERN IN NERVOUS AND IMMUNE SYSTEMS OF AN OPIOID-LIKE ORPHAN RECEPTOR. H. Matthes*, C. Gaveriaux-Ruff, K. Befort, F. Simonin, M.G. Mattei, C. Lemoine, B. Bloch and B. Kieffer. Laboratoire Protéines et Récepteurs Membranaires, C.N.R.S. UPR 9050, ESBS, Université Louis Pasteur, Bld. Sébastien Brandt, F-67400 Illkirch, France.

We have isolated an opioid like receptor cDNA from mouse brain by low stringency hybridization using a μ mouse cDNA as a probe. Sequence analysis of this clone indicated that it contains an open reading frame encoding a 367 amino acid protein which shows high homology to all three opioid receptors, δ, μ and κ and suggests that we have cloned the mouse counterpart of an orphan receptor cDNA isolated from rat and human by other authors during the course of this study. Expression of this cDNA in COS-7 cells did not confer opioid binding properties to the cells supposing that it does not encode an opioid receptor. Mapping studies have allowed us to assign the gene to mouse chromosome 2 region H and to the human chromosome 20 position q13.2-q13.3. *In situ* hybridization revealed that this putative receptor is highly expressed in several mouse brain areas, including the cerebral cortex, thalamus, habenula, hypothalamus, locus coerulus and spinal cord. Using the RT-PCR technology, we have investigated the expression pattern of the transcript in human brain and found it widely represented in most tested brain structures. The transcript was also found in some immune cells.

OPIOIDS: ANATOMY, PHYSIOLOGY AND BEHAVIOR II

536.1

EFFECTS OF INTERFERON ALPHA UPON MORPHINE INDUCED IMMUNOLOGIC CHANGES IN SPINAL CORD-INJURED RATS. N.R.S. Hall*, J.B. Gelderd^ and Maureen P. O'Grady. Dept. of Psychiatry, Univ. of South Florida Coll. of Med., Tampa, FL 33613 and ^Dept. of Human Anatomy, Texas A&M Univ. Coll. of Med., College Station, TX 77843-1114.

Previous studies in our laboratory have revealed that the administration of morphine to spinal cord-injured rats can result in attenuation of a number of immunologic measures, including a significant reduction in spinal cord levels of IL-1. In other studies, we have confirmed that interferon alpha can bind to opioid receptors and that under certain conditions, partially attenuate the behavioral consequences of opioid withdrawal. The present study was designed to assess whether interferon alpha could reverse the immunomodulatory effects of morphine in a spinal cord transection model.

Adult male Sprague Dawley rats were anesthetized and a dorsal midline incision was made in the mid-thoracic region. A laminectomy was performed at the T5-T6 vertebral level, and the spinal cord was transected with a scalpel. A 75 mg pellet of morphine was implanted beneath the skin at the time of surgery. This resulted in plasma levels in excess of 250 ng/ml. Interferon alpha was administered at a concentration of 300 x 10^3 Units per 0.1ml via an Alzet mini-osmotic pump (model # 1003D; mean pumping rate=1.02 microliters/hr, duration= 3 days). While morphine administration resulted in a significant reduction in thymus and spleen weight ($p<0.05$), the administration of interferon alpha failed to modulate this influence. Furthermore, there was no effect of this combination of morphine and interferon alpha upon lymphocyte blastogenesis. Thus, while interferon alpha is capable of inducing behavioral and endocrine changes associated with its opioid properties, these effects do not extend to the assessed measures of the immune system using the described protocols. These studies were supported in part by grants from NIDA (DA05723, DA072450).

536.2

ARE ENDOGENOUS OPIATES INTEGRAL TO THE HEALING PROCESS? O.B. Ilyinsky*, P. Shirley, J. Stray-Gundersen Baylor/UT Southwestern Sports Science Research Center, Dallas, TX 75246

Studies performed in Russia demonstrated that opiates can stimulate nerve fiber growth in cell culture. Direct stimulation of the periaqueductal grey substance increased velocity of wound healing. Transcranial electrical stimulation (TES), used as "electroanalgesia", increased peripheral nerve regeneration and increased skin wound healing by 20% to 25%. To test the hypothesis that endogenous opiates are integral to the healing process, we created full thickness skin wounds ($331 \pm 53 mm^2$) on 172 male outbred Wistar and Sprague-Dawley rats (54-132 days). Rats were randomly separated into 5 groups over 5 experiments including TES (n=59, rats restrained, anode/behind pinnae, cathode/nasal bridge, 30-60 mins on Days 1,2,3,4 post wound, 1.2-1.5mA, 72 Hz), SHAM (n=32, restrained plus electrode placement, no current), OP (n=10, Dalargin a leu-enkephlin analog, 20ug/kg I.P. on Days 1,2,3,4), NAL+TES (n=12, same as TES plus Naloxone 2.5 mg/kg I.P. q 15 mins during TES) and CON (n=59, no treatment to wound). Each experiment had 3 treatments (Exps 1,2,3 included TES, SHAM and CON; Exp 4 TES, OP, CON; and Exp 5 TES, NAL+TES, CON). Two wound diameters were measured on Days 4, 7 and 12 post wounding by micrometer by an investigator blind to rat group. Wound photos were also taken, images digitized and areas calculated by an image analysis program. Results are presented as the differences between different group means (mean(SE)). A Multiple Cell Means analysis was performed using 5 groups, 1st or 2nd wound, younger (<63 days) or older (>63 days), and 2 strains. Significance was assigned at the $p<0.05$. There were no differences between SHAM and CON or between strains. TES wounds were significantly smaller than CON on all Days (D4-5.4%(SE=2.3%), D7-7.9%(2.1%), D12-4.6%(1.1%)). OP wounds were significantly smaller than CON on Day 12 only (7.2%(2.6%)). NAL wounds were significantly larger than CON on all Days (D4 21.7%(4.9%), D7 23.7%(4.5%), D12 5.8%(2.3%)). TES made a greater difference in older rats. These results confirm the involvement of endogenous opioids in the healing process. Further, TES and OP demonstrated potential as a method to augment healing. These results are in agreement with the earlier results, however, the magnitude of the improvement from TES is much less (6% vs. 20%). The fact that TES was more effective in older rats and rats residing in Russia suggests that TES may be more effective where the healing process is suboptimal.

536.3

THE SUPPRESSIVE EFFECT OF NEUROPEPTIDE Y ON FENTANYL-INDUCED MUSCULAR RIGIDITY IN THE RAT. P.W. Lui*, S.J Lai, L.Y. Tsen, M.J. Fu and Samuel H.H. Chan. Dept. of Anesthesiology, Veterans General Hospital-Taipei, and Institute of Pharmacology, School of Medicine, National Yang-Ming University, Taipei, Taiwan 11217, Republic of China.

The present study was to investigate the effect of centrally administered neuropeptide Y (NPY) on fentanyl-induced muscular rigidity in rats. Male Sprague-Dawley rats were anesthetized with ketamine (120 mg/kg, i.p.) and their lungs were mechanically ventilated. Intravenous administration of fentanyl (100 μg/kg) consistently evoked a significant increase in the root mean square of the electromyographic (RMS-EMG) activity recorded from the sacrococcygeal dorsalis lateralis muscle. This implied muscular rigidity was appreciably diminished by intracerebroventricular administration of NPY (4 nmol/2.5 μl). Microinjection of NPY (40 or 160 pmol) into locus coeruleus (LC) dose-dependently inhibited the activation of RMS-EMG by fentanyl. However, LC microinjection of 160 pmol NPY plus antiserum against NPY (NPYAb, 1:20), but not plus normal rabbit serum (NRS), failed to suppress fentanyl-induced EMG activation. In addition, LC microinjection of NPYAb or NRS did not inhibit fentanyl-induced muscular rigidity. Our results suggest that NPY can suppress fentanyl-induced muscular rigidity at the LC, but endogenous NPY may not be involved in the modulation of this phenomenon. Supported by grant from the National Science Council (NSC-84-2331-B075-009, PWL).

536.4

PERIPHERAL ADMINISTRATION OF MORPHINE REDUCES THE CONSEQUENCES OF INFLAMMATION PRODUCED BY TURPENTINE IN ADULT RAT URINARY BLADDER Nicholai Y. Andreev , Natalia I. Dmitrieva, Andrew S.C. Rice* and Stephen B. McMahon. Department of Physiology, St.Thomas's Hospital, London, SE1 7EH, U.K.

We have asked if peripheral opiate treatment can attenuate the visceral hyper-reflexia associated with experimental inflammation of the urinary bladder. In anaesthetised female Wistar rats the urinary bladder was catheterised via the urethra. Bladder motilty was asssesed by continuous intra-vesical pressure measurment during slow bladder filling with saline. The following were derived from the pressure recording: the micturition threshold (MT), the total time during which the bladder generated reflex contractions (TCT) and number of contructions (NC). After control determinations, the bladder was treated with 0.5 ml of 50 % turpentine for 1 hour. After this, the bladder was emptied and motility re-assessed. In all cases the inflammation produced by turpentine led to a hyper-reflexia, observed as a reduction in MT and increase in TCT and NC. The ability of morphine (0.1-0.5 mg/kg) to reverse the hyper-reflexia was then determined by either i.v. or intravesical (i.e. peripheral) administration. The threshold dose for i.v. administration was 0.25 mg/kg. When the same or smaller total amounts of morphine were administered locally, an inhibition of bladder motility was more potent. Thus, 0.1 mg/kg inhibited motility by 46%, compared to no changes after i.v. application of the same dose. All these effects of morphine were naloxone-reversible.

In separate animals the effect of morphine on plasma extravasation in rats' urinary bladder after treatment with turpentine was studied. Morphine (0.5 mg/kg) administered into the bladder significantly reduced the Evan's blue extravasation resulting from turpentine treatment.

These results suggest that opioids are capable of acting peripherally, perhaps on the terminals of bladder afferents, to reduce consequences of inflammation.

536.5

EFFECTS OF NOR-BINALTORPHIMINE (nBNI) AND QUATERNARY ANALOGS OF nBNI ON CI-977-INDUCED DIURESIS AND ANALGESIA. C.A.Paronis*[1], M.Ko[2], K.Sobczyk-Kojiro[3], H.I.Mosberg[3], and J.H.Woods[1,2], Dept. of [1]Pharmacology, [2]Psychology, and [3]Medicinal Chemistry, University of Michigan, Ann Arbor, MI 48109-0632.

Quaternary analogs of the selective *k*-antagonist, nBNI, were synthesized (single quaternary - QnBNI; double quaternary - QQnBNI) and shown in binding studies to be more polar than the parent compound. We examined the *k*-antagonist properties of nBNI, QnBNI, and QQnBNI *in vivo*, postulating that the more polar forms of nBNI would not penetrate the blood brain barrier (BBB). Diuresis, a potentially peripherally mediated response, and analgesia, a centrally mediated response, were measured in rats. The antagonists were injected either s.c. (1-32 mg/kg) or i.c. (1-100 μg) 1 hr before the *k*-agonist, CI-977. CI-977 (0.001-32 mg/kg) dose-dependently increased diuresis; 0.56 mg/kg CI-977 produced a maximum effect (4.7 ml/100g). The CI-977 diuresis dose-effect curve was shifted down in an orderly fashion by either s.c. or i.c. administration of nBNI, QnBNI, or QQnBNI, and no differences among the three nBNI analogs were noted. Analgesia was assessed using a warm-water tail-withdrawal procedure, the control ED_{50} for CI-977 was 0.7 mg/kg. The CI-977 analgesia dose-response curve was shifted to the right in a similar manner after central injections of either nBNI, QnBNI, or QQnBNI (10 μg i.c.). Likewise, systemic injections of nBNI, QnBNI, or QQnBNI (10 mg/kg s.c.) also produced similar rightward shifts of the CI-977 dose-effect curve. Our results suggest that nBNI, QnBNI, and QQnBNI do not differ their capacity to penetrate the BBB. (Supported by PHS Grants DA00254 and DA05653).

536.6

INTERACTION BETWEEN OPIOID RECEPTOR SUBTYPES AND MIANSERIN . C. G. Pick[1]*, M. M. Backer[1] and S. Schreiber[2,1] [1]Department of Anatomy and Anthropology Sackler Sch. of Med., Tel Aviv Univ. Tel Aviv, Israel. [2]Department of Psychiatry Hadassah Univ.Hospital Ein-Karem, Jerusalem, Israel.

Mianserin is a tetracyclic antidepressant with potent 5-HT_1, 5-HT_2 and 5-HT_3 receptor agonist. It has NE properties, due to a presynaptic $\alpha 2$ blockade and a strong antihistamine. In this present study we have examined the analgesic effects of Mianserin alone and in conjunction with opioid subtypes. Mianserin produced analgesia in the mice hotplate assay. This analgesia was antagonized by naloxone. This sensitivity to naloxone indicated that at least some of the analgesic effect of mianserin is mediated by an opioid mechanism of action. Systemic mianserin analgesia was reversed by ß-FNA and nor- BNI, but not nalorphine. The reversal of mianserin analgesia by ß-FNA and nor-BNI and not by the other antagonists, indicated a possible μ and $\kappa 1$ mechanism and argued strongly against either δ or $\kappa 3$ component of analgesia.

536.7

KAPPA OPIOID RECEPTORS IN THE GUINEA PIG HIPPOCAMPUS ARE FUNCTIONALLY COUPLED TO A 4-AP-SENSITIVE POTASSIUM CHANNEL. M. L. Simmons* and C. Chavkin. Dept. of Pharmacology, Box 357280, Univ. of Washington, Seattle, WA 98195.

Kappa opioid receptors in the CA3 region of the hippocampus are localized on mossy fiber terminals. Activation of these receptors inhibits excitatory transmission by inhibiting excitatory amino acid release. Previous work in our lab has shown that kappa receptors in the guinea pig hippocampus are not functionally coupled to L-type or N-type calcium channels, as antagonists of these channels did not occlude the inhibitory effect of a kappa receptor agonist. We have continued these studies in CA3 pyramidal cells using whole-cell voltage-clamp recording. The kappa receptor agonist U69593 (1 μM) produced a 33.3 ± 6.7% (n=6) decrease the amplitude of EPSCs evoked by low-intensity stimulation of stratum granulosum. This inhibition was reversed by 100 nM norbinaltorphimine, a kappa receptor antagonist. The inhibitory effect of U69593 was not blocked by prior bath-application of 300 μM $BaCl_2$, suggesting that kappa receptors are not coupled to inward rectifier potassium channels. Instead, the inhibitory effect of U69593 was blocked by 100 μM 4-aminopyridine (4-AP). These results suggest that kappa receptors on mossy fiber terminals inhibit excitatory amino acid release by increasing the potassium conductance, thereby decreasing the excitability of the presynaptic membrane. DA04123 and Merck Distinguished Scholar Fellowship (predoctoral).

536.8

EFFECTS OF CHRONIC U50,488H ADMINISTRATION ON THE $\kappa 1$ OPIOID RESPONSE IN THE GUINEA PIG HIPPOCAMPUS W. Jin*, G.W. Terman and C. Chavkin. Dept. of Pharmacol., Univ. of Wash., Seattle, WA 98195

Previously studies in our laboratory demonstrated that activation of the $\kappa 1$ opioid receptor by either exogenous or endogenous opioids inhibits dentate granule cell excitability in the guinea pig hippocampus. In the present study, we investigated whether these electrophysiological effects can be modulated by chronic administration of U50, 488H, a κ opioid agonist. Injection of guinea pigs with U50, 488H (s.c.) twice a day in an ascending dosage schedule (10 mg-75 mg/kg) for five days resulted in a tolerance to the drug hypothermic effects. Population spikes of dentate granule cells in the guinea pig hippocampus slice were recorded extracellularly. In naive animals, application of U69, 593, a κ_1 agonist, produced a dose-dependent inhibition of the amplitude of the population spike. The concentration required to produce a half-maximal effect (EC50) was 11 nM. In the tolerant animals, the concentration-response curve was shifted to right (EC50=53 nM), with a decrease of maximal effect. This result suggested that a reduction in the receptor reserve may be involved in the decreasing sensitivity to U69,593. It is known that the endogenous κ opioids released by prolonged tetanic stimulation to the perforant path can inhibit the stimulation-induced long-term potentiation (LTP) at the perforant path-granule cell synapse. We found that the LTP induced by the prolonged tetanic stimulation was significantly enhanced in the tolerant animals, indicating the chronic U50, 488H treatment attenuated the effects of endogenous κ opioids. The LTP induced by a moderate tetanic stimulation (not involved in release of endogenous κ opioid) remained unchanged in the tolerant animals. To test the idea that the NMDA receptors are involved in the development of κ opioid tolerance process, we pretreated animals with MK-801 (0.10 mg/kg, 15 min prior to each injection of U50, 488H), and our preliminary result suggested that MK-801 did not prevent the development of tolerance to either the electrophysiological effect or hypothermic effect of κ_1 opioids. These studies provided a better understanding of the mechanisms underlying κ opioid tolerance in the guinea pig hippocampus. Supported by DA04123.

536.9

(-)-PENTAZOCINE ANALGESIA IS MEDIATED THROUGH KAPPA$_1$ OPIOID RECEPTORS AND IS MODULATED BY AN ANTI-OPIOID SIGMA$_1$ SYSTEM. C.-C. Chien*[#] and G.W. Pasternak. The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Depts. of Neuroscience and Pharmacology, Cornell Univ. Medical College, New York, NY, [#]Cathay General Hospital, Taipei, Taiwan

Pentazocine is a clinically useful pain killer with limited analgesic potency. In thermal antinociceptive assays, like the mouse radiant heat tailflick assay, pentazocine has a maximal analgesia effect of only 30%. When the stereoisomers of pentazocine are tested separately, only (-)-pentazocine produces analgesia. (+)-Pentazocine is inactive. Receptor binding assays show that (-)-pentazocine labels μ and κ_1 receptors with high affinities and σ_1 receptors with moderate affinity whereas (+)pentazocine potently labels σ_1 receptors and has no significant affinity for opioid receptors. (-)-Pentazocine analgesia is antagonized both by the κ_1-selective antagonist norBinaltorphimine (norBNI) and by an antisense oligodeoxynucleotide directed against the cloned kappa receptor (KOR-1), implying a role for κ_1 receptors. The μ-selective antagonist β-funaltrexamine has no effect. Prior studies indicate that κ_1 analgesia mediated by the prototypical κ_1 analgesic U50,488H is significantly modulated by a σ_1 anti-opioid system. (-)-Pentazocine analgesia is similarly affected. When co-administered with the σ antagonist haloperidol, the (-)-pentazocine dose-response curve shifts to the left with a rise in the ceiling response from 30% to 70%. The inactivity of the D_2 antagonist (-)-sulpiride to modulate (-)-pentazocine analgesia confirms the σ_1 specificity of the haloperidol in this system. In conclusion, we show that κ_1 receptors mediate (-)-pentazocine analgesia and this analgesia is modulated by an anti-opioid σ_1 system.

536.10

DISSOCIATION OF THE MOTOR EFFECTS OF (+)-PENTAZOCINE FROM BINDING TO $\sigma 1$ SITES. R.R. Matsumoto*, A.C. Zambon, W.D. Bowen, J.M. Walker, S.L. Patrick, V.N.T. Vo, D.D. Truong, B.R. de Costa and K.C. Rice. University of California Irvine, Irvine, CA 92717; Brown University, Providence, RI 02912; NIDDK, Bethesda, MD 20892.

The purpose of this study was to determine whether a relationship existed between the motor effects produced by (+)-pentazocine (PENT) and its binding to $\sigma 1$ sites. Scatchard analyses revealed decreased [^{3}H]PENT binding in middle aged (5-6 months old) vs. young adult (2-3 months old) rats ($P<0.001$). However, there was no difference between the extent of circling behavior or dystonia produced by microinjection of PENT (9.3 nmol) into the substantia nigra or red nucleus in the two groups of animals. There was also a significant decrease in [^{3}H]PENT binding in rats chronically treated with haloperidol (5 mg/kg, s.c., up to 20 days; $P<0.05$). However, despite the reduction in [^{3}H]PENT binding, there was no difference between the extent of dystonia produced by unilateral intrarubral microinjection of PENT into animals chronically treated with haloperidol vs. saline. The dystonic postures produced by PENT could not be attenuated with the putative σ antagonist BD1047 or the opiate antagonist naloxone. However, (+)-nordihydrocodeinone, partially attenuated the postural effects of PENT ($P<0.03$), despite its very low affinity for $\sigma 1$, $\sigma 2$, or opiate receptors. Taken together with previous studies, the results suggest that [^{3}H]PENT is a potent and selective probe for $\sigma 1$ binding sites *in vitro*, but the *in vivo* effects of PENT cannot be fully attributed to actions at this site.

536.11

PHARMACOLOGY OF DELTA OPIOID RECEPTOR AGONISTS IN THE RAT. M. B. Weinger*, J. S. Stuart, D. Wood, Dept. of Anesthesiology, Univ. Calif. San Diego and VAMC, San Diego, CA 92161.

The use of opiates as analgesics and anesthetics is limited by side-effects such as respiratory depression and muscle rigidity. We are investigating the role of μ, δ, and κ opioid receptors in different opiate endpoints including antinociception (55°C tail dip), sedation (loss of righting), catalepsy, muscle rigidity (tonic EMG activity), and respiration (total body plethysmography and arterial blood gases). Intracerebroventricular (icv) administration of the δ_1-preferring agonist DPDPE produced dose-dependent antinociception yet had no effect on righting or catalepsy except at very high doses. DPDPE had a biphasic respiratory effect with lower doses stimulating and higher doses modestly depressing ventilation. DPDPE alone increased hindlimb muscle tone, but partially antagonized systemic opiate rigidity. These same endpoints were investigated using the δ_2-preferring agonist D-ala^2-deltorphin II (10-120 nmol icv; a gift from P. Schiller) and the non-peptide δ agonist SNC80 (0.8-75 mg/kg subcutaneously; gift of K. Rice and S. Calderon). Coadministration of the δ antagonist naltrindole (NTI; 2-10 nmol icv) was used to confirm the role of δ receptors in the effects observed. NTI reversed DPDPE-induced increases in respiratory rate but did not block high-dose DPDPE ventilatory depression. Deltorphin (DELT) produced significant antinoci-ception without catalepsy or loss of righting. DELT decreased tidal volume but did not affect respiratory rate. DELT had no effect on muscle tone or on opiate rigidity. Systemic administration of SNC80 (75 mg/kg) prominently stimulated ventilation, primarily due to increased respiratory rate. Ongoing studies will evaluate further the hypothesis that δ agonists are effective analgesics with a favorable side-effect profile.

536.12

AN ANTISENSE OLIGONUCLEOTIDE TO THE DELTA-OPIOID RECEPTOR (DOR1) DECREASES BOTH DELTORPHIN II SENSITIVITY AND DOR-IMMUNOREACTIVITY IN THE DORSAL HORN IN MICE. L. S. Stone,[1-3]* M. Riedl,[3] U. Arvidsson,[3] L. Vulchanova,[1+3] K. F. Kitto,[2] J. Lai,[4] E. J. Bilksy,[4] F. Porreca,[4] P. Y. Law,[1-2] R. Elde,[1-3] H. H. Loh,[1-2] and G. L.Wilcox.[1-2] [1]Graduate Program in Neuroscience, Depts. of [2]Pharmacology and [3]Cell Biology & Neuroanatomy, U. of Minnesota, Minneapolis, MN 55455. [4]Dept. of Pharmacology, U. of Arizona, Tuscon AZ 85724.

Previous reports have demonstrated that application of oligonucleotides directed against the delta-opioid receptor can decrease the analgesic potency of delta-, but not mu- or kappa- opioid receptor agonists. However, it has not been shown that the decrease in agonist sensitivity is associated with a decrease in delta-opioid receptor-immunoreactivity (DOR1-ir). We therefore sought to determine agonist effectiveness and DOR1-ir in the same experimental animals. Antisense oligonucleotide directed against DOR1, the corresponding mismatch oligonucleotide and vehicle were injected intrathecally in mice twice a day over a period of three to four days. The animals were then tested for changes in tail-flick latency after intrathecal administration of deltorphin II, transcardially perfused and processed for immunocytochemistry. Antisense-treated animals showed both a decrease in deltorphin II antinociception and a consistently lower intensity of DOR-ir in spinal cord compared to mismatch, vehicle and naive control groups. No change was detected in the immunostaining for the mu-opioid receptor, CGRP, 5-HT or leu-enkephalin, indicating that the treatment was not toxic to primary afferent fibers or descending axons. Ongoing studies examine the effects of antisense oligonucleotides directed against the mu-receptor as well as a region common to all three receptor subtypes. This study demonstrates both the utility and selectivity of molecular lesioning by antisense oligonucleotides *in vivo*. (Supported by Grants from NIDA)

536.13

USE OF δ-ANTISENSE OLIGO IN THE STUDY OF THE TURNOVER OF δ-OPIOID RECEPTORS IN THE SPINAL CORD OF THE MICE. Leon F. Tseng* and Minoru Narita. Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, WI 53226.

Intrathecal (i.t.) pretreatment of male ICR mice with δ-opioid receptor (DOR) antisense oligodeoxynucleotide (AS oligo; 163 pmol), but not mismatch (MM) oligo (163 pmol), daily for 1-3 days caused a time-dependent attenuation of antinociception induced by i.t. administered [D-Ala^2] deltorphin (DT; 6.4 nmol), a DOR agonist, 24 hr after DOR AS oligo injection. Antinociception was measured by the tail-flick test. The DOR-mediated antinociception was not significantly altered after 1 day of i.t. DOR AS oligo pretreatment, but declined progressively after 2 and 3 days of treatment. The experiment was then extended to determine whether the decline in DOR activity for antinociception in the spinal cord in mice treated with DOR AS oligo is due to the stimulation of DORs by endogenously released Met-enkephalin. Concomitant i.t. treatment of mice with naltriben, which blocks DOR, together with DOR AS oligo for 24 hr prevented the attenuation of i.t. DT-induced antinociception produced by DOR AS oligo pretreatment. On the other hand, concomitant i.t. treatment of bestatin or thiorphan, which inhibits the degradation of released Met-enkephalin, with DOR AS oligo for 24 hr enhanced the attenuation of DOR antinociception induced by DOR AS oligo. Furthermore, a combination of pretreatments with DOR AS oligo given i.t. and, 10 min later, β-endorphin given i.c.v., which releases the Met-enkephalin in the spinal cord, dose-dependently attenuated i.t. DT-induced antinociception. These findings suggest that there is spontaneous release of endogenous Met-enkephalin, which stimulates DORs and causes a loss of DOR-mediated antinociception in mice in which the synthesis of DORs has been inhibited by DOR AS oligo (Supported by NIH grant, DA 03811).

536.14

INHIBITION OF PROTEIN KINASE C, BUT NOT PROTEIN KINASE A, BLOCKS THE DEVELOPMENT OF ACUTE ANTINOCICEPTIVE TOLERANCE TO δ-OPIOID RECEPTOR AGONIST IN THE MOUSE Minoru Narita[1], Michiko Narita[1], Hirokazu Mizoguchi[1], Linda K. Vaughn[2]* and Leon F. Tseng[1]. [1]Department of Anesthesiology, Medical College of Wisconsin, [2]Department of Basic Sciences, Marquette University, Milwaukee, WI 53226.

Intrathecal (i.t.) treatment of male ICR mice with δ-opioid receptor (DOR) agonist [D-Ala^2]deltorphin (DT; 6.4 nmol) produced acute antinociceptive tolerance to the subsequent i.t. challenge of DT (6.4 nmol) 3 hr later. Experiments were designed to study the effects of protein kinase C (PKC) inhibitor, calphostin C (CP), and protein kinase A (PKA) inhibitor, KT5720 (KT), on development of DT-induced antinociceptive tolerance. I.t. administration of CP (1-10 ng), which injected alone had no effect on i.t. DT-induced antinociception, dose-dependently blocked the development of DT-induced antinociceptive tolerance. On the other hand, KT did not have any effect on the development of DT-induced tolerance. DOR binding experiments were designed to examine whether the DOR located in the cellular membrane is G-protein-coupled receptors and can be phosphorylated by activation of PKC. Suspensions of the crude synaptic membrane of the mouse spinal cord were incubated with [^{3}H]DSLET for 2 hr at 25°C. DT (0.001-10 μM) dose-dependently displaced [^{3}H]DSLET bindings. [^{3}H]DSLET bindings were inhibited by GTPγs, a non-hydrolyzable GTP analog, indicating that DOR is G-protein coupled. [^{3}H]DSLET bindings were decreased by phorbol 12,13-dibutyrate (PDBu, 0.01-1 μM) dose-dependently, suggesting that phosphorylation of DOR by PKC may be involved in desensitization of DOR. It is concluded that PKC, but not PKA, plays an important role in development of DOR-mediated antinociceptive tolerance in spinal cord of the mouse (Supported by NIH grant, DA 03811).

536.15

INVOLVEMENT OF MET-ENKEPHALIN AND δ_2-OPIOID RECEPTORS IN THE SPINAL CORD IN ANTINOCICEPTION INDUCED BY COLD WATER SWIMMING IN THE MOUSE. Hirokazu Mizoguchi[1], Minoru Narita[1], Raymond M. Quock[2]* and Leon F. Tseng[1]. [1]Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, WI 53226 and [2]Department of Biomedical Science, College of Medicine at Rockford, University of Illinois, Rockford, IL 61107.

Mice exposed to cold water swimming (CWS: 4 °C, 3 min) produced a marked antinociception. Experiments were designed to determine whether Met-enkephalin and what types of opioid receptors in the spinal cord are involved in CWS-induced antinociception in male ICR mice. Antinociception was measured by the tail-flcik test 7 min after CWS. I.t. pretreatment with antibody against Met-enkephalin, which binds the released Met-enkephalin, blocked the CWS-induced antinociception, indicating that the antinociception is mediated by release of Met-enkephalin in the spinal cord. I.t. pretreatment with naltrindole (5 μg, 10 min) or naltriben (10 μg, 10 min), but not 7-benzylidene naltrexone (1 μg, 10 min), CTOP (D-Phe-Cys-Try-D-Try-Orn-Thr-Phe-Thr-NH$_2$, 50 ng, 10 min), or norbinaltorphimine (5 μg, 24 hr), blocked the CWS-induced antinociception, indicating that the antinociception is mediated by δ_2-, but not δ_1-, μ-, or κ-opioid receptors in the spinal cord. In δ_2-opioid receptor binding experiments, suspensions of the crude synaptic membrane of the mouse spinal cord were incubated with [^{3}H]DSLET, a δ_2-opioid receptor radioligand, for 2 hr at 25 °C. DT (0.001-10 μM), Met-enkephalin (0.1-10 μM) dose-dependently displaced [^{3}H]DSLET bindings, indicating that Met-enkephalin, like DT, binds to δ_2-opioid receptors. It is concluded that antinociception induced by CWS is mediated by the release of Met-enkephalin acting on δ_2-opioid receptor in the spinal cord (Supported by NIH grant, DA 03811).

536.16

TOLERANCE TO OPIOID INHIBITION OF cAMP FORMATION REQUIRES ENHANCED PKC- (BUT NOT PKA-) MEDIATED PHOSPHORYLATION. **L. Wang and A.R. Gintzler*. Dept Biochemistry, SUNY Health Science Ctr., Brooklyn, NY 11203, USA.**

The evoked release of myenteric methionine-enkephalin and the stimulated formation of cAMP is regulated by a bimodal, opiate receptor-coupled mechanism. Low doses (nanomolar) enhance evoked transmitter release and second messenger formation whereas higher concentrations (μM) inhibit them. Excitatory opioid actions are mediated by a G_s- like G protein whereas opioid inhibitory responses require a (G_i-like) G protein. Prior, in vivo chronic exposure to morphine can alter the balance between opioid excitatory and inhibitory responses such that the former are predominant. In tolerant/dependent preparations, facilitation of stimulated enkephalin release and cAMP formation is observed with concentrations of opioid that are inhibitory in untreated, opiate naive tissue. The current study demonstrates, in opiate naive and 'addicted' tissue, that the phosphorylation state is a critical determinant of the direction of the opioid regulation of stimulated cAMP formation. In opiate naive preparations, negative opioid modulation of adenylyl cyclase is reversed to enhancement following inhibition of protein phosphatase or activation of PKC. In 'addicted' tissue, activation of PKC, but not PKA, (as a function of chronic in vivo morphine treatment) is required for the reversal of opioid inhibition to enhancement. It is proposed that tolerance to the inhibitory effects of opioids results not only from the loss of negative opioid modulation but from a qualitative change in opioid responsiveness such that excitatory responses are predominant. These changes require enhancement of PKC- (but not PKA-) mediated phosphorylation.

536.17

LEU-ENKEPHALIN MODULATES THE ACTIVITY OF DISTENTION-SENSITIVE NEURONS IN THE DORSAL MOTOR NUCLEUS OF THE VAGUS (DMNV). X. Zhang*, R. Fogel and W.E. Renehan. Division of Gastroenterology, Henry Ford Hospital, Detroit, MI 48202.

It has been suggested that one consequence of the stress-related release of enkephalins is a modulation of gastrointestinal function. It is currently not clear whether the enkephalins exert this influence via a central or peripheral mechanism. Immunocytochemical studies have demonstrated, however, that 1) neurons in the DMNV have opioid receptors and 2) many of the neurons in the hypothalamus, amygdala and nodose ganglion that project tc the DMNV contain enkephalins. The aim of this study was to investigate the effect of local enkephalin administration on the response of individual gut-sensitive DMNV neurons. A multibarrel pipette array was used to record each DMNV neuron's response to duodenal and gastric distention and to administer varying concentrations of Leu-enkephalin (0.1, 1.0 or 10 mM) to the pericellular environment of the recorded cell. The array's recording electrode contained 2.0% Neurobiotin in 1M KCl buffer (resistance 50-70 mΩ), allowing us to label each physiologically-characterized neuron for subsequent three-dimensional reconstruction. A total of 28 distention-sensitive DMNV neurons were characterized and labeled. As we have noted previously, most of the DMNV neurons were inhibited by the distention stimuli. Ten of the distention-sensitive neurons were inhibited by leu-enkephalin injection (6 partial reduction in firing rate, 4 complete inhibition) and 6 neurons had an increase in firing rate (though the spontaneous activity of the excited neurons was inhibited between injections). These results suggest that enkephalins may influence gastrointestinal function by directly modulating the activity of neurons in the DMNV. Supported by NS30083.

536.18

ESTROGEN AND STRESS INTERACT TO REGULATE THE TRANSCRIPTIONAL ACTIVITY OF A PROENKEPHALIN PROMOTER-β-GAL FUSION GENE IN THE HYPOTHALAMUS OF TRANSGENIC MICE. C. A. Priest*, D. Borsook[1], S. E. Hyman[1] and D. W. Pfaff. Laboratory of Neurobiology and Behavior, The Rockefeller University, New York, NY 10021; [1]Harvard Medical School, Boston, MA 02114.

In the hypothalamus, proenkephalin (PE) gene expression is regulated by exposure to estrogen or stress. To examine potential interactions between these systems at the cellular level, the present study utilizes a transgenic mouse line (ENK 1.1; Mol. Endocrinol 6:1502, '92) which expresses a human PE promoter-β-gal fusion gene. Adult male mice (n = 36) were gonadally intact or castrated for 4 wks (CAST) and adult females (n = 28) were ovariectomized for 2 wks (OVX) or were 21 days postpartum. Forty-eight hrs before perfusion, CAST and OVX animals received an s.c. injection of 10 μg estradiol benzoate (EB) or oil, and 4 animals/group received no further treatment. Six hrs before perfusion, remaining animals received an i.p. injection of hypertonic (1.5 M, HYP) or isotonic (0.15 M, ISO) saline. Brains were sectioned at 25 μm and processed for X-gal histochemistry. Expression of the transgene was quantified using computer-assisted image analysis. In the VMH, levels of PE-β-gal expression were highest in gonadally intact males and postpartal dams. In CAST and OVX+EB groups, ISO increased PE-β-gal expression, as compared to uninjected animals; this did not occur in OVX or CAST+EB groups, thus demonstrating a gender-dependent interaction between EB and stress level. HYP suppressed PE-β-gal expression in all groups, as compared to expression following ISO. In the PVN, HYP induced PE-β-gal expression only in intact males and CAST+EB, indicating site-specific regulation of the transgene.

536.19

ENKEPHALIN SUPPRESSES SPONTANEOUS AND NMDA -INDUCED IPSPS IN SEROTONERGIC NEURONS OF THE RAT DORSAL RAPHE NUCLEUS IN VITRO. T. Jolas* and G.K. Aghajanian. Depts. of Psychiatry & Pharmacology, Yale School of Medicine, New Haven, CT 06508.

There is increasing evidence that opioids, in addition to their effects on the dopaminergic system, act also on the serotonergic (5-HT) system. In particular, it has been shown that intra-raphe administration of morphine increases serotonin release in the forebrain of *unanesthetized* rats (Tao and Auerbach, 1994). The purpose of the present study was to test, in dorsal raphe nucleus (DRN) slices, the effect of the opioid peptide met-enkephalin (ENK) on 5-HT neurons. Intracellularly, using KCl (2M) electrodes, these neurons were identified by standard criteria. The effects of ENK (100μM) on spontaneous post-synaptic potentials (PSPs) and membrane potential were measured. To study the PSPs, the cells were manually held at -90mV in current clamp mode and, if the PSPs were too sparse, they were induced with NMDA (20μM). ENK induced a slight hyperpolarization in ~30% of the 5-HT neurons and, in most cases, reduced the number of both spontaneous and NMDA-induced PSPs. ENK also reduced the number of PSPs recorded in some non-serotonergic neurons in the DRN. The PSPs were reduced by bicuculline (100μM) and, in a few cells, by strychnine (2μM) indicating that they are GABAergic and, possibly in some cases, glycinergic inhibitory PSPs (IPSPs). Moreover, they were blocked by TTX (2μM) indicating their impulse-flow dependence.

In conclusion, the results of this study are consistent with the idea that opiates increase serotonin release in the forebrain through a reduction of local inhibitory tone on 5-HT neurons in the DRN.

536.20

ENKEPHALIN SENSITIVE K^+ CHANNELS ON FRESHLY DISSOCIATED RAT AMYGDALA NEURONS. Jonathan E. Freedman*, Yong-Jian Lin and Xueguang Chen. Dept. Pharmaceutical Sciences, Northeastern Univ., Boston, MA 02115.

The amygdala is known to play a role in various opioid associated behaviors, including some subjective components of dependence and withdrawal. We are performing patch-clamp recordings from freshly dissociated rat amygdala neurons, in the presence and absence of opioid drugs. Cells were prepared from the posterior portion of the medial nucleus, or amygdalohippocampal area. Cell-attached recordings with 140 mM KCl as the main charge carrier within the patch pipette were obtained from pyramidally-shaped cells of about 30-40 μm diameter. Single channel currents with a conductance of 130 pS were observed on 8 of 11 cells tested with 10 μM met-enkephalin within the patch pipette. These channels were never observed in 14 control recordings in the absence of enkephalin ($P< 0.001$ by a Fisher exact test). Channel currents were in the inward direction, had reversal potentials consistent with K^+ permeability under our recording conditions, and were inwardly rectifying. The ionic selectivity and receptor pharmacology of this channel have yet to be fully determined, but it seems probable that these channels are activated by opiate receptors. Interestingly, these 130 pS channels in the amygdala appear to be different from the 85 pS K^+ channel which we have previously shown to be activated by D_2-like dopamine receptors in the caudate-putamen.

(Supported by MH-48545.)

SEROTONIN RECEPTORS: EFFECTORS

537.1

MOUSE SEROTONIN 5-HT-5A RECEPTORS ARE NEGATIVELY COUPLED TO ADENYLATE CYCLASE E. RONKEN[1*], R. van DIJK[1], M.van OOSTENBRUGGE[1], L. KODDEN[1], R. GRAILHE[2], R. HEN[2], and B. OLIVIER[1] 1) CNS-Research Solvay Duphar B.V., P.O.Box 900, 1381 DA Weesp, The Netherlands; 2) Dept. of Neurobiology and Behavior, Columbia University, New York, U.S.A.

The recent cloning of serotonin 5-HT-5A and 5-HT-5B receptors has further increased the complexity of 5-HT neurotransmission. 5-HT-5A and 5-HT-5B receptors are closely related on a molecular level and expression of both receptor subtypes seems to be confined to the central nervous system. Binding properties of cloned receptors revealed high affinity towards several ergot alkaloids, notably LSD, as well as to 5-HT and 5CT. Until recently, however, no coupling of these receptors was observed to any known second messenger system. Cloned murine 5-HT-5A receptors were studied using microphysiometry, a technique which measures cell metabolic activity by continuously monitoring acidification of weakly-buffered medium that is perfused over the cells. It was found that 5-HT, 5-CT and methysergide constituted partial agonists, whereas ergotamine and LSD were potent (EC_{50} LSD 18 nM; with respect to 5-CT: 230 nM) and full agonists on 5-HT-5A receptors. Potencies of compounds closely agreed with receptor binding affinity for 5-HT-5A receptors. In subsequent analysis of second messenger systems, 5-HT-5A receptors appeared to be coupled to adenylate cyclase in an inhibitory manner. Initial experimental results were precluded due to Mycoplasma contamination, to which adenylate cyclase appears to be extremely sensitive. After curing the cell lines, however, concentration-dependent inhibition of forskolin-stimulated formation of cAMP was found with 5-HT, 5CT, ergotamine and LSD. In conclusion, cloned mouse 5-HT-5A receptors are inhibitorily coupled to adenylate cyclase, thereby resembling more distant 5-HT receptor congeners, such as the 5-HT-type 1 family. Furthermore, the potent interaction of LSD with the 5-HT-5A receptor provides an additional mechanism through which LSD may exert its hallucinogenic properties.

537.2

PHARMACOLOGICAL CHARACTERIZATION OF THE RECOMBINANT HUMAN 5-HT, RECEPTOR SUBTYPE COUPLED TO ADENYLATE CYCLASE STIMULATION IN A CLONAL CELL LINE. J.M. Zgombick*, N. Adham, J.A. Bard, P.J.-J. Vaysse, R.L. Weinshank and T.A. Branchek. Synaptic Pharmaceutical Corporation, Paramus, NJ 07652.

Functional characterization of the recombinant human 5-HT, receptor coupled to adenylate cyclase stimulation was performed using stably transfected murine fibroblasts (LMtk⁻ cells). The site density of the 5-HT, receptor in clonal cell membranes determined by [^{3}H]5-HT (agonist) and [^{3}H]LSD (antagonist) saturation studies averaged 181 ± 28 and 191 ± 28 fmol/mg protein, respectively, suggesting that $G_{\alpha s}$ is not rate limiting and that all receptors exist in the high affinity state. In intact cells, 5-HT produced a concentration-dependent elevation of intracellular cAMP levels with an EC_{50} value of 70 nM and a maximal response of 5-fold increase above basal levels. The rank order of agonist potency in the second messenger assay paralleled their rank order of binding affinities: 5-CT > 5-HT > 5-MeOT> DPAT > sumatriptan. The potency of agonists to stimulate cAMP production was more than 10-fold lower affinity than their respective binding affinities (K_i values). These data may indicate low efficiency of receptor-effector coupling or alternatively, that the low affinity state of the receptor mediates the stimulatory response. Methiothepin and several ergot alkaloids (i.e. LSD, mesulergine) antagonized the 5-HT-mediated elevation of cAMP levels. The K_b values of the antagonists determined in the second messenger assay were in close agreement with the K_i values of these compounds in the binding assay. Pretreatment with cholera toxin abolished the 5-HT-mediated elevation in cAMP concentration, indicating that the 5-HT, subtype directly interacts with $G_{\alpha s}$ protein(s) to stimulate adenylate cyclase. No changes in inositol phosphate production or in elevation of intracellular calcium concentrations were observed after exposure of stable transfectants to 1 μM 5-HT. Clonal cell lines stably expressing the human 5-HT, receptor will serve as cellular models to study the function and regulation of the 5-HT, subtype, as well as to develop 5-HT,- selective compounds which can be employed to uncover the biological roles and potential therapeutic applications of this novel receptor subtype.

537.3

SEROTONIN AFFECTS sK_{Ca} CHANNEL ACTIVITY IN RAT CORTICAL ASTROCYTES. R.R. Margraf*[1], D.B. Wiehl[1], C.J. Charniga[1], M.-L. Linne[2], H.K. Kimelberg[1], and T.O. Jalonen[1]. [1]Division of Neurosurgery, Albany Medical College, Albany, N.Y. 12208, and [2]Medical School, University of Tampere, FIN-33101 Tampere, Finland.

We have found that serotonin (5-HT) in 1° astrocyte cultures increases intracellular Ca^{++}, measured with Fura-2, and inward single-channel currents. Acutely isolated astrocytes cultured for >24 hrs also respond to 5-HT. No increase in intracellular Ca^{++} is detected in astrocytes < 8 hrs following isolation. The 5-HT-induced single channel inward currents were studied using a cell-attached recording configuration. The L-type Ca^{++} channel blocker, nimodipine, reduced channel activity, but did not completely inhibit the 5-HT-induced response. A transient response to 5-HT was seen in Ca^{++} free media, indicating extracellular Ca^{++} is not necessary for onset of ion channel activity. No enhancement of channel activity was seen when 5-HT was added to cells pre-treated with 500 nM apamin, a channel blocker specific to the small-conductance Ca^{++} activated K^+ channel (sK_{Ca}). In the presence of U73122, a phospholipase C inhibitor, no K^+ channel activation in response to 5-HT was observed. The 5-HT effect was inhibited by 80 nM-10 uM ketanserin, a 5-HT2 antagonist. The $5\text{-}HT_1$ agonist 8-OH-DPAT, and the $5\text{-}HT_3$ agonist 1-(m-phenyl)-biguanidine, did not cause ion channel activation.

In summary, we show that the majority of cultured astrocytes and a small percentage of acutely isolated astrocytes respond to 5-HT with an activation of sK_{Ca} channels which is linked to the release of intracellular Ca^{++} via the G protein and phospholipase C pathway. Ca^{++} influx through voltage-activated Ca^{++} channels may be needed for sustained channel activity. Further work is needed to establish if the absence of a response to 5-HT in most acutely isolated astrocytes results from damage to the $5\text{-}HT_{2A}$ receptor during isolation which is later repaired in culture, or if the receptor is only present in a small number of cortical astrocytes *in situ*.

537.4

5-HT1B RECEPTOR COUPLES TO CARDIAC POTASSIUM CHANNELS. A. Ghavami, M. Baruscotti, P. Blier+*, R. B. Robinson and R. Hen., Department of Pharmacology, Columbia Univ., New York, NY 10032, USA ; +Neurobiological Psychiatry Unit, Mc Gill Univ. Montreal, Canada.

5-HT1B receptors are expressed predominantly on axon terminals and have been shown to inhibit the release of various neurotransmitters including 5-HT and GABA. However the intracellular effectors involved in 5-HT1B-mediated inhibition of transmitter release are unknown. We decided therefore to investigate the possibility that 5-HT1B receptors might couple with ion channels. To be able to express efficiently the 5-HT1B receptor in a wide variety of cells, we constructed a recombinant adenovirus (ad-1B). This virus is replication deficient and expresses under the control of the early cytomegalovirus promoter, the 5-HT1B receptor with an hemagglutinin epitope (tag) at its amino terminal end. Using antibodies against the tag, we were able to detect 5-HT1B receptor on the membranes of various infected primary cells including neurons and myocytes. A small fraction of the neurons were expressing the 5-HT1B receptor while nearly 100% of the cardiac myocytes expressed this protein. Cardiac myocytes were used so that we could compare the 5-HT response to that of the muscarinic agonist carbachol. Neonatal rat ventricular myocytes were voltage clamped and held at -90 mV in a high K (25mM) solution. In a control experiment, 10 non infected cells were exposed to carbachol (10 µM). Carbachol activated an average current of 7.8±3.5 pA/pF (mean±SEM). Nine of these 10 cells were also tested for 5-HT (10 µM) and none responded. In contrast, in 12 infected cells, an inward current was recorded in response to 5-HT; 6 of these cells were also tested for the effect of carbachol and a similar current was detected. 5-HT was tested as first drug in 8 cells and the average current amplitude recorded was 3.3±0.8 pA/pF. The remaining 4 cells were first tested for carbachol and the response was 7.3±2.9 pA/pF. These preliminary data indicate that, when expressed in ventricular myocytes, 5-HT1B receptors can activate an inward current. We are currently investigating the properties of this current to determine if 5-HT and carbachol are activating the same channel and if like the atrial m2 receptor, the 5-HT1B receptor can directly activate ion channels, without cytosolic second messenger intermediates.

537.5

AFFINITY STATES OF $5\text{-}HT_{1A}$ RECEPTOR BINDING SITES ARE CHARACTERIZED ON CRYOSTAT SLICES OF RAT HIPPOCAMPUS AND DORSAL RAPHE. S. Hurt* (1), I. Silverstein (2), H. F. Kung (3) and S. Maayani (2). (1)Dept of Radiochemistry, DuPont NEN Products, Boston, MA, (2)Dept of Anesthesiology, Mount Sinai School of Medicine, NY, NY and (3)Depts. of Radiology and Pharmacology, University of Pennsylvania, Philadelphia, PA

Distinct affinity states have been proposed for a variety of G-protein coupled receptors but have not been demonstrated for naturally expressed receptors in the CNS. We report here on a comparative study of $5\text{-}HT_{1A}$ receptor sites in membrane preparations and in cryostat slices of rat hippocampus and dorsal raphe. The slices were prepared on slides as for radioautography, and radioactivity bound to the slice was assayed by scintillation counting. In the slice, but not the membrane preparation, GTP analogs could induce a complete, rapid (< 10 sec) and temperature-independent loss of high affinity specific binding of the $5\text{-}HT_{1A}$ agonists [^{3}H]-DPAT and [^{3}H]-5-CT, attributable to conversion to the low affinity state. By contrast, binding of [^{3}H]-antagonists MPPF and WY100365 was not reduced in the presence of GTP analogs. In the absence of GTP, the density (B_{max}) of binding sites labeled by the antagonists was significantly higher than the density of agonist-labeled sites. We have defined the high-affinity state as that labeled by agonists in the absence of GTP, and the low-affinity state as that labeled by antagonists in the presence of GTP and have determined the K_i values for $5\text{-}HT_{1A}$ ligands for sites in the high affinity (KH) and low affinity (KL) states. In both brain regions $K_L/K_H > 1$ for agonists and $K_L/K_H \leq 1$ for antagonists (Maayani et al., this volume). We propose that the complete GTP-sensitivity in cryostat slices from rat brain renders it a system of choice for characterization of affinity states of the $5\text{-}HT_{1A}$ receptor (Supported by USPHS GM 34852, MH 48125 and DA 09094)

537.6

INDEPENDENT DEPRESSIVE MECHANISMS OF GABA AND 5-HT1A ON RAT SPINAL AXONS J.Sakuma , J.Ciporen, Y. Saruhashi, J.Abrahams, and W.Young* Department of Neurosurgery, Physiology & Biophysics. NYU Medical Center, 550 First Avenue, New York, NY 10016.

Our laboratory found two serotonergic receptor subtypes (5-HT1A and 5-HT2A) on rat spinal cord axons. One of the receptors, activated by the specific 5-HT2 receptor agonist quipazine, markedly excites axons through calcium dependent mechanisms. The other receptor, activated by the specific 5-HT1A receptor agonist 8-OH-DPAT, depressed axons through yet unknown mechanisms. Our goal is to determine the characteristics and mechanisms of 8-OH-DPAT mediated depression of axonal excitability and conduction. Action potentials (AP) were activated by supramaximal constant current pulse and recorded with micropipettes inserted into the isolated rat dorsal column.

GABA and 8-OH-DPAT significantly depressed action potential amplitudes and increased latencies in cuneate-gracilis fasciculus (CGF) and corticospinal tract (CST). Preliminary results suggest that 5-HT1A receptors are present on CST axons. GABA did not significantly alter conduction velocity whereas 8-OH-DPAT significantly decreased conduction velocity. Ion-selective microelectrode recordings showed that GABA increased extracellular K^+ ($[K^+]e$) while 8-OH-DPAT did not. Likewise, tetraethylammonium (TEA) did not alter 8-OH-DPAT induced depressive effects, suggesting that TEA-sensitive K^+ channels did not mediate the 5-HT1A-induced excitability changes. The Na/K ATPase inhibitor, ouabain, markedly enhanced the depressive effects of GABA. However, ouabain did not alter 8-OH-DPAT's depressive effects. GABA receptor antagonists had no effect on 8-OH-DPAT induced axonal depression; suggesting that the effects were not mediated by GABA release or GABA-gated Cl^- channels.

These findings suggest strongly that GABA and 5-HT1A receptors depress axonal excitability through different and independent mechanisms.

537.7

RELATIVE DRUG EFFICACY OF HALLUCINOGEN VERSUS NON-HALLUCINOGEN AGONISTS IS SIGNAL TRANSDUCTION PATHWAY DEPENDENT. K.A. Berg*[1], S. Maayani[2],and W. P. Clarke[1]. Dept. of Pharmacology[1], Univ. of Texas Health Science Center, San Antonio, Texas 78284 and Dept. of Anesthesiology[2], Mount Sinai School of Medicine, NY, NY 10029.

Activation of $5\text{-}HT_{2C}$ receptors in CHO cells increases accumulation of inositol phosphates (IP) as well as the release of arachidonic acid (AA) via two distinct phospholipases: PLC and PLA_2 (Clarke *et al.*, 1994, Soc. Neurosci. 20: 1158). We are studying the effects of hallucinogen (bufotenin, (±)DOI and d-LSD) vs non-hallucinogen (quipazine and TFMPP) agonists on both of these $5\text{-}HT_{2C}$-mediated pathways. CHO cells stably expressing human $5\text{-}HT_{2C}$ receptors (200 fmol/mg), were pre-labeled with either [^{3}H]-*myo*-inositol or [^{14}C]-AA and IP accumulation and AA release were measured following 10 min of agonist incubation. The results of alkylating experiments with phenoxybenzamine indicated little or no receptor reserve for either response. Agonist potency was similar between the two pathways (see Table). Interestingly, the relative efficacy of the agonists differed depending upon whether activation of PLC or PLA_2 was measured. As shown in the table below (n = 5-9 experiments), the hallucinogens were more efficacious in eliciting AA release than IP accumulation (intrinsic activity (i.a.) ratio of AA/ IP > 1) while the non-hallucinogens were more efficacious on IP accumulation (AA/IP i.a < 1).

DRUG	pEC50		intrinsic activity (i.a.)		AA/PI
	IP	AA release	IP	AA release	(i.a.)
5-HT	7.43 ± 0.1	7.34 ± 0.1	1.0	1.0	1.0
bufotenin	6.50 ± 0.1	6.33 ± 0.1	0.78 ± 0.04	1.03 ± 0.04	1.32
(±)-DOI	6.72 ± 0.1	6.80 ± 0.3	0.61 ± 0.07	0.81 ± 0.08	1.33
d-LSD	7.47 ± 0.1	8.00 ± 0.3	0.17 ± 0.02	0.40 ± 0.13	2.35
quipazine	5.67 ± 0.1	5.80 ± 0.2	0.88 ± 0.14	0.61 ± 0.07	0.69
TFMPP	6.34 ± 0.1	6.31 ± 0.2	1.05 ± 0.13	0.56 ± 0.07	0.53

We suggest that the greater efficacy of hallucinogens to activate the $5\text{-}HT_{2C}$-AA pathway is due to more efficient coupling between the hallucinogen-receptor complex and the G-protein that activates PLA_2 than that with the G-protein that activates PLC. (supported in part by USPHS grants GM 34852 and DA 09094).

538.1

DIFFERENTIAL EFFECTS OF 5-HT1A AND 5-HT1B RECEPTOR AGONISTS ON ESTROGENIC AND ANDROGENIC-INDUCED AGGRESSION. A. Cologer-Clifford and N.G. Simon*. Dept. of Biological Sciences, Lehigh University, Bethlehem, PA 18015.

Male-typical aggression is facilitated by testosterone or its metabolites, while 5-HT1A and 5-HT1B receptor agonists reportedly reduce behavioral expression. We examined the interaction between these hormonal and neurochemical systems. 5-HT1A (8-OH-DPAT) and 5-HT1B (CGS12066B) agonists were administered alone or in combination to castrated CF-1 male mice subcutaneously implanted with either DES (an estrogen) or R1881 (an androgen). All of the agonist treatments reduced offensive aggression without affecting locomotor behaviors in R1881-treated animals. In DES-treated males, only DPAT and CGS+DPAT reduced aggression. Preliminary microinjection studies indicate that the medial preoptic area is an active site for 5-HT modulation of aggression in the presence of DES or R1881. These studies demonstrate a significant interaction between neuroendocrine and neurochemical regulatory systems for aggression.

538.2

EFFECTS ON PATTERNS OF SPONTANEOUS LOCOMOTOR ACTIVITY BY SELECTIVE STIMULATION OF 5-HT_{1A} OR 5-$HT_{2A/C}$ RECEPTORS IN THE RAT. V. Hillegaart, A. Estival and S. Ahlenius*, Biochemical & Behavioral Pharmacology, Astra Arcus AB, Södertälje, Sweden.

We have previously shown that the 5-HT_{1A} receptor agonist 8-OH-DPAT produces a specific pattern of activity, characterised by stereotyped forward locomotion in the periphery of an open-field arena. In the present study, the administration of DOI (0.05-4.0 mg kg^{-1} sc, -20 min) or 8-OH-DPAT (6-400 µg kg^{-1} sc, -20 min) were found to produce this pattern of locomotor activity. Such effects produced by DOI- or 8-OH-DPAT were normalised by pretreatment with the 5-$HT_{2A/C}$ or the 5-HT_{1A} receptor antagonists ritanserin (2 mg kg^{-1} sc, -30 min) and (-) pindolol (2 mg kg^{-1} sc, -30 min), respectively. DOI-induced stereotyped locomotor activity was not antagonised by (-)pindolol pretreatment, nor was 8-OH-DPAT-induced effects antagonised by ritanserin pretreatment, Interestingly, certain aspects of the DOI-induced behavioural stimulation were significantly enhanced by the (-)pindolol treatment. Together with findings on 8-OH-DPAT/DOI interactions, as reported in the literature, this observation supports the notion of interactions between the 5-HT_1 and 5-HT_2 receptor subtypes. The precise nature of such interactions in terms of pre- and post-synaptic location of 5-HT receptors remains to be determined. ***In conclusion:*** Results from this study demonstrate the same stereotyped pattern of locomotor activity after stimulation of 5-HT_{1A} or 5-$HT_{2A/C}$ receptors. By use of selective 5-HT receptor antagonists these effects were shown to be pharmacologically distinct.

538.3

BEHAVIORAL AND HORMONAL RESPONSES TO HALLUCINOGENS FOLLOWING CHRONIC ANTIDEPRESSANT TREATMENT IN RATS K.R. Bonson*, J.W. Buckholtz, D.L.Murphy. Laboratory of Clinical Science 10/3D41, National Institute of Mental Health, Bethesda, MD 20892-1264.

Recent studies have shown that the acute responses to self-administered hallucinogens in humans are increased following chronic administration of tricylic antidepressants or lithium but are decreased after chronic administration of serotonin reuptake inhibitors or MAO inhibitors. Since changes in the functioning of serotonin receptor subtypes may underlie this phenomenon, the present study investigated whether the acute behavioral effects of hallucinogens with different serotonin binding profiles could be altered by the chronic administration of a variety of antidepressants in rats. Following chronic (4 week) administration of desipramine (5 mg/kg/day), clomipramine, (5 mg/kg/day), fluoxetine (5 mg/kg/day), clorgyline (1 mg/kg/day), cycloserine (30 mg/kg/day) or saline, rats in each of the treatment groups were challenged with an acute dose of either DOM (2.5 mg/kg) as a selective 5-HT2 hallucinogen, 5-MeO-DMT (5 mg/kg) as a selective 5-HT1A hallucinogen or LSD (4 mg/kg) as a non-selective 5-HT2/1A hallucinogen. Rats were then observed for emitted behaviors, with temperature measured and trunk blood collected for assessment of hormonal changes. The data indicate that, similar to human responses, the MAO inhibitor clorgyline decreased behavioral responses to all three hallucinogens in rats. Cycloserine, a partial agonist at the serine site on the NMDA receptor complex, reduced the serotonin syndrome induced by 5-MeO-DMT. Other behavioral responses to the hallucinogens were mixed following antidepressant treatment, suggesting that the behaviors observed in rats after the administration of hallucinogens at high doses may not provide a useful model of human hallucinogenic responses. Changes in plasma levels of prolactin, corticosterone and ACTH in response to hallucinogens also indicate a mixed effect of chronic antidepressant administration.

538.4

EFFECTS OF CP-135,807, A POTENT 5HT_{1D} AUTORECEPTOR AGONIST, ON SCHEDULE-CONTROLLED PERFORMANCES IN THE PIGEON. R.S. Mansbach*, C.C. Rovetti and J.E. Macor. Pfizer Central Research, Eastern Point Road, Groton, CT 06340.

CP-135,807 binds with high affinity to central 5HT_{1A} and 5HT_{1D} receptors (10-fold selective for 1D), and in functional studies produces dose-dependent decreases in extracellular 5HT. These and other findings suggest that CP-135,807 may be acting as a terminal 5HT autoreceptor agonist. In an attempt to characterize the behavioral activity of selective 5HT_{1D} ligands, adult male Carneaux pigeons were trained to discriminate i.m. injections of 0.1 mg/kg CP-135,807 from saline under a two-key, fixed ratio schedule of food-reinforced key pecking. CP-135,807 fully and dose-dependently substituted for the training dose. In contrast, little substitution was observed following administration of 8-OH-DPAT, a potent 5HT_{1A} agonist, nor was the training stimulus blocked by WAY 100,635, a selective 5HT_{1A} antagonist. Additional subjects were trained under a multiple schedule of food reinforcement in which responding was punished with mild electric shock in some components and not punished in other components. 8-OH-DPAT produced large increases in punished responding (>500% of control) while having little effect on unpunished responding. In contrast, CP-135,807 produced only modest increases in punished responding in a subset of animals. Taken together, these data suggest that CP-135,807 produces acute psychoactive effects in a species known to possess central 5HT_{1D} receptors, and that these effects cannot be primarily attributed to its activity at 5HT_{1A} sites.

538.5

WAY-100635, A SILENT 5-HT_{1A} RECEPTOR ANTAGONIST HAS ANXIOGENIC EFFECTS IN RATS AND DOES NOT BLOCK STRESS-INDUCED RISES IN STRESS HORMONES. L. Groenink[1], J. Mos[2], J. van der Gugten[1], J. Schipper[2]* and B. Olivier[1,2]. [1]Dept. Psychopharmacology, Utrecht University, P.O.Box 80082, 3508 TB Utrecht and [2]CNS-research Solvay-Duphar B.V., Weesp, The Netherlands.

5-HT_{1A} receptor agonists exert anxiolytic effects in both animals and humans. Moreover, these agonists enhance plasma ACTH, corticosterone and prolactin levels. We studied whether the 5-HT_{1A} receptor antagonist WAY-100635 also affects anxiety and basal stress homone levels in rats. Furthermore, we examined whether WAY-100635 alters stress-induced rises in plasma ACTH, corticosterone and prolactin using the adult ultrasonic vocalisation (AUV) test. In this test, rats are replaced in a cage in which they previously received inescapable shocks. WAY-100635 (0.3 mg/kg sc) significantly enhanced the number of distress vocalisations, whereas higher doses of the drug (1.0 and 3.0 mg/kg sc) did not alter the number of calls. This indicates that WAY-100635 has anxiogenic effects according to a bell-shaped dose-response. Under basal, non-stress conditions, WAY-100635 did not alter plasma ACTH and corticosterone levels. However, the highest dose of WAY-100635 significantly enhanced plasma prolactin levels. Exposure of the rats to the AUV test resulted in significant rises in plasma ACTH, corticosterone and prolactin levels. Prior administration of WAY-100635 did not alter these rises in plasma stress hormone concentrations.

Our results indicate that the supposed silent 5-HT_{1A} receptor antagonist may have anxiogenic effects in rats. Moreover, as the stress-induced rises in plasma ACTH, corticosterone and prolactin levels are not antagonized by a blockade of the 5-HT_{1A} receptor, this receptor is probably not of major importance in the regulation of stress-induced hormone secretion.

538.6

BEHAVIORAL EFFECTS OF (±)-1-(2,5-DIMETHOXY-4-IODOPHENYL)-2-AMINOPROPANE, (DOI) IN THE ELEVATED PLUS-MAZE TEST. C. Bishop-Robinson[1], E. S. Onaivi*[1], N. A. Darmani[2] and E. Sanders-Bush[3]. [1]Dept of Pharmacology, Meharry Med., College, Nashville, TN. [2]Dept of Pharmacology, Kirksville College of Osteopathic Medicine, Kirksville, MO. [3]Dept of Pharmacology, Vanderbilt Univ., Nashville, TN, 37232.

The serotonin (5-HT) system has consistently been implicated in the actions of DOI and other hallucinogens. Recent evidence suggest that the 5-$HT_{2A/2C}$ receptor subtypes may be major targets for such drugs in the CNS. DOI-treated hooded rats (0.1-5.0 mg/kg) and DOI treated ICR mice (0.1-2.0 mg/kg), displayed aversions at lower doses and anti-aversions at higher doses to the open arms of the plus-maze. Mianserin (0.5 mg/kg) and ketanserin (0.1 mg/kg) blocked the anti-aversive behavior, but only mianserin was effective at reversing the aversions produced by the higher doses of DOI in the ICR mice. DOI produced an intense aversion in the DBA/2 and anti-aversion in the C57/BL6 mice to the open arms of the plus-maze. These opposing actions of DOI in the plus-maze may be exploited in studying the neurobehavioral effects of hallucinogens. Since flumazenil was ineffective at blocking the DOI induced changes, it was concluded that the mechanism of DOI induced anxiolysis or anxiogenesis may not involve an action at the benzodiazepine receptors. Supported by NIDA grants to E. S-B-DA 05781 and N. A. D-DA07627; E.S.O-NSF-MRCE # HRD-9255157 and RCMI grant # NIH 5G12RR0303208. C. B-R acknowledges support from MFP-American Psychological Association.

538.7

COMPARATIVE EFFECTS OF 5-HYDROXYTRYPTAMINE$_{1a}$ PARTIAL AGONISTS, FULL AGONISTS, AND ANTAGONISTS IN THE MURINE ELEVATED PLUS MAZE D.R. Helton*, K. Rasmussen, V. P. Rocco, J.P. Tizzano, and M.J. Kallman Lilly Research Laboratories, Greenfield, IN 46140.

Previous studies have reported varied and often inconsistent results concerning the anxiolytic activity of 5-hydroxytryptamine1a (5-HT$_{1a}$) agents on the elevated plus maze (EPM). The present set of experiments profiled the effects of the partial agonists buspirone, (+/-)8-OH-DPAT, (-)8-OH-DPAT, and LY315712, the full 5-HT$_{1a}$ agonists (+)8-OH-DPAT and LY274600, and the selective 5-HT$_{1a}$ antagonist LY297996 on the murine automated EPM. Secondary pharmacology related to sedation was evaluated using home cage activity. (+/-)DPAT (.03-0.3 mg/kg, sc) produced dose-related increases in open arm activity with no evidence of sedation. (+)DPAT increased open arm activity at 0.03 and 0.1 mg/kg followed by loss of effectiveness at 0.3 mg/kg with no sedation at any dose examined. In contrast, (-)DPAT increased open arm activity at 0.03, 0.1, and 0.3 mg/kg, however, sedation was observed at all dose levels. Buspirone (.03-3 mg/kg, sc) produced an increase in open arm activity at 0.3 mg/kg with loss of effect as doses increased (>1 mg/kg) (sedation was also noted at doses>0.3 mg/kg). The full agonist LY274600 (.1-1 mg/kg, sc) also produced dose-related increases in open arm activity with sedation occurring only at doses>3 mg/kg. LY315712 (.03-10 mg/kg, sc) produced bi-phasic effects with increased open arm activity at lower doses and loss of effect as doses increased (>1 mg/kg), with no evidence of sedation. However, the 5-HT$_{1a}$ antagonist LY297996 (.03-3 mg/kg, sc) was ineffective on the maze and did not produce sedation at the doses examined. These results indicate that 5-HT$_{1a}$ partial and full agonists display anxiolytic activity in the murine elevated plus maze. The activity of these agents in this assay may be related to multiple factors including 5-HT$_{1a}$ affinity for pre- and post synaptic receptor stimulation, the degree of intrinsic activity (depending on the extent of receptor reserve), or a combination of these variables.

538.8

A SINGLE PRETREATMENT WITH 8-OH-DPAT REDUCES BEHAVIORAL INDICES OF 5-HT$_{1A}$ RECEPTOR ACTIVATION IN FEMALE RATS. N. Maswood, J. Hines,* and L. Uphouse Department of Biology, Texas Woman's University, Denton, TX. 76204.

Several studies have been designed to examine the effects of prior treatment with 5-HT$_{1A}$ receptor agonists on behavioral indices of receptor activation in male rats. Such studies have led to suggestions that presynaptic, but not postsynaptic, receptors readily undergo agonist modification. In few studies have the effects of prior agonist activation been examined in the female rat. Ovariectomized rats were pretreated with either 0.25 mg/kg 8-OH-DPAT or saline and 7 days later the behavioral effects of the drug were examined. This dose of the drug elicited components of the "serotonin behavioral syndrome" including flattened body posture and forepaw treading. Eating behavior and hypothermia were also present. Hypothermia, incidences of eating behavior, and the duration of flattened body posture were reduced by prior agonist activation while incidences of forepaw treading were unchanged. Thus, behavioral indices of both presynaptic autoreceptor and postsynaptic 5-HT$_{1A}$ receptor activation were reduced following prior agonist treatment.
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538.9

LORDOSIS BEHAVIOR AFTER CO-INFUSION OF NE AND 5-HT INTO THE VMN. S. Maswood*, M. Caldarola-Pastuszka, and L. Uphouse. Department of Biology, Texas Woman's University, Denton, TX. 76204.

Much emphasis has been placed on the lordosis-modulating action of norepinephrine (NE) and serotonin (5-HT) in the ventromedial nucleus of the hypothalamus (VMN). In the present study, the interaction between NE and 5-HT was examined with the prediction that NE might protect against the inhibitory action of 5-HT. After a 5-10 min pretest, intact, proestrous rats were infused bilaterally into the VMN with either 1000 ng of 5-HT per bilateral site or with 1000 ng of 5-HT plus 1000 ng NE. Sexual behavior was monitored for 30 consecutive min after the infusion. When rats were infused with 5-HT alone, there was a decline in the L/M ratio by 5 min after infusion and the decline lasted an additional 10-15 min. Although NE did not prevent the decline in lordosis behavior, NE did reduce the magnitude and duration of decline in the lordosis response.
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538.10

5-HT$_{1A}$ RECEPTOR ACTIVATION AND FEMALE RAT LORDOSIS BEHAVIOR. L. Uphouse*, M. Caldarola-Pastuszka, N. Maswood, and A. Jackson. Department of Biology, Texas Woman's University, Denton, TX. 76204.

In female rats, serotonin 1A (5-HT$_{1A}$) receptor agonists inhibit lordosis behavior within 5-15 min following infusion into the ventromedial nucleus of the hypothalamus (VMN). When, proestrous rats received coinfusions into the VMN with 200 ng 8-OH-DPAT or with 8-OH-DPAT plus the 5-HT$_{1A}$ receptor antagonist, (+) WAY100135, (+) WAY100135 attenuated the lordosis-inhibiting effect of the 5-HT$_{1A}$ agonist. Furthermore, bilateral VMN infusion with 2500 ng (+) WAY100135 facilitated lordosis behavior in suboptimally, hormone-primed ovariectomized rats. These findings strengthen the argument that activation of VMN 5-HT$_{1A}$ receptors is incompatible with lordosis behavior. How 5-HT$_{1A}$ receptor activation is translated into inhibition of lordosis behavior is unclear. However, when ovariectomized, hormone-primed rats were infused bilaterally into the VMN with a 33.3% procaine solution, lordosis behavior declined within 5-15 min. Consequently, it is possible that 5-HT$_{1A}$ receptor agonists inhibit lordosis behavior by reducing the firing of VMN neurons.
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538.11

NON-SELECTIVE 5-HT RECEPTOR AGONISTS AND FEMALE LORDOSIS BEHAVIOR. A. Jackson *, A. Wolf, T. Price, A. Trevino, M. Caldarola-Pastuszka, and L. Uphouse. Department of Biology, Texas Woman's University, Denton, TX. 76204.

Activation of 5-HT$_{1A}$ receptors causes a decline in lordosis behavior while simultaneous activation of 5-HT$_{2A/2C}$ receptors attenuates this decline. In the following study, the ability of two nonselective 5-HT compounds, TFMPP and quipazine (which share agonist action at the 5-HT$_{2C}$ receptor), were evaluated for their ability to protect against the lordosis-inhibiting action of the 5-HT$_{1A}$ agonist, 8-OH-DPAT. Both TFMPP and quipazine, when infused into the ventromedial nucleus of the hypothalamus (VMN), attenuated the effect of 8-OH-DPAT. Alone, neither TFMPP nor quipazine substantially modified lordosis behavior of hormone-primed, sexually receptive rats. However, approximately 50% of hormone primed, nonreceptive rats showed increased sexual behavior following infusion of TFMPP or quipazine into the VMN. These findings support other data indicative of a complex interaction among 5-HT receptor subtypes in the control of lordosis behavior.
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538.12

5-HT$_{2A/2C}$ RECEPTORS AND LORDOSIS BEHAVIOR IN FEMALE RATS. A. Wolf*, M. Caldarola-Pastuszka and L. Uphouse, Texas Woman's University, Department of Biology, Denton, TX 76204.

Infusion of 2000 or 3000 ng DOI (5-HT$_{2A/2C}$ agonist) into the ventromedial nucleus of the hypothalamus (VMN) of non-sexually receptive, hormone-primed (0.5 µg estradiol benzoate) female rats facilitated lordosis behavior. Six of 8 rats infused with 2000 ng DOI showed facilitation while 9 of 11 rats showed facilitation following infusion with 3000 ng DOI. In sexually receptive rats, infusion of the 5-HT$_{2A/2C}$ antagonist, ketanserin (500 ng - 3000 ng ketanserin tartrate), produced a dose dependent reduction in lordosis behavior. However, the drug appeared to be more potent in the ovariectomized than in the intact rats. At the highest dose of ketanserin, 9 of 9 ovariectomized rats showed a reduction in lordosis behavior while only 4 of 6 intact rats showed a decline. Collectively, these findings are consistent with previous assertions that activation of 5-HT$_{2A/2C}$ receptors may facilitate lordosis behavior.
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539.1

SOMATODENDRITIC LOCALIZATION OF THE SEROTONIN 5-HT1A RECEPTOR IN ADULT RAT BRAIN. M. Riad [1], S. Garcia [1], M. Hamon [2] and L. Descarries *[1]. [1] Département de pathologie et CRSN, Université de Montréal, Montréal, Qc, Canada; [2] Neurobiologie Cellulaire et Fonctionnelle, INSERM U288, Paris, France.

An immunocytochemical protocol was developed for the light and electron microscopic visualization of 5-HT1A receptors in adult rat brain. We used specific antibodies raised against a synthetic peptide corresponding to a highly selective portion of the third intracytoplasmic loop of the rat 5-HT1A receptor [El Mestikawy et al, *Neurosci Lett*, 1990; Riad et al, *Neurochem Int*, 1991], and the peroxidase technique. Among several fixation procedures tested, perfusion with 4% paraformaldehyde yielded the best results. In the mesencephalic raphe nuclei, the immunoprecipitate was concentrated at the periphery of neuronal cell bodies and their proximal dendrites, and over small (distal) dendritic branches. In the hippocampus, a similar subcellular distribution was found, involving some interneurons as well as pyramidal and granule cells. No axonal localization (including terminals) was seen, nor any labeling of astroglia. On the basis of these ultrastructural data and light microscopic observations in other brain regions, it was concluded that the 5-HT1A receptor of adult rat CNS is essentially neuronal, and somatodendritic in both its autoreceptor (raphe nuclei) and heteroreceptor locations (e.g. hippocampus). [Supported by FCAR and MRC grant MT-3544].

539.2

EFFECTS OF THE FULL 5-TH$_{1A}$ AGONIST ALNESPIRONE (S 20499) ON 5-HT RELEASE IN DRN- AND MRN-INNERVATED AREAS IN RAT BRAIN

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Alnespirone (S 20499) is a full agonist at pre- and post-synaptic 5-HT$_{1A}$ serotonergic receptors with anxiolytic properties. In vivo, alnespirone inhibits the firing of rat 5-HT dorsal raphe nucleus (DRN) neurones at low doses (ED_{50} = 0.3 mg/kg p.o.). We have studied the effects of alnespirone on 5-HT release using microdialysis in conscious rats. These were implanted with 1.5- or 4-mm-long probes in different forebrain areas and in the DRN. 18-20 h later, the probes were perfused at 0.25 µl/min with artificial CSF containing 1 µM citalopram and 20-min fractions were collected. 5-HT and 5-HIAA were analyzed by HPLC. Basal values of dialysate 5-HT obtained in presence of 1 µM citalopram largely differed between brain regions, in accordance with the density of serotonergic fibers (e.g., basal 5-HT in the DRN was 2.5 times higher than in dorsal hippocampus). A detailed study of the dose-effect relationship has been performed in dorsal striatum, an area receiving afferents exclusively from the DRN. Alnespirone dose-dependently reduced dialysate 5-HT in the range 0.1 mg/kg - 3 mg/kg s.c. The calculated ED_{50} was 0.26 mg/kg. The doses of 1 and 3 mg/kg induced profound and long-lasting (> 200 min) reductions of 5-HT release. The extent and duration of the maximal effect observed (- 75% at 3 mg/kg) suggests full agonistic activity at 5-HT$_{1A}$ receptors controlling release in DRN-innervated areas, as it is comparable to other treatments (TTX, omission of Ca^{2+} ions, etc...) that suppress firing-dependent 5-HT release. Overall, the release-reducing effects of alnespirone were more marked on neurones from the DRN than in those from the MRN, in agreement with electrophysiological data.

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539.3

FURTHER STUDIES ON THE ROLE OF 5-HT$_{1A}$ AUTORECEPTORS IN THE EFFECT OF 5-HT REUPTAKE BLOCKADE ON EXTRACELLULAR 5-HT LEVELS IN THE RAT BRAIN. S. Hjorth* and S. Milano. Inst. of Physiology & Pharmacology, Dept. of Pharmacology, Univ. of Göteborg, Medicinareg. 7, S-413 90 Göteborg, SWEDEN.

The efficacy of antidepressant drugs that block serotonin (5-HT) reuptake may be restrained in the short term by the indirect activation of autoreceptors. In a continuation of our previous studies on this issue, we used *in vivo* microdialysis in anaesthetised rats to further clarify the role of 5-HT$_{1A}$ autoreceptors in the action of 5-HT reuptake inhibitors. Microdialysis probes were placed in the ventral hippocampus and dialysate 5-HT levels determined by HPLC-EC. Citalopram (CIT; 0.5 or 5.0 mg/kg SC) dose-dependently elevated dialysate 5-HT. The 5-HT$_{1A/1B}$ receptor antagonist (-)penbutolol (8 mg/kg SC, 60' after CIT, 5.0 mg/kg, SC) almost doubled 5-HT relative to CIT alone, whereas (+)penbutolol, or a combination of the β_1- and β_2-adrenoceptor blockers betaxolol and ICI118.551 (lacking 5-HT$_1$ receptor affinity) failed to alter the CIT effect. This suggests that the (-)penbutolol-induced augmentation of the CIT response is not mediated by β-adrenoceptors. An autoreceptor dose of the 5-HT$_{1A}$ agonist 8-OH-DPAT (0.025 mg/kg SC) did not modify, whereas the 5-HT$_{1A}$ antagonist WAY100635 (0.3 mg/kg SC) strongly potentiated the CIT-induced elevation of 5-HT. The data confirm the conclusion that indirect stimulation of 5-HT$_{1A}$ autoreceptors - secondary to increased extracellular 5-HT in the raphe cell body areas - limits the overall 5-HT transmission-promoting action of SSRI like CIT. Indeed, recent open studies support the idea, that the clinical efficacy and onset of action of antidepressants that block 5-HT reuptake may be enhanced by concurrent 5-HT$_{1A}$ autoreceptor blockade.

539.4

ANTAGONISM OF SOMATODENDRITIC 5-HT$_{1A}$ AUTORECEPTOR ACTIVATION IN VIVO BY TWO NOVEL 5-HT$_{1A}$ RECEPTOR ANTAGONISTS. R.J. Thielen* N.B. Fangon & A. Frazer. Dept. of Pharmacol., UTHSCSA and Audie L. Murphy Memorial Veterans Hospital, San Antonio, TX 78284.

4-(2'-methoxy-)phenyl-1-[2'-(n-2"-pyridinyl-)p-iodobenzamido-]ethylpiperazine (p-MPPI) and 4-(2'-methoxy-)phenyl-1-[2'-(n-2"-pyridinyl-)p-flouro-benzamido-]ethylpiperazine (p-MPPF) antagonize postsynaptic 5-HT$_{1A}$ receptor activation *in vivo* (Life Sci. 56:PL163, 1995). Many putative 5-HT$_{1A}$ receptor antagonists have been shown to be partial agonists at the somatodendritic 5-HT$_{1A}$ autoreceptor. We report p-MPPI and p-MPPF are antagonists at the somatodendritic 5-HT$_{1A}$ autoreceptor *in vivo*, as measured by 5-HT turnover. 5-HT and 5-HIAA contents in brain regions of the rat were measured by HPLC with electrochemical detection, and their ratio was used as an index of the turnover of 5-HT. Neither p-MPPI nor p-MPPF (10 mg/kg, ip) given alone significantly altered the ratio of 5-HIAA/5-HT in the hippocampus (HIP) (1.0 ± 0.2, saline; 1.1 ± 0.3, p-MPPI; 1.2 ± 0.1, p-MPPF) or striatum (STR) (1.3 ± 0.1, saline; 1.5 ± 0.1, p-MPPI; 1.6 ± 0.1, p-MPPF). The 5-HT$_{1A}$ agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (0.5 mg/kg, sc) reduced the 5-HIAA/5-HT ratio in the HIP and STR by 48 and 33%, respectively ($p<0.05$). p-MPPI and p-MPPF were able to antagonize dose-dependently this reduction in the 5-HIAA/5-HT ratio in the HIP and STR ($p<0.05$) (approximate ED_{50}'s for p-MPPI and p-MPPF, respectively: HIP: 10 and 4 ;STR: 2 and 4 mg/kg). Also, binding of 2 nM [^{3}H]p-MPPF (3 times its K_d) to hippocampal membranes was unaltered by the addition of 100 µM GppNHp to the incubation medium (311 ± 35 and 338 ± 32 fmol/mg protein, in the absence and presence of GppNHp, respectively). Both p-MPPI and p-MPPF act as 5-HT$_{1A}$ antagonists *in vivo* at a response mediated by activation of somatodendritic 5-HT$_{1A}$ autoreceptors (Supported by Research Funds from the VA and USPHS Grant MH48125).

539.5

THE 5-HT$_{1A}$ RECEPTOR ANTAGONIST (S)-UH-301 ENHANCES BOTH THE ACUTE AND CHRONIC EFFECT OF CITALOPRAM ON CENTRAL SEROTONERGIC TRANSMISSION. L. Arborelius*, G.G. Nomikos, P. Hertel, P. Grillner, B. Backlund Höök, U. Hacksell and T.H. Svensson Dept. Physiology & Pharmacology, Div. Pharmacology, Karolinska Institutet, S-171 77 Stockholm, Sweden.

The effects of (S)-UH-301 in combination with acute or chronic administration of the selective serotonin (5-HT) reuptake inhibitor citalopram (CIT) were studied on the activity of 5-HT neurons in the dorsal raphe nucleus, using single cell recording techniques, and on extracellular concentrations of 5-HT in the frontal cortex (FC) of freely moving rats, using microdialysis. Acute administration of CIT (0.3 mg/kg i.v.) significantly decreased the activity of 5-HT cells, an effect that was potently blocked or reversed when (S)-UH-301 (0.25 and 0.5 mg/kg i.v. respectively) was administered before or after CIT. CIT (2.0 mg/kg s.c.) increased 5-HT concentrations in the FC with 186% and (S)-UH-301 (2.5 mg/kg s.c.) alone increased 5-HT by 68%. When (S)-UH-301 was injected 30 min before CIT, 5-HT concentrations increased with 425%. After treatment with CIT (20 mg/kg/day) for 14 days the baseline firing rate was not significantly different from baseline values in rats which had received saline (SAL; 1 ml/kg/day; 0.90 ± 0.12 and 1.23 ± 0.14 Hz). (S)-UH-301 (0.03-0.50 mg/kg i.v.) significantly increased the activity of 5-HT cells in CIT-treated rats, but did not affect 5-HT cells in SAL-treated rats. Biochemically, baseline concentrations of 5-HT in the FC were about 3.5 times higher in chronic CIT-treated rats (1.40 ± 0.23 fmol/min) than in SAL-treated rats (0.39 ± 0.03 fmol/min). In chronic CIT-treated rats administration of (S)-UH-301 increased 5-HT concentrations by 0.78 fmol/min, whereas in SAL-treated rats (S)-UH-301 increased 5-HT by 0.27 fmol/min. Taken together our findings show that (S)-UH-301 can agument both the acute and chronic stimulatory effect of CIT on central serotonergic transmission.

539.6

REGULATION OF 5-HT RELEASE IN 5-HT$_{1B}$ KNOCK-OUT MICE: EXPERIMENTS IN HIPPOCAMPAL, FRONTAL CORTEX AND MIDBRAIN RAPHE SLICES. G. Piñeyro*, N. Castanon+, R. Hen+ and P. Blier. Neurobiological Psychiatry Unit, McGill Univ., Montréal, Canada. +Center for Neurobiology and Behaviour, Columbia Univ., New York, USA

5-HT$_{1B}$ receptors are rodent homologues of human 5-HT$_{1D\beta}$ receptors. Some of these receptors are autoreceptors which negatively regulate 5-HT release in the CNS. In the present study, superfusion experiments were undertaken to assess the regulation of 5-HT release in mice genetically lacking 5-HT$_{1B}$ receptors. Slices were prepared from transgenic and control female mice and preloaded with [^{3}H]5-HT. The electrically-evoked release of 5-HT (S_1) was increased by 189% in hippocampal but not in frontal cortex slices of 5-HT$_{1B}$ knock out mice. The selective 5-HT$_{1B}$ agonist CP-93129 (100 nM) inhibited evoked [^{3}H]5-HT release in hippocampal and cortical slices from wild-type mice but not from 5-HT$_{1B}$ knock-out mice. In contrast, the 5-HT$_{1D/1B}$ agonist sumatriptan (100 nM) did not produce a significant effect in the same projection areas in either group. The non-selective 5-HT agonist 5-carboxyamidotryptamine (5-CT; 100 nM) inhibited evoked [^{3}H]5-HT release by 80% in hippocampal slices and by 60% in cortical slices obtained from both, controls and mutants. These results indicate that in projection areas, 5-CT-sensitive, non-5-HT$_{1B}$, non-5-HT$_{1D}$ receptors, negatively regulate 5-HT release in mutant mice. There was a 254% increase in S_1 values in mesencephalic slices (containing midbrain raphe nuclei) obtained from mutant mice. Interestingly, the 5-HT$_{1B}$ agonist CP-93129 (100 nM) had no effect in midbrain slices obtained from control or transgenic mice. Sumatriptan (100 nM) in contrast, inhibited [^{3}H]5-HT release by 60% in both groups. The latter effect was not blocked by the 5-HT$_{1A}$ antagonist (+)WAY 100135 in neither group of mice. These results support our previous observations indicating that 5-HT$_{1D}$ receptors negatively regulate 5-HT release in rodent raphe nuclei.

539.7

FLUOXETINE-INDUCED INCREASES IN FRONTOCORTICAL 5-HT LEVELS: A MICRODIALYSIS STUDY IN MICE LACKING 5-HT_{1B} RECEPTORS. A-C. Trillat*[1], I. Malagié[1], K. Scearce[2], C. Jacquot[1], R. Hen[2] and A.M. Gardier[1]. 1-Dept. Pharmacol. JE 92-372, Fac. Pharmacie, Univ. Paris Sud, F92296 Chatenay-Malabry, France. 2-Cent. Neurobiol. Behav., Columbia Univ., NY10032, USA.

5-HT_{1B} presynaptic receptors, predominantly located on the terminals of projecting neurons, have been shown to inhibit serotonin release, when activated. This activation has already been suggested to play a role in the long-term effects of specific serotonin reuptake inhibitors (SSRI). Because there are no specific antagonists of these receptors, homozygous mutant mice lacking the gene encoding the 5-HT_{1B} receptors in the central nervous system may represent a useful tool for elucidating further the role of these terminal autoreceptors in the SSRI mechanism of action. In the present report, we compared freely moving mutant mice and their wild-type littermates for the response to an acute injection of an SSRI fluoxetine (Flx, 10 mg/kg i.p.) on the extracellular concentrations of 5-HT as measured in the frontal cortex (FC) by *in vivo* microdialysis coupled to HPLC with amperometric detection {Saudou et al., *Science*, **265** (1994) 1875}. In mutant mice, basal extracellular levels of 5-HT in FC did not differ from those of wild-type mice (in fmol/20µl: 8.51 ± 0.84 and 8.88 ± 0.83, respectively), while basal 5-HIAA efflux was significantly increased (in pmol/20µl: 1.97 ± 0.08 and 1.46 ± 0.09, P<0.001, respectively). An acute 10 mg/kg Flx dose caused similar increases in extracellular 5-HT in FC (expressed as maximal percentage of 5-HT increase over the basal values: 286 ± 50%; P<0.02 and 276 ± 74%; P<0.01, respectively). Extracellular neurotransmitter concentrations measured by *in vivo* microdialysis at nerve terminals are known to reflect a balance between transmitter release and its reuptake. As already reported in rats {Rutter and Auerbach, *J.Pharm.Exp.Ther.* **265** (1993) 1319}, peripheral Flx increases extracellular 5-HT concentrations by decreasing 5-HT release and inhibiting its reuptake. Thus, the present *in vivo* study suggests that mutant mice exhibit unaltered 5-HT release and reuptake control mechanisms. Further studies (using other SSRIs at various doses) need to be performed to confirm the absence of a modulatory role of cortical 5-HT_{1B} presynaptic receptors in the acute effects of peripheral SSRIs.

539.8

THE ROLE OF TERMINAL AND SOMATODENDRITIC 5-HT_{1D} AUTORECEPTORS IN 5-HT RELEASE. J. Sprouse*, L. Reynolds, T. Clarke and H. Rollema. Pfizer Central Research, Groton, CT

The terminal autoreceptor which modulates 5-HT release is of the 5-HT_{1B} or 5-HT_{1D} subtype (depending upon species) and is inhibitory in nature such that extracellular 5-HT decreases in the presence of agonists. This system appears to be under the tonic influence of synaptic 5-HT since antagonists, when administered alone, act to increase 5-HT release (Briley and Moret, Clin. Neuropharmacol. **16**:387, 1993). A mitigating factor in the degree of 5-HT release is thought to be the presence of somatodendritic 5-HT_{1D} autoreceptors (Starkey and Skingle, Neuropharmacology **33**:393, 1994), which when blocked by antagonists promote the release of 5-HT and in turn slow firing rate and reduce impulse flow to the terminals. The present study set about examining the importance of this mechanism with GR-127,935, a recently disclosed 5-HT_{1D} antagonist.

GR-127,935 elevated extracellular 5-HT as measured with microdialysis probes located in guinea pig hypothalamus with a dose of 5 mg/kg sc resulting in a 35% increase above baseline (N = 7). Contrary to the hypothesis stated above, dorsal raphe cell firing as recorded from anesthetized guinea pigs was unaffected by doses of GR-127,935 up to 20 mg/kg iv (N = 4). In these and similar neurons, 8-OH-DPAT (10 ug/kg iv) produced the characteristic decrease in unit activity (84 ± 12 %; N = 5) expected of a 5-HT_{1A} agonist. These data suggest that (1) unlike the terminal autoreceptors, those on cell bodies/ dendrities may not be under tonic control, and/or (2) the amount of 5-HT released by this 5-HT_{1D} antagonist at the cell body autoreceptor is not sufficient to inhibit cell firing.

539.9

INHIBITION OF ADENYLYL CYCLASE ACTIVITY IN THE RAT DORSAL RAPHE REGION MEDIATED BY ACTIVATION OF 5-$HT_{1B\text{-}LIKE}$ RECEPTORS. W.P. Clarke*[1], K.A. Berg[1] and S. Maayani[2]. Dept. of Pharmacology[1], Univ. of Texas Health Science Center, San Antonio, Texas 78284 and Dept. of Anesthesiology[2], Mount Sinai School of Medicine, NY, NY 10029.

Regulation of the activity of serotonergic neurons, located mainly within the raphe nuclei of the brainstem, is in part due to the action of serotonin (5-HT) at somato-dendritic autoreceptors which have been characterized as the 5-HT_{1A} subtype. Serotonergic regulation of 5-HT neuronal activity has been implicated in the etiology of affective disorders such as depression and anxiety. In regions like the hippocampus, postsynaptic 5-HT_{1A} receptor activation results in an increase in K^+ conductance and an inhibition of adenylyl cyclase (AC) activity. However, while it is well known that presynaptic 5-HT_{1A} receptors in the raphe increase K^+ conductance, they do not appear to inhibit adenylyl cyclase. Here we report that activation of a receptor that resembles the 5-HT_{1B} subtype inhibits AC in membranes prepared from the dorsal raphe region.

Cubes (≈2 mm^3) containing the dorsal raphe region from 3-8 rats (male Sprague Dawley) were pooled and homogenized. Adenylyl cyclase activity (the rate of conversion of [^{32}P]-ATP to [^{32}P]-cAMP) was measured in the presence of forskolin (10 µM) and various concentrations of agonists and antagonists. Selective 5-HT_{1A} agonists (±)8-OH-DPAT, (R+)8-OH-DPAT and dipropyl-5-carboxamidotryptamine did not inhibit forskolin-stimulated AC activity (FSAC). In contrast, the 5-HT_1 agonist 5-carboxamidotryptamine (5-CT) produced a 10% inhibition (EC_{50} = 25 nM) which was not affected by spiperone (3 µM), but was antagonized (10-fold shift) by methiothepin (0.3 µM). The rank order of agonist potency was 5-CT > 5-HT > RU 24969 > LSD > CP 93,129 = sumatriptan > CGS 120 66B which correlates well with that for the endogenously expressed 5-$HT_{1B\text{-}like}$ receptor reported in CHO cells (Berg *et al.*, Mol. Pharmacol. 46: 477-484, 1994). These data suggest that in addition to 5-HT_{1A} receptors, 5-HT_{1B} receptors may regulate 5-HT neuronal function in the dorsal raphe. (Supported in part by USPHS grants HD 26437, MH 48125 and GM 34852)

539.10

DIFFERENTIAL ADDRESSING OF 5-HT_{1A} AND 5-HT_{1B} SEROTONIN RECEPTORS IN THE CNS AND IN LLC-PK1 EPITHELIAL CELLS. X. Langlois[1], S. El Mestikawy[1], M. Arpin[2], D. Louvard[2], A. Triller[3]*, M. Hamon[1] and M. Darmon[1].
[1]INSERM U288, [2]Institut Curie and [3]ENS-INSERM, Paris, France.

Specific antibodies raised against selective peptide sequences within the third intracellular loop of the rat 5-HT_{1A} and 5-HT_{1B} receptors were obtained in rabbits. Detailed mapping of the corresponding receptors in rat brain sections using immunoautoradiographic and immunohistochemical techniques confirmed the regional and cellular distributions previously established with specific radioligands. In particular, combined immunocytochemical investigations with these antibodies and in situ hybridization studies allowed the direct demonstration that both 5-HT_{1A} and 5-HT_{1B} receptors are synthesized by serotoninergic neurons within the dorsal raphe nucleus. However, 5-HT_{1A} receptors were exclusively found at the level of their somas and dendrites whereas 5-HT_{1B} receptors appeared to be functionally expressed solely on their terminals. Further studies on the differential addressing of these receptors were carried out in an epithelial cell line from the pig kidney, LLC-PK1, with distinct basolateral and apical domains in the plasma membrane. These cells were transfected with constructed plasmids so as to permanently express either the rat 5-HT_{1A} or the rat 5-HT_{1B} receptors. Immunofluorescence experiments with specific anti-5-HT_{1A} and anti-5-HT_{1B} receptor antibodies and confocal microscopy examination demonstrated that 5-HT_{1A} receptors were localized exclusively in the baso-lateral domain of the cell membrane whereas 5-HT_{1B} receptors were confined to its apical domain. These data support the idea that the message for the differential adressing of 5-HT_{1A} and 5-HT_{1B} receptors is contained within the primary sequence of these receptors. Construction of chimeric receptors is under way in an attempt to identify the peptide sequence(s) responsible for the specific addressing of 5-HT_{1A} and 5-HT_{1B} receptors in LLC-PK1 cells.

SEROTONIN: UPTAKE, FUNCTION

540.1

ANTIDEPRESSANTS INHIBIT THE NEURONAL 5-HT TRANSPORTER IN GLIAL CELLS. IN VIVO AND IN VITRO STUDIES. F. Artigas*, N. Bel, G. Figueras, C. Suñol, M.T. Vilaró, G. Mengod. Dept. of Neurochemistry, C.S.I.C. Barcelona, Spain.

Rat brain astrocytes take up 5-HT with high-affinity (Dave and Kimelberg, *J Neurosci* 14:4972-4986, 1994). We have examined the sensitivity of this glial transporter to antidepressants by exposing primary cortical astrocytic cultures to 100 nM 5-HT (30 min) in presence of the uptake inhibitors citalopram, clomipramine, fluoxetine, fluvoxamine, paroxetine and sertraline. Astrocytes take up 5-HT (ca. 100 fmol/mg protein·min) and metabolize it to 5-HIAA. The addition of 1-10 µM of all uptake inhibitors reduced significantly (31-61 %) the uptake of 5-HT, as measured by the 5-HT + 5-HIAA cell content at the end of the incubation period. The presence of the neuronal 5-HT transporter mRNA in cultured astrocytes was established after extraction of total RNA, reverse transcription, PCR amplification and hybridization with a probe complementary to that of the cloned 5-HT transporter.

In vivo, 5-HT uptake was assessed by perfusing 5-HT (0.1 - 1 µM) through a dialysis probe implanted in frontal cortex of conscious rats and measuring 5-HT at the probe outlet. Recovery of 5-HT by the tissue (uptake + diffusion) was 52±4 % of the infused 5-HT in control rats. Addition of citalopram to the perfusion fluid reduced the tissue 5-HT recovery to 19±2 % suggesting that most 5-HT is removed by high affinity uptake. In 5,7-DHT lesioned rats (200 µg i.c.v., 2 weeks before) the recovery of 5-HT was comparable to that in control animals or in 6-OHDA-lesioned animals (45±2 % vs. 54±3 % and 49±2 %, respectively). Citalopram addition to the perfusion fluid reduced also the *in vivo* 5-HT uptake in 5-HT-denervated animals, thus indicating the presence a non-neuronal uptake for 5-HT sensitive to antidepressants.
(Supported by FIS grant 95/266)

540.2

SPECT Imaging after Paroxetine Challenge Kate M. Bell, M.D.* and Christopher Reist, M.D. Dept.of Psych.,LBVA Hosp., Univ. Ca. Irvine Sch. of Med., Long Beach, CA 90822

Challenge paradigms coupled with SPECT imaging of cerebral blood flow (CBF) have proven useful tools in the study of many neurological and psychiatric disorders. Paroxetine, the potent and selective serotonin reuptake inhibitor, may offer greater specificity than commonly used serotonergic agents to study the function of the serotonergic system. In a pilot study, we examined the changes in CBF in 4 normal controls before and after paroxetine challenge using 99-m-Tc-HMPAO SPECT . Subjects were male, right-handed and aged 32±3 years. SPECT imaging was performed at rest with eyes, ears open and low ambient noise. The second scan was also obtained in the resting state 3 hours after taking 40 mg of paroxetine. Relative to the cerebellar CBF, regions of interest (ROI) before and after paroxetine challenge were compared using paired two-tailed T tests for each ROI. Significant decreases in CBF after paroxetine were seen in the temporal poles (hippocampus, medial and inferior gyri), and the right thalamus. Additional aréas that showed decreases at the trend level were the right superior parietal and left superior frontal lobe. The changes are in agreement with SPECT [^{125}I]-paroxetine binding data and would be consistent with an inhibitory role of serotonin on blood flow.

540.3

CHRONIC FLUOXETINE REVEALS AN INCREASE OF RENIN RELEASE BY 8-OH-DPAT: EVIDENCE AGAINST MEDIATION BY 5-HT_7 RECEPTORS. A. Vicentic*, Q. Li, K. Kunimoto, W. Pinto, T.M. Cabrera, G. Battaglia and L.D. Van de Kar Dept. Pharmacol. Loyola Univ. Chicago, Sch. Med., Maywood, IL 60153.

Previous studies indicate that 5-HT_{1A} agonists increase prolactin but not renin secretion. However, repeated injections of fluoxetine expose a stimulation of renin release by the 5-HT_{1A} agonists 8-OH-DPAT and ipsapirone. Since chronic fluoxetine reduces the 5-HT_{1A} receptor-mediated increases in ACTH and oxytocin, our hypothesis was that the effect of 8-OH-DPAT on renin may be due to activation of 5-HT_7 receptors. Male rats received either saline or fluoxetine injections (10 mg/kg, ip) once daily for 3 days. The 5-HT_2/5-HT_7 antagonist ritanserin (0.1 mg/kg, sc) or vehicle were injected 18 hr after the last fluoxetine injection and 1.5 hr before sacrifice. Rats were challenged with saline or the 5-HT_{1A}/5-HT_7 agonist 8-OH-DPAT (500 μg/kg, sc) 15 min before sacrifice. The dose of ritanserin was sufficient to inhibit both 5-HT_2 and 5-HT_7 receptor-mediated responses. The dose of 8-OH-DPAT, which has a low affinity for 5-HT_2 receptors, but a high affinity for 5-HT_7 receptors (pK_i= ±7.3) was sufficient to activate both 5-HT_{1A} and 5-HT_7 receptors. In fluoxetine but not in saline treated rats, 8-OH-DPAT increased renin release. Ritanserin did not inhibit this effect of 8-OH-DPAT on renin release. Also, fluoxetine potentiated the prolactin response to 8-OH-DPAT. Ritanserin did not inhibit the prolactin response to 8-OH-DPAT, regardless of whether or not the rats were treated with fluoxetine. These data suggest that induction of renin and potentiation of prolactin responses to 8-OH-DPAT, after fluoxetine exposure, are not mediated by 5-HT_7 receptors (supported in part by USPHS MH45812 to LVdK).

540.4

EFFECTS OF SEROTONIN REAGENTS ON BUFOTENINE LEVELS IN THE BRAIN OF *BUFO*. Naokuni Takeda*, Department of Biotechnology, Research and Development Center, COSMO Research Institute, Satte, Saitama, 340-01, Japan

Bufotenine (5-hydroxy-N, N-dimethyltryptamine:BUTN) is a methylated compound detected in urine specimens in psychiatric disorders, such as autism, epilepsy, mental retardation, depression and schizophrenia (Takeda *et al.*, 1995:submitted). The brain of *Bufo* has been known to produce BUTN (Takeda, *Comp. Biochem. Physiol.* 107C:275, 1994: *Soc. for Neurosci.* 19:1171, 1993; 20: 1165, 1994). To get pharmacological clues to the etiology of such human psychiatric disorders, the brains of *Bufo* were used as an animnal model. To examine the regulations of BUTN in the brain, serotonin reagents were injected directly into the brain by microdialysis. These were *p*-chlorophenylalanine (*p*-CPA) and ritanserin. Both *p*-CPA and ritanserin caused a decrease in the levels of BUTN, compared with sham operated controls. The level of BUTN was related to that of serotonin. The effects were greater in *p*-CPA than in ritanserin. Reduced levels of BUTN were also found in blood and urine specimens, confirming the origin of BUTN in the brain.

540.5

ORIGIN OF THE SEROTONERGIC INNERVATION TO THE DORSOLATERAL HYPOTHALAMUS IDENTIFIED BY RETROGRADE TRANSPORT AND DOUBLE IMMUNOCYTOCHEMISTRY. V. Ljubic-Thibal, A.J. Morin, E. Hamel and M. Diksic*. Montreal Neurological Inst and Hosp, and Dept. Neurology and Neurosurgery, McGill Univ, Montréal, QC, Canada H3A 2B4

Recent observations from our laboratory suggest that the serotonergic neurons in the lateral wings of the dorsal raphe nucleus (LW-DRN) project ipsilaterally to the dorsolateral hypothalamus (DLH). Selective lesioning of DLH serotonergic terminals results in increased levels in the ipsilateral LW-DRN of the serotonin-synthesizing enzyme, tryptophan-hydroxylase (TPH) and of newly synthesized serotonin, as reflected by selective accumulation of α[^{3}H]methyl-serotonin (α[^{3}H]M5-HT) in this area following systemic injection of its precursor α[^{3}H]M-tryptophan (α[^{3}H]MTrp) (*Biochem Pharm* 49:633, 1995; and Ljubic-Thibal et al., unpublished). In order to better identify the cells of origin of these DLH serotonergic projections, we injected α[^{3}H]MTrp (25μCi, SA 10Ci/mmol in 0.05 μl CSF) or Cholera toxin ß subunit (CTb iontophoretically at 2 μA for 30 min) into the DLH (from Bregma -3.0; 1.0 lateral; 8.1 from brain surface). CTb sections were also processed for TPH immunocytochemistry. Following injection of α[^{3}H]MTrp (10 days) or CTb (5 days), retrogradely labelled neurons were primary confined to the ipsilateral DRN. Some of the retrogradely labelled CTb neurons were also immunopositive for TPH and these were localized more specifically in the upper part of the LW-DRN. These results confirm that serotonergic neurons in the lateral aspect of the DRN project ipsilaterally to the hypothalamus (O'Hearn & Molliver, *Brain Res Bull* 13:709, 1984). Further, they show that part of the DLH serotonergic innervation originates in the lateral wings of the DRN, an area which appears particularly vulnerable to alterations in the terminal field of the DLH. Supported by MRC of Canada and NINDS-USA.

540.6

IMMUNOCYTOCHEMICAL DETECTION OF SEROTONIN AND SEROTONIN TRANSPORTER IN THE HUMAN SMALL INTESTINE. S. B. Bledsoe*, S. K. F. Chong, and F. C. Zhou, Depts. Anatomy and Pediatrics, Indiana Univ. Sch. Med., Indianapolis, IN 46202

We previously showed that an antibody against multiple-antigenic (MAP) form peptide 315 of serotonin (5-HT) transporter (5-HTT315) can identify the 5-HT-like fibers in the brain (Bledsoe and Zhou, 1994). Currently, we report that using antibodies to 5-HT and 5-HTT315, we are able to identify 5-HT producing cells and 5-HT transporter bearing cells in the small intestines.

Human small intestine samples were obtained through biopsy and fixed in 4% paraformaldehyde for 40μm sectioning and staining. The rabbit 5-HTT315 and mouse polyclonal 5-HT (against Limulus hemocyanin conjugated 5-HT) antibodies were used for indirect immunocytochemical staining. Large columnar 5-HT-immunoreactive (im) cells were observed in the mucosal crypt or in the villi. Many 5-HT-im cells are argentaffine cell-like with apexes facing the lumens. When secretion of 5-HT occurs it should have quick access to the lumen.

The 5-HTT-im was observed in columnar cells, and located in the mucosal crypt and villi. The morphology of the 5-HTT315-im cells is similar to the 5-HT-im cells, but smaller in number. These data indicate that both 5-HT producing cells and 5-HT transporters bearing cells exist in crypts and villi of human small intestine. The existence of these two types of cells suggest that 5-HT can be produced and taken back into human small intestine. The exact role of the 5-HT in the small intestine, or the purpose of conservation or elimination of 5-HT in the GI tract is unknown. It is also unknown whether the same cells which produce the 5-HT are responsible for the uptake of the 5-HT. Study is in progress to identify the two populations of cells which produce and/or take up 5-HT (Support NIMH 50602 to Zhou).

540.7

SEROTONIN INNERVATION IN THE RAT SUBSTANTIA NIGRA: QUANTITATIVE DATA AT THE LIGHT AND ELECTRON MICROSCOPIC LEVELS. H. Moukhles*, O. Bosler, J.P. Bolam, A. Vallée, D. Umbriaco M. Geffard and G. Doucet. Département de pathologie and CRSN, Université de Montréal, Montréal, Qc, Canada

To further understand the role of serotonin (5-HT) in the neural circuitry of substantia nigra (SN), we have quantified the number of 5-HT axonal varicosities, the proportion of such varicosities bearing a synaptic specialization and their repartition among potential targets in the *pars reticulata* (SNr) and *pars compacta* (SNc) of the rat. Serotonin axonal varicosities, counted at the light microscopic level following in vitro [^{3}H]5-HT uptake/storage and radioautography, amounted to 8 x 10^6/mm^3 in the SNr and 6 x 10^6/mm^3 in the SNc. Their synaptic incidence, determined at the electron microscopic level following immunolabeling with a monoclonal antibody against a 5-HT-glutaraldehyde-protein conjugate, was in the order of 65-100% in the SNr and 35-50% in the SNc. The vast majority (93%) of these synapses were on dendrites. In dual labeling experiments, with the combined tyrosine hydroxylase immunolabeling of dopamine (DA) neurons, 19 % of the 5-HT synaptic contacts in the SNr were on DA dendrites and 6% were on a type of dendrites (MT) identified by its peculiarly high cytoplasmic density of microtubules. The mean diameter of the 5-HT terminals and the mean length of their synaptic contacts were smaller on DA dendrites and larger on MT dendrites than on unidentified dendrites, whereas the width of the DA dendritic targets was larger than that of unlabeled dendritic targets. The present data suggest that 1) 5-HT innervation of the SN is largely synaptic; 2) DA dendrites are among the synaptic targets, and 3) the nature of the synaptic targets is an important determinant of the dimension of 5-HT varicosities and length of synaptic contacts. (Supported by the MRC and FRSQ).

540.8

PRE- AND POST-SYNAPTIC INHIBITION BY SEROTONIN IN RAT SUPRACHIASMATIC NUCLEUS (SCN) *IN VITRO*. Z. G. JIANG* Y. Q. YANG AND C.N. ALLEN Center for Research on Occupational and Environmental Toxicology and Department of Physiology, Oregon Health Sciences University, Portland, OR 97201.

Serotoninergic transmission has anatomically and functionally been implicated in the SCN biological clock mechanisms in mammals, but the cellular actions of serotonin in the SCN remain largely unknown. Using a whole-cell recording technique on horizontal brain slices from circadian rhythm-monitored rats, we tested several serotonin analogs in a total of 109 cells in the ventral, VIP-positive part of the SCN. It was found that: 1) serotonin (1-100 μM), 5-CT (0.1-10 μM) or 8-OH-DPAT (1-30 μM) dose-dependently caused an outward current (5-100 pA) at -60 mV in 30 (27%) neurons throughout the 24 hr cycle. 2) The outward current was associated with an increase in input conductance, was enhanced by depolarization, reduced and reversed its polarity with hyperpolarization, and was blocked by Ba (1 mM), ritanserin (3 μM) or mesulergine (3 μM), but not by TTX (1 μM) or pindolol (3 μM). 3) The optic nerve stimulation-evoked EPSC was reduced by 10-70% (6 of 10 cells), and the focal stimulation-evoked and spontaneous IPSCs were inhibited by serotonin agonists in 20% of cells ($n \geq 10$). 4) Both extrinsic glutamate-induced inward currents and GABA-induced outward currents were unaffected by serotonin (10 μM $n \geq 4$). In conclusion, serotonin regulates the SCN neurons by both pre- and post-synaptic inhibition mechanisms; the latter may involve activation of 5-HT_7 receptors and then opening of a potassium channel. (Supported by grant AG10794)

540.9

INHIBITORY ROLE OF SEROTONIN IN THE OPTIC TECTUM OF THE FROG.
A.A. Malayev*, and E.A. Debski, School of Biological Sciences, University of Kentucky, Lexington, KY 40506

Serotonin induces membrane hyperpolarization in neurons of the optic tectum of *Rana pipiens* (Malayev & Debski, *Neurosci Abstr.* 20:1704). Using the blind patch-clamp technique in brain slices, we have examined the mechanism of this effect and its implication for tectal functioning.

External application of serotonin (100 μM) in the presence of TTX and the absence of external Ca^{2+} induced outward current in tectal neurons clamped at -50mV. This effect could be mimicked by α-methyl-serotonin (10 μM), the mammalian 5-HT_2 receptor agonist. Voltage-ramp experiments showed that the activated conductance had a linear I-V curve with a reversal potential close to E_{K+}. Including the Ca^{2+} chelator BAPTA (20 mM) in the patch-pipette did not eliminate the serotonin-activated conductance, showing that elevation of free intracellular Ca^{2+} was not necessary for current activation. However, the presence of GTP-γ-S (500 μM) in the pipette resulted in the irreversible activation of this conductance, suggesting the involvement of G-proteins. The effect of serotonin on tectal activity was examined by inducing synaptic current in layer 6 patch-clamped neurons by acute stimulation in layer 9, the retinorecipient layer. External application of serotonin inhibited only the delayed induced current (in 8 out of 8 cells) and did not have any effect on the monosynaptic response (in 3 out of 3 cells). This effect could again be mimicked by α-methyl-serotonin (in 7 out of 7 cells). These data show that serotonin might inhibit neuronal activity in the optic tectum associated with processing of the visual signal. Supported by NSF grant IBN-9209651.

540.10

SEROTONIN MODULATES SYNAPTIC TRANSMISSION AT THE CENTRAL AMYGDALA NUCLEUS.
H. Zheng* and M.C. Schiess, Neurology,UTMB, Galveston, TX 77555-0539

The purpose of this study was to determine the serotonin (5-HT) receptor subtypes mediating effects on postsynaptic transmission at the central amygdala nucleus (ACe) in a 500 μM coronal *in vitro* slice preparation. Intracellular current clamp recordings from ACe neurons with stimulation (15V) of presumed amygdalo-fugal fibers within the slice typically evoked a waveform consisting of a CNQX (10μM) sensitive fast (f) EPSP, a bicuculline (30μM) sensitive f-IPSP, followed by an AP5 (50μM) sensitive slow (s) EPSP at -60 mV. 10μM 5-HT reversibly reduced the f-IPSP and s-EPSP in $26/31_N$ neurons (84%); with no effect on synaptic transmission in $5/31_N$ (16%). Ketanserin, 5-$HT_{2/1C}$ antagonist, and NAN-190, 5-HT_{1A} antagonist, had no effect alone or in inhibiting serotonin effects on the f-IPSP and s-EPSP. The full 5-HT_{1A}agonist, 8-OH-DPAT reduced the NMDA mediated s-EPSP 50% of the time or had no effect. 2-methyl 5-HT, 5-HT_3 agonist, at 10μM reproduced the effects of 5-HT on reducing the f-IPSP and s-EPSP. Additionally, MDL-72222, 5-HT_3 antagonist, blocked the effects of 5-HT on synaptic transmission. These results suggest that the identified 5-HT effects on reducing a $GABA_A$ mediated f-IPSP and an NMDA mediated s-EPSP can be partially but not solely explained by 5-HT_3 receptor activity.
Supported by UTMB Small Grant to MCS.

540.11

TOPOGRAPHIC ORGANIZATION OF THE RAT DORSAL RAPHE (DR) WITH RESPECT TO MODALITY SPECIFIC SENSORY TARGETS: A COMPUTER ASSISTED THREE DIMENSIONAL ANALYSIS
M.L. Kirifides*, D.M. Devilbiss, R.C.S. Lin and B.D. Waterhouse, Dept. of Anatomy and Neurobiology. MCP/Hahnemann Univ., Phila., PA 19102.

Previous investigations have demonstrated a crude topographic organization of the DR with respect to efferent targets in the mammalian brain. The present report describes a computer-based image analysis of the distribution and neurochemical identity of neurons within sub-regions of the DR that project to different cortical and subcortical sensory targets. Fluorescent retrograde tracers were used to identify neurons within the DR which project to sites along the ascending somatosensory trigeminal and visual pathways. Projection neurons were further identified immunohistochemically using antibodies directed against serotonin, dopamine, or GABA. Three dimensional representations of the data were created by manually or automatically scanning 40 μm coronal brainstem sections and serially aligning arrays of retrogradely or immunohistochemically labeled cells. DR boundaries and other landmark structures with respect to a three dimensional biological coordinate system were maintained by a computer (C.A.R.P., Biographics, Inc. Winston-Salem, N.C.). Labeled cells were represented by color-coded spheres according to injection site or neurochemical identity. Thus, multiple experimental cases could be displayed simultaneously; thereby, revealing spatial relationships between functionally or chemically related subsets of DR neurons. We found that in comparison to conventional analytical procedures C.A.R.P. provided an accurate and efficient method of acquiring and manipulating a large database of neuroanatomical information. Overall, this analysis clarifies the spatial relationships between modality and neurochemical-specific clusters of cells within discrete sub-regions of the DR nucleus. (Supported by NIDA DA 05117 and NINDS 32461 to BDW)

540.12

ULTRASTRUCTURAL FEATURES OF BRAIN SEROTONIN PERIKARYA AND NERVE TERMINALS AFTER LONG TREATMENT REGIMENS WITH DEXFENFLURAMINE.
M. Kalia* and N.P.O'Malley, Dept. of Neurosurgery, Jefferson Medical College of Thomas Jefferson University, Philadelphia PA 19107.

Light microscopy of immunostained serotonergic perikarya and nerve terminals reveals the presence of serotonin (5HT) but provides few morphological clues about cellular integrity. Previous studies from our laboratory have employed retrograde tracing and double immuno labeling and have demonstrated the presence of 5HT in animals treated with dexfenfluramine (DFF). In this study we examined 5HT neurons in animals treated with 16 mg/kg of DFF for 21 days. Post treatment survival periods ranged from 18 hr. to 21 days. A polyclonal serotonin antibody and standard immunocytochemical (ICC) methods were used for staining prior to embedding in plastic. Serotonergic profiles were first identified under the light microscope and serial ultra thin sections of the selected area were collected on fomvar-coated grids, stained and examined on a JOEL 100CX electron microscope at an accelerating voltage of 80K. The labeling was densest in the superficial parts of the section and was visualized as an electron dense reaction product. Examination of serial sections permitted us to follow a serotonergic neuron once the labeled profile was identified. We examined the brains of 10 experimental and 10 control rats. The ultrastructure of serotonergic immunoreactive profiles was indistinguishable in the two groups. We found no ultrastructural evidence of disruption of neuronal morphology in animals treated with DFF. Since we examined transmitter identified neurons we can definitively conclude that dexfenfluramine produces no neuropathology in brain serotonergic neurons. Supported by IRIS.

540.13

DETERMINANTS OF HUMAN PLATELET AND PLASMA SEROTONIN.
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A series of studies on the determinants of human platelet (PLT) and platelet-poor plasma (PPP) serotonin have been carried out. We have measured PLT serotonin (5HT) in patients with idiopathic thrombocytopenia (ITP), PLT and PPP 5HT in subjects receiving oral 5HT loads, and PPP 5HT in a large number of adult normal control subjects (N=21).

When measured PLT 5HT levels of ITP patients were expressed as ng/billion PLTs, an inverse relationship between level and PLT count was observed, suggesting that PLTs load 5HT throughout their circulating lifespan. No increases in PLT or PPP 5HT were observed after an oral load of 5HT (0.25 mg/kg) in normal subjects or in patients medicated with fluoxetine, suggesting that little luminal 5HT passes through the intestine into the blood stream. In addition, a meal does not appear to result in increased levels of 5HT in the general circulation, even in the presence of potent 5HT reuptake inhibitors. Finally, the mean PPP 5HT in normal subjects was 160 ± 80 pg/ml, even lower than our previous estimation of 300-500 pg/ml and in agreement with the recent report of Beck et al. (BBRC, 196:260-266). The relevance of the findings to physiological models of PLT, PPP, and gut 5HTwill be discussed, and the implications with respect to possible roles of 5HT in homeostasis, immune response, emesis and digestive processes will be considered.

540.14

BURST-LIKE FIRING PATTERN IN A SUB-POPULATION OF PRESUMED 5-HT NEURONES IN THE RAT DORSAL AND MEDIAN RAPHE NUCLEI.
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It is generally accepted that 5-HT neurones in the dorsal (DRN) and median raphe nuclei (MRN) are slow firing (0.1- to 3 Hz) with a regular pattern. Here, we report the results of an *in vivo* electrophysiological study suggesting that a sub-population of raphe 5-HT neurones have additional neurophysiological characteristics. Extracellular and intracellular recordings were made in male Sprague-Dawley rats anaesthetised with chloral hydrate, Saffan, or chloral hydrate and ketamine. Extracellular or intracellular potentials were recorded with glass or metal microelectrodes using conventional electrophysiological methods. Antidromic stimulations were performed from the medial forebrain bundle (MFB).

Presumed 5-HT neurones recorded extracellularly were sorted into two categories: (i) classical 5-HT neurones firing only single spikes in a regular pattern as described previously, and (ii) neurones in which, during the otherwise regular firing pattern, some of the expected single spikes were replaced by a doublet or triplet of spikes. Spikes in this burst-like firing mode (spikes in doublets or triplets) were very close together (2.4 to 11.5 ms), and occurred with a diminishing spike amplitude. The proportion of spikes in doublets to single spikes ranged from 5 to 95% of the total spikes displayed. Burst-firing raphe neurones were detected with both glass and metal electrodes, and regardless of the type of anaesthetic used. Burst-firing neurones were inhibited by 8-OH-DPAT and were stimulated antidromically from the MFB. The bursting pattern occurred in 82 of 295 neurones recorded from the DRN, while it was much less frequent in MRN. The presence of burst-firing raphe neurones with 5-HT neurone-like properties was confirmed by *in vivo* intracellular recordings. Our findings indicate the presence of a previously unrecognised sub-population of 5-HT neurones with a burst-like firing pattern.

540.15

5-HT FACILITATION OF TRIGEMINAL MOTONEURONAL EXCITABILITY VIA MULTIPLE EFFECTS ON INTRINSIC MEMBRANE CONDUCTANCES.
C.-F. Hsiao*, P.R. Trueblood and S.H. Chandler. Department of Physiological Science, UCLA, Los Angeles, CA 90095

To determine the ionic mechanism(s) responsible for 5-HT facilitation of trigeminal motoneuronal (TMN) discharge during reflex and cortically induced rhythmical jaw movements in the guinea pig we investigated the effects of 5-HT application on TMN intrinsic membrane conductances in brainstem slices from guinea pigs. Bath application of 5-HT (10-30 μM) depolarized motoneurons in current clamp and produced an inward holding current in voltage clamp mode. These effects were associated with an increase in membrane resistance and persisted in TTX, and low Ca^{2+}/Mn^{2+}-containing solutions as well as in the presence of certain K^+ channel blockers such as 4-AP and TEA. Extrapolated instantaneous current-voltage relationships obtained before and at the peak of the 5-HT response from holding potentials between -50 and -65 mV intersected at approximately -88 mV which was slightly negative to the predicted E_K, suggesting that the $I_{5\text{-}HT}$ was partially due to a reduction in a leakage K^+ conductance. Ba^{2+} (500 μM) reduced the steady inward 5-HT current and increase in input resistance. However, a residual $I_{5\text{-}HT}$ remained. To determine if the residual $I_{5\text{-}HT}$ was related to the presence of a hyperpolarization-activated current I_h, we studied the effects of 5-HT on the cesium-sensitive I_h. 5-HT enhanced the peak amplitude of this current. However, in solutions containing a mixture of Ba^{2+} and Cs^+ a residual small $I_{5\text{-}HT}$ was still present which was not associated with a change in input resistance. The data suggest that the previously demonstrated facilitation of TMN discharge by 5-HT during oral-motor behaviors occurs through a combination of a reduction in a leakage K^+ current, an increase in a Cs^+-sensitive I_h and an increase in an unidentified inward current. Supported by NIH RO1 DE 06193 and DE 04166

540.16

SEROTONIN REDUCES AFTERHYPERPOLARIZATION IN HIPPOCAMPUS BY INHIBITING CALCIUM-INDUCED CALCIUM RELEASE.
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We have previously shown that serotonin acting on 5-H4 receptors increases excitability in hippocampus by inhibiting a slow calcium-activated afterhyperpolarization (AHP). Calcium-induced calcium release (CICR) has been proposed to modulate intracellular calcium transients in hippocampus. Therefore we have now examined the role of CICR in the generation of the AHP as well as its possible regulation by serotonin.

Intracellular recordings were obtained from the CA1 region of the rat hippocampus in *in vitro* brain slices. Caffeine, but not adenosine antagonists nor the phosphodiesterase inhibitor IBMX, enhanced the AHP triggered by 1-20 action potentials. In agreement with these results, administration of the calcium release channel blockers dantrolene and ruthenium red or of the intracellular calcium pump inhibitor thapsigargin reduced the amplitude of the AHP and blocked the effect of caffeine. These results indicate that CICR plays an important role in the generation of the AHP in this area.

Interestingly, the ability of serotonin to inhibit the AHP was dramatically reduced or abolished when CICR was prevented from contributing to the AHP by using either dantrolene, ruthenium red or thapsigargin. In contrast, the ability of serotonin to reduce the AHP was increased when the contribution of CICR to the AHP was enhanced by using caffeine. These results indicate that CICR plays an important role in the generation of the AHP in the CA1 region and constitutes the specific target that is regulated by 5-HT4 receptors to reduce the AHP and increase membrane excitability. Supported by MH 43985.

INTERACTIONS BETWEEN NEUROTRANSMITTERS III

541.1

DOPAMINERGIC AXON TERMINALS ARE ABSOLUTELY REQUIRED FOR THE TIGHT FUNCTIONAL COUPLING BETWEEN *EAA* AND *DA* RECEPTORS
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In extensively washed synaptic membrane preparations from rat frontal cortex, the *in vitro* addition of either the D_1 (SKF 38393) or the D_2 (LY 171555) specific agonists markedly decreased the apparent affinity (Kd) of [^{3}H]-MK801 specific binding. In the same membrane preparation, the concentration of L-glutamate required to produce half maximal enhancement of [^{3}H]-MK801 binding was approximately the same both in the presence or in the absence of dopaminergic drugs.
I.c.v. administrations of the neurotoxin 6-OHDA resulted in a dramatic reduction of DA frontal cortex levels, whilst repeated administrations (21 consecutive days) with either the D_1 (SCH 23390) or the D_2 (YM 09151-2) antagonists failed to change amine (and metabolite) contents. Repeated administrations with D_1 receptor blocker selectively increased the Bmax values of [^{3}H]-SCH 23390 binding while [^{3}H]-Spiroperidol binding was increased both by repeated administrations of YM 09151-2 and by i.c.v. injection of 6-OHDA. Although chronic D_2 blockade and 6-OHDA consistentely increased D_2 receptor number, in extensively washed synaptic plasma membranes of rats repeatedly administered with YM 09151-2 but not with 6-OHDA [^{3}H]-MK801 binding was increased. It is concluded that the effects of NMDA receptor activation could not be directely mediated by stimulation of DA release, but are highly dependent upon the presence of DA axon terminals.

541.2

FAST INHIBITION OF *N*-METHYL-D-ASPARTATE (NMDA) RECEPTOR FUNCTION: AN ATYPICAL EFFECT OF DOPAMINE.
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A novel, possibly non-metabotropic action of dopamine (DA) in the modulation of excitatory amino acid (EAA) function was observed in neurons isolated from the chick retina and from the rat thalamus and grown in culture. Two assays were used: EAA-mediated ^{3}H-GABA release and whole-cell patch-clamp current recordings. DA (200 μM) inhibited ^{3}H-GABA release induced by L-GLU and NMDA, but not by kainate. This effect of DA was mimicked by (+)SKF-38393, a specific DA1 receptor agonist, but not by 8-Br-cAMP (5 mM) or by forskolin (20 μM). Also, the inhibition could not be prevented by the antagonists (+)SCH-23390 (DA1), haloperidol or spiroperidol (DA2), each at 10 μM. The direct effects of DA and (+)SKF-38393 on NMDA-induced currents were measured using a motorized flowpipe array, fast-perfusion method. DA (100-200 μM) and (+)SKF-38393 (50 μM) partially blocked the current induced by NMDA (50-100 μM with glycine 10 μM), but not that induced by kainate. The effect was stereospecific: (-)SKF-38393 did not modulate ^{3}H-GABA release and was much less potent in blocking the NMDA-induced current. In 8 thalamic neurons, (+)SKF-38393 reduced this current by 52±3%; this blockade was unaffected by (+)SCH-23390 (10μM). The blockade developed in less than 0.5 s and reversed at a similar rate upon removal of the dopaminergic agonist. These fast kinetics are consistent with a hypothesis that a putative DA site may be closely coupled to the NMDA receptor-channel. (Supported by CNPq, CAPES, CEPEG, FAPERJ, Finep-UMAB Mol. Pharm. Train. Prog.; USPHS Grants NS25296 and ES05730).

541.3

DOPAMINE-GLUTAMATE INTERACTIONS IN THE REGULATION OF PROTEIN PHOSPHORYLATION IN THE NUCLEUS ACCUMBENS.
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Medium spiny neurons of the nucleus accumbens are innervated by mesolimbic dopaminergic projections from the ventral tegmental area and by glutamatergic neurons projecting from cortex and hippocampus. There is considerable evidence to indicate that the actions of dopamine and glutamate on accumbens neurons underlie many of the acute and chronic effects of various drugs of abuse. We are examining possible interactions between dopaminergic and glutamatergic systems in the regulation of protein phosphorylation in the nucleus accumbens. Neurons of the nucleus accumbens abundantly express DARPP-32, a *d*opamine and c*A*MP-*r*egulated *p*hospho*p*rotein (Mr=*32*kD), which is converted into a potent inhibitor of protein phosphatase-1 when phosphorylated by cAMP-dependent protein kinase (PKA). Incubation of rat nucleus accumbens slices with dopamine (DA)(100 μM) or a D_1 receptor agonist (SKF 38393, 1 μM) increases DARPP-32 phosphorylation by several-fold. DA-induced phosphorylation of DARPP-32 is inhibited by incubation with AMPA (1-100 μM), an agonist at AMPA-type glutamate receptors. This effect is reversed by pretreatment of slices with CNQX (50 μM), a selective antagonist. In agreement with studies in neostriatum (Halpain et al, *Nature*, 343:369), NMDA also blocks DA-induced phosphorylation of DARPP-32 in accumbens slices; this effect is reversed by the NMDA receptor antagonist, MK-801. Similar regulation of the phosphorylation of protein phosphatase inhibitor-1, another inhibitor of protein phosphatase-1, has also been observed in the nucleus accumbens. Thus, both AMPA- and NMDA-type receptors apparently participate in glutamate-mediated inhibition of DARPP-32 phosphorylation in the nucleus accumbens, most likely by mediating calcium-dependent activation of a phosphatase (calcineurin) which dephosphorylates DARPP-32. (Supported by MH40899 to P.G. and a NARSAD Young Investigator Award to G.S.)

541.4

CO-EXPRESSION OF STRIATAL DOPAMINE RECEPTOR SUBTYPES AND EXCITATORY AMINO ACID SUBUNITS.
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Nigrostriatal dopamine (DA) modulates the corticostriatal glutamatergic (EAA) afferent responses. This interaction may be mediated through activation of specific DA and EAA receptor subtype combinations located predominantly on the postsynaptic medium-spiny striatal projection neuron. We examined the cellular expression patterns for D_{1A}, D_2, or D_3 DA receptors versus ionotropic EAA subunits; NMDA-R_1 and non-NMDA ($GluR_1$, $GluR_2$) by detection of transcript abundance, expression of encoded proteins, and analysis of binding profiles using concurrent fluorescent localization paradigms. The results demonstrate: (1) mRNA transcripts do not always reflect protein or binding patterns of the receptor, especially for the $GluR_1$ subunit, (2) all three DA receptor mRNAs are found in medium-sized striatal neurons, positive for all three encoded EAA subunit proteins, (3) $GluR_{2/3}$ and NMDA-R_1 protein containing neurons also possess viable ligand recognition sites for the D_2 DA receptor subfamily, (4) D_2 and D_3 protein levels are detected in neurons which bind the NMDA receptor toxin, conantokin-G. The data suggest a morphological "promiscuity" for these receptors in that all combinations of DA subtypes and EAA subunits can be co-expressed in the medium-sized striatal neuron populations. This supports physiological data (*Proc. Nat. Acad. Sci. USA* **90**:9576-9580, 1993) that showed that postsynaptic responses can be predicted on the basis of co-activation of selective pairs of DA and EAA receptors in medium-sized striatal neurons. This work was supported in part by USPHS NS 23079 and NS 32277 to MAA.

541.5

CELLULAR ELECTROPHYSIOLOGICAL AND NEUROCHEMICAL ASSESSMENT OF HIPPOCAMPAL 5-HT and NMDA FUNCTION FOLLOWING REPEATED FENFLURAMINE ADMINISTRATION. A. Dugar*, K. Dowhower, and J.M. Lakoski. Departments of Pharmacology and Anesthesia, The Pennsylvania State University College of Medicine, Hershey, PA 17033

Fenfluramine, clinically used as a potent anorectic agent for treatment of obesity, stimulates the release and inhibits the reuptake of serotonin (5-HT) resulting in decreased levels of this neurotransmitter. *N*-methyl-d-aspartate (NMDA) antagonists have been demonstrated to protect against depletions of 5-HT produced by structurally similar substituted amphetamines. Therefore, electrophysiological and neurochemical studies of interactions between 5-HT (including the 5-HT_{1A} subtype) and NMDA receptors may provide insight into fenfluramine-mediated responses.

Adult male Sprague-Dawley rats were injected with *d,l*-fenfluramine (FEN; 12.5 mg/kg, i.p., 2x daily for 4 days) or with vehicle (saline). At 24 hr following the last injection, *in vivo* microiontophoresis in a chloral hydrate anesthetized preparation was performed in the CA1 and CA3 hippocampal subfields using 5-HT, 8-OH-DPAT (selective 5-HT_{1A} agonist), NMDA and MK-801 (noncompetitive NMDA antagonist). A second group was dissected, with the frontal cortex and hippocampus used for receptor binding analyses and autoradiographic localization of 5-HT_{1A} sites ($[^3H]$8-OH-DPAT). Electrophysiological results showed that NMDA and 5-HT exerted excitatory and inhibitory effects in the hippocampus, respectively, independent of drug treatment. In FEN treatment versus vehicle treatment groups, cells showed a supersensitivity to application of 5-HT. Binding studies suggested decreased receptor density, without a concomitant change in affinity, of 5-HT_{1A} sites in the frontal cortex. In conclusion, repeated FEN administration results in a physiological supersensitivity to 5-HT which may be mediated, in part, by a down-regulation of 5-HT_{1A} receptors. Using physiological and neurochemical approaches, the effects of pretreatment with MK-801 on FEN-induced responses are being evaluated to provide further insight into 5-HT and NMDA interactions.

541.6

ANTAGONISTS AT THE GLYCINE MODULATORY SITE OF THE NMDA RECEPTOR REVERSE NEUROKININ$_1$ RECEPTOR AGONIST FACILITATION OF NMDA RECEPTOR EVOKED ACTIVITY IN THE RAT DORSAL HORN. P.A. Heppenstall, S.M. Fleetwood-Walker* and T. Dickinson, Department of Preclinical Veterinary Sciences, University of Edinburgh, Edinburgh, U.K.

Evidence that substance P (SP) and glutamate coexist in fine primary afferent terminals, and that NK_1 and NMDA receptors interact synergistically [Randic, M. *et al.*, J. Physiol. **63**:265-286 (1992)] suggests a potential role for these receptors in the central hyperexcitability that is brought about by sustained activity in nociceptive afferents. Indications that SP may have more than a simple direct action in acute nociception are supported by evidence that glycine is released from local inhibitory interneurons by SP [Maehara, T. *et al.*, Regul. Peptides Suppl. **1**, S102 (1992)]. Following sustained activity, glycine might now co-activate the glycine recognition site of the NMDA receptor (Gly_{NMDA}) and contribute to the NK_1 and NMDA interaction. We have investigated this hypothesis using extracellular recordings form laminae III-V dorsal horn neurons of anaesthetised rats.

Ionophoresis of the selective NMDA agonist 1-aminocyclobutane-cis-1,3-dicarboxylic acid (ACBD) produced a 945±343% increase in the firing rate. The highly selective NK1 receptor agonist acetyl-[Arg^6,Sar^9,$Met(O_2)^{11}$]-SP_{6-11} facilitated this response by 223±37%, but had no effect when applied without ACBD at the same current. In the presence of the Gly_{NMDA} antagonists 7-chlorothiokynurenic acid or L701,252 this increment was reduced by 89±30% and 98±29% while the antagonists had no effect on ACBD evoked activity alone.

This suggests that the co-operation between NMDA and NK_1 receptors is mediated, in part, by the glycine site of the NMDA receptor, perhaps by means of glycine, released from an interneuron in response to substance P.

541.7

INTRACORTICAL INFUSION OF NMDA RECEPTOR ANTAGONISTS: NEUROCHEMICAL EFFECTS . P.H.Hutson*,F. Murray and L.J.Bristow
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Systemic administration of non competitive but not glutamate or glycine site antagonists for the NMDA receptor activate mesocorticolimbic DA pathways.However, cortical DA metabolism was increased following the local infusion of the glutamate site antagonist CPP into the medial prefrontal cortex (mPFC) (Hata et.al. 1990).In the present study, rats were implanted under isoflurane anaesthesia with a unilateral guide cannula ,into the mPFC (A +3.7mm; L 1.0mm; H -3.0mm).48h later infusion of CPP (1.5ug/0.5uL in 1min) increased DA metabolism(as indicated b y [DOPAC]+[HVA]/[DA]) ipsilaterally in mPFC (187% of control), n.accumbens (148% of control), amygdala (146% of control), VTA (150% of control) but not striatum (110% of control) 2h later.These effects were still present 4h after CPP infusion. In contrast ,infusion of the glycine/NMDA site antagonist 5,7-dichlorokynurenic acid (1.5ug/0.5ul in 1 min) at the same coordinates did not significantly increase DA or 5-HT metabolism in these brain regions at any time upto 4h. Results provide further evidence that antagonists acting at distinct sites on the NMDA receptor complex may differentially modulate mesocorticolimbic DA function.
Hata , N. et.al. (1990) Neurosci. Lett. 120, 101-104.

541.8

MODULATION OF NMDA RECEPTORS EXPRESSED IN *XENOPUS* OOCYTES BY SEROTONIN T. Blank*, R. Zwart and J. Spiess, Dept. Molec. Neuroendocrinology , MPI for Exp. Med., Hermann-Rein Str. 3, 37075 Goettingen, FRG

Using *Xenopus* oocytes injected with rat brain total RNA we investigated the effect of 5-HT (5-hydroxytryptamine) on NMDA (N-methyl-D-aspartate) receptor-mediated responses. Two electrode voltage clamp recordings showed that application of 5-HT (200 nM) for 1 min caused a transient potentiation of the NMDA response. After reaching a maximum (175 ± 6.5 %, mean ± SEM, n = 7) in approximately 4 min, the current amplitude had returned to the initial control value within 28 min. Similar enhancement was obtained by application of the PKC activator phorbol 12-myristate 13-acetate (PMA, 10 nM) also for 1 min. No effect was produced by the PKA activator forskolin (20 µM). 5-HT and PMA mediated potentiation of the NMDA responses was strongly reduced by 6 min pretreatment with the PKC inhibitor sphingosine (10 µM). The 5-HT_2 receptor agonist, alpha-methylserotonin (200 nM), mimicked the effects of 5-HT. If 5-HT (200 nM) was coapplied with ketanserin (100 µM), a selective 5-HT_{2A}/5-HT_{2C} receptor blocker, the potentiation of NMDA receptor-induced responses was almost completely abolished. These results demonstrate the involvement of 5-HT_2 receptors in serotonergic modulation of NMDA induced responses most likely via phosphoinositol (PI) hydrolysis and subsequent stimulation of PKC by its physiological activator diacylglycerol (DAG).

541.9

HISTAMINE SLOWS DESENSITIZATION OF NMDA RECEPTOR-MEDIATED ION CURRENT IN RAT CORTICAL NEURONS. R. Zwart*, T. Blank and J. Spiess. Max Planck Institute for Exp. Med., Dept. Molec. Neuroendocrinol., Hermann-Rein-Str. 3, D-37075 Goettingen, F.R.G.

Histamine may play a physiological role in modulating NMDA receptor (NMDAR) function in rat hippocampus, most likely by acting directly at an extracellular site on the NMDAR (Science 261,104-106,1993; Neuron 11,1-8,1993). The mechanism of action of histamine potentiation is not known. Glycine and spermine potentiate NMDAR-mediated ion currents by regulating agonist-induced desensitization kinetics of the receptor.

We investigated the effects of histamine on NMDAR-mediated ion currents in whole-cell voltage clamped rat cortical neurons in primary culture. In a subpopulation of the cells tested (19/33), histamine (10-100 µM) enhanced the amplitude and slowed the desensitization of the NMDA-induced responses in a concentration-dependent manner. Steady-state currents were relatively more potentiated than the initial peak currents, indicating that histamine reduces the degree of agonist-induced desensitization. The effects of histamine on steady state desentization and rate of recovery from desensitization are currently being investigated. In another subpopulation of cortical neurons (13/33), histamine did not have any effect. In one cell, a large potentiation of the NMDA-induced inward current was observed, whereas the kinetics of desensitization remained unaffected.

The results indicate that histamine may play a physiological role in modulating NMDAR-mediated responses in a subpopulation of cortical neurons. The histamine-induced potentiation is most likely due to altered receptor desensitization properties. *Xenopus* oocyte expression studies revealed that histamine potentiation of NMDAR-mediated ion currents strongly depends on the subunits constituting the receptor (Mol. Pharmacol. 46,531-541,1994). The variability in effects observed may be explained by the existence of multiple isoforms of NMDARs in cortical neurons.

541.10

NMDA RECEPTOR INDUCED CURRENTS AND VOLTAGES AT NEOCORTICAL NEURONS: FACILITATION BY RECEPTORS COUPLED TO PHOSPHOLIPASE C. F-J. Kong and R.S. Neuman*. Faculty of Medicine, Memorial University, St. John's, Newfoundland, Canada A1B 3V6.

Using sharp electrodes or whole cell recording, we have examined the actions of carbachol, 1S,3R-ACPD, 5-HT and phenylephrine on neurons, likely pyramidal cells, in slices of cortex prepared from 11-14 day old rats. Perfusion of these agonists: 1) depolarized the membrane or produced an inward current; 2) increased the amplitude and/or duration of the aspartate induced inward current/depolarization. Facilitation of the aspartate response typically appeared earlier than effects on resting membrane potential and current. The concentration-response (C-R) relationship for aspartate was examined with and without 1S,3R-ACPD as this agonist does not exhibit desensitization. 1S,3R ACPD shifted the aspartate C-R curve to the left and increased the maximum response. Facilitation of the aspartate response was observed less frequently in whole cell compared to sharp electrode recordings. However, the addition of 0.1-0.3 mM GTP to the whole cell electrode greatly increased the incidence of facilitation.

Addition of inositol triphosphate (IP_3) to the whole cell electrode after recording the control aspartate responses revealed an enhancement of the aspartate induced inward current. Moreover, the carbachol induced facilitation observed with sharp electrodes was gradually eliminated following intracellular iontophoresis of heparin, an IP_3 receptor antagonist. In whole cell recordings perfusing the recording electrode with heparin also eliminated the carbachol facilitation.

These observations suggest that facilitation of NMDA receptor mediated response in cortical neurons results from an IP_3 receptor mediated release of intracellular Ca^{2+}. Supported by the Canadian MRC

541.11

GABA REDUCES THE INITIAL PHASE OF NMDA-INDUCED ELEVATIONS IN INTRANEURONAL CALCIUM. M.L. Koenig*, J.P. Sanders, and J.L. Meyerhoff. Div. Neuroscience, Walter Reed Army Inst. Research, Washington, DC 20307-5100.

We have investigated the hypothesis that one of the mechanisms by which GABA exerts antiepileptic effects is by reducing the excitatory effects of glutamate mediated by the NMDA receptor. Neurons derived from the forebrains of fetal (E15) rats were loaded with the fluorescent Ca^{2+} indicator indo-1-AM, and changes in intraneuronal calcium ($[Ca]_i$) were monitored using the ACAS 570C confocal laser cytometer (Meridian Instr., Okemos, MI). As has been previously reported by Segal (Hippocampus 3: 229, 1993) and, more recently, by Reichling et al. (J. Physiol. 476: 411, 1994), we find that GABA itself elicits an immediate but small and transient increase in $[Ca]_i$ in cultured fetal neurons. The increase in $[Ca]_i$ is dose-dependent and is abolished in the absence of extracellular Ca^{2+} suggesting that GABA is promoting an influx of Ca^{2+} in these neurons. When GABA (10 μM) is applied two min. prior to NMDA (5 μM), the initial rise in $[Ca]_i$ generated by the glutamate agonist is reduced by 50% from 518 ± 35 nM (n = 31) to 223 ± 44 nM (n = 26) ($p < 0.001$). Since GABA does not appear to affect post-peak $[Ca]_i$ levels, we are investigating the potential effectiveness, in this model, of longer acting GABAergic drugs as well as drugs which reduce the rate of catabolism of the inhibitory amino acid.

541.12

N-METHYL-D-ASPARTATE-INDUCED INCREASED LEVELS OF ENDOGENOUS ADENOSINE ARE ENHANCED BY INHIBITION OF ADENOSINE DEAMINASE AND ADENOSINE TRANSPORT IN RAT STRIATUM. S. M. Delaney* and J. D. Geiger. Dept. of Pharmacol., U. of Manitoba, Winnipeg, R3E 0W3, Canada.

Excitatory amino acid stimulation within the CNS can evoke increases in levels of endogenous adenosine which, in turn, may lead to neuroprotection. We tested whether inhibition of both adenosine metabolism and transport could potentiate such increases in adenosine. Unilateral microinjections of N-methyl-D-aspartate (NMDA) into striatum of rats subsequently killed by high-energy focused microwave irradiation (10 kW, 1.25 s) significantly increased *in vivo* levels of endogenous adenosine (data expressed as % of uninjected contralateral striatum). At a dose of 25 nmoles NMDA, levels of adenosine were 263 ± 16%. Adenosine levels in control rats injected with vehicle (50 mM TRIS-HCl pH 7.4) were 126 ± 28%. Inhibitors of adenosine deaminase (deoxycoformycin, DCF) and nucleoside transport (dilazep, DLZP) at doses that did not affect levels of endogenous adenosine, potentiated NMDA-induced increases in adenosine levels to 426 ± 60%. In the presence of DCF and DLZP, NMDA dose-dependently increased levels of adenosine from 166 ± 34% at 10 nmoles to 622 ± 119% at 100 nmoles. MK801 at a dose (4 mg/kg i.p.) that did not significantly affect levels of adenosine (140 ± 18%) completely blocked NMDA-induced increases in levels of endogenous adenosine (110 ± 12%). Inhibitors of adenosine metabolism and transport may provide therapeutic benefit by potentiating excitatory amino acid-induced increases in levels of endogenous adenosine. (Supported by a grant from the Medical Research Council of Canada.)

541.13

DOPAMINE-NMDA INTERACTIONS IN THE MODULATION OF LOCOMOTOR ACTIVITY AND ONE-TRIAL INHIBITORY AVOIDANCE RESPONSES. A.Mele*, C.Castellano, A.Felici, S.Cabib, S.Caccia, and A.Oliverio. Dipartimento di Gen. e Biol. Mol., Università di Roma I; Istituto di Psicobiologia e Psicofarmacologia del C.N.R. and Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy.

This study was aimed to further elucidate possible interactions between the two systems in modulating locomotor activity and memory consolidation. Systemic administrations of both DA agonists and NMDA antagonists enhance locomotor activity of NMRI mice in a dose dependent fashion. On the contrary DA agonists and NMDA antagonists have an opposite effect in the response to the one-trial inhibitory avoidance paradigm. Both D1 and D2 agonists, in fact, enhance memory consolidation, while NMDA antagonists inhibit it. We have also shown that both D1 (SKF38393) and D2 (Quinpirole) agonists, at ineffective doses, 5.0 and 0.25 mg/kg respectively, attenuate the impairing effects of NMDA antagonists in the one-trial inhibitory avoidance. Only high, sedative, doses of the D2 antagonist (sulpiride 30mg/kg), instead, are able to antagonize MK-801 induced hyperlocomotion. To further elucidate the mechanisms of interaction between the two systems we repeatedly treated the animals with DA antagonists in order to verify whether treatments that induce an increased response to DA agonists were able to elicit changes in the response to NMDA antagonists in the two behavioral assays. Repeated administrations of the D1 antagonist SCH23390 (0.5 mg/kg) were able to induce changes in the response to CPP and MK-801 in the one trial inhibitory avoidance, but not in the locomotor activity test. Repeated administrations of D2 antagonists haloperidol (4 mg/kg) or sulpiride (125 mg/kg), instead, were able to induce an enhanced response to MK-801 in both behavioral tests.

541.14

EFFECTS OF GABA AND DOPAMINE ON MEMBRANE PROPERTIES OF THE FIRST BASALAR MOTONEURONE OF THE LOCUST. L. S. Prothero, J. C. McLelland, J. A. David and R. M. Pitman*. University of St. Andrews, St.Andrews, Scotland, U. K. KY16 8LB.

GABA is an important inhibitory neurotransmitter in the insect nervous system, while dopamine is widely distributed in the insect CNS and can influence membrane properties of insect neurones. There is some evidence indicating that dopamine (DA) may modulate the magnitude of GABA responses. To investigate this possibility, interactions between responses mediated by these two neurotransmitter candidates have been studied on the first basalar motoneurone of the locust (*Schistocerca gregaria*) using two types of preparation. In the first, recordings were made from the motoneurone within its ganglionic environment, while in the second, recordings were made from isolated basalar motoneurone somata. GABA was focally pressure-applied to the neurone from a micropipette, while DA was added to the experimental chamber. GABA evoked a dose-dependent hyperpolarization which was accompanied with a marked increase in membrane conductance. Application of dopamine alone (≥ 10^{-4}M) resulted in membrane depolarization which could be associated with a relatively small increase in membrane conductance. GABA responses were potentiated in the presence of DA (10^{-5}M). The magnitude of this effect was too large to be attributed to any shift in membrane potential away from the GABA reversal potential, suggesting that dopamine has a selective modulatory effect upon responses mediated by GABA. Supported by the BBSRC, U.K..

541.15

DOPAMINE D_2 RECEPTOR ACTIVATION AND INHIBITION AFFECTS GABA AND $GABA_A$ RECEPTOR β_2/β_3 SUBUNIT IMMUNOREACTIVITIES IN THE RAT PITUITARY INTERMEDIATE LOBE. S.A. Sands*, A.L. De Blas, and B.M. Chronwall. School of Biological Sciences, University of Missouri-Kansas City, Kansas City, Missouri, 64108.

Melanotropes posses dopamine D_2, $GABA_A$ and $GABA_B$ receptors and are innervated by axons containing dopamine and GABA. Stimulation of D_2 receptors decreases melanotrope proliferation, POMC mRNA levels, and calcium channel activity and protein expression. This study was conducted to test the hypothesis that dopamine receptor stimulation also regulates $GABA_A$ receptor protein in vivo. Chronic treatments with haloperidol, a dopamine D_2 receptor antagonist, or bromocriptine, a receptor agonist, and immunohistochemistry for the β_2/β_3 subunits of the $GABA_A$ receptor showed that receptor subunit immunoreactivity increased in overall average optical density after haloperidol treatment, whereas it decreased after bromocriptine treatment. Thus, the β_2/β_3 subunit of the $GABA_A$ receptor seems to be similarly regulated to POMC and calcium channels, suggesting an interaction between the signalling pathways within the melanotrope. In a corollary experiment the presynaptic compartment was assayed by immunohistochemistry using antisera for GABA and glutamate decarboxylase (GAD). Following haloperidol treatment the number of GABA and GAD containing axons decreased. After bromocriptine treatment, GAD and GABA immunoreactive axons did not change. The mechanism of this response is, however, different from that of the postsynaptic response; it could be at the level of hypothalmic neuronal perikarya or axons and terminals.

Supported by NIH grant NS 28019 to BMCh.

541.16

DOPAMINE EXERTS BOTH SYNAPTIC AND NONSYNAPTIC INFLUENCES ON GABAERGIC NEURONS IN NEOSTRIATUM. L.G. Harsing, Jr.* and M.J. Zigmond. Department of Neuroscience, University of Pittsburgh, Pittsburgh PA 15260.

Dopaminergic neurons are known to make synaptic contacts onto GABAergic neurons of the neostriatum. However, there is reason to believe that DA also exerts nonsynaptic influences on many striatal targets, and we wished to know whether this might also be true for GABAergic targets. We prepared striatal slices (350 μm) from rats, preloaded them with [^{3}H]GABA (29 pM), superfused with standard Krebs bicarbonate buffer containing aminooxyacetic acid (0.1 mM) and nipecotic acid (0.1 mM), and measured tritium overflow during electrical stimulation (8 Hz, bipolar) an index of GABA release. The D_2 receptor antagonist (-)sulpiride (0.1-10 μM) increased GABA release 2-fold. This effect was completely abolished by the DA synthesis inhibitor 3-iodotyrosine (2 mM), even though no decline in the tissue level of DA could be detected. This indicates an inhibitory influence of endogenous DA released from a pool with a rapid turnover. GABA release evoked by high K^+ was also stimulated by sulpiride in the presence of TTX, suggesting that DA can exert an inhibitory influence on GABA via an action on GABAergic terminals. Since axoaxonic synapses are not thought to exist in this region, we assume that these effects are nonsynaptic. The D_1 receptor antagonist (+)SCH-23390 (0.1-10 μM) failed to alter electrically-evoked GABA release when given alone, but reduced GABA efflux in the presence of sulpiride. Collectively, our findings suggest a model whereby DA exerts an excitatory influence on GABA release via D_1 receptors (synaptic?), which is modulated nonsynaptically by inhibitory D_2 receptors on the same neurons. (Supported in part by USPHS grant NS19608 and Fogarty International Center fellowship NS05270.)

541.17

DOPAMINERGIC REGULATION OF EXTRACELLULAR GABA LEVELS IN THE PREFRONTAL CORTEX OF THE RAT. A.C. Grobin* and A.Y. Deutch. Depts. of Psychiatry and Pharmacology, Yale Univ. Sch. Medicine, New Haven, CT 06510.

Dopamine (DA) axons synapse with GABA interneurons in the prefrontal cortex (PFC) providing an anatomical basis for pdopaminergic regulation of GABA function. *In vitro* studies have reported that DA agonists stimulate GABA release. We have previously shown that administration of apomorphine (APO), a mixed D_1/D_2 agonist, augments extracellular GABA levels *in vivo*. This effect is observed when APO is given systemically or applied locally through the microdialysis probe. To further characterize this effect *in vivo*, specific D_1-like and D_2-like agonists were administered to male Sprague-Dawley rats and GABA levels determined in the PFC using *in vivo* microdialysis. Additionally, the D_2 antagonist sulpiride was infused locally prior to systemic administration of APO to explore the nature of the APO-induced increase in GABA levels. Local administration of the D_1 agonist SKF 38393 (0.02-2 mM) did not change extracellular GABA levels. Local administration of the D_2 agonist quinpirole (0.01-100 μM) resulted in a dose-dependent increase in extracellular GABA. Local application of 20 μM sulpiride blocked the increase in GABA levels elicited by systemic APO (0.5 mg/kg). These data suggest that DA increases GABA release in the PFC predominantly through a D_2-like receptor. This report provides the first *in vivo* characterization of DA regulation of GABA release in the PFC. These studies were supported by MH-45124 and GM-07324.

541.18

D1- AND AMPA-MEDIATED STRIATAL AND NIGRAL GABA EFFLUX IN AWAKE RATS. E.M. Byrnes* and I.P. Bruno, Dept. of Psychology and Neuroscience Program, Ohio State University, Columbus, OH 43210

The mediation of striatonigral GABA efflux by D1 and AMPA receptor ligands was determined in awake rats using in vivo microdialysis with repeated insertions/perfusions. Intrastriatal perfusion with AMPA (100 μM) increased striatal GABA efflux (50-175%). Intrastriatal perfusion with the full D1 agonist SKF 81297 led to bidirectional effects depending upon dose; a low dose (10 μM) decreased striatal GABA efflux (40-60%) whereas a higher dose (100 μM) increased GABA efflux (75-150%). Each of these effects of SKF 81297 was blocked by local perfusion with the D1 antagonist SCH 23390 (10 μM). The bidirectional effects of the D1 agonist on striatal GABA efflux were not secondary to dose-related differences in motoric behavior. Concurrent intrastriatal perfusion of the low dose of SKF 81297 attenuated the AMPA-mediated GABA efflux whereas there was no obvious interaction between AMPA and the higher dose of SKF 81297. Intrastriatal perfusion with AMPA and/or SKF 81297 had no systematic effect on GABA efflux in substantia nigra. These results highlight the complex dose/drug modulation of striatal GABA efflux in awake animals and suggest a potential dissociation, under certain conditions, between striatal and nigra GABA efflux. The effects of ligands perfused directly into the nigra will also be presented.

541.19

INTRASTRIATAL *c-fos* ANTISENSE OLIGONUCLEOTIDE DECREASES GABA RELEASE IN THE SUBSTANTIA NIGRA BUT NOT IN THE GLOBUS PALLIDUS. AN *IN VIVO* MICRODIALYSIS STUDY IN THE AWAKE RAT. R. RIMONDINI[1], W. SOMMER*[1,3], W. T. O'CONNOR[2], A. HANSSON[1,3], K. FUXE[1], U. UNGERSTEDT[2]. Department of Neuroscience[1] and Pharmacology[2], Karolinska Institutet S171-77, Sweden and The Max-Delbrück Centre for Molecular Medicine[3], Berlin-Buch, Germany.

We employed the antisense phosphorothioate oligonucleotide 5'-GAACATCATGGTCGT-3' to selectively "knock down" c-*fos* expression in rat striatum. C-*fos* is one of the first transcribed immediate early genes after striatal dopamine D_1 receptor activation. The induction of c-*fos* is believed to indicate activation of signal transduction cascades with subsequent alterations in neuronal transmission. In the present study we combined the antisense approach with *in vivo* microdialysis to investigate the effects of intrastriatal infusion of the c-*fos* antisense oligionucleotide on GABA release in the substantia nigra and the globus pallidus which represent the terminal sites of the dopamine regulated direct and indirect striatal efferent GABA pathways. Rats were implanted with an injection canula in the right striatum and one microdialysis probe (0.5mm, O.D.; 1mm membrane length) was implanted into the ipsilateral substantia nigra and another (2mm) into the ipsilateral globus pallidus. Basal dialysate GABA levels were 7.49 +/- 0.37nM and 9.50 +/- 0.7nM in the substantia nigra and globus pallidus respectively. Intrastriatal infusion of the c-*fos* antisense (1mM in 2μL) did not influence dialysate GABA levels in the globus pallidus but profoundly decreased (-42 +/- 13%) GABA levels in the ipsilateral substantia nigra within 60min compared to the sham and sense control This finding demonstrates the importance of c-*fos* expression in the mechanism of dopamine D_1 receptor signal transduction in the regulation of striatonigral GABA transmission and strengthens the evidence for a differential dopamine D_1 and D_2 receptor mediated regulation of the direct and indirect striatal efferent GABA pathways.

541.20

MUSCARINIC REDUCTION OF GABAERGIC SYNAPTIC POTENTIALS RESULTS IN DISINHIBITION OF THE EARLY-EPSP IN NEOCORTEX. V. B. Aramakis*, A. E. Bandrowski, E. E. Guthrie and J. H. Ashe. Depts. of Neuroscience and Psychology, Univ. of California, Riverside, CA 92521.

The ability of muscarinic actions of acetylcholine (ACh) to modulate amino acid-mediated synaptic potentials was examined in the *in vitro* rat auditory cortex, using whole-cell recording techniques. Synaptic potentials [i.e., the early-EPSP (AMPA/kainate), early-IPSP ($GABA_A$), and late-IPSP ($GABA_B$)] were elicited in layer III pyramidal neurons following stimulation of deep gray matter. Methacholine (MCh), a muscarinic agonist, applied by either bath (10-50 μM) or iontophoresis (40-80 nA) produces either an increase or decrease of the amplitude of the early-EPSP. MCh-induced increase in amplitude occurs only when overt GABAergic IPSPs are also elicited (n=18/20). The magnitude of the excitatory synaptic potentials is governed by the degree of temporal overlap of IPSPs (Metherate & Ashe, J. Physiol., 481, 331-348, 1994). Thus, we hypothesized that the increase in early-EPSP amplitude observed in the presence of MCh is due to disinhibition and is not a result of direct facilitation of the early-EPSP itself. This view was supported by the findings that: 1) MCh produces a decrease in the amplitude of pharmacologically isolated GABAergic IPSPs (i.e., elicited in the presence of excitatory amino acid antagonists, n=5/5) and 2) when the early-EPSP is elicited with low intensity stimulation, resulting in no apparent or a weak IPSP, its amplitude is decreased rather than increased by MCh (n=17/18). These findings suggest that fast thalamocortical transmission, via the early-EPSP, is regulated by GABA-mediated mechanisms that are susceptible to modulation by muscarinic actions of ACh. Supported by NSF (IBN 9310582).

541.21

PERFUSION WITH N-METHYL-D-ASPARTATE IN RAT VENTRAL TEGMENTAL AREA BIPHASICALLY REGULATES LOCAL GABA RELEASE AND MESOSTRIATAL DOPAMINE RELEASE *IN VIVO*. A DUAL MICRODIALYSIS PROBE STUDY. W.T. O'Connor*, T. Wang+, E. French+ and U. Ungerstedt. Department of Physiology and Pharmacology, Division of Pharmacology, Karolinska Institute, Stockholm S171-77, Sweden and +Department of Pharmacology University of Arizona, College of Medicine, Tucson AZ 85724, Arizona, USA.

The effect of local perfusion with the glutamate receptor agonist N-Methyl-D-Aspartate (NMDA) in the Ventral Tegmental Area (VTA) on GABA release locally in VTA and dopamine release in the nucleus accumbens was investigated using the dual microdialysis probe approach in the halothane anaesthetized rat. One microdialysis probe was implanted in the VTA and a second probe in the ipsilateral nucleus accumbens. Local perfusion with NMDA into the VTA elicited a dose related biphasic response and a strong correlation between the ability of NMDA to increase local GABA release in the VTA and dopamine release in the nucleus accumbens was observed. Local perfusion with the 30 and 100μM doses of NMDA in the VTA was associated with an increase in both GABA release in the VTA and dopamine release in the nucleus accumbens. While the highest 300μM dose of NMDA was associated with a profound reduction in both GABA release in the VTA and dopamine release in the nucleus accumbens. In addition, the NMDA (100μM) induced increase in dopamine release in the nucleus accumbens was abolished following local perfusion with the noncompetitive NMDA receptor antagonist PCP (100μM) into the VTA. Taken together, the data suggest that NMDA receptors are located on both the A_{10} dopamine cell body and the GABA interneuron in the VTA and that both neurons display a similar sensitivity to NMDA receptor stimulation i.e. excitation at lower doses and a depolarization blockade at higher doses of NMDA. These data strengthen the evidence for a dynamic regulation of mesolimbic GABA and dopamine transmission by cortical glutamate.

542.1

ANATOMICAL ORGANIZATION OF THE PREGANGLIONIC SYMPATHETIC NEURONS OF THE RAT SPINAL CORD. W. J. Anderson* and Gail Bennett. Indiana University School of Medicine, Terre Haute, IN, 47809.

In the spinal cord, It has been shown that the neurons are grouped into columns which run in a longitudinal direction. Motoneuronal cell columns are somatotopic in nature, and previous studies have demonstrated the existence between these columns. In addition to this, the main dendritic organization of Motoneuronal cell columns runs longitudinally where the formation of dendritc bundles take place. This study was performed to evaluate the anatomic and organizational similarities between motoneuronal and sympathetic neurons. Immunocytochemical techniques using antibodies to choline acetyltransferase (ChAT), 5-hydroxytryptamine (5-HT), tyrosine hydroxylase (TH), thyrotrophic Releasing Factor (TRH), neuropeptide Y, and gamma aminobutyric acid (GABA) were studied utilizing single and double immunocytochemistry. Ultrastructural studies using TME of the entire sympathetic spinal cord were studied in the horizontal and sagittal plane. The results of this study indicate a fifth column of preganglionic sympathetic groups previously described with dendritic interactions among all columns. In addition, small cholinergic interneurons interacted among all columns. Specificity of supraspinal and spinal axons innervated the interomediolateral cell column and dendrite branches between the various columns. Ultrastructurally, dendro-dendritic, dendrosomatic, and somato-somatic gap type junctions were frequent. These results support two potential functions: 1. electrotonic interactions between neuronal membranes for more efficient sympathetic coordination; 2. a dendritic substrate for spinal and supraspinal control.

542.2

VIP AND PACAP POTENTIATE THE GLUTAMATE-EVOKED RELEASE OF ARACHIDONIC ACID FROM MOUSE CORTICAL NEURONS

N. Stella and P.J. Magistretti* Institut de Physiologie, Université de Lausanne, Switzerland.

Neurons containing VIP in the rodent cerebral cortex constitute a homogeneous population of bipolar, radially-oriented interneurons which can be activated by specific glutamatergic cortical afferents.

In the present study, we examined the potential interaction between VIP and glutamate on the release of arachidonic acid (AA) in primary cultures of cortical neurons. We observed that the release of AA evoked by glutamate is potentiated by VIP (EC_{50} = 1 μM) and its analogue PACAP (EC_{50} = 0.7 nM). This effect is mediated through PACAP I receptors and is dependent on the related formation of cAMP and PKA activation. Experiments in which the INDO-1 fluorescent dye was used show that activation of PACAP I receptors does not enhance the glutamate-evoked increase in intracellular calcium; this observation clearly indicates that the cAMP-PKA pathway potentiates the glutamate-evoked release of AA at a level downstream of the glutamate receptor-mediated increase in intracellular calcium permeability. Indeed, when intracellular calcium levels are raised in a receptor-independent manner by applying ionomycin, the ensuing AA release is still potentiated by the cAMP-PKA pathway.

Since VIP inhibits AA release evoked by glutamate in astrocytes [Stella et al. (1994) *J. Neurosci.* 14:568-575], the data reported show that VIP and glutamate interact in a synergistic and cell-specific manner on cortical neurons to increase AA release. Since AA enhances glutamatergic neurotransmission, these results suggest that VIP could amplify glutamate-mediated synaptic efficacy.

542.3

THE EFFECTS OF RESERPINIZATION ON MK801-INDUCED LOCOMOTION ARE TIME- AND DOSE-DEPENDANT. R.E. Steinpreis* and J.J. Panos. Department of Psychology, University of Wisconsin-Milwaukee, Milwaukee, WI 53211.

Depletion of catecholamines with reserpine has been reported to both increase and decrease MK801-induced locomotor activity in rats. In the present study, the effects of different doses of MK801 on locomotor activity was examined at three time periods after reserpinization, using a between groups design. For each dose of MK801, groups of rats received either vehicle, MK801 without reserpine, or reserpine with MK801 administered either 6, 24 or 48 hours later. Locomotor activity was gathered by computer for 150 minutes once MK801 (or vehicle) had been administered. The effects of reserpinization were dependent on the dose of MK801, such that reserpine attenuated the locomotor effects of the low dose of MK801 and enhanced MK801-induced locomotor effects at the high dose of MK801. Furthermore, the 6 hour delay after reserpinization produced the largest increases in activity and the longer delays produced smaller increases, regardless of the dose of MK801. In conclusion, the directionality of reserpine's effect on MK801-induced locomotion are dependent on the dose of MK801 and the magnitude of reserpine's effects are dependent on the time lag between reserpinization and subsequent administration of MK801.

542.4

DOPAMINE MODULATION OF GABA- AND GLUTAMATE-INDUCED CHANGES OF VENTRAL PALLIDAL NEURONAL ACTIVITY. A.G. Karczmar*, P.I. Johnson and T.C. Napier. Dept. Pharmacol., Loyola Univ. Chicago, Sch. Med., Maywood, IL 60153.

Microiontophoresis was used to investigate the possible influence of dopamine (DA) on GABA- and glutamate-induced responses of ventral pallidal (VP) neurons recorded extracellularly from chloral hydrate-anesthetized rats. DA modulation was determined by comparing its ability to alter amino acid-induced activity ("signal") against spontaneous firing ("noise"). An ejection current-response curve (5 to 100 nA) was generated for DA to determine the current level which produced 50% of the maximum DA-induced response (ECur50) and subthreshold current (STh), i.e. one that did not alter baseline firing rates. When co-iontophoresed with GABA or glutamate, ECur50 DA altered the signal-to-noise ratio in 27 of the 34 VP neurons tested; STh DA altered signal-to-noise ratio in 33 of 45. Co-iontophoresis of DA with GABA generally diminished the inhibitory effects of GABA on VP neuronal firing as 8 of 10 neurons tested with ECur50 DA and 14 of 16 neurons tested with STh DA showed signal-to-noise ratio decreases (-20±8% and -26±6%, respectively). The remaining 4 neurons showed increased signal-to-noise ratios. When DA was co-iontophoresed with glutamate, 12 of 17 neurons in each of the ECur50 or STh DA categories showed a signal-to-noise ratio decrease (-54±33% and -102±68%, respectively) whereas the remaining neurons displayed increases (137±119% and 64±22%, respectively). These data demonstrate that when DA alters both spontaneous firing and responses evoked by GABA or glutamate, the evoked responses are affected to a larger extent. DA substantially alters GABA- and glutamate-evoked responses even at ejection currents that are below those necessary to change spontaneous firing. Thus, it appears that DA is a potent modulator of GABA and glutamate neurotransmission in the VP. Work supported by USPHSGs DA05651 to PIJ and MH45180 to TCN.

542.5

EFFECTS OF MORPHINE AND/OR COCAINE CO-IONTOPHORESED WITH GLUTAMATE ON VENTRAL PALLIDAL NEURONAL ACTIVITY. J-X. Liao*, P.I. Johnson and T.C. Napier. Dept. Pharmacol., Loyola Univ. Chicago, Sch. Med., Maywood, IL 60153.

Microiontophoresis was used to investigate the possible influence of morphine and/or cocaine on glutamate-evoked responses of ventral pallidal (VP) neurons recorded extracellularly from chloral hydrate-anesthetized rats. Morphine and cocaine modulation was determined by comparing their ability to alter glutamate-induced activity ("signal") against spontaneous firing ("noise"). An ejection current-response curve (2 to 128 nA) was generated for morphine and cocaine to determine the current level which produced 50% of the maximum drug-induced response (ECur50) and subthreshold current (STh), i.e. one that did not alter baseline firing rates. Of the neurons tested for drug sensitivity, 80%(12/15) were sensitive to morphine, 81% (13/16) were sensitive to cocaine, and 69% (9/13) were sensitive to both morphine and cocaine. When each drug was co-iontophoresed with glutamate, both increases and decreases in the signal-to-noise ratio were seen. These changes were independent of any drug-induced changes in spontaneous firing rate. ECur50 morphine altered the signal-to-noise ratio in 80% (4/5) of the neurons tested whereas ECur50 cocaine co-iontophoresis altered signal-to-noise ratio in 86% (6/7). STh morphine altered the signal-to-noise ratio in 83% (5/6) of tested neurons and STh cocaine altered it in 86% (6/7). Preliminary results with GABA indicate that morphine and cocaine also are able to alter GABA signal-to-noise ratios. These data suggest that morphine and cocaine regulate glutamate-evoked responses in the VP to a larger extent than the spontaneous firing rate. In addition, both drugs appear to be potent VP neuromodulators as subthreshold currents of each drug proved capable of affecting the glutamate signal. Work supported by USPHSGs DA05651 to PIJ and MH45180 to TCN.

542.6

GLUTAMIC ACID DECARBOXYLASE (GAD) NERVE TERMINALS INNERVATE PERIVASCULAR NEURONS AND MICROVESSELS IN THE RAT CEREBRAL CORTEX. E. Vaucher* and E. Hamel. Montreal Neurological Inst, McGill Univ, Montréal, Canada, H3A 2B4.

Recently, we reported that basal forebrain neurons innervate cortical microvessels in the rat frontoparietal and perirhinal cortices (Vaucher & Hamel, *Soc Neurosci Abs*, #37.4, 1994). Based on ultrastructural morphometric analyses, we suggested that not only cholinergic but also GABAergic neurons were involved in this innervation. Here, we investigated by light (LM) and electron microscopy (EM) immunocytochemistry the associations of GAD neuronal elements with local microvessels in both cortical subdivisions in the rat (n=3). We used GAD-6 monoclonal antibody (Boehringer Mannheim) which, as reported previously (Escalpez et al, 1994, *J Neurosci* 14:1834), labeled prominently nerve terminals. On 30 μm-thick sections, few GAD immunostained neurons were seen while a high density of immunoreactive puncta was observed, of which several surrounded intracortical perikarya and their apical dendrites. On 1 μm-thick semithin sections, immunoreactive punctate structures were apposed to small vessels and to numerous unlabeled (and occasionnally GAD immunolabeled) cell soma contacting intraparenchymal vessels. Innervation of cell soma was confirmed at the EM level and GAD terminals were found to be highly synaptic. In both cortical areas, 20% of the perivascular GAD terminals (contained within 3 μm from the vessel wall, n=364) were very close (< 0.25 μm) to the blood vessels. In the perirhinal cortex, the terminals appeared slightly closer to vessels and smaller in size than their counterparts in the frontoparietal cortex (respectively, distance: 0.95 ± 0.07 and 1.11 ± 0.05 μm, area: 0.37 ± 0.02 and 0.46 ± 0.02 μm^2). These results confirm that GAD neurons establish close relationships with cortical microvessels and suggest that GABA may have a modulatory role by acting on perivascular cell bodies which neurochemical content should be investigated. Further lesions of the substantia innominata will be performed to confirm the basal forebrain origin of these GAD fibers. Supported by the MRC of Canada and Le Ministère de la Recherche et de l'Espace, France.

542.7

CALCIUM-SILENT CHOLINERGIC RECEPTORS AND BRADYKININ CO-MODULATE CYTOPLASMIC CALCIUM.
Jay S. Coggan* and Stuart H. Thompson. Hopkins Marine Station, Department of Biological Sciences, Stanford University, Pacific Grove, CA 93950.

Intracellular Ca signals $(Ca)_i$ evoked by bradykinin (Bk, 10 nM) and carbachol (Cb, 1 mM) together were studied in N1E-115 neuroblastoma cells loaded with the Ca indicator fura-2. Bk and Cb both cause release of Ca from internal stores, but the Cb signal is also associated with Ca influx. When Bk and Cb were applied together, the responses were characterized by Ca release and Ca influx. The Bk and Cb-related Ca influx signal persisted in the presence of pirenzepine (200 nM) and atropine (1 μM) but was blocked by curare (10 μM) and pertussis toxin (0.5 μg/ml). The Bk and Cb Ca influx was also observed in cells that do not respond to Cb alone. Calcium-silent cholinergic receptors, therefore, modulated $(Ca)_i$ in the presence of Bk. Marked cross desensitization of Bk receptors by Cb was also observed. The inhibition is most pronounced when the cells have been primed with a two minute dose of Cb 10 minutes before application of Bk. The cross desensitization is reversed by curare and occurs after Cb responses have fully recovered from homologous desensitization.
Supported by NIH NS14519 (ST) and NS14519 (JC).

542.8

CALCIUM RELEASE FROM INTRACELLULAR CALCIUM STORES BY ACH AND ATP RECEPTORS IN THE EMBRYONIC CHICK RETINA.
Y. Sakaki and M. Yamashita.* Dept. Physiol., Osaka Univ. Med. Sch., Suita, Osaka 565, Japan

The signal transduction pathways from muscarinic acetylcholine (mACh) receptors and adenosine triphosphate (ATP) purinoceptors (P_{2U}) to intracellular Ca^{2+} stores were studied in embryonic day 3 chick retinae with Ca^{2+}-sensitive fluorescent dye Fura-2 AM.

Ca^{2+} responses to carbamylcholine (CCh) and ATP were enhanced by the pre-treatment with LiCl (1 mM, 30 min), while pertussis toxin (PTX) treatment (250 ng/ml, 30 min) affected neither of the two responses. These results suggest the involvements of IP_3 and PTX-insensitive G-protein in the mACh and ATP signal transduction cascades. Thapsigargin (Tg) pre-treatment (250 nM, 12 min) completely blocked both of the responses. The ATP response was abolished after successive applications of CCh in a Ca^{2+}- free bath solution. However, the simultaneous application of CCh and ATP induced an increase in $[Ca^{2+}]_i$, even when no Ca^{2+} responses appeared after multiple applications of CCh or ATP. These interactions between CCh and ATP could suggest that the two pathways have some cross talk during the Ca^{2+} mobilization.

An influx of extracellular Ca^{2+} occurred after the retina was bathed in the Ca^{2+}- free solution and this influx was enhanced by Tg (500 nM, 12 min). The addition of Zn^{2+} or La^{3+} to the bath solution abolished this Ca^{2+} influx, suggesting that the embryonic retinal cell has Zn^{2+}- and La^{3+}- sensitive Ca^{2+} replenishment mechanisms that can be triggered by the depletion of intracellular Ca^{2+} stores.

Refs.;
Yamashita, M. et al. (1994). *J. Neurobiol.* **25**: 1144-1153.
Sugioka, M. and Yamashita, M. (1994). *Soc. Neurosci. Abstr.* **20**: 561.

542.9

ALTERATIONS OF STRIATAL AND CSF NEUROTRANSMITTER METABOLISM FOLLOWING LEVODOPA.
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Levodopa (LD) markedly improves Parkinsonism, but its long-term use is often associated with problems such as response fluctuations and dyskinesias. For these, changes in striatal dopaminergic mechanisms have been suspected, though influences on other neurotransmitter systems might be involved. We studied effects from 5 days of LD (50 mg/kg i.p. after carbidopa, 2.5 mg/kg i.p.) on dopamine (DA) and other striatal monoamine neurotransmitters in rabbit brain and foramen magnum CSF. HPLC analysis revealed the expected increases of DA and its metabolites, as well as a 2X increase of norepinephrine (NE) concentration. Though serotonin (5-HT) was unchanged, 5-hydroxytryptophan and 5-hydroxyindoleacetic acid (5-HIAA) concentrations were elevated 2X and 6X versus vehicle-treated controls. Enhanced turnover of 5-HT was suggested by an increased 5-HIAA/5-HT ratio. After LD administration, CSF showed increased DA, NE, 5-HIAA, and epinephrine concentrations. These results suggested that, in addition to its dopaminergic effects, LD therapy has influences on striatal NE and 5-HT metabolism which might contribute to outcomes of dopaminergic therapy.

542.10

PURINE-DOPAMINE RELATIONSHIPS IN STRIATUM: XANTHOSINE AND GUANOSINE.
P.A. LeWitt*, D.A. Loeffler, A.J. DeMaggio, P.E. Milbury, W.R. Matson, P.L. Juneau. Clinical Neuroscience Program, Sinai Hospital, Detroit, MI 48235, and ESA, Inc., Chelmsford, MA 01824-4171.

The co-localization of striatal adenosine and dopamine (DA) receptors and their functional interactions are known links between purine and dopaminergic systems. Another relationship is suggested our findings in CSF from unmedicated Parkinson's Disease patients, that xanthine and homovanillic acid concentrations are highly correlated. Also, CSF xanthosine concentration is increased 3X as compared to controls. To explore the link between DA and purine metabolites, we produced DA depletion (with reserpine, 2 mg/kg i.p. 5 days) and DA enhancement (with levodopa, 50 mg/kg i.p. x 5 days) in rabbits. These manipulations led to 63% increase and 26% decrease, respectively, in striatal xanthosine concentrations. Both reserpine and levodopa treatments reduced striatal guanosine concentration by approximately 50%.

Xanthosine is an intermediate in the metabolism of guanosine, adenosine, and inosine nucleotides. Our observations suggest that dopaminergic stimulation regulates striatal xanthosine and, possibly, other purine metabolites. The physiological significance of these striatal purine changes and their relationship to monoaminergic neurotransmission remains to be learned.

542.11

CCK-8 AND THE DOPAMINE D_2 RECEPTOR ANTAGONIST RACLOPRIDE INDUCE FOS-LIKE IMMUNOREACTIVITY IN THE SHELL PART OF THE RAT NUCLEUS ACCUMBENS VIA DIFFERENT MECHANISMS.
Xi-Ming Li, Ulla-Britt Finnman, Xiao-Jun Xu*▲ and Kjell Fuxe. Department of Neuroscience; ▲Department of Neurophysiology at Huddinge Hospital, Karolinska Institute; Stockholm, Sweden

Induction of neuronal Fos-like immunoreactivity (IR) in the rat brain after *in vivo* treatment with cholecystokinin octapeptide (CCK-8) and the dopamine (DA) D_2 receptor antagonist raclopride was demonstrated. *In vivo* treatment with the neuropeptide CCK-8 (0.01, 0.1 and 1 nmol/rat, i. c. v) or the D_2 antagonist raclopride (0.1, 0.5 and 1 mg/kg, i. p.) alone increased the Fos-like ir profiles in a dose-dependent way in the shell part of the rat nucleus accumbens (AcbSh). Combined treatment with CCK-8 (0.1 nmol/rat) and raclopride (0.5 mg/kg) caused significant additive increases in the Fos-like ir profiles in the AcbSh. In the central caudate-putamen, the medial olfactory tubercle, and the frontal cerebral cortex where either compound alone was weakly active or inactive, the combined treatment with both compounds led to a significant induction of neuronal Fos-like ir profiles. These results suggest that the blockade of D_2 and activation of CCK receptor transduction lines can induce Fos-like IR and furthermore produce long-term regulation of gene expression in the striatum via different mechanisms. The observed synergistic and additive effects of CCK-8 and raclopride on the induction of neuronal Fos-like IR may be the result of an antagonistically CCK receptor regulation of D_2 receptors.

542.12

NITRIC OXIDE INDUCES RELEASE OF HYPOTHALMIC VASOPRESSIN AND CORTICOTROPIN RELEASING FACTOR *in vitro* AND *in vivo*.
T.F.W.Horn*, F.E.Bloom and J.Raber, The Scripps Research Institute, 10666 N. Torrey Pines Rd., La Jolla, CA 92037, U.S.A.

Nitric oxide (NO) may modulate the central and peripheral release pattern of arginine-vasopressin (AVP) and corticotropin releasing factor (CRF). Therefore we investigated the direct effect of NO-containing perfusion buffer (gassed with 5% NO/95% N_2) on AVP and CRF release from hypothalamic slices in an *in vitro* superfusion paradigm (samples collected at 15-min intervals). After a 90-min wash period, the first control sample was taken and the perfusion buffer switched to NO-containing medium for two consecutive sample periods followed by a final control period. This sampling procedure was repeated after 30 min. The applied NO concentration in the superfusion chambers measured by a NO-sensitive electrode reached plateau levels of approx. 3 μM. NO induced a significant increase in AVP-release during both NO-pulses. CRF release occurred delayed and was significantly increased during the second pulse of NO only. The effect was blocked in the presence of 10 mM cobalt chloride, indicating the involvement of Ca^{2+} or 50 μM dandrolene, an inhibitor of intracellular calcium mobilization. Hemoglobin (100 μg/ml) acting as a NO-scavenger abolished the effect of NO on both, AVP and CRF. The NO-induced AVP release is not required for the later changes in CRF since the same delayed effect of NO was seen in AVP- deficient Brattleboro rats. Furthermore, to confirm the results for AVP *in vivo* microdialysis with NO containing medium within the paraventricular (PVN) and supraoptic nucleus (SON) of urethane-anaesthetized rats were performed. Both, PVN- and SON- microdialysis samples (30-min) displayed increased levels of AVP during perfusion with NO-containing medium as compared to control perfusions. Our data provide the first direct evidence that NO can act locally as a positive modulator of central AVP release. By local modulation of central vasopressin release NO might also affect physiological parameters such as body temperature. We will now extend our studies to investigate the role of NO in central thermoregulation and febrile thermogenesis. (Supp. by MH47680, HFSPO, DAAD)

542.13

ELECTRICAL STIMULATION OF THE PREFRONTAL CORTEX INCREASES EXTRACELLULAR GLUTAMATE IN THE VENTRAL TEGMENTAL AREA. Z.L. Rossetti* and R.A. Wise, CSBN, Concordia University, Montrèal, Quebéc, Canada H3G 1M8.

The ventral tegmental areta (VTA) receives a major excitatory projection from the medial prefrontal cortex (PFC). Changes in this excitatory drive may underly the response of VTA cells to drugs or external stimuli. To study the function of this excitatory pathway *in vivo*, we measured by microdialysis the extracellular concentrations of glutamate (GLU) in the VTA in response to electrical stimulation of the PFC. Following baseline stabilization, rats previously implanted with a bipolar electrode in the PFC and with a guide cannula in the VTA, were stimulated at 100 µA (60 Hz, p.w. 0.1 msec, duration 1 sec, inter-stimulus interval 10 sec) for 10 min. Extracellular GLU was measured in 10-min samples (1 µl/min) by HPLC with electrochemical detection, following OPA/β-ME derivatization. Basal dialysate concentrations of GLU were 1.54±0.18 µM (mean±SE). Electrical stimulation elevated extracellular GLU to 165±22% (n=7) of baseline. The stimulation-associated GLU response was no longer present when the probe was perfused with tetrodotoxin (10 µM), suggesting that the increase in GLU was of neuronal origin. Fluorescence retrograde staining showed that cells projecting to the area of the probe originated in the vicinity of the PFC electrode. These results provide direct functional evidence of an excitatory input to the VTA from the PFC and suggest that microdialysis coupled with electrical stimulation may constitute a useful tool to study glutamatergic function *in vivo*.

542.14

INHIBITORY EFFECT OF NEUROPEPTIDE Y ON CATECHOLAMINE SYNTHESIS IN RAT PHEOCHROMOCYTOMA (PC12) CELLS. L. A. McCullough and T. C. Westfall*. Dept. of Pharmacol. and Physiol. Science, Saint Louis Univ. Health Sciences Center, St. Louis, MO 63104.

In rat PC12 cells differentiated with nerve growth factor (NGF), neuropeptide Y (NPY) inhibited depolarization-stimulated catecholamine synthesis as determined by *in situ* measurement of dopa production in the presence of the decarboxylase inhibitor NSD-1015. The inhibition was concentration-dependent, with 1 µM NPY producing a 50% attenuation of the depolarization-induced increase in dopa production. The inhibitory effect of NPY was prevented by pretreatment with pertussis toxin, suggesting the involvement of a GTP-binding protein of the G_i or G_o subtype. The NPY analog [Leu^{31},Pro^{34}]NPY also caused inhibition of dopa production, but was less potent than NPY itself, while NPY 13-36 and PYY had no significant effect. This pattern is most consistent with the involvement of the Y3 NPY receptor subtype. In PC12 cells differentiated to a mature chromaffin phenotype with dexamethasone, NPY also caused a concentration-dependent inhibition of dopa production with equal efficacy as in NGF-differentiated cells, although with reduced potency. PYY was again without effect in dexamethasone-differentiated cells. NPY had no effect on basal dopa production in NGF-differentiated PC12 cells or on depolarization-stimulated dopa production in undifferentiated cells. In addition, NPY was effective at inhibiting dopa production in NGF-differentiated cells following stimulation with 0.5 µM forskolin. These results indicate that NPY, which is co-stored and co-released with catecholamines in the central and peripheral nervous systems, can modulate catecholamine synthesis by inhibiting tyrosine hydroxylation in addition to its modulatory effects on catecholamine release.

542.15

MECHANISM UNDERLYING DOPAMINE RECEPTOR MEDIATED INHIBITION OF NEUROPEPTIDE Y (NPY) RELEASE FROM PHEOCHROMOCYTOMA (PC12) CELLS. Guihua Cao, Devin Houston* and Thomas C. Westfall. Dept. of Pharmacol. and Physiol. Science, Saint Louis Univ. Health Sciences Center, St. Louis, MO 63104.

We have previously observed that the mixed dopamine agonist, apomorphine, inhibits the evoked release of neuropeptide Y (NPY) from both striatal slices and nerve growth factor (NGF) differentiated PC12 cells (Soc. Neurosci. Abs.:19:1173, 1993; 20:1560, 1994). In the present experiments we have carried out a further pharmacological characterization of this inhibition in PC12 cells. PC12 cells were cultured in 6-well plates and differentiated with NGF for 5 days. NPY in the release buffer and PC12 cells were measured using radioimmunoassay. Apomorphine produced a concentration-dependent (10^{-7}-10^{-5} M) inhibition of potassium (K^+)-evoked (50 mM) release from PC12 cells. This effect was attenuated by pertussis toxin (50 ng/ml for 20 hr), suggesting that the involvement of a GTP-binding protein of the G_i or G_o subtype. The inhibitory effect of apomorphine was blocked by the specific dopamine D_2 receptor antagonist, eticlopride, and mimicked by the dopamine D_2 agonist, quinpirole. These results are consistent with the dopamine inhibitory effect being mediated by a dopamine D_2 receptor. Other studies have investigated the signaling mechanisms which mediate this inhibitory effect. We have observed that the N-type calcium channel antagonist omega-conotoxin also decreases the K^+-evoked release of NPY but the effect of omega-conotoxin and apomorphine were not additive. These results are consistent with apomorphine inhibiting the release of NPY via inhibition of Ca^{2+} influx. It is concluded that the inhibitory effect of apomorphine on NPY release involves G-proteins and is mediated via dopamine D_2 receptors which inhibit Ca^{2+} influx. (Supported by HL-26319 and HL-35202).

542.16

ALPHA-ADRENERGIC RECEPTOR MEDIATED MODULATION OF ACTIVATION OF ADENYLYL CYCLASE BY VASOACTIVE INTESTINAL PEPTIDE (VIP) IN RAT CORTICAL CULTURES. Y. Li and P.A. Rosenberg*. Dept. of Neurol., Children's Hospital & Harvard Medical School, Boston MA 02115.

We have shown previously that catecholamines, acting through beta adrenergic receptors, and VIP, stimulate intracellular cAMP accumulation in astrocytes, release of cAMP, and extracellular hydrolysis of cAMP to adenosine (*J. Neurosci.* 14: 2953-2965; *Soc.Neurosci.Abstr.* 20: 10). Release of cAMP from astrocytes and extracellular hydrolysis to adenosine might be a general mechanism by which neuromodulators positively coupled to adenylyl cyclase might act. With this in mind, we investigated the interactions of alpha adrenergic receptors with VIP, because of the well-documented synergism between alpha1-adrenergic receptors and VIP that has been demonstrated in the cortical slice preparation (Magistretti, *Proc.Natl.Acad.Sci.* 78: 6535-6539).

Norepinephrine plus atenolol (to block activation of beta-adrenergic receptors) produced little consistent effect on VIP evoked cAMP accumulation in mixed cultures of neurons and astrocytes. In contrast, in relatively pure cultures of astrocytes, norepinephrine plus atenolol caused a large reduction in the response to VIP. This effect of norepinephrine plus atenolol could be mimicked by the alpha2 -adrenergic antagonist clonidine, and could be blocked by yohimbine, establishing it as an alpha2-adrenergic receptor mediated response. We found no evidence for an alpha1-adrenergic receptor mediated effect potentiating the response to VIP. The discrepancy between the results obtained with cortical slices and with cortical cultures is unexplained, but the diminished effect of alpha2-adrenergic agonists in the mixed cultures of neurons and astrocytes suggest that the presence of neurons somehow modulates the alpha-adrenergic response in astrocytes.

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542.17

NEUROMODULATORY ROLE OF ADENOSINE IN NUCLEUS ACCUMBENS-MEDIATED BEHAVIORS. H. J. Normile*, A. Del Villar, K. A. Martens and R. A. Barraco. Wayne State University School of Medicine, Detroit, MI 48202.

We've previously shown that bilateral microinjections of adenosine analogs into the nucleus accumbens (ACB) affects inhibitory avoidance memory and locomotor activity via activation of adenosine A_1 and A_{2a} receptors, respectively. Numerous in vitro studies have suggested that the behavioral effects of adenosine and its analogs are due to a functional interaction between adenosine, its receptors and other CNS transmitter systems. The present experiment represents our initial inquiry into the CNS systems which may mediate adenosine's effects on behavior by examining the effects of adenosine A_1 and A_{2a} receptor activation in the ACB on inhibitory avoidance memory and locomotor activity of mice pretreated IP with drugs that alter cholinergic and dopaminergic activity. Intra-ACB injections of a selective adenosine A_1 receptor agonist, CPA, combined with the muscarinic receptor antagonist, scopolamine, significantly impaired inhibitory avoidance memory at doses that failed to affect performance when administered alone. Intra-ACB injections of a selective adenosine A_{2a} receptor agonist, CGS 21680, combined with the dopamine D2/D3 receptor agonist quinpirole, suppressed locomotor activity at high CGS 21680 doses, whereas low doses of CGS 21680 attenuated the suppressant effects of quinpirole on locomotor activity. These findings suggest that endogenous adenosine may functionally interact with the cholinergic and dopaminergic systems in the control of ACB-mediated behaviors. (Supported in part by MBRS #RR08167 and the Joseph Young, Sr. Research Fund).

542.18

SYMPATHOLYTIC ACTIVITY OF ADENOSINE AGONISTS IS DEPENDENT ON STIMULATION FREQUENCY. C. E. Crosson* and T. Gray. Department of Ophthalmology and Visual Sciences, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Prejunctional adenosine receptors have been identified on sympathetic nerve fibers in several systems. In this study, the effect of adenosine agonists on ^{3}H-norepinephrine release from the isolated iris/ciliary body (ICBs) of New Zealand White rabbits was examined. Rabbits were sacrificed by an overdose of sodium pentobarbital, the eyes enucleated, and the ICBs dissected free. ICBs were loaded with ^{3}H-norepinephrine for 60 minutes, washed, and samples collected at one minute intervals. To evoke ^{3}H-norepinephrine release, ICBs received electrical stimulation (10 V) at 5 and 20 Hz. At 5 Hz stimulation, the addition of the relatively selective adenosine A_1 agonist N^6-cyclohexaladenosine (CHA) at concentrations from 10^{-9} to 10^{-6} M did not significantly alter the release of ^{3}H-norepinephrine as measured by the changes in stimulus ratios. However, when tissues were stimulated at 20 Hz, the addition of CHA produced significant dose-related inhibition of norepinephrine release, with an IC_{50} of 4.8 µM. The adenosine A_2 agonist CV-1808 did not significantly alter 20 Hz-stimulated ^{3}H-norepinephrine release. Preincubation with the adenosine receptor antagonist 8-cyclopentol-1,3-dimethylxanthine (CPT) reversed the suppression of ^{3}H-norepinephrine release induced by CHA. These studies provide evidence that adenosine A_1 agonists can modulate sympathetic function of the ICB. However, the expression of this response was dependent on the frequency of electrical stimulation to induce norepinephrine release. Hence, prejunctional adenosine receptors may act as circuit breakers to limit neurotransmission at high frequencies.

542.19

CAFFEINE INDUCES BIPHASIC CHANGES IN BEHAVIOUR AND STRIATAL IMMEDIATE EARLYGENE EXPRESSION Bertil B. Fredholm*, George Nomikos and Per Svenningsson Dept. Physiology and Pharmacology, Karolinska Institutet, Stockholm,Sweden.

Caffeine is known to increase the expression of c-*fos*, c-*jun*, and *jun* B mRNA in doses above 50 mg/kg i.p. These doses tend to depress motor behaviour, whereas lower doses (below 50 mg/kg) stimulate. Caffeine was given by i.p. injection and motor behavior recorded in an automatic activity meter. Animals were killed between 0.5 and 8 hrs after administration of caffeine. IEG expression was examined by *in situ* hybridization. At a dose (25 mg/kg) which caused increased motor behaviour caffeine decreased the expression of NGFI-A, NGFI-B and *jun* B. The effect was significant 4 and 8 hrs after caffeine injection. The decrease in NGFI-A was confined to the striatopallidal neurons. At higher doses there was an increase observed in both striatopallidal and striatonigral neurons. The effect of low dose caffeine was mimicked by the D_2 agonist quinpirole (1 and 3 mg/kg), but it was not blocked by the D_2 antagonist raclopride(2 mg/kg). The results also show that a single dose of caffeine can have diametrically opposing effects on immediate early gene expression and motor behaviour depending upon dose. The data also indicate that co-existing adenosine A_{2A} and dopamine D_2 receptors have functionally important opposing actions which cannot all be explained by a direct receptor-receptor interaction.

542.20

Nitric oxide mediated neuromodulation in post-hypoxic myoclonus. D.D. Truong,* T.Q. Vu, R.R. Matsumoto, A.G. Kanthasamy, Parkinson & Movement Disorders Laboratory, Dept. of Neurology, Univ. of California, Irvine, CA 92717.

Nitric oxide is a neuromodulator which has been implicated in excitatory amino acid mediated cellular responses. Since overactivity of excitatory amino acids has been linked to neurological consequences following hypoxia or ischemia, we evaluated the role of nitric oxide in post-hypoxic myoclonus. Following 7-10 min of cardiac arrest, rats exhibit audiogenic myoclonus, which has neurological features that resemble post-hypoxic myoclonus in humans. The nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 300mg/kg, i.p) was administered to cardiac arrested rats and the audiogenic myoclonus was quantitated at 30 min intervals for three hrs. L-NAME significantly ($p<0.01$) attenuated audiogenic myoclonus as compared to pretreatment. The response was rapid and lasted more than three hrs. L-NAME also ameliorated seizure threshold in the post-operative period (1-3 days) of the cardiac arrest surgery. To determine the direct relationship between nitric oxide and myoclonus, the nitric oxide donor isosorbide dinitrite (ISDN, 300 mg/kg i.p) was administered. ISDN significantly ($p<0.05$) accentuated the myoclonus score. These results suggest that nitric oxide plays a neuromodulatory role in the expression of post-hypoxic myoclonus. Experiments are in progress to identify direct/indirect neuromodulation of nitric oxide in the pathogenetic mechanism of post-hypoxic myoclonus. (Supported by Myoclonus Research Foundation).

542.21

LOCALIZATION OF CHOLINERGIC AND GABAERGIC NEURONS IN THE RETICULAR NUCLEUS OF THE THALAMUS AND THEIR PROJECTIONS TO THE MEDIODORSAL NUCLEUS OF THE THALAMUS IN THE CAT. L. Gritti*, M. Mariotti and M. Mancia Institute of Human Physiology II, University of Milan, I-20133 Milano, Italy.

The distribution of cholinergic and GABAergic neurons was examined in the Reticular nucleus of the thalamus (RE) of cats with a sequential immunohistochemical staining procedure using BDHC (benzidine dihydrochloride) for GAD (glutamic acid decarboxylase) after using DAB (diaminobenzidine) for ChAT (choline-acetyl-transferase). ChAT-immunoreactive cells were found among GAD-immunoreactive cells in the anterior part of the RE. ChAT+ cells were less numerous than GAD+ cells (in a ratio of 1:20). Efferent projections from the two cell types to the intermediate part of the Mediodorsal nucleus (MD) of the thalamus were examined by retrograde transport of horseradish peroxidase conjugated with wheat germ agglutinin (WGA-HRP) combined with immunohistochemical revelation of GAD and ChAT. Of the large number of cells projecting to the intermediate part of MD, GAD+ neurons represent a significant proportion (>69%) and ChAT+ neurons a very small proportion (<4.0%). More than 70% of the ChAT+ cells and less than 18% of the GAD+ cells located in the RE were retrogradely labelled. These results indicate that the cholinergic neurons lying amongst the GABAergic neurons of the anterior part of the RE may exert an influence on the neurons located in the intermediate part of the MD nucleus of the thalamus. They might also constitute, as suggested by Mancia and Otero-Costas (1973), the chemo-anatomical substrate whereby the disfacilitatory effect of the midbrain reticular formation could act on thalamo-cortical neurons.

UPTAKE AND TRANSPORTERS: CATECHOLAMINES II

543.1

INHIBITION OF DOPAMINE TRANSPORT BY HYDROPHOBIC CATIONS. M.R. Rollin and J.C. Schaeffer*. Dept. of Chemistry, California State University, Northridge, CA 91330.

Many dopamine transport inhibitors are hydrophobic cations at physiological pH and probably bind to the dopamine transporter through ion pair formation with an anionic center located in a hydrophobic region. Secondary amine derivatives of phenylethylamine and related compounds were evaluated for their ability to block ^{3}H-BTCP binding to a strong inhibitory site on the dopamine carrier in rat brain membranes and to inhibit ^{3}H-DA accumulation by synaptosomes. Secondary amines containing two large hydrophobic groups were very potent inhibitors in both assay systems. Structural changes in the hydrophobic substituents occasionally decreased inhibitor strength suggesting unfavorable steric interactions. Comparison of the inhibition curves for all the inhibitors tested in each assay system indicated the presence of normal sensitivity inhibitor and high sensitivity inhibitor classes. Simple reversible bimolecular associations can explain the normal sensitivity inhibitors, but the high sensitivity inhibitors appear to involve inhibitor/transporter interactions of greater complexity. Because assay dependent relative inhibitor potency differences, complex assay/sensitivity class patterns and both competitive and non-competitive inhibition were observed, transport inhibition by hydrophobic cations probably involves multiple transporter sites/conformations and their interactions.

543.2

LONG-TERM OVARIECTOMY INCREASES EXPRESSION OF THE DOPAMINE TRANSPORTER GENE IN THE RAT *SUBSTANTIA NIGRA*. R.Bossé, R.Rivest and Thérèse Di Paolo*. School of Pharmacy, Laval University, Québec, Québec G1K 7P4 and Department of Molecular Endocrinology, Laval University Medical Center, Sainte-Foy, Québec, G1V 4G2, CANADA.

Removal of dopamine (DA) from the synaptic cleft is principally done via an uptake mechanism involving the DA transporter (DAT). We have previously shown gender and sex steroid effects on rat brain DAT. We recently described decreased striatal DAT levels in ovariectomized rats. Menopause is characterized by lowered estrogen levels. Increased onset of schizophrenia and tardive dyskinesia is also observed in post-menopausal women. To further investigate the mechanisms involved in DAT regulation and their impact on CNS changes occuring at menopause, we used ovariectomy as a model of decreased gonadal function. The effect of short-term ovariectomy (ST-OVX, 2 weeks) and long-term (LT-OVX, 3 months) and its possible antagonism with a 17-ß estradiol treatment (10 µg E_2, b.i.d., 2 weeks before sacrifice) on rat *substantia nigra pars compacta* (SNc) and ventral tegmental area (VTA) DAT gene expression was investigated. 30 female Sprague-Dawley rats were divided into 5 groups: (1) intact rats at random stages of the estrous cycle (CTRL), (2) ST-OVX,(3) ST-OVX+E_2, (4) LT-OVX and (5) LT-OVX+E_2. *In situ* hybridization was used to estimate levels of the DAT mRNA. ST-OVX did not affect SNc DAT mRNA levels whereas LT-OVX led to a 36% ($p<0.05$) increase. E_2 treatment partially restored DAT mRNA levels toward control values in LT-OVX rats but has no effect in ST-OVX rats. Similar changes occured in the VTA. For comparison, we recently showed that LT-OVX decreases striatal DAT density by 35%. Increased expression of the DAT was observed in the SNc and VTA following gonadal hormone withdrawal whereas the striatal DAT protein is decreased. This suggests various levels and/or mechanisms of control of this transporter. *Supported by the MRC of Canada.*

543.3

ESTROGEN MODULATION OF SYNAPTOSOMAL DOPAMINE RELEASE. T.L. Thompson* and R.L. Moss. UT Southwestern Med. Ctr., Dallas, TX 75235.

We have shown previously that the ovarian steroid estrogen has short-term, presumably non-genomic, mediated membrane effects on dopaminergic activity in the nucleus accumbens (J. Neurochem. 62:1750-1756, 1994). Estrogen rapidly potentiates depolarization induced dopamine (DA) release from the nucleus accumbens in vivo and this effect is not sensitive to nomifensine (NOM) treatment. To determine whether estrogen directly interacts with the NOM sensitive-site on the DA transporter, we examined the ability of estrogen to alter 3H-DA uptake and release using a rat synaptosomal preparation. Synaptosomes prepared from ovariectomized rats were preloaded with 3H-DA in the presence or absence of NOM (μM-mM) and/or 17β-estradiol (μM-mM). NOM inhibited 3H-DA uptake in a dose dependent manner. Pre-exposure to 17β-estradiol for 15-45min. had no effect on 3H-DA uptake measured over 1 or 5 min., nor was it able to alter NOM mediated inhibition of uptake. Stimulated 3H-DA release was measured from preloaded synaptosomes in response to K+ (60mM, 1 or 5min.) following exposure to buffer or 17β-estradiol (10-35min., pre.inc.) Basal DA release was significantly attenuated in the presence of the steroid. In addition, K+-stimulated release was consistantly lower following exposure to 17β-estradiol. Interestingly, NOM treatment resulted in a similar attentuation of basal and K+-stimulated released. Synaptosomes which were exposed to 17β-estradiol and NOM responded as those treated with either agent alone. While it is apparent that 17β-estradiol can interact at the presynaptic membrane to modulate DA release, these preliminary data suggest it may be acting at a site distinct from the the NOM sensitive-site on the DA transporter. Experiments are currently underway to identify the precise site of steroid action. Supported by MH 47418.

543.4

DOPAMINE TRANSPORTER ACTIVITY IN MIDBRAIN NEURONS IS DIRECTLY CORRELATED WITH VULNERABILITY TO MPTP TOXICITY: RELATIONSHIP TO CALBINDIN-D28k. M.K. Sanghera*, R.B. Simerly and D.C. German, Dept Psychiat, UT Southwestern Med Cntr, Dallas, TX 75235-9070, and Div Neurosci, Oregon Regional Primate Res Cntr, Beaverton, OR 97006.

The dopamine transporter (DAT) is the site at which MPP^+, the neurotoxic metabolite of MPTP, gains access to dopaminergic (DA) neurons. The midbrain DA neurons in the C57BL/6 mouse that contain the calcium-binding protein, calbindin-D28k (CALB), are less vulnerable to degeneration than the DA neurons that lack CALB (Liang et al., 1995). The purpose of the present study was to examine the relationship between CALB and DAT activity in midbrain DA neurons. Male C57BL/6 mice were examined using combined in situ hybridization with a rat DAT cDNA riboprobe, and CALB immunohistochemistry. Four sections were examined through the midbrain DA cell complex (nuclei A8, A9 and A10). There were varying amounts of DAT mRNA activity over the CALB-containing DA neurons; some CALB-containing neurons exhibited high DAT mRNA, and some exhibited low DAT mRNA. There was a positive correlation between DAT mRNA levels and regions where cells are located that are vulnerable to MPTP-induced cell death. These data suggest that both CALB and low DAT activity are important in protecting DA neurons from MPTP-induced cell death.

543.5

PROTEIN KINASE ACTIVITY MODULATES DOPAMINE TRANSPORTER FUNCTION. R.A. Huff*+, R.A. Vaughan#, M.J. Kuhar#, and G. Uhl+@. +Molec. Neurobiol. & #Nsci. Branches, IRP, NIDA, NIH; @Depts. Neurol.&Neurosci., JHUSM , Baltimore, MD 21224.

Dopaminergic neurotransmission is terminated by presynaptic reuptake of dopamine by the dopamine transporter (DAT). We have previously reported that protein kinase C (PKC) activation can alter dopamine transport Vmax in transiently-expressing COS cells (Eur. J. Pharmacol., 268, 115-119). We now report that activation of PKC with the active phorbol ester PMA in LLC-PK1 cells that stably express DAT cDNA decreases dopamine uptake Vmax values by 35%; parallel studies in transiently-expressing COS cells yield 49% decreases. No decrease in uptake was observed when either cell line was treated with the inactive phorbol ester 4α-PDD. Neither PMA nor 4α-PDD changed transporter number, as assayed by radioligand transporter binding. General transport process toxicities were not observed as Na^+/Cl^- dependent transport of alanine and the facilitated transport of leucine were unaffected in LLC-PK1 cells and only modestly altered in COS cells. Treatment with the protein kinase A inhibitor H-89 decreased dopamine transport Vmax by 80% in LLC-PK1 and 61% in COS cells, but neither effect was antagonized by 8-bromo cyclic AMP. Initial results of DAT labeling with [^{32}P]orthophosphate followed by immunoprecipitation suggest that some of the PKC effects may result from direct transporter phosphorylation. Replication of these effects, and their absence in site-directed transporter mutants devoid of specific potential phosphorylation sites, would support a significant role for transporter phosphorylation in regulation of dopaminergic neurotransmission.

543.6

TRANSGENIC MICE WITH DIFFERENTIAL DOPAMINE TRANSPORTER EXPRESSION: PSYCHOSTIMULANT RESPONSES AND NEUROTOXIC CONSEQUENCES. D.M. Donovan*#, L.L. Miner#, I. Sora#, J. Mulle#, S. Prezedborski@, V. Jackson-Lewis@, S. Izenwasser$, R. Rothman+, C. Dersch+ & G.R. Uhl!#. Molec.Neurobiol.#, +Clin.Pharm., $Preclin.Pharm. Branches; @Col.-Pres.Univ.; !Depts.Neurosci&Neurol, JHUSM,Balto,MD 21224.

The dopamine transporter (DAT) has been centrally implicated in rewarding effects of cocaine and in the selective dopaminergic neurotoxicity mediated by MPP+. Transgenic mice regionally overexpressing a variant dopamine transporter in catecholaminergic neurons via the 4.8Kb tyrosine hydroxylase promoter reveal DAT overexpression in catecholaminergic cells and their processes as characterized via Northern blot analyses, immunohistochemistry, ligand binding and dopamine uptake. Sensitivity to MPP+ intoxication through MPTP administration is observed in both transgenic and wild type littermate controls. The DAT transgenic animals display differences from wild type littermate controls in habituation to locomotor apparatus and in conditioned place preference mediated by both cocaine and, in initial experiments, morphine.

543.7

SPECIES-AND BRAIN-REGION SPECIFIC DOPAMINE TRANSPORTERS: IMMUNOLOGICAL AND GLYCOSYLATION CHARACTERIZATION M.T. McCoy*, V.L. Brown, R.A. Vaughan and M.J. Kuhar Neuroscience Branch, NIDA Intramural Program, Baltimore, MD 21224

Dopamine transporters from the caudate nucleus of three species (mouse, dog, and human) and from rat nucleus accumbens, frontal cortex and midbrain were analyzed for glycosylation and for cross-reactivity to antisera generated against rat dopamine transporter peptide sequences. For the immunological characterization, samples were photoaffinity labeled with [^{125}I]DEEP and were immunoprecipitated with four different DAT anti-peptide antibodies. DATs from all species were immunoprecipitated with all four sera, although with varying effectiveness. Compared to rat DATs, precipitation of mouse DATs was most similar and precipitation of human DATs was least similar, presumably reflecting species differences in primary amino acid sequence. The transporters obtained from various brain regions of the rat were immunoprecipitated with a profile similar to the striatal DATs. Deglycosylation studies were done by immunoprecipitating photolabeled DATs followed by enzyme treatment and polyacrylamide gel electrophoresis. Samples from all species and all brain regions were sensitive to both N-glycanase and neuraminidase, and demonstrated about the same degree of M_r loss as rat striatal DATs due to these enzymes. The transporters from the various species and regions displayed slightly different molecular weights, and no transporters were found with substantially lower molecular weights, indicating that if incompletely glycosylated forms are present (e.g., in the midbrain cell bodies) they do not become photolabeled. This shows that DATs from cell body regions as well as terminals are capable of ligand binding and possess mature carbohydrate structures.

543.8

NMDA AND KAINIC ACID INCREASE THE VELOCITY OF DOPAMINE TRANSPORT IN RAT STRIATAL SUSPENSIONS AS MEASURED USING ROTATING DISK ELECTRODE VOLTAMMETRY. S. M. Welch* and J. B. Justice, Jr., Dept. of Chemistry, Emory University, Atlanta, GA 30322.

Rotating disk electrode voltammetry is an electroanalytical technique which has been used to measure kinetics of dopamine transport in suspensions of rat striatal tissue (Meiergerd and Schenk, *J. Neurochem.* 63, 1683, 1994). In the present study, the technique was utilized to examine the effects of glutamate agonists on the initial velocity (v_0) of dopamine (DA) uptake in rat striatal suspensions. DA was added to suspensions of striatal tissue obtained from male Wistar rats and the clearance of DA from the solution was monitored by a glassy carbon rotating disk electrode at an applied potential of +450 mV vs Ag/AgCl. The v_0 was obtained from the slope of a linear regression performed on the first ten seconds of the clearance profile. The glutamate receptor agonists N-methyl-D-aspartic acid (NMDA) and kainic acid were found to increase initial rates of dopamine uptake. At an added DA concentration of 2 μM, NMDA (200 μM) increased v_0 from 548 +/- 28 pmoles/sec/g tissue wet weight (control, n = 6) to 803 +/- 63 pmoles/sec/g tissue (n= 4). The increase was reversed by the competitive NMDA receptor antagonist AP5 (200 μM). In addition, 10 μM kainic acid increased v_0 from 505 +/- 34 pmoles/sec/g (control, n = 6) to 702 +/- 71 pmoles/sec/g (n = 8). These results suggest that glutamate may play a role in regulation of the striatal dopamine transporter.

543.9

NEUROPEPTIDE Y INCREASES DOPAMINE UPTAKE IN VITRO. A. C. Thompson* & J. B. Justice, Jr. Department of Chemistry, Emory University, Atlanta, GA 30322.

Rotating disk electrode (RDE) voltammetry was used to measure the initial velocity (V_0) of DA uptake in nucleus accumbens suspensions (300 µl, 37°C) prepared from male Wistar rats. V_0 was calculated by linear regression on the slope of the decreasing current that followed a 2 µM DA addition. Neuropeptide Y (NPY at 1,10, or 100 pM), or vehicle, was added approximately 100 s prior to the 2 µM DA and the effect of NPY on the V_0 of DA uptake into the tissue was determined. V_0 was normalized to the DA concentration (µg/mg tissue) for data analysis.

A significant NPY dose dependent increase in the rate of DA uptake was observed ($p < 0.002$). After treatment with 100 pM NPY, V_0 was 1467±260 pmol/s/(µg/mg) DA (n=9) and was nearly 3-fold greater than the V_0 after vehicle treatment (506±89 pmol/s/(µg/mg) DA, n=9). Treatment with 1 pM and 10 pM NPY produced intermediate responses with V_0 of 824±123 (n=10) and 1164±131 (n=9) pmol/s/(µg/mg) DA, respectively.

These results suggest that NPY may regulate DA neurotransmission in the nucleus accumbens by increasing the rate of DA reuptake.

543.10

PROTEIN KINASE C ACTIVATORS DECREASE DOPAMINE UPTAKE INTO STRIATAL SYNAPTOSOMES. B.J. Copeland*, N.H. Neff, and M. Hadjiconstantinou. Departments of Psychiatry, Pharmacology and The Neuroscience Program, The Ohio State University College of Medicine, Columbus, Ohio, 43210

Recent evidence from cloning and transfected cell lines suggests the dopamine transporter (DAT) is modulated by second messenger-dependent pathways. To investigate this possibility, crude synaptosomes were prepared from the mouse striatum and incubated with the protein kinase C (PKC) activators phorbol 12-myristate 13-acetate (PMA; 10 μM), or *sn*-1,2 dioctanoylglycerol (DiC_8; 5 μM) for 6 minutes at 37°C. PMA and DiC_8 significantly decreased the uptake of ^{3}H-dopamine into synaptosomes. Kinetic analysis showed a decrease in the apparent V_{max} of the transporter without effect upon K_m. The inactive phorbol ester 4α-PMA (10 μM) had no effect. Washoff of PMA and DiC_8 failed to reverse the decrease in uptake, and no changes in ^{3}H-mazindol binding to the DAT were observed with either compound. The addition of the protein phosphatase inhibitor, okadaic acid (OKA; 500 nM), further decreased the uptake observed with DiC_8. These findings support a role for PKC in the modulation of the dopamine transporter activity, and suggest phosphorylation may be a regulatory mechanism by which transport function is altered.

543.11

NOMIFENSINE AS AN ENHANCER OF L-DOPA STIMULATED DOPAMINE RELEASE FROM SUPERFUSED STRIATAL TISSUE FRAGMENTS K. Xu, B. Liu, J.L. McDermott* and D.E. Dluzen. Dept. of Anatomy, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272-0095

In this report, we examined the modulatory effects of Nomifensine (NMF), a dopamine (DA) reuptake blocker, on L-Dihydroxyphenylalanine (L-DOPA) evoked DA release from superfused corpus striatal (CS) tissue fragments of male rats. In Experiment 1, we tested the effects of L-DOPA infusions (5*u*M) in the presence of different doses of NMF upon DA release rates. We found that a statistically significant increase ($p<0.01$) in L-DOPA stimulated DA release was obtained when 1 m*M* of NMF was used. By contrast, 10 *u*M or 100 *u*M of NMF failed to alter L-DOPA evoked DA release rates. In Experiment 2, we combined NMF (1 m*M*) with decreasing doses of L- DOPA (5, 1, 0.1 *u*M) in order to further study the effect of NMF upon DA neuronal release. Our data showed that, 50 fold reductions in L-DOPA (0.1 *u*M) can be used to produce equivalent release rates in the presence of this uptake inhibitor. At the same time, with a reduction in L-DOPA, there was a corresponding decrease in 3,4-Dihydroxyphenylacetic Acid (DOPAC), which was also statistically significant ($p<0.01$). These data demonstrate that NMF, a DA uptake blocker, can be used to enhance the capacity of L-DOPA to evoke DA release from CS tissue fragments, and reduce the L-DOPA doses while maintaining equivalent L-DOPA evoked DA release rates.

543.12

RAPID MEASUREMENT OF BIDIRECTIONAL NOREPINEPHRINE AND DOPAMINE TRANSPORT IN LLC-NET CELLS USING ROTATING DISK ELECTRODE VOLTAMMETRY. W. B. Burnette*, K. S. Danek, C. G. Trowbridge, and J. B. Justice, Jr. Department of Chemistry, Emory University, Atlanta, GA 30322.

The electrochemical technique of rotating disk electrode voltammetry (RDEV) has been applied to the measurement of the inward and outward transport of norepinephrine (NE) and dopamine (DA) in LLC-NET cells, which express the NE transporter (NET). Initial rates (v_0) of inward transport were estimated from the first 10 seconds of data following the addition of substrate, and v_0 of efflux from cells pre-loaded with 1 μM substrate were estimated from the first 20 seconds of data following the addition of 1 μM d-amphetamine (d-AMPH).

The kinetics of transport of DA into LLC-NET cells were studied using RDEV. The v_0 of transport was measured at 30 DA concentrations ranging from 98 nM to 15.7 μM. Estimates of K_M and V_{max} were 1.75+/-0.4 μM and 2.76+/-0.19 fmol/min/cell, respectively (mean+/-sem). Experiments were also performed in which two additions of 1 μM NE or DA were made to the same preparation, and either vehicle,1 μM d-AMPH,or 1 μM tyramine was added in between. The ratio of the initial rates measured after each substrate addition (v_02/v_01) was decreased from 1.08+/-0.03 to 0.51+/-0.05 for DA, and from 0.91+/-0.03 to 0.39+/-0.05 for NE in the presence of d-AMPH. A 30% increase in the v_0 of inward DA transport was observed when 1 μM tyramine was preloaded, demonstrating trans acceleration of inward DA transport. The initial rates of inward transport of 1 μM NE and DA in control experiments were 330+/-33 and 522+/-44 amol/min/cell, respectively. The initial rates of efflux from cells preloaded with 1 μM substrate following the addition of 1 μM d-AMPH were 56.6+/-4.8 and 54.6+/-2.0 amol/min/cell for DAand NE, respectively. These results demonstrate the feasibility of measuring catecholamine transport in a heterologous expression system using RDEV, and further show the effects of alternative substrates for the NET on rates of bidirectional transport of NE and DA.

543.13

ANTISENSE OLIGONUCLEOTIDE INHIBITS NOREPINEPHRINE TRANSPORTER mRNA TRANSLATION IN PC12 CELLS. Qi Xiao* and S. M. Tejani-Butt. Dept. of Psychiatry, Univ. of Penn. Sch. of Med., Phila., PA 19104.

The norepinephrine transporter (NET) is located on NE neurons and terminals and is responsible for the reuptake of NE that is released following nerve stimulation. Since PC12 cells express NET, they are an useful *in vitro* model system for studying presynaptic NE neuronal activity. Previous studies in PC12 cells have identified two PC12 membrane proteins with a relative mass of approximately 32 kDa and 53 kDa, which may represent components of the NET. Antisense inhibiton of gene expression has become a widely used strategy for regulating the expression of specific genes. The present study used sequence-specific antisense oligonucleotides (ODNs) complementary to NET mRNA, and determined whether this intervention would inhibit NET mRNA translation in PC12 cells. PC12 cells were cultured in RPMI medium 1640 and total RNA was isolated. RNA translation in ^{35}S-methionine-supplemented rabbit reticulocyte lysate was performed at 30°C with 15 ug of total RNA and 100 uM of antisense or sense ODN for 60 min. Samples were separated on 12 % SDS-PAGE and exposed overnight to Kodak XAR film. Antisense ODNs had significant inhibitory effects on NET mRNA translation. A decrease in translation products were observed which corresponded to approximately 32 kDa and 53 kDa respectively. In contrast, 100 uM sense ODN had no effect on NET mRNA translation. The results indicate that the antisense ODN approach may be useful for studying the regulation of NET gene expression. (Research funds from USPHS grant NS 31699).

543.14

PHENYLEPHRINE INTERACTIONS WITH NOREPINEPHRINE AND DOPAMINE TRANSPORTERS. A.M. Etgen*, G.B. Karkanias, Z. Kogen and A. Fleischmann. Depts. Neurosci. & Psychiat., Albert Einstein Coll. Med., Bronx, NY 10461

These experiments examined the influence of the α_1-adrenergic agonist and sympathomimetic agent phenylephrine (PHE) on monoamine efflux and uptake in hypothalamic and striatal preparations. At 1.0 µM, PHE promoted calcium-independent efflux of norepinephrine from hypothalamic slices. In hypothalamic synaptosomes PHE produced a concentration-dependent inhibition of ^{3}H-norepinephrine uptake (IC_{50}≈90 nM) which was neither blocked by the α_1-adrenergic antagonist prazosin nor mimicked by the α_1 agonist methoxamine. PHE also inhibited synaptosomal uptake of ^{3}H-dopamine (IC_{50}≈7 µM), and it promoted efflux of both norepinephrine and dopamine from synaptosomes. PHE had no effect on ^{3}H-γ-aminobutyric acid uptake into hypothalamic synaptosomes. To determine whether PHE can interact with the dopamine transporter we examined the ability of PHE to block ^{3}H-dopamine uptake in synaptosomes from rat striatum. PHE produced a dose-dependent blockade of ^{3}H-dopamine uptake in striatal synaptosomes (IC_{50}≈ 40 µM). To eliminate any uptake through the norepinephrine transporter we coincubated striatal synaptosomes with 100 µM desmethylimipramine, a selective inhibitor of the norepinephrine transporter. Under these conditions PHE produced a concentration-dependent inhibition of ^{3}H-dopamine uptake (IC_{50}≈ 30 µM). These findings suggest that the sympathomimetic agent PHE can act at the dopamine as well as the norepinephrine transporter. Supported by MH41414, RSDA MH00636, and T32 NS07183.

543.15

INHIBITION OF VESICULAR MONOAMINE TRANSPORT BY TREATMENT OF PC12 CELLS WITH PHORBOL ESTER, PMA N. Nakanishi*, [1]H. Watanabe, [1]H. Hasegawa, [2]T. Ueha, S. Katoh, N. Minami. Dept. of Biochem., and [2]Dept. of Physiol., Meikai Univ. Sch. Dent., Sakado, Saitama 350-02, Japan, and [1]Dept. of Bioscience, Nishi-Tokyo Univ., Uenohara, Yamanashi 409-01, Japan.

We reported that cAMP down-regulates cellular uptake of catecholamine by inhibiting vesicular monoamine transport process (J. Neurochem. 64, 600, 1995). This effect of cAMP was mediated by protein kinase A pathway. Since protein kinase A and protein kinase C have close relationship in the signal transduction pathway, we examine the effect of phorbol 12-myristate 13 acetate (PMA) on vesicular amine transport to clarify a role of protein kinase C in regulation of the amine transport. Activity of the vesicular amine transport was measured as 5HT uptake by digitonin-permeabilized PC12 cells. When cells were treated with PMA (1 μM) for 30 min prior to the digitonin-permeabilization, vesicular 5HT uptake was decreased to about 50% of the control value, whereas the PMA addition into the uptake assay mixture by already permeabilized cells showed no effect. The inhibitory effect of PMA was disappeared when the intact cells were treated with both PMA and K252a, a protein kinase inhibitor. These results suggest an inhibitory effect of protein kinase C on vesicular monoamine transport.

543.16

LOCALIZATION OF NORADRENALINE TRANSPORTER mRNA EXPRESSION IN THE HUMAN LOCUS COERULEUS. C. Eymin[1], Y. Charnay[1,2], B. Greggio[1] and C. Bouras[1,3]*. [1]Dpt of Psychiatry, University of Geneva, Switzerland; [2]CNRS, France and [3]Dpt of Neurobiology, Mount Sinai School of Medicine, New-York, USA.

High affinity reuptake of transmitters via selective Na^+/Cl^--dependent membrane transporters are thought to play a major role in the regulation of the synaptic activity. Furthermore, there is evidence that the monoamine transporters represent the main target of certain classes of antidepressants and drugs such as cocaine. The distribution of the noradrenaline transporter mRNA was examined in the human dorsolateral pontine tegmentum, using *in situ* hybridization histochemistry. Three [^{35}S]-labeled oligonucleotide probes complementary to the nucleotide sequence encoding the amino acids 36-51, 186-197 and 579-591 of the human noradrenaline transporter mRNA were used. These sequences showed no homology with other membrane transporters or receptors sequences published in the GenBank. Analysis of film autoradiograms showed that noradrenaline transporter mRNA was expressed through the whole extent of the locus coeruleus complex. The largest population of labeled cells was seen in the nucleus locus coeruleus proper whereas few scattered labeled cells were visualized in its ventral subdivision, namely the locus subcoeruleus area. No hybridization signal was detected in the serotonergic cell groups including the median and the dorsal raphe nuclei. Thus, these anatomical findings suggest that cells expressing noradrenaline transporter mRNA in the human brainstem are predominantly, if not exclusively, concentrated within areas known to contain the largest collections of noradrenergic neurons.

543.17

THE VESICULAR MONOAMINE TRANSPORTER, IN CONTRAST TO THE DOPAMINE TRANSPORTER, IS NOT REGULATED FOLLOWING CHRONIC COCAINE SELF-ADMINISTRATION. J.M. Wilson*, M.E. Carroll and S.J. Kish. Clarke Institute of Psychiatry, Toronto, Ontario and Department of Psychiatry, University of Minnesota, Minneapolis, MN.

While various neuronal markers have been used as indices of nerve terminal integrity, many are susceptible to regulatory processes which confound the quantification of nerve terminal loss. This study examined the possible regulation of the vesicular monoamine transporter (VMAT2) in rat brain. A paradigm of chronic, unlimited access to self-administration of cocaine was used. This paradigm has previously demonstrated marked up- and down-regulation of the dopamine transporter (DAT; as determined by [^{3}H]WIN 35,428 binding) during cocaine access and withdrawal (Wilson, et al., J. Neurosci., 14: 2966, 1994). From the same rat brain samples, sequential brain sections were retained for quantitative autoradiographic analysis of VMAT2. Following a 20 min. preincubation at 25°C in 50 mM sodium phosphate buffer (pH 7.7), brain sections were incubated for 40 min. with 5nM [^{3}H]TBZOH (plus 2μM TBZ for non-specific binding). Specific [^{3}H]TBZOH binding was detected in DA (striatum, nucleus accumbens, olfactory tubercle), 5-HT (dorsal raphe) and NA (hypothalamus) rich areas of control rat brain. Following chronic, unlimited access to self-administration of cocaine, no changes in [^{3}H]TBZOH binding to VMAT2 were detected, either on the last day of cocaine access or after 3 weeks drug withdrawal. This is in contrast to DAT which was upregulated (+65% striatum, +69% nucleus accumbens) on the last day of cocaine access and down regulated (-30% nucleus accumbens) after cocaine withdrawal. The present data support the suggestion of Vander Borght, et al. (Neurosci, in press) that VMAT2 is not regulated by chronic perturbation of the DA system, and that consequently VMAT2 might serve as an accurate indicator of nerve terminal integrity. In addition, the lack of reduction in [^{3}H]TBZOH binding suggest that chronic cocaine exposure does not result in actual degeneration of DAergic nerve terminals in rat brain. (Supported by US NIDA DA 7182).

543.18

DISCRIMINATIVE STIMULUS EFFECTS OF ANTIDEPRESSANTS IN METHAMPHETAMINE-TRAINED SQUIRREL MONKEYS. D.M. Grech* H. R.D. Spealman and J. Bergman, Harvard Medical School

New England Regional Primate Res. Center, Southborough, MA 01772-9102.

The discriminative stimulus effects of a number of clinically useful antidepressants were studied in squirrel monkeys trained to discriminate 0.30 mg/kg methamphetamine (MA) from saline using conventional drug discrimination procedures. Substitution tests were initially completed with MA (0.03-1.0 mg/kg), prior to testing a number of chemically diverse drugs such as Nomifensine (0.10-3.0 mg/kg), Bupropion (0.30-3.0 mg/kg), Trazadone (1-17.8 mg/kg), Doxepin (0.30-5.6 mg/kg), Fluoxetine (1-17.8 mg/kg), Citalopram (1-17.8 mg/kg) and Sertraline (1-10 mg/kg). For comparison purposes, a number of tricyclic antidepressants (TCA's) were also studied in these monkeys. Fluoxetine, Citalopram, Sertraline and the TCA's, drugs which primarily inhibit NE or 5-HT uptake, failed to substitute for MA. Partial substitution (>60% MA-lever responding) for MA was obtained with Nisoxetine, Trazadone and Doxepin, whereas Bupropion and Nomifensine fully substituted for MA in all subjects. These results suggest that antidepressants which function as DA uptake inhibitors share discriminative stimulus properties with MA. However, our results with Doxepin and Trazadone suggest that NE and 5-HT systems may also be involved in mediating the MA-like effects of some clinically important antidepressants. This research was supported by grants DA 03774, RR00168, DA 00499 and MH 07568.

543.19

PHARMACOLOGY AND DISTRIBUTION OF NOREPINEPHRINE TRANSPORTERS (NET) IN HUMAN LOCUS COERULEUS (LC) AND RAPHE NUCLEI. G.A. Ordway*, C. A. Stockmeier, G.W. Cason, and V. Klimek. Dept. Psychiatry & Human Behavior, University of Mississippi Med. Ctr., Jackson, MS 39126, and Dept. Psychiatry, Case Western Reserve University, Cleveland, OH, 44106.

NET are a site of action for many antidepressant drugs, e.g. tricyclic antidepressants, and drugs of abuse, e.g. cocaine. [^{3}H]Nisoxetine (^{3}H-NIS) is a unique NET ligand that does not bind to intracellular sites. The distribution of ^{3}H-NIS binding to NET along the rostral-caudal axes of LC and dorsal and median raphe was measured autoradiographically in 7 normal human subjects post-mortem. Binding to NET was differentially distributed along the LC axis and correlated positively with numbers of neuromelanin-containing (noradrenergic) cells along the LC axis within individuals. Surprisingly, densities of NET in the dorsal and median raphe were similar to that in the LC. ^{3}H-NIS binding to NET was observed along the entire rostral-caudal extent of the dorsal and median raphe nuclei. The possibility that ^{3}H-NIS binds to serotonin transporters in the raphe was studied by autoradiographically assessing the inhibition of ^{3}H-NIS binding by desipramine, imipramine or citalopram. The order of potency of these drugs for inhibition of ^{3}H-NIS binding was identical in the LC and dorsal and median raphe nuclei, and was characteristic of binding to NET (desipramine > imipramine > citalopram). Thus, high levels of NET occurs in the LC as well as in the raphe of the human. Drugs which are selective for NET vs. serotonin transporters may achieve specificity with respect to their target proteins, but physiologically even highly selective NET drugs will bind with high capacity in the dorsal and median raphe nuclei. (Supported by MH46992 and MH45488).

543.20

RESERPINE DECREASES NOREPINEPHRINE TRANSPORTER mRNA EXPRESSION IN THE ADRENAL MEDULLA BY AN INNERVATION-INDEPENDENT MECHANISM J.F. Cubells[1,3]*, J.W. Jahng[2,3], H. Baker[2,3], B.T. Volpe[2,3], G.P. Smith[1], S.S. Das[3], and T.H. Joh[1,2,3]. Depts. of [1]Psychiatry, [2]Neurology, & [3]Burke Med. Res. Inst., Cornell U. Med. Coll. White Plains, NY 10605.

Reserpine administration decreases levels of norepinephrine transporter (NET) mRNA and simultaneously increases levels of tyrosine hydroxylase (TH) mRNA in the adrenal medulla (AM; Cubells, et al., 1995, J. Neurochem., in press). To investigate the mechanism underlying this effect, we administered reserpine (10 mg/kg s.c., 24 h premortem) or 20% ascorbic acid vehicle to rats that had undergone unilateral splanchnicotomy, and measured relative levels of NET mRNA and TH mRNA in the AM using quantitative *in situ* hybridization. Splanchnicotomy did not alter the effect of reserpine on levels of NET mRNA, but diminished the reserpine-induced increase in TH mRNA by 75%. Innervation-independent mechanisms therefore mediated the reserpine-induced decrease in NET mRNA, as well as a small component of the increase in TH mRNA. We therefore postulated that a reserpine-induced increase in levels of adrenocorticotropin (ACTH; Lowy et al., 1990, Biol. Psychiatry 27: 546) mediated the effect of reserpine on NET expression in the AM. In preliminary experiments, ACTH 1-24 (Acthar Gel, 4 U/day s.c. for 5 d) diminished NET mRNA levels in the AM of hypophysectomized rats. We hypothesize that ACTH regulates the expression of NET mRNA in the AM, presumably by stimulating adrenocortical steroid production, *Supported by a Reader's Digest Fellowship, Dept. of Psychiatry, CUMC (J.F.C). and NIH grants MH24285 (T.H.J.) and MH00149 (G.P.S).*

543.21

IBOGAINE INHIBITS BIOGENIC AMINE TRANSPORTERS. C. Messer* and G. Rudnick. Dept. Pharmacology, Yale University School of Medicine, New Haven, CT, 06510.

Ibogaine, a plant indole alkaloid, has psychoactive properties and is a putative anti-addictive agent. Although several potential targets have been identified, the molecular target(s) mediating the psychoactive and possible anti-addictive properties of ibogaine are unknown. Cells from a porcine kidney cell line (LLC-PK_1) were stably transfected with transporters for each of the biogenic amines. Using [^{3}H]dopamine as a substrate for the dopamine and norepinephrine transporters, and [^{3}H]serotonin as a substrate for the serotonin transporter, ibogaine was shown to specifically inhibit transport at all three biogenic amine transporters. This inhibition was significantly higher for the serotonin transporter (K_i = 10 μM), than for either the norepinephrine or dopamine transporters (K_i = 86 μM). The results of binding studies using a crude membrane preparation taken from the transfected LLC-PK_1 cells showed that ibogaine's ability to inhibit transport is closely paralleled by its ability to inhibit the binding of 2B-carbomethoxy-3B-(4-[^{125}I]iodophenyl)tropane, a cocaine analog, for each of the biogenic amine transporters. It has been estimated that peak brain concentrations of ibogaine reach 100 μM following pharmacologically effective doses in rodents (Glick, S.D. et al., Brain Res. 628:201-8.) making the biogenic amine transporters potential molecular targets for the actions of ibogaine.

543.22

EFFECTS OF NERVE GROWTH FACTOR ON EXPRESSION OF VESICULAR AMINE TRANSPORTERS IN PC-12 CELLS. J. A. Near* and C. R. Adamson. Medical Sciences Program, Indiana University School of Medicine, Bloomington, IN 47405

Tritiated dihydrotetrabenazine ([^{3}H]TBZOH) binds with high affinity (K_d=2-10 nM) to a vesicular amine transporter found in rat brain and sympathetic nerves ($rVMAT_2$), and with much lower affinity (K_d = 200-500 nM) to a similar transporter present in PC-12 cells and in normal rat adrenal ($rVMAT_1$). PC12 cells treated with nerve growth factor (NGF) acquire a sympathetic neuron-like phenotype. The purpose of the present work was to determine if NGF treatment had any effect on the relative or absolute levels of expression of the two transporters in PC12 cells. NGF treatment led to growth of neurite-like processes in 60-85% of the cells, compared with <2% in controls. Computerized nonlinear least squares curve fitting analysis of [^{3}H]TBZOH binding to a crude membrane fraction from control and NGF treated cells revealed that the best fit was to a two site model in all cases. Less than 5% of the total binding activity could be attributed to higher affinity sites, and the capacity of these sites (0.2 pmol/mg protein) was unaffected by NGF treatment. Lower affinity binding capacity in both treated and untreated cells increased from 15 pmol/mg protein in controls to 24 pmol/mg protein after NGF treatment. NGF treatment had no significant effect on apparent dissociation constants for either class of binding sites. We conclude from these results that NGF treatment of PC12 cells may increase the level of expression of the transporter already present in untreated cells, but that a vesicular amine transporter with higher affinity for [^{3}H]TBZOH does not become the major form expressed after such treatment.

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543.23

THE EFFECT OF DIABETES ON CATECHOLAMINE TRANSPORTER GENE EXPRESSION. M.D. Brot*[1], P.Szot[2], A. Zavosh[1,3], A.L. McCall[4], and D. Figlewicz Lattemann.[1,3] Divs. of Endocrin/Metab[1] and GRECC[2], Seattle VA Medical Center, Depts. of Psychology[3], and Psychiatry and Behav Sci.[2], UW, Seattle, WA 98195; and Medicine Service, Portland VA Med. Center[4], Portland, OR 97207.

Dopamine (DA) and norepinephrine (NE) transporters take up their respective catecholamines from the synaptic cleft, thus inactivating their signal. Exogenous insulin treatment differentially affects these transporters, increasing DA reuptake while decreasing NE uptake. There is a paucity of research on the relationship between catecholamine transporters and endogenous insulin, such as in diabetes. In the present study, we evaluated the coordinated regulation of NE and DA neuronal function by measuring gene expression for the DAT, NET; and for tyrosine hydroxylase (TH), the rate limiting enzyme for NE and DA synthesis.

Rats treated with 65 mg/kg streptozotocin became diabetic [(Diabetics vs control): Glucose levels: 538±15 vs 138±9 mg/dl; Insulin levels: 11±3 vs 52±5 uU/ml.] After 7 days, TH and NET mRNA were assayed in the locus coeruleus and TH and DAT mRNA were assayed in the substantia nigra compacta/ventral tegmental area by *in situ* hybridization. No difference in TH or NET mRNA was found in NE neurons. However, in DA neurons of diabetic rats, a trend ($p=0.15$) for decreased levels of DAT mRNA (OD: 0.450±.015 vs. 0.487±.015) and a significant ($p<0.05$) decrease of TH mRNA (OD: 0.288±.026 vs. 0.378±.037) were observed. These results suggest differential effects of diabetes (hypoinsulinemia) on CNS NE and DA neurons and provide molecular evidence for the altered dopaminergic function which has been observed in clinical and experimental diabetes.

BEHAVIORAL PHARMACOLOGY I

544.1

NITRIC OXIDE INHIBITION ENHANCES PARADOXICAL SLEEP IN CATS. I. de Andrés,* M. Garzón, B. Gomez, N. Fernandez and G. Dieguez. Dpts. Morfología and Fisiología. Universidad Autónoma. Madrid 28029, Spain.

Previous studies examining the effects of nitric oxide synthase (NOS) inhibitors on cortical evoked responses and the sleep-wakefulness states of rats and rabbits have indicated that nitric oxide may have a role in arousal and in slow wave sleep (SWS) mechanisms. In this study, we have investigated the modifications in the sleep-wakefulness states of cats after systemic administration of Nω-nitro-L-arginine methyl ester (L-NAME), a NOS inhibitor. Five cats with electrodes for EEG, EMG, EOG and PGO chronic recordings were used. A 30 mg/kg dose of L-NAME was administered via i.p. in all animals; in addition, the effects of 5, 15, and 60 mg/kg were tested in three of them. The different doses, given in a randomized order, were separated by intervals of at least 15 days. Eight hour sleep recordings were obtained the day before (baseline) and in the early (days 0, 1 and 2) and late (day 10) periods after the administration of the different L-NAME doses. Comparisons among baseline and the different experimental days (Anovas for repeated measures and paired t-tests) showed that starting in the second hour after L-NAME administration, all doses effectively produced a significant increase in paradoxical sleep (PS). The PS enhancement on day 0 was similar with the different doses. However, long-lasting effects were observed with the intermediate doses (15 and 30 mg/kg) since a significant increase in PS was also detected on days 1 and 2 after L-NAME administration. The different treatments also increased SWS, but this increase only reached statistically significant values on day 0 after the 15 mg/kg dose. Sleep changes were accompanied by moderate decreases in wakefulness and/or drowsiness. These results indicate that the main effect of L-NAME in the range of doses used is to facilite PS, thus suggesting that nitric oxide may participate in PS mechanisms.

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544.2

ENHANCED SPECIFIC ANORECTIC ACTIVITY OF THE NITRIC OXIDE SYNTHETASE INHIBITOR, L-NAME, IN OBESE ZUCKER RATS. A. Stricker-Krongrad*, B. Beck and C. Burlet. INSERM U 308. 38, rue Lionnois. 54000 Nancy. France

The presence of a nitric oxide synthetase (NOS) was demonstrated in the rat brain. It has been recently shown that administration of NOS inhibitors reduces food intake in mammals. These results suggest that NO might be involved in the mechanisms controlling feeding behavior. To test this hypothesis, we administered L-NAME (L-N^G-Nitro Arginine Methyl Ester) intraperitoneally (IP) and intracerebroventricularly (ICV) at dark onset in obese Zucker rats, a genetic model of hyperphagia and obesity. Lean Zucker rats served as controls. The feeding behavior was recorded via a complete automatic system. L-NAME (IP, 0, 10, 25 and 50 mg/kg) induced a dose-dependent decrease in food intake in the obese rats (MED: 0.50 ± 0.06 mg/kg ; ED_{50}: 3.50 ± 0.46 mg/kg), but remained ineffective in the lean rats whatever the dose used. These anorectic properties were well-translated into the picture of the microstructure of feeding behavior. L-NAME (IP) induced decreases in meal duration (-85 %), in meal number (-64 %) and in time spent to eat (-93 %) in the obese rats. In the lean rats, decreases in meal duration (-46 %), in meal number (-44 %) and in time spent to eat (-65 %) were balanced by an increase in meal size (+62 %), leaving food intake unaffected. ICV L-NAME (10 μg) reproduced the same effects in the obese rats, but lean rats still remained insensitive. This study clearly demonstrates that the activity of the NOS inhibitor L-NAME is enhanced in the obese Zucker rat and that this action is most probably located into its brain. This indicates that NO could be implicated into the expression of hyperphagia.

544.3

MECHANISM OF ACTION OF NITRIC OXIDE (NO) DONOR-INDUCED PENILE ERECTION AND YAWNING. M.R. Melis* & A. Argiolas. Bernard. B. Brodie Dept. Neurosci., Cagliari Univ., 09124 Cagliari, Italy.

The effect of NO donors sodium nitroprusside, hydroxylamine and isoamyl nitrite injected into a lateral ventricle (i.c.v.) or in the paraventricular nucleus of the hypothalamus (PVN) on penile erection and yawning was studied in male rats. After i.c.v. injection, only isoamyl nitrite (10-100 μg) induced these behavioral responses, while the others induced behavioral changes, i.e. motor hyperactivity and convulsions, that masked the above responses. In contrast, the NO donors (10-50 μg) induced penile erection and yawning when injected in the PVN. The NO donor responses were prevented by the guanylate cyclase inhibitors methylene blue and LY 83583 and by the oxytocin antagonist $d(CH_2)_5$-Tyr(Me)-Orn^8-vasotocin given i.c.v. but not in the PVN. The NO scavenger hemoglobin was ineffective given i.c.v. or in the PVN. 8-bromo-guanosine 3':5'-cyclic monophosphate (8-Br-cGMP) (10-100 μg) did not induce penile erection and yawning when injected i.c.v. or in the PVN. These results show that NO donors induce penile erection and yawning by activating central oxytocinergic transmission in the PVN with a cGMP-independent mechanism.

544.4

THE NITRIC OXIDE SYNTHASE INHIBITORS N^G-NITRO-L-ARGININE METHYL ESTER AND N^G-NITRO-L-ARGININE ARE ANXIOLYTIC AND ARE DEVOID OF BENZODIAZEPINE SIDE-EFFECTS IN RATS. R.W. Dunn*, R.A. Del Vecchio and S. LaMarca. Ohmeda PPD Inc., 100 Mountain Ave., Murray Hill, NJ 07974.

N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-nitro-L-arginine (L-NOARG) inhibited cerebellar rat neuronal nitric oxide synthase (NOS) activity with IC_{50} values of 0.90 and 0.48 uM, respectively. Bolus iv administration of either L-NAME (54.8 and 100 mg/kg) or L-NOARG (30 mg/kg) significantly ($p<0.05$) disinhibited lick-shock conflict responding in water deprived rats, similar to the benzodiazepine (BZD) diazepam (0.3 and 0.5 mg/kg). The stereoisomer N^G-nitro-D-arginine methyl ester (D-NAME; 100 mg/kg, iv) was inactive in the lick-shock assay. L-arginine (50 mg/kg, iv), a substrate for NOS, antagonized the anxiolytic effect of L-NAME. The BZD antagonist flumazenil (10 mg/kg, iv), which completely reversed the disinhibitory activity of diazepam, had no effect on the anxiolytic effect of L-NAME. In addition, diazepam impaired both learning in the step-down passive avoidance paradigm and motor coordination in the rotorod assay, while L-NAME and L-NOARG did not. These results show that inhibition of NOS resulted in disinhibition of conflict behavior, indicative of anxiolytic activity. Unlike diazepam, the NOS inhibitors did not produce impairments in either learning or motor coordination. Therefore, NOS inhibitors may represent a novel class of anxiolytic agents which are devoid of BZD side-effects.

544.5

RECIPROCAL BEHAVIORAL CROSS-SENSITIZATION BETWEEN PHENCYCLIDINE AND (-) BUT NOT (+)PENTAZOCINE. X. Xu and E.F. Domino*. Dept. of Pharmacology, University of Michigan, Ann Arbor, MI 48109-0630 and Department of Psychology, Grand Valley State University, Allendale, MI 49401.

Phencyclidine, a noncompetitive NMDA antagonist and also a *sigma* ligand, produces behavioral sensitization. Our previous study suggests that phencyclidine-induced behavioral sensitization may not be mediated only by the NMDA receptor complex. The present research investigated the locomotor stimulant effects of the *sigma* ligand (+) and the opioid ligand (-)pentazocine, and the possibility of reciprocal cross sensitization with the two enantiomers in phencyclidine sensitized animals. Total locomotor activity and ambulation of adult female Sprague Dawley rats were assessed with an automatic photoelectric system. The acute administration of either isomer of pentazocine in doses of 10-32 mg/kg i.p. produced slight locomotor stimulation. Repeated administration of (+)pentazocine did not produce behavioral sensitization. On the other hand, repeated injections of the (-) enantiomer produced significant locomotor sensitization. (-)Pentazocine sensitized rats showed cross-sensitization to phencyclidine. Phencyclidine sensitized rats showed cross-sensitization to (-)pentazocine. This unexpected observation requires further research. (Supported in part by the Psychopharmacology Research Fund 361024.)

544.6

EFFECTS OF PHENCYCLIDINE (PCP) AND MK-801 ON SENSORIMOTOR GATING IN CD-1 MICE. P. Curzon* and M.W. Decker. Neuroscience Research, D-47W, AP-9A, Pharmaceutical Products Division, Abbott Laboratories, 100 Abbott Park Rd., Abbott Park, IL 60064-3500

Abuse of PCP, which binds in the NMDA receptor channel, or d-amphetamine, an indirect dopamine (DA) agonist, produces psychotomimetic states similar to schizophrenia. Schizophrenics have a deficit in sensorimotor gating, as measured by prepulse inhibition (PPI). In rats, compounds binding to the PCP site and DA agonists disrupt PPI of acoustic startle. In contrast to the large number of studies on the pharmacology of PPI conducted in rats, relatively little work has been done with mice. In order to develop a mouse model for testing potential antipsychotics, we tested the effects of PCP (1.1-36.0 μmol/kg) and the PCP site ligand, MK-801 (0.03-3.0 μmol/kg), on PPI in mice and determined if the neuroleptic, haloperidol, could reverse the MK-801 effect. Because PCP also binds to sigma receptors, we tested the sigma ligand (+)-3-PPP.

Male CD-1 mice (28-32 g) were tested in San Diego Instruments, SR chambers. The mice received trials in which a 120 dB (40 ms) acoustic startle stimulus was presented alone and trials in which an acoustic (20 ms) prepulse stimulus between 70 and 85 dB was given 100 ms prior to the startle stimulus. Presentation of the prepulse reduced the startle response. This PPI was analyzed by ANOVA.

Significant reductions ($p<.05$) in PPI were observed following treatment with MK-801 (0.9 and 3.0 μmol/kg) or PCP (11.0 μmol/kg) i.p. Preliminary findings with mice treated with haloperidol (0.13-1.3 μmol/kg) 15 min after MK-801 (0.9 μmol/kg) revealed no significant reversal of the MK-801 disruption. In contrast to PCP, (+)-3-PPP (117.0 μmol/kg) significantly enhanced PPI ($p<.05$).

The similarities of the effects of PCP and MK-801 on PPI in mice in the current study and the effects previously reported with these agents in rats suggest that a mouse model may be viable. However, further pharmacological evaluation of this mouse model with different agents needs to be carried out.

544.7

POST-TRIAL MUSCARINIC RECEPTOR BLOCKADE DOES NOT AFFECT THE RETEST PROFILE OF MICE IN THE ELEVATED PLUS-MAZE TEST OF ANXIETY. R.J. Rodgers*, J.C. Cole, N.J.T. Johnson, C. Dewar and G.R. Kidd, Ethopharmacol. Lab., Dept. Psychology, University of Leeds, LS2 9JT, U.K.

The elevated plus-maze test is the most popular animal model of anxiety currently in use. It is based on the natural aversion of rodents for open spaces, has been validated for rats and mice and is bidirectionally sensitive to pharmacological and non-pharmacological manipulations of anxiety. One of the most intriguing aspects of this test, but one which limits its application, is the change in behavioural baseline with repeated testing. On re-exposure, mice and rats generally show an exaggerated avoidance of the open arms and a *reduction* in the efficacy of diazepam and similar anxiolytics. To assess the possible involvement of muscarinic receptor mechanisms in the test-retest change in behavioural baseline, mice were treated with saline, 0.1 or 1.0 mg/kg scopolamine HBr immediately after an initial 5min exposure to a standard elevated plus-maze (post-trial scopolamine). Upon 24h retest, control animals showed a retest profile comprising reductions in open arm measures and increases in aspects of risk assessment and immobility: this profile was not altered by scopolamine. The study was repeated using an initial 2 min exposure period. A very robust test-retest effect was observed using this revised procedure, while other aspects of the data confirmed that animals were acquiring considerable knowledge of the maze during the first half of a normal 5min exposure period. However, post-trial scopolamine was again ineffective in altering this effect of prior maze experience. While further research is required, these data suggest that muscarinic receptor mechanisms are not involved in the mechanisms underlying the between-trials changes in plus-maze behaviour.

544.8

BEHAVIORAL EVIDENCE FOR THE MODULATION OF (+)MK-801-INDUCED HYPERLOCOMOTION BY SIGMA LIGANDS IN MONOAMINE-DEPLETED MICE. S. Okuyama*, Y. Imagawa and K. Tomizawa. 1st Laboratory, Medicinal Research Laboratories, Taisho Pharmaceutical Co., Ltd., 1-403 Yoshino-cho, Ohmiya, Saitama 330, Japan.

The selective non-competitive N-methyl-D-aspartate (NMDA) antagonist (+)-5-methyl-20,11-dihydro-5H-dibenzo(a, d)cyclohepten ((+)MK-801) induced a dose-dependent increase in locomotor activity in mice pretreated with a combination of reserpine and alpha-methyl-para-tyrosine (alpha-MT). A selective and potent sigma receptor "antagonist" NE-100 (N, N-dipropyl -2- [4 - methoxy - 3 - (2 - phenylethoxy) phenyl] - ethylamine monohydrochloride), which did not affect spontaneous locomotor activity per se, did not inhibit the locomotor stimulatory effects of (+)MK-801. Sulpiride, a dopamine D2 receptor antagonist, and clozapine, a dopamine D4 receptor antagonist, which decreased spontaneous locomotor activity, did not inhibit the locomotor stimulatory effects of (+)MK-801. The sigma receptor "agonists" (+)N-allynormetazocine ((+)SKF10,047), (+)pentazocine and (+)-3-(3-hydroxyphenyl)-N-(1-propyl)piperidine ((+)3-PPP), which did not affect spontaneous locomotor activity per se, dose-dependently enhanced the hyperlocomotion induced by (+)MK-801. The enhancement of (+)MK-801-induced hyperlocomotion by (+)SKF10047, (+)pentazocine and (+)3-PPP was completely blocked by NE-100. The enhancement of (+) MK-801-induced hyperlocomotion by (+)pentazocine was not affected by the treatment of with sulpiride and clozapine. These findings indicate that sigma ligands can markedly attenuate NMDA antagonist-induced behavior, suggesting that a major physiological role of sigma receptors might be to modulate the function of the NMDA receptor ion channel complex in vivo.

544.9

EFFECT OF INTRA-AMYGDALA ADMINISTRATION OF BETA ADRENERGIC AGONISTS ON THE BEHAVIOR OF RATS UNDER A DRL SCHEDULE. S. Frith, J. Wilkins, N. Leidenheimer*, and J. M. O'Donnell, Dept. of Pharmacology, LSU Med. Ctr., Shreveport, LA.

Previous work has suggested that nuclei within the amygdaloid complex may be important in the mediation of the actions of antidepressant drugs, particularly those involving beta adrenergic receptors. In order to examine the role of these receptors in mediating antidepressant-like behavioral activity, the actions of intra-amygdala and i.c.v. infusion of the nonselective beta adrenergic agonist isoproterenol and the beta-2 selective agonist clenbuterol on differential-reinforcement-of-low-rate (DRL) behavior were determined. Doses of 10 and 30 ug of isoproterenol, administered i.c.v., produced antidepressant-like effects on DRL behavior, reducing response rate and increasing reinforcement rate. By contrast, a total dose of 60 ug (30 ug in a total volume of 1 ul, bilaterally) of isoproterenol was required to produce antidepressant-like effects when it was administered directly into the amygdala. Similarly, 30 ug clenbuterol, administered i.c.v., reduced response rate and increased reinforcement rate of rats under the DRL schedule, while a total dose of 60 ug (30 ug bilaterally) was ineffective when administered directly into the amygdala. All antidepressant-like effects of the beta adrenergic agonists were antagonized by propranolol, suggesting mediation of the agonist-induced behavioral effects by beta adrenergic receptors. The weak actions of isoproterenol and clenbuterol following intra-amygdala infusion, compared to their actions following i.c.v. administration, suggest that beta adrenergic receptors in the amygdala are not a major site of action mediating the antidepressant-like effects of beta adrenergic agonists on DRL behavior. (Supported by grants from NIMH.)

544.10

BEHAVIORAL EFFECTS OF INTRA-AMYGDALA BENZODIAZEPINES IN MICE. F.E. Lotrich and E.J. Gallaher*. Oregon Health Sciences University and VA Medical Center, Portland OR 97201.

Benzodiazepines (BZs) have many effects: anxiolysis, hypnotic sedation, muscle relaxation, ataxia, seizure protection, and amnesia. Selective breeding has led to mice which are sensitive or resistant to some effects and not others. To determine the neurochemical bases underlying the behavioral differences, a necessary prerequisite is the identification of neuroanatomic loci contributing to each behavior. The amygdala may be involved in mediating anxiety and is currently being investigated as a locus for some of the effects of BZs.

Mice from the DHP-1 line (diazepam high performance) were prepared with bilateral guide cannulae above the basolateral nucleus of the amygdala using stereotaxic surgery. After recovery, chlordiazepoxide (CDP; 200 mM in 0.5 μL) was infused, and the mice were examined for anxiolysis using the plus maze. Several other effects were also examined: muscle relaxation, ataxia, locomotor activation, and protection against PTZ-induced seizures. Initial results suggest that intra-amygdala CDP in mice has anxiolytic effects ($p<0.01$) in the plus maze. The percentage of open entries following vehicle infusion was 13.6% ± 11, while following bilateral CDP infusion it was 35.6% ± 7. No ataxic or muscle relaxant effects were found. The advantage of using mice is that there is an increasingly large database of murine genetics. These studies are currently being extended to DS and DR mice, which have been shown to differ in sensitivity to ataxia and anxiolysis.

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544.11

PROGESTERONE-BSA AND 3α-ANDROSTANEDIOL-BSA CONJUGATES AFFECT ESTROUS BEHAVIOR WHEN APPLIED TO THE MBH AND POA. C.A. Frye*, K.R. Van Keuren, P.N. Rao and M.S. Erskine, Department of Biology, Boston University, Boston, MA 02215

We investigated whether progesterone (P) and 5α-androstane-3α,17β-diol (3α-Diol) have membrane-mediated effects on lordosis behavior in the MBH and POA. Ovariectomized (ovx) rats with bilateral guide cannula over the medial basal hypothalamus (MBH) were primed with 17β-estradiol (E_2). After a pre-test for sexual receptivity, animals received implants of 3α-Diol conjugated to BSA (3α-Diol:BSA), free 3α-Diol diluted with BSA (3α-Diol&BSA), P conjugated to BSA (P:BSA), free P diluted with BSA (P&BSA) or control BSA and were retested 5, 30, 60, 90, 120, 150, 180 and 210 min later. In contrast to control BSA, P:BSA and P&BSA implanted animals, those that received 3α-Diol:BSA and 3α-Diol&BSA showed facilitated lordosis. In Exp 2, additional E_2-treated animals were implanted with guide cannulae aimed over the preoptic area (POA), a site implicated in the inhibition rather than the facilitation of lordosis. In contrast to control BSA, 3α:BSA and 3α&BSA implanted animals, receptivity increased within 5 minutes of application of P:BSA and P&BSA implants to the POA. Additional ovx animals were tested for effects of systemic hormone treatments on the efficacy of the MBH and POA implants. When P was given to E_2-primed rats, 3α-Diol:BSA and 3α-Diol&BSA applied to the MBH or POA inhibited sexual receptivity; when 3α-Diol was given s.c., 3α-Diol:BSA had facilitatory effects in both areas. P:BSA and P&BSA implants in the MBH or POA of E_2 and P-primed rats did not further facilitate lordosis, while in E_2 and 3α-Diol primed rats, receptivity was not facilitated by either type of P implant. 3α-Diol and P may act in part at the neuronal membrane in the MBH and POA.

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544.12

BIPHASIC EFFECTS OF ALLOPREGNANOLONE ON INTRAMALE AGGRESSION IN MICE. J.F. DeBold*, H. Barros, S. So and K.A. Miczek, Psychology Department, Tufts University, Medford, MA 02155.

Ethanol, benzodiazepines and neuroactive steroids all have allosteric effects of neural $GABA_A$ receptors. This shared mechanism of action may be the basis for their shared behavioral effects, such as sedation and anxiolysis. In addition, benzodiazepines and ethanol both can have stimulatory effects on intramale aggression in mice. However, neuroactive steroids have not been extensively examined for their effects on aggressive behavior. The following experiment was designed to determine if a potent neuroactive steroid, 5α-pregnan 3α-ol, 20-one (allopregnanolone), alters aggressive behavior in male mice in the same dose response pattern as alcohol.

Adult male CFW mice were pair-housed with a female and three weeks later were observed for their behavioral response to an unfamiliar group-housed male mouse introduced into their home cage. After establishing baseline levels of aggression, the effects of allopregnanolone (1-30 mg/kg, ip) on the aggressive behavior of the male residents were measured. Mice treated with 10 mg/kg allopregnanolone showed an enhancement of aggressive behavior toward intruders, and a suppression of attack bites at the 30 mg/kg dose. Alcohol has a similar biphasic dose response effect on aggressive behavior with 1.0-1.7 g/kg, po, increasing aggression and 3.0 g/kg reducing it. Since both ethanol and allopregnanolone can have modulatory effects on the $GABA_A$ receptor complex, this suggests that their similar behavioral effects may have a similar mechanistic basis.

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544.13

PREGNANOLONE-INDUCED HYPNOSIS AND HYPOTHERMIA ARE MEDIATED BY DIFFERENT MECHANISMS. S.-W. Chen*, M. F. Davies and G. H. Loew. Molecular Research Institute, Palo Alto, CA 94304.

The endogenous neurosteroid-pregnanolone (5β-pregnan-3α-ol-20-one) has anxiolytic, anticonvulsant and hyperphagic activities that are mediated by $GABA_A$ receptors. Because other modulators of the $GABA_A$ receptor system such as benzodiazepines share these activities and affect body temperature, this study examines effects of pregnanolone on body temperature and locomotor activity. After given pregnanolone (0 to 20 mg/kg) ip, the locomotor activity and body temperature were measured in male rats for 60 min. Pregnanolone dose-dependently reduced activity to a level previously observed for flunitrazepam. However, unlike flunitrazepam, pregnanolone caused minimal hypothermia. The structure-related analog, 5α-pregnan-3β-ol-20-one (10 mg/kg), had no activity and did not reverse the sedative effect which is consistent with lack antagonism of other known effects of pregnanolone. However, it reversed the hypothermic effect of pregnanolone. Picrotoxin, the $GABA_A$ receptor antagonist, at the dose of 0.5 mg/kg, had no effect by itself, but also reversed the hypothermic effect. These results indicate that the pregnanolone effect on body temperature may involve $GABA_A$ receptor similar to those initiating other effects, while the effect on hypnosis seems to be mediated by other mechanisms. Further investigation of other putative neurosteroid full and partial agonists will also be presented.

544.14

EFFECTS OF THE DYNORPHIN-A(1-8) ANALOG E2078 IN RHESUS MONKEYS. J.A. Vivian*, E.R. Butelman, J.H. Woods. Department of Pharmacology, University of Michigan Medical School, Ann Arbor, MI 48109-0632.

E2078 {[N-methyl-Tyr[1],N-methyl-Arg[7],D-Leu[8]]dynorphin-A(1-8)ethylamide} is a systemically active dynorphin-A analog with reported analgesic and diuretic effects in humans and rodents. The purpose of the present study was to characterize E2078 effects on food-maintained responding and diuresis in rhesus monkeys (*Macaca mulatta*). In monkeys trained to respond under a multiple-cycle FR30 schedule for food reinforcement, E2078 (0.001-0.032 mg/kg s.c.) dose-dependently decreased responding. The rate-suppressant effects were antagonized by the kappa selective antagonist nor-binaltorphimine (nor-BNI; 3.2 mg/kg s.c.). After nor-BNI administration, the potencies of both E2078 and the kappa receptor agonist U69,593 (0.001-0.1 mg/kg s.c.) were reduced for at least 5 weeks in this assay. E2078 (0.056-0.56 mg/kg s.c.) dose-dependently increased urine volume (ca. 400%). This effect was prevented by the opioid antagonist quadazocine (1.0 mg/kg s.c.) as well as nor-BNI (3.2 mg/kg). The effects of nor-BNI were again long-lasting, with the diuretic effects of E2078 and U50,488 returning to pre-nor-BNI levels after approximately 4 weeks. The results suggest that the dynorphin analog E2078 produces robust kappa-agonist effects in rhesus monkeys following systemic administration. Supported by USPHS Grants DA 00254 and DA 07268.

544.15

EFFECTS OF DOPAMINE D3 RECEPTOR LIGANDS ON LOCOMOTOR ACTIVITY AND REWARD IN THE RAT

T. Kling-Petersen*[1], E. Ljung[1], L. Wollter[1] and K. Svensson[2] [1]Dept. of Pharmacology, Medicinaregatan 7, 413 90 Göteborg, Sweden and [2]CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001, ISA.

Dopamine D3 selective agonists, like R-(+)-7-OH-DPAT produce a significant increase in reward thresholds over a wide dose range (0.094 - 96.0 nmol/kg, s.c.) while producing a decrease in locomotor activity over the same doserange in male, Spague Dawley rats. Similarly, the dopamine D3 antagonist U99194A (Haadsma-Svensson, et al. *J Med Chem* **1995**, *In press*) produces an increase in locomotor activity (25.- 200 µmol/kg, s.c.) while a weak increase in reward thresholds (3.5 - 50.0 µmol/kg, s.c.) are seen in the ICSS model. U99194A is also capable of producing positive Conditioned Place Preference (12.5 - 100.0 µmol/kg, s.c.).

R-(+)-7-OH-DPAT, at doses that per se produced a increase in reward threshold, significantly enhanced d-amphetamines facilitatory effect on brain stimulation reward when administered in combination with d-amphetamine (0.25 or 1.0 mg/kg, s.c.). The same facilitatory effect is seen in the locomotor activity model. U99194A is also capable of enhancing the facilitatoty effect of d-amphetamine (0.25 mg/kg, s.c. but not 1.0 mg/kg, s.c.) in the ICSS model, while U99194A produce a tendency to block the locomotor activity enhancing effects of d-amphetamine.

The present data support the hypothesis (Svensson, K. et al, J Neural Transm, 95, 71, 1994) that the functional D3 receptor is a postsynaptic receptor with a psychomotor inhibitory function on both behavior and reward.

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION: OTHER I

545.1

LOSS OF INPUT FROM THE BED NUCLEUS OF THE STRIA TERMINALIS TO THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS AFTER ADRENALECTOMY.

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Limbic structures, such as the hippocampus and amygdala, exert their influence on stress regulation predominantly by means of a projection to the bed nucleus of the stria terminalis (BNST), which in turn projects to the hypothalamic paraventricular nucleus (PVH). To evaluate the significance of the projection of the BNST to the PVH and in particular to the corticotropin-releasing hormone (CRH) neurons we investigated this projection in normal and adrenalectomized (ADX) Wistar rats. The latter were used for immunocytochemical detection of CRH, both on light microscopical and electron microscopical levels. The (length) density of labelled fibres and terminals in the PVH was quantified in 6 untreated and 7 ADX rats with similar injection sites in the medio-ventral BNST. Remarkably, the density was statistically significantly smaller in the whole PVH and all subdivisions of ADX rats (PVH: 1.17 ± 0.17 10^{-3} μ/μ^3) than in untreated rats (PVH: 2.59 ± 0.12 10^{-3} μ/μ^3; $p<0.004$). In addition, the relative density of fibers projecting to the CRH-rich part of the PVH was statistically significantly smaller in ADX rats (0.60 ± 0.03) than in untreated rats (1.10 ± 0.0; $p<0.01$) whereas the other subdivisions of the PVH showed no changes in relative density. Electron microscopical investigations showed no labelled synapses on CRH neurons and thus confirmed the absence of input from the BNST to CRH neurons after ADX. These results indicate a loss of input to the CRH neurons from the BNST following ADX and suggest a large stress-related plasticity at the level of the PVH.

545.2

DISRUPTION OF CATECHOLAMINERGIC AFFERENTS DOES NOT ATTENUATE ACUTE FOOTSHOCK-INDUCED ACTIVATION OF STRESS-RELATED HYPOTHALAMIC NEUROSECRETORY NEURONS.

H.-Y. Li*, A. Ericsson and P.E. Sawchenko. The Salk Institute, La Jolla, Ca 92037

We showed previously that intermittent footshock induced the expression of the immediate-early gene, *c-fos*, in parvocellular CRF, and, secondarily, in magnocellular oxytocin and autonomic-related projection neurons of the paraventricular nucleus of the hypothalamus (PVH). In extrahypothalamic areas, footshock-responsive cell groups included ones associated with the limbic region of the telencephalon, somatosensory and nociceptive information processing, and brainstem catecholamine neurons. Since catecholaminergic neurons in the nucleus of the solitary tract and ventrolateral medulla comprised the dominant loci of footshock-responsive cells that were anatomically identified as projecting to the PVH, we evaluated these as a candidate afferent mediator of hypothalamic neuroendocrine responses. Using a retractable wire microknife, unilateral transections were placed in the coronal plane at the ponto-medullary junction. After 7 days' recovery, and 7 days' adaptation to the shock chamber, animals were exposed to a single footshock session (60 shocks of 1 mA current, 1 msec. duration, delivered randomly over 30 min), and sacrificed 2 hr later. Despite massive depletion of the aminergic innervation on the ipsilateral side, shock-induced Fos expression was comparable in strength and distribution in the PVH on both sides of the brain. This lesion did, however, result in a substantial reduction of Fos expression in the NTS and ventrolateral medulla on the ipsilateral side. These results contrast with those seen in response to a systemic cytokine (interleukin-1) challenge, where identical cuts ablated the Fos response in the ipsilateral PVH, but left the ipsilateral medullary induction comparable to that seen on the contralateral side. We conclude that in the footshock model, activation of medullary aminergic neurons is a secondary consequence of stress, and is mediated via a *descending* projection transected by our ablation. Stress-induced activation of medullary aminergic neurons does not necessarily imply an involvement of these cell groups in driving endocrine hypothalamic responses to a particular stressor.

545.3

AN INTEGRATIVE EXAMINATION OF STRESS EFFECTS ON THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

D.L. Helmreich*, M.I. Morano, S.J. Watson, Mental Health Research Institute, Univ. of Michigan, Ann Arbor MI, 48109

Activation of the hypothalamic-pituitary-adrenal (HPA) axis by stress is a well documented response used by most mammals to maintain homeostasis after a challenge, and the impact of stress on individual components of the HPA axis has been previously characterized by us and other researchers. In the current study, we sought to determine the effects of stress on multiple components of the HPA axis, including secretagogue regulation at the level of the paraventricular nucleus of the hypothalamus (PVN) and receptor regulation at the level of hippocampus, within an *individual* animal in order to create an integrated view of stress effects on the HPA axis. To this end, adult male rats were subjected to either 5 days of a daily session of 1 hr of restraint stress (n=6), or 5 days of a daily session of non-habituating stressors (n=6). Unhandled animals (n=6) served as controls. On day 6, animals were euthanized via rapid decapitation; the brain, pituitary, and median eminence were quickly removed from each animal and frozen. Five days of non-habituating stress resulted in an increase in adrenal weight, and both stress paradigms resulted in a decrease in thymus weight. Preliminary results also suggest that 5 days of non-habituating stress resulted in an increase in mineralocorticoid receptor mRNA in the CA3 region of the hippocampus. Evaluation of these results, in concert with other means and ratios from individual animals, should provide a comprehensive and integrative view of the impact of stress on the HPA axis.

545.4

GABA AND GLUTAMATERGIC LOCAL NETWORKS CONTROL CRF NEURON ACTIVITY IN ORGANOTYPIC CULTURES OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS.

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More than 60% of total synaptic connections within the paraventricular nucleus are of intrinsic (intranuclear) origin (Kiss et al. 1983). We have now investigated the role of this local circuitry in the control of CRF neuron activity. Paraventricular nuclei were microdissected from 200-400 μm thick hypothalamic slices of 5 days old rat pups and then organotypic slice cultures were prepared that retained a well preserved cytoarchitectonic organization of the CRF neuron population and its local environment in chemically defined medium for up to 5 weeks. Ultrastructural immuno-cytochemistry revealed that a large number of presynaptic boutons were immunoreactive with monoclonal antibodies raised against GABA or glutamate. The pharmacological block of $GABA_A$ receptor by bicucullin (20 μM) produced a robust (up to six fold) increase in CRF hormone release from the culture. This effect was completely abolished by the glutamate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). Similarly, electrophysiological analyses of extracellular recordings from the CRF neuron subdivision showed that bicucullin markedly enhanced evoked EPSPs and resulted in the occurrence of spontaneous bursting activity. The effect of bicucullin was entirely reversed by CNQX (20 μM). We conclude that the functional activity of CRF neurons in these cultures is under a tonic inhibitory influence of an intrinsic GABA-ergic circuit. Suppression of GABA-ergic transmission appears to have a permissive role in inducting an increased activity of CRF neurons that is driven by excitatory glutamatergic synapses.

545.5

EFFECT OF MICROINJECTION OF MUSCIMOL INTO DORSOMEDIAL OR PARAVENTRICULAR HYPOTHALAMIC NUCLEUS ON STRESS-INDUCED INCREASES IN PLASMA ACTH IN RATS. S.M. Morin*, E.H. Stotz-Potter, and J.A. DiMicco. Dept of Pharmacol and Toxicol. and Prog. in Med. Neurobio., Indiana Univ. Sch. of Med., Indpls, IN 46202

Acute stress evokes large increases in heart rate (HR) and smaller increases in blood pressure (BP), and stimulates the release of adrenocorticotrophic hormone (ACTH). We have shown that microinjection of the $GABA_A$ agonist muscimol (MUS) 80 pmol into the dorsomedial hypothalamus (DMH) prior to air stress nearly abolishes these cardiovascular changes while similar injection into the paraventricular nucleus (PVN) has no effect. Here, we examined the effect of microinjection of MUS into either the PVN or the DMH on stress-induced release of ACTH which is thought to result from activation of CRF containing projections from the PVN. Rats, instrumented for the monitoring of HR and BP and for blood collection, received bilateral injection of either 80 pmol MUS or 100 nl saline vehicle (VEH) into the DMH (n=3) or the PVN (n=3) and were immediately placed in a restraining tube and subjected to 10 min of air stress. Blood samples were taken prior to microinjection and 10 min after the start of air stress. All injections were shown histologically to be in or within 200 μm of the target nucleus. As reported previously, injection of MUS into the DMH markedly attenuated stress-induced increases in HR (-71%) and BP (-88%), while injection of MUS into the PVN had no effect. Baseline plasma ACTH levels ranged from 16-84 pg/ml. Stress increased plasma ACTH levels by 275 ± 15 pg/ml after injection of VEH into the DMH, but by only 71 ± 25 pg/ml after injection of MUS (p=0.03; paired t-test). In rats receiving injections into the PVN, stress increased plasma ACTH levels by 180 ± 52 pg/ml after VEH, and by 117 ± 38 pg/ml after MUS (p>0.05; NS by paired t-test). Thus, stimulation of inhibitory $GABA_A$ receptors in the DMH suppresses both the cardiovascular and neuroendocrine effects of acute stress in rats. (Supported by USPHS Grant NS 19883 and American Heart Ass'n., Indiana Affiliate)

545.6

DIMINISHED "IN VIVO" HYPOTHALAMO-PITUITARY-ADRENAL (HPA) AXIS RESPONSE DURING MIDDLE AGE: EVIDENCE FOR AN ADRENAL IMPAIRMENT. M. O. Suescun, A. N. Chisari, M. E. Carino, A. Giovambattista and E. Spinedi*. Neuroendocrine Unit, IMBICE, 1900 La Plata, Argentina.

The aim of the present work was to determine which level of the HPA axis could be mainly implicated in the decreased HPA axis function during middle-age. For this purpose, 2- and 15-month-old male mice were i.p. treated with: a) 0.1 μg ACTH, b) 0.5 μg CRH, c) 0.3 IU insulin (INS) or d) vehicle alone. Mice were then killed by decapitation at 20 min (ACTH), 30 min (CRH) or 45 min (INS) after treatment. Trunk blood was collected and plasma ACTH (IRMA) and corticosterone (B) (RIA) levels were determined. The results indicate that there were not age-related differences in basal (vehicle-injected mice, from both groups, killed at either 20, 30 or 45 min after treatment) plasma levels of both ACTH (mean ± SEM of 10-12 mice per group; 76.08 ± 13.73 vs. 82.78 ± 11.79 pg/ml of plasma in 2- and 15-month-old mice, respectively) and B (6.07 ± 1.05 vs. 4.35 ± 1.21 μg/dl of plasma, respectively). Plasma ACTH and B levels were significantly ($P < 0.05$ or less) higher than the respective baseline, regardless the group, after the treatment with either stress stimulus and, we found not significant age-dependent differences in plasma ACTH concentrations after each stress treatment. Conversely, the adrenal response varied in an age-related fashion. In fact, B released in plasma after the injection of either ACTH, CRH or INS was significantly ($P < 0.05$) higher in 2-month-old (mean ± SEM, n = 8 mice per group; 17.23 ± 0.47, 19.94 ± 1.75 and 23.86 ± 1.01, respectively) than in 15-month-old mice (8.97 ± 2.03, 10.27 ± 1.59 and 15.31 ± 1.49, respectively). Our results clearly indicate that middle-aged mice have impaired HPA axis response after the stimulation of either the hypothalamic, pituitary or adrenal level. Since full corticotrope responses were found to both INS and CRH stimuli, it remains to be determined whether adrenal ACTH receptor number or/and binding characteristics are responsible for such an impairment. (Supported by CONICET)

545.7

ALTERATIONS OF NINE NEUROPEPTIDERGIC NERVE FIBERS IN THE EXTERNAL ZONE (EZ) OF THE MEDIAN EMINENCE (ME) OF THE RAT FOLLOWING SHORT TO LONG TERM ADRENALECTOMY (ADX). M. Yang,* G. Cao, X. Zhao, K. Yang and Z. Lu. West China Univ, of Med. Sci., Chengdu, Sichuan, 610041., P.R. China.

In vivo and in vitro studies have confirmed that cholecystokinin octapeptide (CCK-8), corticotropin releasing factor (CRF), methionine-enkephalin (m-ENK), oxytocin (OT), somatostatin (SOM), substance P (SP), vasopressin (VP), and vasoactive intestinal peptides (VIP) are directly or indirectly involved in the regulation of release of ACTH during stress. The density of these neuropeptides containing nerve fibers, except CRF and VP, has not been completely studied following ADX. Using immunocytochemistry combined with Micro Image Analysis System we examined the density of nine neuropeptidergic nerve fibers in the EZ of the ME of adult male rats at 7, 21, 40, and 70 days following ADX. There was low density of the above neuropeptides containing nerve fibers in the EZ of the ME of the normal rats, except CRF and SOM containing nerve fibers that were of high density. The density of the nerve fibers containing VP increased significantly at 7 days; m-ENK and neurotensin (NT) increased significantly at 70 days after ADX. Nerve fibers containing CCK-8 and SP decreased significantly at 7 days while SOM decreased at 40 days. Nerve fibers containing CRF decreased dramatically at 7 days and returned to normal levels at 21 days after ADX; Nerve fibers containing OT and VIP were not altered. Overall, the response of these neuropeptidergic nerve fibers to ADX varied considerably. Nerve fibers containing CRF, AVP, CCK-8 and SP were altered rapidly and those fibers containing NT, m-ENK and SOM were altered slowly following ADX. The results suggest that in addition to CRF and AVP, other neuropeptides including CCK-8, NT, SOM, m-ENK, and SP are neurotransmitters/ neuromodulators involved in the hypothalamo-pituitary-adrenal axis regulation, and may be involved in regulation of ACTH release during stress.

545.8

NEONATAL QUIPAZINE ADMINISTRATION DECREASES THE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) RESPONSE TO RESTRAINT STRESS IN ADULT RATS. B.M. Tannenbaum[1]*, S. Weaver[2], M.J. Meaney[1]. [1]McGill Univ., Douglas Hosp. Res. Ctr., Montreal, Canada H4H 1R3. [2]Dept Animal Sci., Univ. of Alberta, Edmonton, Canada

Serotonergic input has been postulated to be involved in the upregulation of hippocampal glucocorticoid receptor (GR) levels in rats handled neonatally. Quipazine application to hippocampal cell cultures has been shown to increase GR levels (Mitchell et al., 1990, J. Neurosci. 10:1745). The increased GR levels are associated with a decreased HPA response to restraint in adult animals handled as neonates. In this study we tested whether *in vivo* quipazine application mimics the effects of neonatal handling on the response to restraint stress later in life. Male rat pups were injected either with 15 mg/kg (s.c.) quipazine dimaleate dissolved in sterile saline or saline alone from postnatal day 1-7. At 78 days of age animals were implanted with jugular catheters to obtain measures of plasma adrenocorticotropic hormone (ACTH) before, during and following the termination of a 20 min. period of restraint. Quipazine-treated rats secreted significantly ($p < 0.05$) less ACTH at 20 min. following the termination of restraint and had a trend towards lower ACTH levels at mid-restraint. Overall, integrated ACTH responses were significantly reduced in quipazine-treated animals. The results of this experiment demonstrate that neonatal quipazine administration does effect permanent changes in the response to restraint in adult rats and supports the importance of serotonergic inputs during development on the adult response to stress.

545.9

CHRONIC SOCIAL STRESS ALTERS BOTH HYPOTHALAMIC AND EXTRA-HYPOTHALAMIC VASOPRESSIN mRNA LEVELS IN RAT BRAIN. D.S. Albeck*, D.C. Blanchard, R.J. Blanchard, J. Nikulina, C.R. McKittrick, B.S. McEwen and R.R. Sakai. Rockefeller University, New York, NY 10021, University of Hawaii, Honolulu, HI 96822 and University of Pennsylvania, PA 19104

Dominance hierarchies are quickly established among male rats in a mixed sex colony (5 males, 2 females). The subordinate animals show physiological and behavioral effects of stress in these colonies. The subordinates can be divided into two groups, subordinate responders (SRS) and subordinate non-responders (NRS). This division is based upon the animal's ability or inability to show an increase in plasma corticosterone in response to a novel stressor (1 hr restraint stress). Using *in situ* hybridization we examined relative levels of vasopressin (AVP) mRNA levels in hypothalamic and extra-hypothalamic nuclei.

AVP mRNA levels in magnocellular regions did not vary as a function of behavioral rank. In contrast AVP mRNA expression per cell in the parvocellular region of the paraventricular nucleus was significantly greater in SRS rats than in NRS rats, while the total number of cells expressing AVP mRNA did not differ. In the medial amygdala AVP mRNA expression level per cell did not change, but the NRS rats showed significantly fewer AVP mRNA positive cells than when compared to control and dominant rats.

Paraventricular vasopressin projections to the median eminence act synergistically with corticotropin-releasing hormone as ACTH secretagogues. The current results suggest that in NRS rats this synergy is dysfunctional, possibly affecting the rats ability to show an increase in plasma corticosterone in response to a novel stressor. Behavioral consequences of disrupting vasopressin content of the medial amygdala are currently unclear although NRS rats exhibit behavioral deficits in risk assessment, overall motor activity and sexual behavior. [Supported by: NRSA 1F32 MH 10232-01A1 (DA), NARSAD (RRS), NSF BNS9111524 (DCB) and MH41256 (BMc)].

545.10

EXPOSURE TO STRESS EARLY IN DEVELOPMENT ALTERS HPA AXIS FUNCTIONING OF ADULT CONGENITAL LEARNED HELPLESS (cLH)RATS. J.A. King[1]* and E. Edwards[2]. [1]Department of Psychiatry, Behavioral Sciences Division, University of Massachusetts Medical School, Worcester, MA 01655; [2]Department of Pharmaceutical Sciences, Pharmacology & Toxicology, University of Maryland, Baltimore MD 21201.

This study examined the effects of early stressors (at postnatal day 7 day 14 and day 21) on the HPA axis functioning of cLH adult rats at baseline condition and after exposure to 40 minutes of electric footshock. Corticosterone (CORT) plasma levels were monitored in these rats exposed during development to maternal deprivation (MD) stress and to social stress (CS). Unstressed cLH rats have CORT basal levels that were similar to controls (cNLH rats also not stressed during development). In response to early exposure to both CS and MD, there was a step-wise increase in CORT levels of cLH rats with age of presentation to either CS and MD (day 7, 49% ↑; day 14, 84% ↑; and day 21, 543 % ↑). In response to acute footshock, adult cLH rats displayed a normal stress response if they had been exposed to CS and MD on day 7 and day 14. However, exposure to either stressor at day 21 resulted in a significant desensitization of the HPA axis (unstressed cLH rats after footshock: 40 μg/dl CORT; day 21 CS stressed cLH rats after footshock: 24.7 μg/dl). These data are consistent with our findings of low baseline levels of ACTH in cLH rats stressed at day 21 with a hypersensitive ACTH response to acute stress in adulthood. Our results suggest that exposure to stress during development has long term consequences such as an altered functionality of the HPA axis in adulthood which is specially evident in response to challenge.

545.11

AFFILIATIVE BEHAVIORS AND ENDOCRINE RESPONSES IN PERIADOLESCENT RATS. F. Cirulli*, M. L. Terranova and G. Laviola, Behav. Pathophysiology Sect., Lab. FOS, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161, Rome, Italy.

Adrenal hormones have been shown to modulate social bonding in several mammalian species. In the developing rat, periadolescence [postnatal day (PND) 32-43] is characterized by elevated levels of affiliative and playful social interactions which have been suggested to underlie bond formation and the establishment of socio-sexual roles. Aim of this study was to examine the relationship between hypothalamic-pituitary-adrenal (HPA) axis activity and behavioral responses following separation and reunion of familiar and unfamiliar periadolescent rats. Sprague-Dawley rats have been kept in pairs with a same sex littermate from the time of weaning (PND 21). On PND 33 the members of each pair were separated for a 24-hr period, and randomly assigned to three groups: a) immediately sacrificed (SEP); b) reunited for 30 min with their previous cagemate (FAM); c) reunited for 30 min with an unfamiliar conspecific of the same age and sex (UNF). During the reunion both social and non-social behaviors were scored. Overall, higher levels of rough-and-tumble play were observed in encounters between UNF subjects, and this was more evident in the male group. UNF females were more involved in affiliative behaviors than in cage exploration than FAM. Blood corticosterone (CORT) levels measured at the end of the separation period did not differ from base and were higher in females than in males. Following reunion, all subjects showed increased CORT levels. Degree of familiarity did not affect hormonal changes in males, while UNF females showed a lower CORT elevation than FAM subjects. Overall, no direct relationship was found between behavioral and hormonal parameters in males, while data suggest a negative correlation between affiliative behaviors and HPA axis activation in females.

545.12

EFFECT OF ACTH TREATMENT TO AMINOGLUTETHIMIDE TREATED RATS ON THEIR HPA AXIS RESPONSES TO STRESS. Y.N. Wong, and A.P. D'mello*. Dept of Pharmaceutics, Philadelphia College of Pharmacy and Science, Philadelphia, PA 19104.

The existence of stress induced facilitation has been revealed by the transient removal of corticosterone (B) response during the first stressor and the consequent sensitization of ACTH and B responses to a second stressor. Stress stimulates the secretion of a variety of ACTH and B secretagogues making it difficult to delineate the possible contribution of each factor to stress induced facilitation. The objective of the present study was to examine the specific role of ACTH in stress induced facilitation. Male Sprague-Dawley rats whose B responses to stress were transiently inhibited with aminoglutethimide (AG), were subjected either to an 1 hour physical immobilization stressor (IMM), or administered a single 50 µg/kg s.c. dose of ACTH. Twenty four hours later both groups of rats were subjected to the mild stress of a saline injection (INJ). Plasma ACTH and B were measured 15 and 30 minutes after the injection. As previously demonstrated, AG+IMM pre-treatment on day 1 caused hypersecretion of both B and ACTH to the INJ on day 2. However, the AG+ACTH pre-treatment on day 1 caused the hypersecretion of only B on day 2. The magnitude of B hypersecretion caused by both pre-treatments were similar. These results suggest that ACTH secreted during the first stressor might be responsible for stress induced facilitation of B responses to a subsequent stressor.

545.13

ESTROGEN ALTERS NEUROENDOCRINE RESPONSES TO PSYCHOLOGICAL STRESS IN THE RAT. E.W. Bingaman*, L.D. Van de Kar, J.M. Yracheta, Q. Li, T.S. Gray. Dept. of Cell Biol., Neurobiol., and Anatomy and Dept. of Pharmacol. and Exp. Ther., Loyola Univ. of Chicago, Maywood, IL 60153.

Circulating androgens differentially influence various neuroendocrine and behavioral responses to conditioned stress. This study tested whether estrogen modulates several important parameters of the conditioned stress response. Male and female Sprague/Dawley rats were used in this study. Female rats were ovariectomized using halothane anesthesia. These animals received either estrogen replacement (OVX+E) or a sham incision (OVX). Two weeks later, animals were subjected to a conditioned stress paradigm. On three consecutive days, experimental rats were placed in a chamber for 10 minutes and then received a 10 second footshock. On the fourth day, rats were placed in the chamber but did not receive a shock. Control rats were treated identically but were never shocked. The corticosterone ($p<.05$) and prolactin ($p<.01$) responses to conditioned stress were significantly potentiated in OVX+E rats compared to OVX female and intact male rats. However, The ACTH response to conditioned stress was significantly ($p<.01$) higher in OVX rats compared to intact males and OVX+E females. The stress-induced increase in plasma renin concentration was potentiated ($p<.05$) in OVX+E rats compared to intact males while the stress-induced increase in plasma renin activity was lower ($p<.05$) in OVX rats compared to OVX + E females. This suggests differences in both renin and renin substrate. Behavioral responses to conditioned stress did not differ among males, OVX females, and OVX+E females. These data indicate that estrogen mediates several, but not all, aspects of the response to psychological stress. Supported by NIH NS20041.

545.14

MECHANISMS OF ACUTE STRESSOR-INDUCED CHANGES IN CORTICOSTEROID BINDING GLOBULIN (CBG). T. Deak*, M. Fleshner, J. L. Meriwether, L. Goehler, L. R. Watkins, R. L. Spencer & S. F. Maier, Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

CBG is a protein responsible for the serum transport of corticosterone (CORT). Past research has demonstrated that exposure to chronic stressors or chronic high levels of glucocorticoids (GC's) causes a substantial decrease in serum levels of CBG. We have recently demonstrated that this effect can also be observed in rats exposed to a single session of inescapable shock (IS; see Fleshner et al., companion abstract). We now report that the mechanism of CBG downregulation following IS is independent of GC regulation. In all experiments, a baseline blood sample was taken from the tail vein (400 µl in <2 min), rats were exposed to 100 tail shocks (5 sec, 1.6 mA, 1 min ITI), & a second blood sample was taken 24 hours post shock. Both serum CORT & CBG were measured in each experiment (by RIA & single-point binding assay, respectively). Neither bilateral adrenalectomy (with chronic basal CORT replacement) nor i.p. RU 486 (10 mg/kg), a type II GC receptor antagonist, had any effect upon IS-induced decreases in CBG. Likewise, an injection of CORT (2.5 mg/kg i.p.) which produces stress levels of CORT was not sufficient to reduce CBG. In addition, we investigated whether autonomic innervation of the liver, the primary source of CBG, is necessary for the IS-induced decrease in CBG. Disruption of neither parasympathetic nor sympathetic innervation of the liver was able to block the IS-induced decrease in CBG. Alternative mechanisms of CBG regulation will be discussed, as well as the implications for circulating levels of corticosteroids. Supported by NIH Grant MH45045 & Undergraduate Research Opportunities Program.

545.15

A LONG-TERM INCREASE IN BASAL LEVELS OF CORTICOSTERONE AND A DECREASE IN CORTICOTROPIN-BINDING GLOBULIN FOLLOWING ACUTE STRESSOR EXPOSURE. **M. Fleshner*, T. Deak, R. L. Spencer, M. L. Laudenslager, L. R. Watkins, & S. F. Maier, Dept. Psych, Univ. CO, Boulder, CO 80309. [1]Dept. Psychiatry, U. CO. Health Sci. Cntr., Denver, CO.**

Adrenal glucocorticoids play an important role in mediating many of the behavioral and physiological effects of exposure to stressors. Focus has been primarily on the acute stress-induced rise in glucocorticoids (corticosterone in the rat). There are reports, however, that exposure to chronic stressors can produce an increase in basal corticosterone and a decrease in corticotropin-binding globulin (CBG). These changes occur subsequent to the stress-induced rise in corticosterone. The following experiments examined whether exposure to an acute stessor (100 5-s inescapable tail shocks; IS) could also produce long-term changes in basal corticosterone (RIA) and CBG (competitive binding assay). We report that rats (n = 6/grp) exposed to a single session of IS show increases in basal total serum corticosterone which persists 48-96 hours after IS termination. The increase is present only at the diurnal trough (AM). CBG levels are also decreased for 24-48 hours. The decrease is present at both the diurnal peak (PM) as well as trough (AM). These changes also result in an increase in the percent and amount of unbound or free corticosterone (biologically active). Thus glucocorticoid sensitive targets are exposed to high levels of free corticosterone for several days after IS termination. The long-term increase in free corticosterone reported here may play an important role in mediating some the effects produced by IS, as well as those produced by other acute stressors. NIH-MH45045.

545.16

EFFECT OF LOCUS COERULEUS LESIONS ON THE ACTIVITY ON THE HYPOTHALAMUS-PITUITARY-ADRENAL (HPA) AXIS. D.R. Ziegler* and J.P. Herman, Dept. of Anatomy and Neurobiology, University of Kentucky Medical Center, Lexington, KY 40536-0084.

Recent studies have shown that activation of the HPA stress axis stimulates activity in the locus coeruleus (LC), which, in turn, leads to noradrenergic excitation of the forebrain and increased arousal. We have investigated whether this relationship is reciprocal, that is, does the LC influence the HPA axis response to stress? Naive, male Sprague-Dawley rats were microinjected in the locus coeruleus with either 6-hydroxydopamine (with ascorbic acid in saline) or vehicle. After a 7-10 day recovery period, the lesion and sham groups were further partitioned into stressed and control (handling) groups with stressed subjects undergoing a chronic variable stressor regimen applied twice a day for 2 weeks. The morning after the last day of stress/handling, all subjects were sacrificed and thymus, adrenal glands, trunk blood, and brains were collected. Data on body weight gain, adrenal hypertrophy, and thymic involution all showed a trend toward a significant difference between stressed and control subjects; however, this response was less robust than has been observed in other experiments using a 4 stress week regimen. Plasma ACTH and CORT as well as the tissue data failed to show a significant interaction between stress and lesion factors. However, it appears that LC lesions, regardless of stress or handling, elevated plasma CORT and lead to adrenal hypertrophy. Single-trial open-field behavior (immobility, ambulation, rearing, defecation) was not substantially influenced by either stress or lesion. Forthcoming data will reveal whether effects of LC lesion are exerted on central components of HPA regulation (corticotropin releasing hormone, glucocorticoid receptor and mineralocorticoid receptor protein and mRNA). Supported by MH49698.

545.17

CONNECTIONS BETWEEN THE AMYGDALOID CENTRAL NUCLEUS AND THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS: A COMBINED RETROGRADE-ANTEROGRADE TRACING STUDY. C.M. Prewitt* and J.P. Herman. Dept. of Anatomy and Neurobiology, Univ. of Kentucky Medical Center, Lexington, KY 40536-0084.

The central nucleus of the amygdala (ACE) has been shown to affect regulation of the HPA stress axis. Bilateral lesion of ACE decreases basal CRH mRNA in paraventricular nucleus (PVN) and stress-induced release of ACTH. However, there is scant evidence to support direct projections from the ACE to the PVN region. The present studies were designed to assess potential bisynaptic forebrain circuits relaying information from the ACE to the PVN. Rats were injected with 2% fluorogold (FG) (retrograde tracer) in the PVN and with 2% PHA-L (anterograde tracer) into the ACE. Tracer distribution was revealed by immunohistochemistry. PHA-L injections were mostly confined within the lateral part of ACE; the major forebrain projection fields were primarily relegated to the juxtacapsular, ventrolateral and posterolateral subnuclei of the bed nucleus of the stria terminalis (BNST), with some labeling seen in the lateral and dorsomedial hypothalamus. No anterograde labeling was seen in the PVN. Retrograde labeling from PVN injections labeled numerous hypothalamic and preoptic nuclei and the ventromedial and posterior intermediate subdivisions of the BNST. FG labeling in the amygdala was confined to the medial division; no retrogradely labeled cells were seen in the ACE. Appositions of PHA-L fibers to FG-labeled neurons were very rare, generally being localized in the ventral region of the posterolateral BNST. No appositions were seen in hypothalamic nuclei. These data suggest that despite apparent action of the ACE on the HPA axis, connections between the ACE and PVN are unlikely to form bisynaptic connections through the BNST or hypothalamus. Supported by MH49698.

545.18

HPA AXIS AND ANXIETY IN ADOLESCENT PREGNANCY. A. Chicz-DeMet*, B. Simon, T.D. Wu, H.C. Cunanan, H.A. Cornett, D. Lee, A.F. Johnson, T.D. Thai, M.S. Scott, and T. Fisher. Dept. Psychiatry & Human Behavior, UC Irvine, CA 92717.

Anxiety and stress often disturb the hypothalamic-pituitary-adrenal (HPA) axis and may contribute to poor birth outcome. Fifty-five expectant adolescents were administered the Symptom Checklist 90 Revised questionnaire during the third trimester of pregnancy to assess anxiety and stress. As expected, significant correlations were found between plasma β-endorphin and ACTH ($r=0.568$, $p<0.001$), and ACTH and cortisol ($r=0.423$, $p<0.001$) in expectant mothers. Prepartum anxiety scores were correlated with maternal distress indices (Global Severity Index, $r=0.905$, $p<0.001$); Positive Symptom Total, $r=0.812$, $p<0.001$), maternal infection during pregnancy ($r=0.401$, $p=0.002$), and fetal heart rate during labor ($r=0.378$, $p=0.004$). Anxiety ratings and distress indices, however, were not associated with stress hormones. Prepartum anxiety for mothers with preterm deliveries ($N=5$) was 70% lower than for mothers with term births. Six month postpartum decreases in anxiety scores ($N=38$) were not significant and may be attributed to the appreciably low prepartum anxiety scores. The results support a putative role of anxiety as a risk factor in pregnancy, and at labor and delivery, but fail to confirm a relationship between anxiety and stress hormone disregulation in this small sample.

545.19

REVERSAL OF NEUROENDOCRINE EFFECTS OF CHRONIC STRESS BY THE TRANSCENDENTAL MEDITATION TECHNIQUE D.K. Levitsky, K.G. Walton*, C.R.K. MacLean, N.D. C. Pugh, S.W. Wenneberg, R.H. Schneider, J.V. Mandarino, S. Hillis[2], R. Waziri[1]. Departments of Physiology and Chemistry, Maharishi International University, Fairfield, IA 52557 and Departments of [1]Psychiatry and [2]Statistics and Actuarial Science, University of Iowa, Iowa City, IA 52242

A previous cross-sectional study comparing long-term practitioners of Transcendental Meditation (TM) to matched controls showed highly significant differences in urinary variables reflecting neuroendocrine function. In particular, TM practitioners had decreased excretion of urinary free cortisol, aldosterone, vanillylmandelic acid, zinc, calcium and sodium, and increased excretion of dehydroepiandrosterone and nighttime 5-hydroxyindoleacetic acid (5-HIAA). In addition, TM practitioners had reduced scores on tests of anxiety and mood disturbance. These and other results suggested that the differences were due to reversal of the effects of stress by the TM program. The current prospective, random-assignment study attempted to verify this hypothesis. Healthy, Caucasian males (18-32 y) were randomly assigned to either TM or a stress education control (SEC). Before and after four months' practice of the assigned stress management program, three contiguous 8-hour urine collections were taken and psychological self-report tests sensitive to level of psychosocial stress were administered. Urine samples were analyzed for 5-HIAA by spectrophotometry, for adrenocortical steroids by RIA and for Na, K, Ca, Mg and Zn by atomic absorption spectrometry. Fourteen out of 15 key variables changed in the direction predicted for the reversal of effects of chronic stress in the TM group, whereas 7 out of the 15 changed in a direction opposite to that predicted for reversal of the effects of chronic stress in the SEC group. If changes produced by TM are cumulative over time as these and other results suggest, then the present results provide strong confirmation that TM reverses the neuroendocrine effects of chronic stress related to function of the HPA axis. *Supported by NIH Research Grant #1 R15HL 40495 01A1, and NIH Grant #RR59, Clinical Research Centers Branch.*

545.20

SPONTANEOUS VARIABILITY of the HUMAN WAKING EEG and ACTH, CORTISOL and GLUCOSE BLOOD CONCENTRATIONS W.G. Sannita*, D. Giglioli, L. Lopez, S. Massimilla, G. Murialdo. DISM-Neurophysiopathology and DISEM, University, Genoa, Italy; Center for Cerebral Neurophysiology, CNR, Genoa, Italy; Dept. of Psychiatry, SUNY, Stony Brook, NY, USA.

Pathological unbalancements and the exogenous administration of ACTH or glucocorticoids induce neuropsychological and EEG modifications congruent with the effects of substitutive hormone treatment or ACTH-fragments and with the evidence of steroid action on neurons. The spontaneous fluctuations of the quantitative (power spectral analysis) waking resting EEG and plasma ACTH, cortisol and glucose concentrations were studied in 8 male volunteers (24.5± 1.7 yr.) EEG recordings and blood samples (6 per subject) were obtained at pre-defined intervals during a 6-h experimental session. Glucose, cortisol and ACTH concentrations (hexokinase-G6P-DH essay or RIA) were within normal ranges, respectively 3.37-5.6 mmol/L, 7.8-47.2 nmol/L and 3.1-17.6 pmol/L, without pulsatile changes or correlation among variables. Systematic trend over time were not observed, with the exception of a consistent decrease of plasma cortisol. Changes in the neuropsychological status (including the visuo-spatial function) were not detected. The signal power in the 6.5-14.0 Hz frequency interval (corresponding to the "alpha" rhythm) of the EEG changed in phase with, and was correlated to the fluctuations of ACTH in the absence of cortisol/glucose EEG effects. Two subgroups of subjects with lower or higher EEG power values and ACTH levels respectively were identified, with cut-off between subgroups at approximately 8-10 pmol/L, therefore suggesting some influence of the neuro-hormonal status on the between-subject EEG variability. The correlation of ACTH and EEG appeared independent of glucose availability and not mediated through cortisol action; a role of common factors active on, and pacing the spontaneous and physiological variations in both ACTH and EEG (such as the corticotropin releasing factor) is conceivable.

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION: CRF

546.1

REGULATION OF CORTICOTROPIN-RELEASING HORMONE (CRH) RECEPTOR mRNA IN THE RAT BRAIN AND PITUITARY BY GLUCOCORTICOIDS AND STRESS. **Shinya Makino[1]*, Jay Schulkin[1], Mark A. Smith[2], Karel Pacák[3], Miklós Palkovits[4], and Philip W. Gold[1]. Clinical Neuroendocrinology Branch[1], Biological Psychiatry Branch[2] and Laboratory of Cell Biology[4], NIMH, and Clinical Neuroscience Branch[3], NINDS, Bethesda, MD 20892.**

We used in situ hybridization to measure CRH receptor (CRH-R) mRNA levels in the paraventricular nucleus (PVN), anterior pituitary (AP), amygdala and bed nucleus of the stria terminalis (BNST) under several conditions. Systemic corticosterone (CORT) treatment decreased CRH-R mRNA in the PVN, AP, and lateral and basolateral nucleus of the amygdala (BLA). Acute (2 hrs) and repeated (2 hrs daily, 14 days) immobilization stress increased CRH-R mRNA in the PVN and decreased it in the AP, but did not affect CRH-R mRNA in the BLA. Adrenalectomized rats with CORT pellet replacement had higher levels of CRH-R mRNA both in the PVN and AP than sham rats following stress. Brainstem hemisection which damaged ascending catecholaminergic fibers attenuated immobilization stress-induced upregulation of CRH-R mRNA ipsilaterally in the PVN. None of the treatments affected CRH-R mRNA levels in the central and medial nucleus of the amygdala or the BNST. These results suggest that high concentrations of CORT inhibit CRH-R mRNA transcription in the PVN and AP. However, stress input can override such inhibitory effects and thus upregulate CRH-R mRNA in the PVN. The extrahypothalamic regions such as amygdala and BNST may have different sensitivities to CORT or CRH for the regulation of CRH-R mRNA.

546.2

MEASUREMENT OF RAT CRH mRNA EXPRESSION DURING HEMORRHAGE USING QUANTITATIVE PCR AND CAPILLARY ELECTROPHORESIS. W.J. Gdula, D.N. Darlington, J.M. Butler, B.R. McCord and D.S. Gann*. Dept. of Surgery, Univ. of Maryland Sch. of Med., Baltimore, MD 21201 and FBI Laboratory, Forensic Science Research Unit, FBI Academy, Quantico, VA 22135.

We have developed a method to measure changes in mRNA in specific brain nuclei. This method converts extracted mRNA to cDNA by reverse-transcription and amplifies cDNA by polymerase chain reaction (PCR). To control for PCR efficiency between samples, known amounts of competitor cDNA (constructed to have the same primer hybridization sites, but of a different size) are co-amplified with the wild-type cDNA. To test this method, eight Sprague Dawley rats (300g) received a 20ml/kg hemorrhage or sham hemorrhage and the paraventricular nuclei were punched (1.5mm diameter x 2mm) at 2h. Total RNA was extracted, converted to cDNA, spiked with competitive template and amplified for 28 cycles. The products were separated by capillary electrophoresis and measured using laser induced fluorescence of intercalating dye. We found that hemorrhage elevated CRH mRNA (3.51pg/4punches vs. 3.01pg/4punches; sham). This is the first application of quantitative PCR for measuring mRNA in brain areas after hemorrhage and confirms previous findings from our laboratory. This study was supported by GM46540 and GM08414.

546.3

CHANGES IN CRH AND AVP mRNA AFTER ADRENALECTOMY, WATER DEPRIVATION AND HEMORRHAGE IN THE RAT. E. J. Weeks, D. N. Darlington, M. Tehrani and D. S. Gann. Depts. of Surg. and Physiol., U. of MD Sch. of Med., Baltimore, MD 21201.

To determine if CRH and AVP mRNA change after adrenalectomy (ADX), water deprivation (DEP) or hemorrhage (HEM), the paraventricular (PVN) and supraoptic (SON) nuclei of 48 S-D rats were punched and snap-frozen after 3days ADX, 3days DEP or 20ml/kg HEM. Total RNA was extracted, reverse transcribed to cDNA and the cDNA for CRH, AVP and the constitutively expressed glyceraldehyde phosphate dehydrogenase (GAPDH) were amplified by PCR. PCR products were measured by UV detection after HPLC and analyzed as areas under the curve. To semi-quantitatively estimate mRNA changes and to control for PCR efficiency, CRH and AVP products were expressed as ratios to the externally amplified GAPDH products. ADX led to a rise in PVN CRH mRNA (0.464±0.079 vs sham 0.178±0.083). DEP led to a rise in AVP mRNA in the SON (1.12±0.317 vs. Cont. 0.573±0.114) but not PVN (0.145±0.085 vs. Cont. 0.250±0.183). HEM led to a rise in CRH mRNA that peaked by 4hrs and returned to normal by 8, 12 and 24hrs. AVP mRNA did not change after HEM in PVN or SON. Utilizing a highly sensitive method of detection, HEM, which increases both plasma ACTH and AVP, increases mRNA for CRH but not AVP. Supported by GM46540 and GM08414.

546.4

FETAL ETHANOL EFFECTS ON HYPOTHALAMIC CRF mRNA LEVELS FOLLOWING STRESS IN DEXAMETHASONE (DEX) TREATED ANIMALS. J.A. Osborn, C.K. Kim, W.K. YU, L. Herbert, R.T. Zoeller and J. Weinberg*. Department of Anatomy, Univ. of British Columbia, Vancouver, V6T 1Z3 Canada.

Rodents prenatally exposed to ethanol (E) demonstrate hypothamic-pituitary-adrenal (HPA) hyperresponsiveness to stressors compared to controls. We have shown that E animals are less sensitive than controls to DEX suppression of the HPA stress response, with differential effects in males and females which are dependent on circadian rhythm. The present study investigated the possibility that hypothalamic corticotropin releasing factor (CRF) and/or vasopressin (AVP) mRNA levels may be differentially altered following stress in E males and females.

Sprague-Dawley male and female offspring from (E), pair-fed (PF), and ad lib-fed control (C) groups were tested at 80-140 days of age. Three hr post saline or DEX injection, animals were exposed to 1 min ether stress and decapitated 1 hr later. Plasma corticosterone (CORT) levels were determined. In situ hybridization was performed using ^{35}S-labeled cDNA oligonucleotide probes for AVP and CRF in the paraventricular nucleus of the hypothalmus.

DEX injected males had significantly lower plasma CORT levels and lower CRF mRNA levels than saline injected males; there were no differences among E, PF and C males. In contrast, CRF mRNA levels were significantly higher in E than PF females and marginally higher in E than C females. DEX injected females had significantly lower plasma CORT levels than saline injected females; there were no differences among E, PF and C females. There were no differneces in AVP mRNA levels among E, PF and C males or females. These data extend our previous work demonstrating as sex difference in sensitivity to DEX suppression in E animals and suggest that altered CRF biosynthesis in E animals may play a role in mediating HPA hyperresponsiveness. Supported by AA07789 NIAAA (J.W.), AA08887 NIAAA (R.T.Z.) and a Studentship, MRC of Canada (J.A.O.).

546.5

SPECIFIC IMMUNOTARGETTED LESIONS OF CRF OR AVP NEURONS OF THE PVN AFFECT CORTICOTROPE FUNCTION BUT NOT LH SECRETION IN LACTATING RATS. A. Burlet*, C-D. Walker, P. Tankosic, F.J.Tilders, D.Lavallée. INSERM U308, University of Nancy, France and McGill University, Douglas Hospital Research Center, Montreal, Canada.

Lactation in the rat is associated with elevated corticosterone (B) release, blunted adrenocortical responses to stress and inhibition of LH secretion. Since tonic activation of the adrenocortical axis might mediate LH suppression, we tested the effect of specific lesions of CRF or AVP neurons of the hypothalamic paraventricular nucleus (PVN) on 1) the inhibitory action of suckling on LH release and 2) the ACTH response to hypotensive stress during lactation. Ovariectomized females were injected over the PVN on day (d) 2 of lactation with ricin A (TX) associated to either a monoclonal anti-CRF (CRF-TX), anti-AVP (AVP-TX) antibody or to non-specific IgGs (IgG-TX). On d7, the ability of suckling to decrease LH release was measured after 24h of pup separation. On d10 and d12, ACTH and B responses to nitroprusside infusion were measured with (d10) or without (d12) pups. Hypothalamic CRF and AVP peptide and mRNA content were determined by immunocytochemistry and *in situ* hybridization, respectively. Effective PVN lesions were confirmed by a specific decrease in both peptide and mRNA levels in CRF-TX and AVP-TX groups. In AVP-TX group, CRF mRNA levels were decreased in addition to AVP mRNA, suggesting an enhanced sensitivity of a CRF/AVP-containing cell population during lactation. Suckling-induced LH decrease was not affected by either CRF or AVP lesions. However, restoration of normal ACTH responses to stress following pup separation was prevented in CRF-TX group compared to AVP-TX or IgG-TX groups, suggesting a preferential role for CRF neurons in the "reactivation" of the adrenocortical axis after lactation. The ability of PVN CRF and AVP neurons to functionally compensate for specific lesions during lactation remains to be established.(Funded by MRC Canada-CNRS).

546.6

DIFFERENTIAL EXPRESSION OF *FOS/JUN* FAMILY IN THE PARAVENTRICULAR NUCLEUS (PVN) INDUCED BY STRESS: ITS RELATIONSHIP TO CORTICOTROPIN-RELEASING FACTOR (CRF) GENE TRANSCRIPTION. T. Imaki, T. Shibasaki, N. Chikada, S. Harada, M. Naruse, H. Demura and J. Imaki*. Dept. of Medicine, Tokyo Women's Medical College, and Dept. of Anatomy, Nippon Medical School, Tokyo, 162, Japan

Stressful stimuli evokes the transcriptional activation of CRF gene and the *fos/jun* family. To determine a possible relationship between *fos/jun* expression and CRF gene transcription by stress, we have analyzed the expression of *fos/jun* family mRNA, and CRF heteronucleous RNA (hnRNA), which reflects gene transcription, after stress using *in situ* hybridization. Male Wistar rats were perfused transcardially 0, 5, 10, 15, 30, 60, and 120 min after beginning of 30 min-restraint. Brain sections were hybridized with ^{35}S-labeled cRNA probes. Restraint stress induced rapid (within 5 min) and transient increases in *c-fos* mRNA and CRF hnRNA expressions in the PVN, with peak expression occurring at 30 min. The induction of *jun-B* was seen later than that of *c-fos* mRNA and CRF hnRNA. By contrast, *jun-D and c-jun*, which were constitutively expressed in the PVN, increased slightly following stress. These results suggest that differential expression of *fos/jun* gene occur in the PVN neuron following stress. None of the *fos/jun* gene studied was induced before CRF hnRNA expression, suggesting that *fos/jun* gene may not directly activate CRF gene transcription in the PVN.

546.7

CORTICOTROPIN RELEASING HORMONE (CRH) REGULATION OF ANTERIOR PITUITARY (AP) PROOPIOMELANOCORTIN (POMC) MRNA LEVELS IN FETAL SHEEP IS GESTATIONAL AGE DEPENDENT. D. A. Myers*. Dept. Physiol., Coll. Med. Univ. of Oklahoma, Health Sci. Center, Oklahoma City, OK 73190.

AVP and CRH regulate AP ACTH secretion in fetal and adult sheep. AVP and CRH regulation of AP POMC expression in fetal sheep has not been examined with respect to development. Neither CRH nor AVP increases POMC mRNA levels in AP from adult (Endo 132:1692) or near term fetal sheep (J.Endo.143:199). During late gestation in fetal sheep, paraventricular nuclei/ adrenocortical-dependent morpholgic maturation of AP corticotropes occurs. The purpose of this study was to examine AVP and CRH modulation of POMC mRNA levels at two fetal ages: 120-122 days of gestation (dG), before the onset of fetal adrenocortical/corticotrope (A/C) maturation (n=10 fetuses), and 142-144 dG, after A/C maturation (n=4 fetuses; 147 dG=term). Fetal AP were enzymatically dispersed. After 48 hours of culture, cells were challenged (18h) with CRH, AVP or both (10^{-6} to 10^{-12}M). POMC mRNA levels were also determined at dispersion, and 24 h post-dispersion for each group. At 120-122dG, CRH but not AVP increased POMC mRNA; AVP had no effect on CRH induced increase in POMC mRNA. At 142-144 dG, neither CRH nor AVP affected POMC mRNA levels, similar to reports for adult or late gestation sheep. At 120-122 dG, there was a small, but non-significant increase in POMC mRNA levels during the initial 24 h post-dispersion. At 142-144dG, POMC mRNA levels increased significantly during the initial 24 h post-dispersion. The results indicate that CRH regulation of ovine POMC gene expression may be dependent on 1) development, and 2) the level of adrenocortical activity. Supported by NIH R29 HD31127-01A1.

546.8

DEVELOPMENT OF AN IMPROVED LIGAND FOR CRF RECEPTOR CHARACTERIZATION USING A RADIOLABELLED CRF ANTAGONIST M.H. Perrin, S. Sutton, J. Gulyas, D. Lovejoy* , J.E. Rivier, and W.W. Vale. The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037

In many receptor systems, labelled antagonists have advantages over agonists for the characterization of receptor structure and function. Astressin is a CRF receptor antagonist with greater potency than any previous peptide antagonist (Rivier et.al., 1995 Ann. Mtg Soc. Neurosci). The CRF receptors belong to the super-family of G-protein coupled receptors. In this super-family, the binding of agonists is usually to two sites (high and low affinity), whereas that of antagonists is mainly to one site. In our previous CRF receptor studies we have used the radiolabelled CRF agonist, ^{125}I-(Nle^{21}, Tyr^{32})-ovine CRF, for characterization of the natural and cloned (CRF-RA and CRF-RB) receptors. We describe here the binding of a potent CRF antagonist, Tyr^{12}-astressin, for use as a tracer for characterizing CRF receptors. Iodinated Tyr^{12}-astressin binds to cloned CRF-RA stably expressed in CHO cells with a Kd=1.8nM; there are approximately 4x as many receptors when measured using the labelled antagonist as measured by the labelled agonist. The radiolabelled antagonist is equally well bound at 4, 18, and 37 C, reaches steady state at 20 C within 60 min and is stable for 120 min. Micromolar concentrations of guanyl nucleotides displace the labelled agonist bound to the cloned receptor but fail to displace the labelled antagonist. Both agonists and antagonists competitively displace the labelled antagonist in a dose dependent fashion in good agreement with their biological potencies. Astressin also binds with nanomolar affinity to the second cloned receptor, CRF-RB. This antagonist should prove of use for the characterization of CRF receptors and their ligands.

546.9

STIMULATORY INFLUENCE OF EtOH ON ACTH SECRETION: ROLE OF VASOPRESSIN AND CORTICOTROPIN-RELEASING FACTOR.
Catherine Rivier* and Soon Lee. The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037.

The mechanisms through which EtOH stimulates ACTH secretion remain elusive. Though the primary role of the hypothalamic peptide corticotropin-releasing factor (CRF) is recognized, we do not know the level(s) at which EtOH alters CRF-dependent pathways. In addition the participation of vasopressin (VP), a peptide also released by EtOH and that regulates ACTH release, is unclear. Using an intronic probe, we measured significant increases in CRF heteronuclear RNA levels 20-40 min after injection of EtOH (3 g/kg, ip). At these times, gene expression of *c-fos* and NGFI-B was already markedly increased. CRF, CRF receptors, and VP mRNA levels were then measured 3 h after EtOH administration. Possibly because of the sizable preexisting pool of CRF mRNA in the cell body, only modest and not statistically significant increases were observed in EtOH-treated rats. On the other hand, gene expression of CRF receptors and VP was markedly elevated. To determine if the EtOH-induced stimulation of VP neurons was important in the subsequent activation of the HPA axis, we injected VP antibodies 5 min prior to EtOH administration. At doses of the antiserum that completely abolishes ACTH released by large doses of exogenous VP, the ACTH response to the acute injection of EtOH (1-3 g/kg) was significantly blunted by removing endogenous VP.

In conclusion, we have shown that acute EtOH administration up-regulates VP gene expression and CRF gene transcription in the hypothalamus, and that VP released into the portal and/or general circulation is important for the activation of the corticotrophs. These results indicate that EtOH exerts a rapid stimulatory influence on the activity of hypothalamic neurons that are important for ACTH secretion. Because hypothalamic CRF stimulates the activity of its own neurons, the appearance of CRF receptors in the hypothalamus suggests the potential presence of amplifying mechanisms through which CRF, and possibly VP, may prolong the effect of alcohol on the brain.

546.10

POSITIVE GLUCOCORTICOID REGULATION OF CORTICOTROPIN-RELEASING HORMONE GENE EXPRESSION.
A.F. Seasholtz*, R. Regentin, and H. Guardiola-Diaz, Mental Health Research Institute and Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109.

Corticotropin-releasing hormone (CRH) is the major hypothalamic releasing factor in the mammalian stress response. CRH is also expressed in other regions of the brain and in a variety of peripheral tissues where its role is less well understood. The endogenous CRH gene has been shown to be negatively regulated by glucocorticoids in the hypothalamus while it is positively regulated by glucocorticoids in human placental trophoblasts. In order to more systematically evaluate the role and mode of action of glucocorticoids on CRH gene expression, we have utilized CRH gene transfer experiments in cell lines. In transfected PC-12 cells, a human CRH-reporter fusion gene is positively regulated by glucocorticoids and this positive glucocorticoid regulation acts synergistically with the cAMP-mediated increase in CRH-reporter activity. The positive glucocorticoid regulation is half maximal at 5-10 nM dexamethasone and requires treatment for at least 24 hours. Studies with RU-38486 demonstrate that the glucocorticoid receptor (GR) is required. However, deletion analysis has shown that several putative GR DNA binding sites in the CRH 5' flanking DNA are not required for the positive regulation. These results suggest that positive glucocorticoid regulation of CRH-reporter expression in PC-12 cells is not mediated by DNA binding, but may involve induction of a new trans-acting protein or protein-protein interactions involving the activated GR. This work was supported by grants to A.F.S. from NARSAD and NIH DK42730.

546.11

TREADMILL-RUNNING EXERCISE ACTIVATES BRAIN TRANSCRIPTION OF CRF AND ITS TYPE 1 RECEPTOR IN THE RAT.
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The present study was aimed at investigating the effects of treadmill-running exercise on the transcriptional activity of corticotropin-releasing factor (CRF) and its type 1 receptor (CRF_1 R) in the brain of male rats killed during and following a 90-minute session of exercise. Transcriptions of CRF heteronuclear (hn)RNA and CRF_1 R mRNA were assessed on brain sections using intronic and exonic *in situ* hybridization histochemistry. Exercise led to a rapid and transient expression of the CRF gene; 30 minutes after the onset of exercise, a positive signal for CRF hnRNA was detectable in both the parvocellular division (parvo) of the hypothalamic paraventricular nucleus (PVH) and in the inferior olivary nucleus (ION). These positive signals, which were not perceivable in resting control rats, vanished soon after the completion of exercise. Exercise also induced the transcription of the gene encoding for the CRF_1 R. The transcription was observed 90 and 180 minutes after the end of exercise in the parvo PVH. There was no basal expression of CRF_1 R mRNA during exercise or in resting control rats. The presence of CRF hnRNA in the parvo PVH and ION of exercised rats suggests that exercise can activate expression of the CRF gene in neurons involved in the control of both hypothalamic-pituitary-adrenal axis and motricity. Meanwhile, the post-exercise induction of CRF_1 R mRNA in the parvo PVH provides evidence that exercise may influence the CRF neuroendocrine function by acting at the CRF site of action.

546.12

ESTROGEN POTENTIATES THE STRESS-INDUCED INCREASE OF CRH mRNA IN THE PVN.
R.Y. Yukhananov* and R.J. Handa. Department of Cell Biology, Neurobiology and Anatomy, Loyola University Medical Center, Maywood, IL 60153

Gonadal steroids, in addition to the their role in the control of reproduction appear to be important regulators of the neuroendocrine stress responses. It has been shown recently that estrogen (E) may potentiate the stress-induced secretion of ACTH and corticosterone in rats and this effect is mediated at the hypothalamic level. To further clarify the mechanism of E action we have determined by *in situ hybridization* the levels of CRH mRNA in the parvocellular part of the paraventricular nucleus (PVN) following two types of stressor: injection of hyperosmotic sodium chloride (1.5 M NaCl, 10 ml/kg) and novelty (open field 15 min). Gonadectomized (GDX) male rats were implanted with silastic capsules containing E, dihydrotestosterone propionate (DHTP) or empty (placebo). CRH mRNA levels were determined by counting the number of silver grains over individual cells on slides coated with photographic emulsion. Neither E, nor DHTP pretreatment produce any changes in the levels of CRH mRNA of the home cage control. Injection of 1.5 M NaCl significantly increased the CRH mRNA levels in sham-operated, placebo- or E-treated but not DHTP-treated rats. Following novelty, however, only E-treated rats expressed significantly higher levels of CRH mRNA as compared to non-stressed control. Thus, estrogen potentiates the increase of the CRH mRNA following psychological stressor (novelty), but not following injection of hyperosmotic sodium chloride. These data suggest that estrogen potentiates neuroendocrine stress responses by influencing CRH in a stress specific fashion. The regulatory pathways mediating these effects remains to be determined. Supported by BNS 9408890 and USPHS AA08696.

546.13

CASTRATION INCREASES CORTICOTROPIN RELEASING HORMONE (CRH) mRNA BUT NOT GLUCOCORTICOID RECEPTOR (GR) mRNA IN THE PARAVENTRICULAR N. OF THE HYPOTHALAMUS.
R.J. Handa*, G.J. Heina, A.H. Nagahara, J.E. Kerr. Dept. of Cell Biology, Neurobiology and Anatomy. Loyola Univ., Chicago, Stritch School of Medicine. Maywood, IL 60153.

Previous studies have demonstrated that gonadectomy (GDX) of male rats will increase the ACTH and corticosterone response to stress (Handa et al, 1994). These changes are accompanied by increases in hypothalamic CRH levels (Almeida et al, 1992; Bingaman et al, 1994). In this study, we tested the hypothesis that GDX-induced changes in hormone secretion are associated with changes in CRH mRNA or GR mRNA levels in the PVN. Male Sprague Dawley rats (3 months old) were used. Animals were GDX'd or GDX'd and implanted with two Silastic capsules containing dihydrotestosterone propionate (DHTP; 2 cm long) at the time of GDX. Control rats were left intact. Rats were sacrificed 3 weeks after GDX, and brains were removed and analyzed for CRH mRNA and GR mRNA levels. Some animals in each group were adrenalectomized (ADX'd) 4 days prior to sacrifice or ADX'd and injected daily with the specific GR agonist, RU28362 (40 ug/KG BW in sesame oil). CRH mRNA and GR mRNA were measured using in situ hybridization with ^{35}S labelled cRNA probes against rat CRH mRNA and GR mRNAs. Increases in CRH mRNA ($p<0.01$) were detected in GDX animals. DHTP treatment did not reduce these levels to that of the intact. There was no effect of GDX on GR mRNA levels. Furthermore, ADX increased CRH mRNA levels and RU28362 treatment of ADX rats prevented this increase. There was no interaction between ADX and GDX on CRH or GR mRNA levels. These data are consistent with GDX induced increases in hormone secretion and suggest that increases in CRH mRNA, but not alterations in GR mediated feedback regulation, are responsible for the previously observed neuroendocrine changes. Supported by NSF IBN 94-08890

546.14

MOLECULAR AND PHARMACOLOGICAL CHARACTERIZATION OF A CRF RECEPTOR CONSTITUTIVELY EXPRESSED BY THE HUMAN NEUROBLASTOMA CELL LINE IMR-32.
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Radioligand binding, functional and cloning studies have revealed that the human neuroblastoma cell line, designated IMR-32, constitutively expresses a single g-coupled CRF receptor gene product. Pharmacological evaluation revealed this receptor binds [125-I]oCRF in a saturable and reversible manner with an apparent dissociation constant Kd of 130 pM and a maximum binding capacity, Bmax of 45 pmol/mg protein. In addition, it is coupled to adenylcyclase as determined by its ability to stimulate the formation of the second messenger cAMP and shift [125-I]oCRF binding in the presence GTP analog, GMPPNP. Both binding and cAMP formation were blocked by the competitive peptide antagonist, a-helical CRF. At the molecular level, these cells express only a single form of CRF receptor message RNA which appears to be homologous to a recently cloned CRF receptor (Chen et al., (1993) Proc. Natl. Acad. Sci. (USA) *90*, 8967 - 8971).

546.15

DIVERSE SUBCELLULAR DISTRIBUTIONS OF THE CRF-BINDING PROTEIN IN RAT BRAIN AND PITUITARY. C.A. Peto, W. Vale and P.E. Sawchenko*. The Salk Institute, La Jolla, CA 92037.

The CRF-binding protein (CRF-BP) is a 37 kDa protein, distinct from CRF receptors, that is nonetheless capable of binding the peptide with high affinity and reversibly neutralizing its biological actions. The CRF-BP has been localized in anterior pituitary, where it coexists in a subset of corticotropes, and in brain, where it shows limited overlap with cellular sites of CRF expression. Preembedding immunoperoxidase staining methods were used to permit ultrastructural analyses that might provide insight into the still obscure functions of the CRF-BP. In anterior pituitary, CRF-BP-ir was detected diffusely in the cytoplasm of cells morphologically similar to corticotropes. In these, reaction product was not detected in secretory granules, but rather was most pronounced in secondary lysosomes and multivesicular bodies. In brain, marked regional differences in the subcellular pattern of CRF-BP staining were evident. In isocortex, where colocalization of BP and peptide is rare, BP-ir was distributed in perikarya, dendrites and axons in a manner similar to that of a prototypic neuropeptide. This included nondescript reaction product deposition in cytoplasm, dendrites and axon terminals, with the latter commonly engaging in synaptic contacts with unlabeled dendritic profiles. In the oval subnucleus of the bed nucleus of the stria terminalis, a site that contains overlapping accumulations of CRF-BP-ir varicosities and CRF-ir perikarya, BP staining was restricted to vesicle-laden varicosities that only very rarely engaged in synaptic contacts. Instead, these were most commonly directly apposed to unlabeled axon varicosities and terminals. In the nucleus raphe magnus, a site at which CRF and its BP coexist extensively in perikarya, CRF-BP-ir displayed a subcellular pattern of localization much like that seen in the anterior pituitary. The results are compatible with the view that the function of the CRF-BP may vary in different cellular contexts. In cellular targets of CRF (anterior pituitary) or in neurons in which peptide and BP coexist (raphe), the CRF-BP may play a role in processing and degradation of CRF and/or ligand-receptor complexes. In some other areas of the CNS, the BP seems positioned to serve as a transmitter/modulator at conventional synapses, and in still others as a paracrine modulator of local CRF effects.

546.16

STIMULATION OF SEROTONINERGIC SYSTEM CAUSES TRANSCRIPTIONAL ACTIVATION OF CRF AND ITS RECEPTOR SELECTIVELY WITHIN THE PARAVENTRICULAR NUCLEUS (PVN) OF THE RAT HYPOTHALAMUS. S. Rivest*, N. Laflamme, S. Boyetto, and D. Richard. Molecular Endocrinology Lab., CHUL Research Center and Dept. of Physiology, Laval University, Québec, Canada, G1V 4G2.

The present study investigated the effect of intraperitoneal (i.p.) administration of the indirect serotoninergic (5-HT) agonist dexfenfluramine (FEN) on the transcriptional activity of corticotropin-releasing factor (CRF) and its receptor (CRF_1 R) in the brains of conscious male rats via *in situ* hybridization histochemistry using both intronic and exonic probe technology. FEN induced a rapid and wide neuronal activation as indicated by the strong signal of c-*fos* mRNA in numerous structures throughout the brain 30 and 60 min after treatment. In the parvocellular division of the PVN, the large majority of CRF-immunoreactive (ir) perikarya displayed a positive signal for the mRNA encoding c-*fos*. Colocalization between CRF-ir neurons and c-*fos* positive cells was not detected in any other regions. This selective activation of PVN CRF neurons was also confirmed by the presence of CRF primary transcript; 30 min after i.p. injection of the indirect 5-HT agonist, a positive signal for CRF heteronuclear (hn)RNA was observed specifically in the parvoPVN. Moreover, transcription of the gene encoding the receptor for CRF was highly stimulated in the PVN following 5-HT activation Although this hypothalamic nucleus exhibited a barely detectable signal under basal conditions, FEN induced a strong signal of CRF_1 R mRNA in the parvoPVN. Interestingly, CRF-ir neurons displayed a positive signal for the mRNA encoding the CRF_1 R 3 and 6 h after systemic treatment with the indirect 5-HT agonist. These results indicate that although FEN can generate a wide neuronal activation throughout the brain, this 5-HT agonist triggers the activity of CRF neurons selectively in the parvocellular division of the PVN, a mechanism possibly related to the activity of hypothalamic-pituitary-adrenal axis. Induction of CRF_1 R mRNA in CRF cells of the PVN indicates that neuroencodrine CRF neurons can be targeted by CNS CRF under 5-HT stimulation.

Supported by the MRC of Canada

NEUROENDOCRINE REGULATION: HYPOTHALAMIC PEPTIDES

547.1

IMPACT OF INSULIN ON NEUROPEPTIDE Y (NPY) IN THE HYPOTHALAMIC ARCUATE AND PARAVENTRICULAR NUCLEI IN VIVO AND IN VITRO. I. Silva, J. Wang, M. Felber, A. Akabayashi* and S.F. Leibowitz. The Rockefeller University, New York, N. Y. 10021.

Circulating insulin has been shown to control the gene expression and synthesis of neuropeptide Y (NPY) in the hypothalamus. This peptide, in a projection from the arcuate nucleus (ARC) to the paraventricular nucleus (PVN), may have a role in energy balance, and insulin may contribute to this process via its impact on NPY. These studies examined insulin's effect on hypothalamic NPY in the rat using immunocytochemistry and radioimmunoassay. In in vitro studies, treatment with insulin (25-100 μg/ml) over a 4 h period dose-dependently decreased NPY release from cultures of neonatal rat hypothalamic cells (-33% at 100 μg/ml, $p<0.05$). In studies of hypothalamic fragments, insulin (100 μg/ml) markedly inhibited, by 50-85% ($p<0.01$), the release of NPY from both the hypothalamic mediodorsal region (MDH - including the paraventricular nucleus, PVN) and the hypothalamic mediobasal fragment (MBH - including the arcuate nucleus, ARC). After intracerebroventricular injection of insulin (80 mU/10 μl) in intact animals during the light (inactive) period, NPY-immunoreactive fiber densities were reliably decreased in the PVN and ARC. Insulin had no impact on NPY immunoreactivity in the dorsomedial nucleus. These results agree with other studies in showing that insulin inhibits NPY synthesis and/or release in the areas of the ARC and PVN, where NPY is believed to modulate feeding behavior.

547.2

INSULIN INHIBITS GALANIN IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS: IN VIVO AND IN VITRO STUDIES. J. Wang*, D. Andrews, H. Liu and S.F. Leibowitz. The Rockefeller University, N.Y., N.Y. 10021.

Insulin controls the synthesis of neurochemicals in the brain. Recent work indicates that insulin may inhibit gene expression and production of the peptide, galanin (GAL), which potentiates feeding behavior. The present studies examined insulin's effect on hypothalamic GAL in the rat using immunocytochemistry and radioimmunoassay. In in vitro studies, treatment with insulin (25-100 μg/ml) over a 4 h period dose-dependently decreased GAL release from cultures of neonatal rat hypothalamic cells (-65% at 100 μg/ml, $p<0.05$). In investigations of hypothalamic fragments, insulin (100 μg/ml) inhibited, by 67% ($p<0.05$), K^+-evoked release of GAL from the mediodorsal region of the hypothalamus (MDH - including paraventricular nucleus, PVN); it had no impact on GAL release from the mediobasal fragment (MBH - including arcuate nucleus, ARC). After intracerebroventricular injection of insulin (80 mU/10 μl) into intact animals during the light (inactive) period, GAL-immunoreactive fiber densities were decreased in the PVN and ARC. However, insulin had no impact on GAL-ir neurons in the dorsomedial nucleus and ARC, while in the PVN, GAL-staining neurons were not evident in either insulin-treated or control subjects. These results demonstrate a strong and site-specific inhibitory effect of insulin on GAL neurons, particularly in the mediodorsal hypothalamus or PVN area. This confirms previous results (Tang et. al., Neurosci. Abstr., 1994) showing an inhibition of GAL gene expression and peptide production in the PVN.

547.3

BOMBESIN ACTS IN THE SUPRACHIASMATIC NUCLEUS TO AFFECT CIRCADIAN CHANGES IN TUBEROINFUNDIBULAR DOPAMINERGIC NEURON ACTIVITY AND PROLACTIN SECRETION. L.M. Mai* and J.T. Pan. Dept. Physiol. and Anat., Natl. Yang-Ming Univ. Taipei, Taiwan, R.O.C.

We have previously shown that central administration of bombesin can block the afternoon prolactin surge (Neuroendocrinology 57:40-44, 1993). A circadian change in the activity of tuberoinfundibular dopaminergic neurons, which coincides with the onset of the prolactin surge, has also been observed (Neuroendocrinology 60:520-526, 1994). Whether bombesin acts on the tuberoinfundibular dopaminergic neuron to affect the prolactin surge and where does bombesin act in the brain were the focuses of this study. Bombesin (0.75 μg/3 μl/rat) was first injected into the lateral cerebroventricle of estrogen-primed ovariectomized rats at 1200 h through preimplanted cannulae. Both circadian changes of prolactin and tuberoinfundibular dopaminergic neuron activity were blocked by the treatment. In suprachiasmatic nuclei-lesioned rats, however, both rhythms were absent, and the injection of bombesin exhibited no significant effect either. Using microinjection of bombesin (50 ng/0.2 μl) bilaterally into either the suprachiasmatic or arcuate nuclei at 1200 h, only the former injection effectively blocked both rhythmic changes in tuberoinfundibular dopaminergic neuron activity and prolactin secretion. We conclude that bombesin may act on the rhythm generation center, the suprachiasmatic nucleus, to exert its effect to disrupt the tuberoinfundibular dopaminergic neuron activity and the prolactin surge.

547.4

DISTRIBUTION OF VASOACTIVE INTESTINAL PEPTIDE-POSITIVE FIBERS IN THE ARCUATE NUCLEUS AND ITS EFFECT ON DORSOMEDIAL ARCUATE NEURON ACTIVITY IN BRAIN SLICES. S.K. Huang, J.Y.H. Chan* and J.T. Pan. Inst. Physiol., Natl. Yang-Ming Univ., Taipei, Taiwan

Vasoactive intestinal peptide (VIP) has been proposed as one of the prolactin-releasing factors, and it also acts as both central and peripheral neurotransmitter and neuromodulator. The present study investigated the distribution and action of VIP in the hypothalamus of ovariectomized Sprague-Dawley rats implanted with estrogen capsules. Using immunohistochemical staining method, VIP-positive neurons were found in the paraventricular and suprachiasmatic nuclei as previously reported by others. Dense VIP-immunoreactive fibers, however, were found in the arcuate nucleus extruding perpendicular from the border of the third ventricle, which distribution overlapped with that of the putative tuberoinfundibular dopaminergic neurons in the arcuate nucleus as doubled-labeled with tyrosine hydroxylase antibody. Using single-unit recording of dorsomedial arcuate neurons in brain slices of ovariectomized rats, VIP was shown to significantly activate the firing rates of a majority of neurons recorded (65.4%, 17/26 neurons). These results provide both anatomical and pharmacological evidence indicating that VIP may play an important role in the arcuate nucleus and in the regulation of tuberoinfundibular dopaminergic neurons.

547.5

ISOLATION, AND CHARACTERIZATION OF THE CHICKEN PACAP/GHRH-LIKE GENE. J.E. McRory, S.L. Krueckl, K.R. von Schalburg and N.M. Sherwood*. Department of Biology, University of Victoria, B.C. Canada, V8W 2Y2.

In chickens the physiology of growth hormone (GH) secretion is well documented. However, a true chicken GH-releasing hormone (GHRH) has not yet been identified. We have isolated and sequenced a gene and cDNA which encodes for a precursor molecule containing two neuropeptides. One of these peptides, based on sequence identity, is pituitary adenylate cyclase activating polypeptide (PACAP). The other neuropeptide could be either GHRH or PRP (PACAP-related peptide). The chicken PACAP gene is 6145bp long and contains an intron-exon structure comparable to other members of the glucagon superfamily. Present in the chicken PACAP promoter are tentative CAAT and TATAA concensus sites 65bp upstream of a potential start site. CAAT and TATAA sites have not been reported for other PACAP genes. The role of the full length and truncated PACAP and GHRH peptides to release GH *in vivo* and *in vitro* will be presented.

547.6

A FIXED TISSUE SLICE PREPARATION FOR EXAMINING DENDRITIC ARCHITECTURE OF NEUROENDOCRINE NEURONS IN THE RAT ARCUATE NUCLEUS. S.C. Danzer*, N.T. McMullen and N.E. Rance. Departments of Cell Biology and Anatomy, and Pathology, University of Arizona College of Medicine, Tucson, AZ 85724.

Previous morphologic studies of dendrites in the rat arcuate nucleus have been accomplished through Golgi techniques. We have developed a technique to examine the dendritic morphology of arcuate neuroendocrine neurons by combining fluorogold labeling and intracellular injection of neurons in a fixed tissue slice preparation. Fluorogold, when injected IP, labeled arcuate neuroendocrine cells with projections to the fenestrated capillaries of the median eminence, where the blood-brain barrier is leaky. Arcuate neuroendocrine cells containing fluorogold were visualized under epifluorescent illumination in 200-500 um tissue slices. The labeled cells were then impaled and filled, under visual control, with glass microelectrodes containing a combination of 0.5% lucifer yellow, 1% biotinylated lucifer yellow, and 2.5% biocytin. After post-fixation, the slices were processed with an ABC/DAB reaction to obtain a dark, Golgi-like fill for subsequent analysis. Preliminary results show that arcuate neuroendocrine neurons tend to be either unipolar, or multipolar with 2-4 primary dendrites. The dendritic processes are sparsely to moderately spiny, and are generally confined to the arcuate nucleus. Axons are frequently beaded and project toward the median eminence. The combination of these techniques gives reliable, complete, and permanent visualization of the morphology of hypothalamic neuroendocrine neurons in the rat arcuate nucleus. (Supported by NIH Grant AG09214 and the Flinn Foundation of Arizona)

547.7

GALANIN MESSENGER RNA AND PEPTIDE LEVELS ARE DECREASED IN RADIOTHYROIDECTOMY-INDUCED PITUITARY ADENOMAS. J.F. Hyde*, K.W. Drake and J.P. Moore, Jr. Dept. of Anatomy and Neurobiology, University of Kentucky College of Medicine, Lexington, KY 40536.

We have shown that galanin gene expression is markedly increased in lactotroph (estrogen-induced) and somatotroph (GHRH-induced) adenomas. The objective of this study was to examine galanin gene expression in the development of thyrotroph adenomas. Male C57BL mice were injected with 200 µCi of iodine-131 (I-131) or saline. The development of radiothyroidectomy-induced pituitary hyperplasia/adenomas was examined at 3, 6, 9 and 12 months after I-131 injection. After 3 months, the anterior pituitaries of I-131-treated mice were 2-fold larger than control pituitaries, and plasma TSH levels were increased more than 20-fold. However, galanin peptide levels in the anterior pituitary of I-131-treated mice were decreased 80%; no changes in hypothalamic galanin peptide concentrations were detected. Treatment with estradiol increased galanin peptide concentrations in the anterior pituitaries of all mice, however, galanin peptide levels in I-131-treated mice remained 50% lower than controls. Treatment with tri-iodothyronine selectively increased pituitary galanin peptide levels in I-131-treated mice, but these levels still remained lower than controls. Plasma TSH levels and anterior pituitary size continued to increase in I-131-treated mice and were more than 100-fold and 10-fold higher, respectively, than controls by 12 months. Galanin peptide concentrations in the anterior pituitary remained depressed throughout this study. Galanin mRNA levels in the anterior pituitary were estimated by RT-PCR, and were significantly lower in I-131-treated mice. We conclude that galanin peptide concentrations are decreased in a tissue-specific manner during the onset and development of I-131-induced thyrotroph adenomas due to a decrease in galanin mRNA levels. Unlike other pituitary tumors examined, galanin does not appear to facilitate thyrotroph hyperplasia. (Supported by DK-45981 and HD-07436)

547.8

INCREASED GALANIN SECRETORY ACTIVITY AND CELL NUMBER IN THE ANTERIOR PITUITARY OF hGHRH TRANSGENIC MICE. J.P. Moore, Jr.* and J.F. Hyde. Department of Anatomy and Neurobiology, University of Kentucky College of Medicine, Lexington, KY 40536.

We recently reported that galanin mRNA and peptide levels, and immunoreactive cell numbers are increased within the anterior pituitary gland of the human growth hormone-releasing hormone (hGHRH) transgenic mouse. In order to determine if these observed increases in galanin gene expression are related to changes in peptide secretion, we employed the cell immunoblot assay (Kendall and Hymer, 1987) which allows for the detection of peptide secretion from single cells *in vitro*. Anterior pituitary glands from six month old male hGHRH transgenic and non-transgenic mice were incubated with 0.25% trypsin to prepare a dispersed cell suspension. The cell suspension was then dispensed, in a monolayer, onto an Immobilon PVDF membrane equilibrated in HEPES-buffered DMEM, and maintained at 37°C in a 95% air/5% CO_2 incubator for six hours. The cells were then fixed with 4% paraformaldehyde, rinsed with phosphate buffer, and stained for galanin immunoreactivity using diaminobenzidine as the chromogen. This method allows for the visualization of the secretory products from single cells as they are adsorbed onto the membranes. Utilizing an NIH Image Software system, we observed that 1) increased numbers of cells from the pituitaries of hGHRH mice secrete galanin as compared to pituitary cells from non-transgenic siblings, and 2) the amount of immunoreactive galanin peptide secreted from cells from hGHRH transgenic mice was increased as compared to non-transgenic siblings. These data show that increased galanin secretion from pituitary cells of hGHRH transgenic mice parallels the increase of galanin gene expression. Moreover, the cell immunoblot assay will allow us to determine the pituitary cell type(s) secreting galanin. (Supported by DK-45981 and HD-07436)

547.9

MODULATION OF GAP JUNCTIONAL INTERCELLULAR COMMUNICATION IN CULTURED LHRH NEURONS. D.F. Matesic*, T. Hayashi, J.E. Trosko and J.A. Germak. Dept. of Pediatrics & Human Development, Michigan State Univ., East Lansing, MI 48824.

Rhythmic secretion of luteinizing hormone-releasing hormone (LHRH) by hypothalamic LHRH neurons controls the onset of puberty and reproduction. One potential means for coordinating LHRH secretion is via electrotonic or chemical signals transferred through gap junctions. The LHRH-secreting neuronal cell line, GT1-7[1], which has been shown to be dye coupled[2,3] and to express the gap junction protein subtype, connexin 26(Cx26)[3], was used to study regulatory mechanisms of gap junctional intercellular communication. Using fluorescence recovery after photobleaching (FRAP), we found that treatment of GT1-7 neurons for 24 hr with 2µM forskolin or 100 µM dibutyryl cAMP + 100 µM IBMX increased the percentage of dye coupled cells from 12-15% in untreated cultures to 30-40%, which was maintained up to at least 48 hr. Subsequent treatment of cells with the gap junction inhibitors, octanol or dieldrin, reduced the level of coupling to below the level in untreated cells, supporting the hypothesis that the observed increase in dye coupling was mediated by gap junctions. Western blot analysis of forskolin-or dibutyryl cAMP + IBMX-treated cells showed no increase in the intensity of Cx26 protein. RT-PCR analysis showed no increase in Cx26 mRNA levels following 6, 12 or 24 hr treatment with dibutyryl cAMP + IBMX. These results suggest that increased Cx26 expression did not account for the observed increase in dye coupling. Alternative mechanisms include changes in Cx26 localization or assembly, increased numbers of cell-cell contacts, or induction of expression of other connexin subtypes. Supported by NSF IBN 94-07267 [1]Neuron 5: 1, 1990; [2]PNAS 89: 4149, 1992; [3]Neuroendocrinology 58: 485, 1993.

547.10

PROGESTERONE (PROG) STIMULATES THE RELEASE OF GONADOTROPIN-RELEASING HORMONE (GnRH) FROM GT1-1 NEURONS, BUT NOT VIA THE $GABA_A$ RECEPTOR AS DOES ITS METABOLITE 3α-HYDROXY-5α-PREGNAN-20-ONE (TH PROG). M. El-Etr*, Y. Akwa, R.J. Fiddes, K. Shazand, P. Robel, E.E. Baulieu. INSERM U.33, 94276 Bicêtre, France.

We previously reported that TH PROG (10nM-10µM) stimulates within 3min the release of GnRH from immortalized hypothalamic GT1-1 neurons, through an interaction with the $GABA_A$ receptor (R) since bicuculline and picrotoxin inhibit this effect (El-Etr et al., Proc.Natl.Acad.Sci.USA, in press). Here we report that surprisingly PROG also stimulates the release of GnRH, but with a markedly different profile of action since exclusively in the micromolar range concentrations, with a six time lower potency and a lag of 20min. This effect cannot be related to the conversion of PROG to TH PROG, which can be performed by GT1-1 cells, as $GABA_A$ R antagonists do not counteract PROG action. In order to define the mechanism of action of PROG, the antagonistic effect of RU486 was tested and RT-PCR experiments were carried out to look for intracellular PROG R mRNA. The effect on GnRH release of the PROG-3-BSA conjugate, which does not cross cell membranes, was also investigated, as well as the role of calcium and sodium channels.

547.11

OPIOID PEPTIDES REGULATE GnRH RELEASE IN VITRO

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Opioid peptides inhibit GnRH release in mammals. In birds, the effects of these peptides on the GnRH system is unclear. Previous experiments in our lab have shown a dose-related inhibition of cGnRH-I release in vitro with exposure to enkephalin. This study investigated effects of β-endorphin (END) on cGnRH-I release from hypothalamic slices in vitro. Longitudinal slices (5mmx1mmx3mm) spanning preoptic area to mediobasal hypothalamus (POA-MBH) taken from adult male Japanese quail (Coturnix japonica) were placed in chambers (Endotronics Perifusion System; slices from one brain in a chamber with a total of 6 chambers) and equilibrated in Medium 199 (Biofluids, Inc) for two hours. Baseline samples (5 min collection time) and a norepinphrine (NE; 10^{-8}; RBI) challenge were conducted during this time. END (10^{-7}; Sigma) was added to the Medium 199 through the rest of the experiment. END exposure resulted in reduced ($p< 0.01$) baseline release of cGnRH-I and in decreased ($p< 0.01$) NE stimulated cGnRH-I release. In a separate experiment, a dose-response relationship was observed with END concentrations from 10^{-5} to 10^{-11}. Decreased cGnRH-I release was reflected in reduced pulse amplitude, with no change in frequency. These results provide evidence for inhibition of cGnRH-I release by the opioid peptide END, similar to enkephalin. Further, the reduction in NE-stimulated release of cGnRH-1 also suggests a direct effect of END on both GnRH and NE neurons. Supported by USDA #88-37242-2913 and NRI#92-37203-7742 (MAO).

547.12

Dexamethasone Does Not Alter Basal Corticotropin-Releasing Factor (CRF) Produced by Rat Amygdaloid Neurons in Culture. JW Kasckow*, JJ Mulchahey, †DG Parkes, CD Kilts, P Lambert, MJ Owens, ED Risby, MD Stipetic, CB Nemeroff. Emory U. Sch. Med., Dept. Psychiatry, Atlanta, GA 30322; †Howard Florey Inst. of Exp. Physiol. and Med., U. Melbourne, Australia, 3052.

Amygdalar and hypothalamic neurons have been postulated to play a role in the stress response. Glucocorticoids negatively regulate CRF containing hypothalalmic cells; however, it is not known whether similar effects on CRF containing cells in the amygdala occur. The effects of dexamethasone (DEX) on CRF in primary rat amygdalar cultures derived from E19 pups were evaluated. The amygdala was dissected bilaterally and dissociated with 0.25% trypsin. Cells were plated at a density of 10^6 cells/well in DMEM, 10% fetal bovine serum, 14 mM glucose, 15 mM $NaHCO_3$, 5 mM Hepes and pen/strep. At 17 days, triplicate sets of cells were treated 12 hours with either forskolin (3, 10, 30 µM) or DEX (4, 20, 100 nM). Supernatants were collected and cells were harvested with 0.1% NP-40. CRF secretion and content was measured via radioimmunoassay, expressed as mean ± standard error, and statistical significance was determined at the $P<0.05$ level by Duncan's Multiple Range test. Forskolin produced significant increases in CRF intracellular content at 30 µM: 27.9 ± 0.2 vs. 22.8 ± 1.6 pg/well in controls. Significant increases in CRF secretion occurred at doses as low as 3 µM: 17.6 ± 0.7 vs. 9.1 ± 0.2 pg/well in controls. DEX at all three doses failed to alter secreted and intracellular levels of CRF. Intracellular content of CRF in the presence of DEX (4, 20, 100 nM) was determined in primary rat hypothalamic cultures raised under identical conditions mentioned above. A significant decrease in CRF content occurred at 100 nM: 25.2 ± 0.7 vs. 31.3 ± 2.8 pg/well in controls indicating that the failure of the amygdalar cultures to respond was not an artifact of the culture methods. Further characterization of these amygdalar cultures is under way in order to investigate their responsiveness to known stimulators and inhibitors of CRF involved in the stress response. Supported by NIMH MH-42088. Dr. Kasckow is a Pfizer awardee and Dr. Parkes is a CJ Martin Fellow.

547.13

EFFECTS OF ESTRADIOL ON LUTEINIZING HORMONE-RELEASING HORMONE IN SK-N-SH NEUROBLASTOMA CELLS.

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The SK-N-SH human neuroblastoma cells are investigated as a model to study the opioids-LHRH-ovarian steroids interactions at the neuronal level. Our previous study showed that the SK-N-SH cells express LHRH mRNA, and that the LHRH mRNA level and LHRH concentration decreased during neuronal differentiation induced by retinoic acid (RA, 10 µM). Recently we have found that the LHRH mRNA level decreased after 6-day treatment with 5 nM of estradiol (E). Quantitative densitometric analysis showed that E further potentiated the RA-induced decrease in the LHRH mRNA level (20.13 OD x mm² - no treatment, 8.75 and 4.69 OD x mm² after RA and RA+E, respectively). The concentrations of LHRH were measured after RA and/or E treatments. Marked reduction in LHRH level occured on day 1 but was most significant on day 6 (from control value of 4.30 ± 1.62 to 0.53 ± 0.08 ng/mg protein after RA, to 0.42 ± 0.06 ng/mg protein after E, and to 0.61 ± 0.18 ng/mg protein after RA+E). Preliminary findings suggest the presence of E receptors mRNA in SK-N-SH cells; the expression of the mRNA coding for E receptors seems to be lower in cells exposured to RA. We conclude that E has a profound attenuating effect on the LHRH system in undifferentiated as well as differentiated SK-N-SH neuroblastoma cells. [Supported by the grants from URC (ISU) and NIH-IDeA]

547.14

FLUORESCENT ANALOGUES OF GROWTH HORMONE-RELEASING FACTOR: NEW TOOLS FOR PROBING GRF RECEPTORS.

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Highly sensitive techniques, such as flow cytofluorometry and confocal fluorescence microscopy imaging, make possible the characterization of receptors and their cellular/subcellular localization in real time, in response to various stimuli. With this in mind, we have designed fluorescent analogues of GRF, to probe the pituitary GRF receptor. Since the side chain of Lys^{12} and Lys^{21} are important receptor-binding pharmacophores of GRF in this tissue, their ϵ-amino function could not be used for fluorescein labeling. Analogues of hGRF(1-31)NH_2 and hGRF(1-44)NH_2 bearing a Lys^{31} were selected, because structural modifications within the segment 30-44 have less influence on GRF bioactivity. Fluorescein was also coupled to the α-amino group of hGRF(1-29)NH_2 and hGRF(1-44)-NH_2. [N^{ϵ}-5-carboxyfluoresceinyl(CF)-Lys^{31}]hGRF(1-31)NH_2 and [N^{ϵ}-5-CF-Lys^{31}]hGRF(1-44)NH_2 exhibited receptor binding affinity and adenylate cyclase activity comparable to that of hGRF(1-29)NH_2 in rat adenopituitary, while these parameters were decreased for [N^{α}-fluorescein-5-thiocarbamyl(FTC)]hGRF(1-29)NH_2 and [N^{α}-FTC]hGRF(1-44)NH2. The availability of high affinity fluorescent agonists of GRF receptors will be of great value to study the behavior of GRF and GRF-GRF receptor complexes in physiological and pathological conditions. Supported by the Sandoz Foundation for Gerontological Research.

547.15

AFFERENT SYNAPSES ON ESTROGEN RECEPTOR-IMMUNOREACTIVE NEURONS IN THE ANTEROVENTRAL PERIVENTRICULAR NUCLEUS OF THE FEMALE RAT: ELECTRON MICROSCOPY.

R.E. Watson Jr.*, B.E.Maley, M.G. Engle, and M.C. Langub, Jr. Dept. of Anatomy and Neurobiology, University of Kentucky Med. Ctr., Lexington, KY 40536-0084.

The anteroventral periventricular nucleus (AVPv) in the female rat is critical to the steroid-mediated control of the preovulatory gonadotropin surge. It is believed that together with steroidal influences, neurochemical systems including substance P (SP) and catecholamine (tyrosine hydroxylase [TH]) producing neurons play integral roles in this neuroendocrine event. We hypothesized that steroidal and SP and catecholamine signals converge upon estrogen receptor-immunoreactive (ER-ir) neurons in the AVPv. Previous light level immunocytochemical data indicated that in the AVPv, SP- and TH-ir fibers are in position to synaptically contact ER-ir neurons. This study was conducted to assess the potential for such interaction at the ultrastructural level. Sections through the AVPv were immunostained sequentially for ER using monoclonal antibody H222 (Abbott Laboratories) with tetramethylbenzidine (TMB) as chromogen, and either rabbit anti-SP [1: 4000] or rabbit anti-TH [1: 5000; Eugene Tech] using diaminobenzidine (DAB) as chromogen. Ultrastructurally, ER-ir neurons contained intranuclear TMB crystals, while DAB reaction product was within labeled axonal terminals. The majority of DAB-labeled synaptic terminals were axodendritic; however, axosomatic contacts were present on both ER- and non-ER-ir neurons. SP-ir axosomatic synapses were upon 8.5% of ER-ir neurons and 13% of non-ER-ir neurons. Nine percent of ER-ir neurons and 9.2% of non-ER-ir neurons had TH-ir axosomatic synapses. These results provide morphological evidence for SP and catecholamine regulation of ER-ir neurons in the AVPv through axosomatic synapses and suggest a potential anatomical mechanism through which these compounds could modulate gonadotropin secretion. Supported by NIH Grant HD29050 (REW).

547.16

LOCALIZATION OF NATRIURETIC PEPTIDE RECEPTOR mRNAS IN THE RAT BRAIN.

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Atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP) transduce signals primarily by high-affinity binding to the extracellular domains of membrane-bound guanylate cyclases, resulting in mobilization of cyclic GMP. The A-type natriuretic peptide receptor (NPR-A) selectively binds ANP while the B-type natriuretic peptide receptor (NPR-B) displays high selectivity for CNP. A third natriuretic peptide receptor (NPR-C) can bind both ANP and CNP but does not have the catalytic guanylate cyclase domain and has been postulated to be a "clearance" receptor. The present study employed probes designed to target the 5' extracellular ligand binding domain known to be different in all three receptor species. NPR-A and NPR-B were analyzed by *in situ* hybridization histochemistry. Strong hybridization signal for NPR-A mRNA was present only in the medial habenula, subfornical organ, area postrema, and the olfactory bulb. Notably, NPR-A mRNA was expressed also in several white matter tracts, suggestive of glial origin. In contrast, NPR-B mRNA expression appeared to be predominantly neuronal, being heavily localized in the subfornical organ, hypothalamic neuroendocrine neurons, olfactory system, and motor nuclei of cranial nerves. The distribution of NPR-C mRNA was assessed by polymerase chain reaction. This species was most abundant in cortex and hypothalamus. The results of the present study suggest the capacity for natriuretic peptide systems to exert regulatory control on a wide variety of central nervous system functions and provide the framework for biological action of the peptides in clearly defined neural circuits. Supported by the American Heart Association, Kentucky Affiliate, MH49698 and HD29050.

547.17

REPRODUCTIVE STATE CHANGES NADPH-DIAPHORASE IN THE PARAVENTRICULAR NUCLEUS AND THE SUPRAOPTIC NUCLEUS OF RATS B.C.Woodside*, B. Robinson and S. Amir, Centre for Studies in Neurobiology, Psychology Dept., Concordia University, Montreal, Qué., Canada.

Neurons in both the paraventricular nucleus (PVN) and the supraoptic nucleus (SON) stain for NADPH-diaphorase, a histochemical marker of nitric oxide synthase. Nitric oxide itself has been implicated in the control of corticotropin releasing hormone (CRH) and oxytocin release from neurosecretory cells in the PVN and SON as well as in the neural plasticity seen in these areas. Because changes in reproductive state are associated with modulation of CRH and oxytocin release as well as morphological changes in neurons in the PVN and SON, we determined whether NADPH-diaphorase staining density would also vary as a function of reproductive state. The reproductive conditions examined were: longterm ovex; Day 21 of pregnancy; Day 12 of lactation; and the diestrous and estrous days of the estrus cycle. All animals were anaesthetised with Somnotol (60mg/kg) and then perfused transcardially with saline and 4% paraformaldehyde. Brains were removed, postfixed for 18 hours, sectioned and then processed for NADPH-diaphorase histochemistry. NADPH-diaphorase staining in both the PVN and the SON was dramatically increased in late pregnant females compared with all other groups. The activity of oxytocinergic neurons is enhanced both during late pregnancy and lactation, thus it is unlikely that the increase in NADPH-diaphorase observed only in late pregnancy simply reflects an increase in neuronal activity.

547.18

NEURAL ENDOPEPTIDASE 24.15 COLOCALIZES TO THE NUCLEUS IN DISCRETE NEURAL POPULATIONS Bindiya Moorjani*, T. J. Wu, Marc J. Glucksman and James L. Roberts, Fishberg Research Center for Neurobiology, Mount Sinai Medical Center, New York, NY 10029.

The metalloendopeptidase EC 3.4.24.15 (EP24.15) is very active in the extracellular space, where it is purported to metabolize various biologically active peptide hormones. However, it is also localized in the nucleus of neurons and glia, where its function is unknown. Preliminary studies indicated that salt-loading of rats leads to a dramatic shift in the localization of EP24.15 from the nucleus to the cytoplasm in magnocellular neurons of the hypothalamic paraventricular nucleus. To further address the distribution and cellular compartmentalization of EP24.15 in the hypothalamus we examined the localization of EP24.15 in identified neurons under various hormonal states. Female rats at various stages of the estrous cycle were transcardially perfused with 4% paraformaldehyde, and the brains post-fixed for 4 h in the same fixative. Free-floating vibratome sections were processed for single (DAB, EP24.15 only) or double labeling for EP24.15 and GnRH or Tyrosine Hydroxylase (TH). Single-labeling with EP24.15 antisera demonstrated a cellular or nuclear localization. In the double labeled sections, 50% of GnRH-or TH- positive neurons were observed to have a nuclear localization of the enzyme. The present results showing a heterogenous nuclear localization within defined neuronal populations suggest that the location and its activity may be dependent on the neuronal status.

NEURAL-IMMUNE INTERACTIONS: DEPRESSION AND STRESS

548.1

CHEMICAL INDUCED TUMORIGENESIS AND IMMUNE FUNCTION IN TRANSGENIC ANIMALS WITH IMPAIRED TYPE II GLUCOCORTICOID RECEPTOR (GR) GENE EXPRESSION. **J.M.C. Blom*, N. Barden, D. Viglienghi, B. Silveri and G. Racagni. Center of Neuropharmacology, Univ. of Milan, 20133 Milan, Italy, and Molecular Psychogenetics, CHUL, Québec, Canada.**

Recently a transgenic animal model dealing with the neuroendocrine aspects of depression has been developed, and is characterized by partial knockout of GR gene expression, caused by GR antisense RNA expression (Pepin et al. 1992, Nature 355:725). These transgenic mice display a hyperactive hypothalamic-pituitary adrenal (HPA) axis and dysfunctional glucocorticoid inhibitory feedback similar to that seen in 60% of depressed patients. Based on the fact that glucocorticoids affect the immune system and that both depression as well as immunosuppression play a potential important role in the onset and progress of cancer, the hypothesis was tested that dysruption of GR gene expression, accompanied by alterations in immune functioning, may result in enhanced susceptibility to chemically induced tumorigenesis. Adult female transgenic mice and intact controls were injected s.c. with the chemical carcinogen 9,10 dimethylbenzanthracene (DMBA, 50 mg/kg) or with the vehicle (DMSO). 73% of the transgenic mice developed tumors within three weeks post-injection, while only 23% of the controls displayed tumors 8 weeks after treatment. None of the mice injected with the vehicle developed tumors. Furthermore, tumor incidence correlated positively with altered immune responsiveness, as measured by lymphocyte cell number and lymphoproliferative assays. These results indicate that suppression of the immune system enhances susceptibility to chemically-induced tumors, and that disruption of the glucocorticoid negative feedback loop and the HPA-immune axis can increase the risk of developing cancer if coincident with a carcinogenic event.

548.2

GLUCOCORTICOID-INDUCED PROGRAMMED-CELL DEATH (APOPTOSIS) IS INHIBITED IN TRANSGENIC MICE EXPRESSING TYPE II GLUCOCORTICOID RECEPTOR (GR) ANTISENSE RNA: DOWN-REGULATION OF Bcl-2 AND INTERLEUKIN 2 (IL-2R) RECEPTOR OVER-EXPRESSION. M.C. Morale[2], G. Bartoloni[2], F. Italia[2], F. Gallo[2], N. Barden[4] and B. Marchetti[1*], Depts. Pharmacol and Pathology, Catania, IRCS Inst. Mental Retard. and Brain Aging, Troina (EN) Italy, and Mol.Psychogenetics, CHUL, Quebec, Canada.

A major factor in the development of thymocytes is the induction of, or the protection from, programmed cell death (apoptosis). The susceptibility of lymphocytes to glucocorticoid-induced apoptosis is part of a thymic maturational program and is known to depend on the maturation stage (immature thymocyte are particularly sensitive), the activation stage (strongly activated T-cells become resistant), local lymphokine concentration, the sex steroid hormone milieu, and most likely by other as yet undefined factors present in the thymic microenvironment. We have recently documented a number of developmental alterations and permanent effects on T-cell function in transgenic mice expressing a type II GR antisense RNA construct (Endocrinology, in press). In the present study we have investigated whether alterations in the processes involved in T-cell selection accompain the post-natal development of transgenic mice with impaired GR function. For this aim, thymus glands from intact and transgenic mice were processed at different time-intervals during post-natal ontogeny (5-60 days) and DNA fragmentation, the distribution of the oncogene bcl-2 (a candidate protein implicated in the onset of apoptosis), and two markers of T-cell proliferation (Ki67 and PCNA), were correlated to the acquisition of IL-2R inducibility. Flow cytometric and internucleosomal DNA cleavage analyses revealed a significant inhibition of apoptosis in the thymus of transgenic mice. The immunohistochemical staining pattern of Ki67 and PCNA was markedly increased in the face of a down-regulation of Bcl-2. Moreover, over expression of IL-2R accompanied the development of thymocyte responsiveness. These results provide experimental support for the concept that expression and regulation of GR at the thymus gland level constitute a crucial developmental step for the acquisition of the T-cell repertoire.

548.3

STRESS MAY SUPPRESS SPECIFIC IMMUNITY BY STIMULATING MACROPHAGES. **Brennan, F.X.*, M. Fleshner, T. Deak, L. R. Watkins, & S. F. Maier, *Dept Psych, Wilkes Univ.; Dept. Psych, Univ. CO, Boulder, CO 80309.**

Stressor exposure may activate macrophages (MØs). This activation could increase innate immunity. However, MØ activation is also suppressive of other aspects of immunity, such as T-cell proliferation to ConA or alloantigen (Fleshner et al., J. Neuroimmunology, 56, 1995). We investigated whether stress-induced MØ activation could contribute to stress-induced decreases in specific immunity, i.e., *in vivo* antibody production to KLH. Two approaches were taken: 1) Block MØ function *in vivo* prior to stress; or 2) Activate MØs *in vivo* in the absence of stress. MØ or IL-1 activity were suppressed *in vivo* with either gadolinium chloride (7.5 mg/kg; $GdCl_3$; a phagocyte inhibitor) or IL-1 receptor antagonist (100mg/kg; IL-1RA). Rats (12/gr) were injected with either $GdCl_3$ or saline. Twenty-four hrs after injection, rats were immunized with KLH (200 µg) and exposed to either 100 5-s inescapable tail shocks (IS) or returned to their home cage. IL-1RA was administered (12/gr) after IS + KLH. MØ stimulation studies involved zymosan, LPS, or vehicle injection (12/grp) given before KLH. The Ig response to KLH was measured on days 5, 7, 10, 14 and 21 after immunization using ELISA. Preliminary results suggest that MØ blockade can inhibit IS-induced suppression of the anti-KLH Ig response, whereas MØ stimulation in the absence of IS suppresses the anti-KLH Ig response. NIH-MH45045.

548.4

IMPAIRED NATURAL KILLER CELL RESPONSES IN A GENETIC ANIMAL MODEL OF DEPRESSION *Elliot M. Friedman*[1], Michael R. Irwin[1], and David H. Overstreet[2], (1) Department of Psychiatry, University of California, San Diego and San Diego Veterans Affairs Medical Center, San Diego, CA 92161; (2) Skipper Bowles Center for Alcoholic Studies and Department of Psychiatry, University of North Carolina, Chapel Hill, NC, 27599.*

Studies examining the relationship between major depression and immune function have yielded mixed results; decreases in natural killer (NK) activity, for example, have been reported, but these changes are neither consistent nor limited to depression. Moreover, this relationship is complex, involving variables such as sleep, neuroendocrine activity, and age, and examination of the contributions of these variables to immune dysfunction in depression will require animal models.

The Flinders Sensitive Line (FSL) rat exhibits multiple behavioral and physiological abnormalities reminiscent of major depression, including sleep disturbance, reduced behavioral activity, and hypersensitivity to cholinergic agonists. We report here that these animals also exhibit reductions in NK activity.

Rats were rapidly decapitated and the spleens were removed and teased apart. Spleen NK cytotoxicity against target YAC-1 mouse lymphoma cells was determined in a standard 3-hour chromium release assay. NK number was determined by flow cytometry using a specific mouse anti-rat NK monoclonal antibody. There were no significant differences in absolute NK lytic activity among the three strains of rats, but the FSL rats had significantly more splenic NK cells than FRL rats [$p<.05$]. When lytic activity was adjusted for NK cell number, the FSL rats exhibited significantly less lytic activity per cell than FRL rats [$p<.05$].

Compared to control animals, the FSL rats exhibited depression-like decreases in cellular immune responses under non-stressful conditions. These results provide support for the FSL rats as a valuable model for the study of the physiological substrates of major depression.

548.5

DIFFERENTIAL CORTICOSTERONE RESPONSES TO VARIOUS STIMULI IN LEWIS AND FISCHER 344 RATS. T. Bienen, L. J. Grota* and D. L. Felten. Depts. of Neurobiol. and Anat. and Psychiatry, Univ. of Rochester, NY 14642

Lewis/N (L) and Fischer-344 (F344) rats are widely used to study the interactions between the nervous, immune, and endocrine systems. It has been reported that L rats, in comparison to F344 rats, have an attenuated hypothalamic-pituitary-adrenal (HPA) axis response to a variety of challenges. This is believed to be due to a defect in central CRH production. The purpose of this study was to investigate the responsiveness of the HPA axis in individual male L and F344 rats (200-350 grams) to a variety of treatments.

The rats were implanted with an indwelling inferior vena cava catheter and allowed to recover for 7 days. Baseline samples were taken each day before treatment. Blood samples were drawn at 15, 30, 45, 60, and 90 min. after treatment with ACTH$_{1-24}$ (500 ng/kg, day 1), stress (exposure to a new environment and ip puncture using a 26G needle, day 2), nicotine (0.01 µg/kg, day 3), and saline (day 5). After LPS treatment (60 µg/kg, day 4) samples were taken at 30 min., 1 hr, 2 hrs, 4 hrs, 6 hrs, and 24 hrs. Serum was assayed for corticosterone (CS) by RIA.

There were no significant strain differences in baseline CS levels. Both L and F344 rats responded to ACTH, stress, nicotine, and LPS with CS levels returning to baseline by the end of the sampling period. F344 rats showed significantly higher CS responses to stress at 15, 30, and 60 min., to nicotine at 15 and 30 min., and to LPS at 60 and 120 min. in comparison to L rats. We observed no significant differences between L and F344 rats in CS responses to ACTH and saline.

It is concluded that L rats are less responsive to stress and nicotine, but only slightly less responsive to LPS than F344 rats. This suggests that male L rats are capable of mounting a substantial CS response to an immunological challenge. (This work was supported by R37MH42076 and a Markey Charitable Trust Award.)

548.6

ENHANCEMENT OF IMMUNE FUNCTION BY ACUTE STRESS. F. S. Dhabhar* & B. S. McEwen. Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY 10021.

Immune cell trafficking is crucial to the performance of the surveillance and effector functions of the immune system. We have previously demonstrated that acute stress induces rapid, and large magnitude changes in the distribution of peripheral blood leukocytes in the rat. We have also shown that the effects of stress on leukocyte distribution are mediated by the adrenal steroid, corticosterone, acting through the Type II (glucocorticoid) receptor.

In the present series of studies we examined the skin as a target organ for the stress-induced redistribution of leukocytes. Our results suggest that acute stress induces an influx of leukocytes into the skin. In order to determine the functional immunologic consequences of these changes in leukocyte distribution, we examined the effects of acute stress on a cell-mediated immune response (delayed type hypersensitivity (DTH)) induced in the skin. Briefly, DTH was induced by challenging the dorsal surface of the right pinna of previously sensitized animals with 2,4-dinitro-1-fluorobenzene. The course of inflammation was monitored by measuring increases in pinna thickness, and by histological quantification of leukocytes infiltrating the site of challenge. We observed that the stress-induced redistribution of leukocytes to the skin was accompanied by a significant increase in the rate, magnitude, and duration of the DTH reaction observed in the skin of stressed animals compared to unstressed controls. These results suggest that under certain conditions, acute stress may enhance immune surveillance and increase the capacity of the immune system to respond to challenge. (The MacArthur Foundation & The Arthur Vining Davis Foundation.)

548.7

LABORATORY AND NATURALISTIC STRESSORS INFLUENCE IMMUNE FUNCTIONING. M.D. Zaharia, A.V. Ravindran, J. Griffiths, G.J. Remington*, Z. Merali & H. Anisman. Dept Psychology, Carleton University & Dept of Psychiatry, Royal Ottawa Hospital, Ottawa, Ont. Canada and Neuropsychopharmacol. Res. Unit, Clarke Inst. of Psychiatry, Toronto, Ont. Canada.

Severe naturalistic and modest laboratory stressors have been shown to influence immune functioning. While the former have been associated with immunosuppression (reduced cell proliferation, natural killer cytotoxicity), the latter may provoke an immunoenhancement. In the present investigation we demonstrated that, among university students, laboratory stressors (e.g., mathematical challenge, stress interview) increased the number of NK cells without affecting either CD3, CD4, CD8 or CD19 cells. With a more profound naturalistic stressor (final academic exams), NK cell number increased to a greater extent, and an increase of CD3, CD4 and CD19 cells was also evident. In addition, the laboratory stressors provoked a marked increase of cell proliferation in response to both PHA and Con A. A still greater increase of cell proliferation was associated with the stress of the academic examination. The variations of lymphocyte numbers, as well as the stress-related alterations of cell proliferation, were unrelated to variations of plasma cortisol or ACTH. Likewise, it did not appear that the individual's previous stressor history impacted on the immune measures. However, the variations of cell numbers induced by the laboratory stressors tended to be more pronounced among dysthymic patients (i.e., chronic, low-grade depression) than in non-depressed university students. The results are related to the possibility that depressive illness and stress experiences in non-depressed subjects may be associated with activation of some aspects of the immune response.

548.8

MODULATION OF THE RECURRENCE OF HERPES LABIALIS BY EXPERIMENTALLY INDUCED DISGUST. A. Buske-Kirschbaum, A. Geiben, I. Gutberlet, C. Wermke, K.-M. Pirke, C. Kirschbaum, H. Lehnert* and D. H. Hellhammer. Center for Psychobiology and Psychosomatic Research, University of Trier, D-54286 Trier, Germany.

The present study was designed to determine whether emotional processes such as disgust might lead to altered immune function in patients which, as a result, is followed by manifestation of HSV symptoms. Healthy subjects (n=18) with with recurrent herpes simples virus (HSV) infection were recruited who (1) show more than five infections/year and (2) who suffer from HSV symptoms only after experience of strong disgust due to dirty dishes (glasses, plates). They were randomly distributed to two treatment groups. The experimental group (n=9) was exposed to five slides showing dirty glasses or plates. After presenting the aversive objects visually, the volunteers were confronted with the real objects previously presented by the slides. The control group (n=9) was treated identically with the difference being that these subjects were confronted with neutral slides as well as with neutral objects. Blood samples were obtained five minutes before and 30 minutes as well as two days after stimulus presentation. Furthermore, all subjects were examined for HSV symptoms by a physician two days after the experiment. Saliva was obtained at regular intervals before and during stimulus presentation. Moreover, two additional saliva samples were collected during the medical examination two days after the treatment. Two days after presentation of the aversive objects, four experimental subjects showed HSV symptoms. No symptoms, however, could be determined in control subjects. Furthermore, elevated tumor necrosis factor (TNF-α) and suppressed cortisol concentrations were observed on day 3 in the experimental group. However, no significantly altered distribution of lymphocyte subpopulations was found after stimulus presentation after 30 minutes or two days, respectively. These data suggest that psychological processes such as the experience of disgust against specific objects can lead to alteration of immune function which might be accompanied by HSV recurrence in sensitive patients.

548.9

DEPRESSIVE-LIKE BEHAVIOR AND INTERLEUKIN-6 (IL-6) DURING AUTOIMMUNE DISEASE. B. Sakić, H. Szechtman and JA Denburg. Depts Biomed Sci and Medicine, McMaster Univ, Hamilton, Ont, CANADA L8N 3Z5.

We suggested previously that a component of autoimmunity-associated behavioral profile (AABP) in MRL-lpr mice is depressive-like behavior. The present study examines this suggestion by employing a novel behavioral paradigm: the sucrose preference test, which had been proposed to measure decreased sensitivity to rewards, a core symptom of depression. A second aim of the study was to identify immune factors which may play a role in AABP. In 5 and 12 week old autoimmune MRL-lpr mice, the concentration-intake curve for sucrose was shifted, compared to the control MRL +/+ substrain. Pretreatment with an immunosuppressive agent obliterated the substrain difference, indicating involvement of autoimmune/inflammatory mechanisms and suggesting that autoimmune disease alters responsivity to "hedonic" stimulation. Considering that reduced sucrose intake in the MRL-lpr substrain was present before marked autoantibody production, their reduced sucrose preference is probably related to other immune factor(s). Mice with high titres of serum IL-6 showed low sucrose intake compared to mice without IL-6 bioactivity (due to immunosuppressive treatment). Results suggest that decreased sensitivity to reward precedes severe symptoms of autoimmunity, and chronic hyperproduction of IL-6 may be a factor in pathogenesis of AABP. (BS is OMHF Postdoctorate Fellow. Supported by NSERC)

548.10

SEVERE LIFE STRESS PREDICTS EARLY HIV DISEASE PROGRESSION AND LONGITUDINAL CHANGES IN IMMUNE STATUS

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Changes in components of immune physiology have been associated with some forms of stress in animals and humans. However, it is unknown whether stress can impact on the course of HIV disease. To test the hypothesis that life stress can influence HIV disease progression, we are conducting prospective studies in HIV-infected men. Using several different disease endpoints (e.g., CDC stage and clinical symptoms) , our data reveal that severe life stress increased the likelihood of disease progression by nearly four-fold as well as the severity of early disease progression. Stress was also associated with alteration in lymphocyte subsets comprising natural killer (NK) and cytotoxic T lymphocytes (CTL). These changes were most notable in combination with co-occuring depressive symptoms. This longitudinal, prospective study provides some of the first evidence that severe life stress may influence the course of both HIV disease and immunity. Ongoing study will provide an opportunity to determine the effects of severe stress on more advanced stages of HIV disease progression.

548.11

IMMUNE CORRELATES IN SUBTYPES OF DEPRESSIVE DISORDER. A.V. Ravindran*, J. Griffiths, Z. Merali and H. Anisman. Dept of Psychiatry, Royal Ottawa Hospital, and School of Psychology, University of Ottawa, Ottawa, Ontario, Canada.

Major depressive disorder has been associated with immune alterations, including reduced mitogen-induced cell proliferation, diminished natural killer (NK) cell activity, as well as variations of several lymphocyte subsets. These immune alterations may be related to the severity of the depression or may be secondary to symptoms that characterize the disorder (e.g., anorexia, sleep disturbances, increased stress perception). In the present investigation we demonstrate that plasma endocrines and lymphocyte subsets vary across subtypes of depression (major depression, atypical depression, and dysthymia). Plasma cortisol did not differ between groups, whereas plasma ACTH levels were modestly increased in major depression. As well, analysis of platelet serotonin receptors revealed an increase of paroxetine binding sites in major depressive patients. The number of circulating NK cells was increased in all depressive groups, while in both the major and atypical depressives the total lymphocytes, CD3 (pan T), CD8 (suppressor/cytotoxic) and CD19 (pan B) cells were increased as well. Dysthymia was also associated with increased variability and levels of IL1β in mitogen stimulated lymphocytes. In nondepressed patients stressors of increasing severity provoked a similar pattern of immunological change. The data are consistent with the proposition that depression may be associated with activation of some aspects of immune activity, and that such effects vary as a function of the depressive subtype. The possibility is considered that immune alterations may be related to the stress/anhedonia associated depression.

548.12

CYTOKINE-INDUCED ENDOCRINE, NEUROCHEMICAL AND BEHAVIORAL ALTERATIONS. S. Lacosta*, Z. Merali, and H. Anisman. Dept of Psychology, Carleton University & University of Ottawa, Ottawa, Ontario, Canada.

It has been hypothesized that cytokines, such as interleukin (IL)-1β and IL-2 may serve as a link between the immune and central nervous systems. Essentially, it is thought that cytokines secreted from activated immune cells may gain access to the brain, albeit to a limited extent, and may thus come to affect behavior. Moreover, it has been hypothesized that the brain may translate cytokine activation much as if it were a stressor. In the present investigation it was demonstrated that systemic administration of IL-1 and IL-2 (0.2-1.6 ug) elicited different behavioral and neurochemical changes. In particular, IL-1 influenced exploratory patterns, consumption of highly palatable diets and anxiety on a plus maze. In addition, this cytokine provoked an increase of plasma corticosterone and ACTH, and altered the levels and turnover of norepinephrine (NE) and dopamine (DA) in several brain regions. In contrast, while IL-2 elicited anhedonic-like effects, acute systemic administration of this cytokine did not elicit anxiety, or increases in plasma corticosterone and ACTH. Treatment with IL-2 was, however, found to influence hypothalamic and mesolimbic catecholamine activity. It appears that in addition to hypothalamic NE and DA variations, treatment with IL-1 and IL-2 may affect monoamine activity in several mesocorticolimbic sites. While differential neurochemical and endocrine effects are elicited by the two cytokines, both induce patterns of behavior which, although not entirely congruent with one another, are typically associated with stressors.

548.13

CORTICOTROPIN-RELEASING FACTOR TREATMENT UPREGULATES INTERLEUKIN-1 RECEPTORS IN THE MOUSE PITUITARY: REVERSAL BY DEXAMETHASONE. T. Takao*, C. Tojo, T.Nishioka, K. Hashimoto and E. B. De Souza. Second Department of Internal Medicine, Kochi Medical School, Nankoku 783, Japan (T.T., C.T., T.N., K.H.) and Neurocrine Biosciences, Inc., San Diego, CA 92121(E.B.D.S.)

The cytokine interleukin-1 (IL-1) alters a variety of immune, central nervous system and neuroendocrine activities characteristic of an integrator of the brain-endocrine-immune response to stress. In order to further characterize the mechanisms involved in stress-induced regulation of IL-1 receptors, we examined specific iodine-125-labeled human interleukin-1α ([^{125}I]IL-1α) binding following treatment with corticotropin-releasing factor (CRF) and dexamethasone (DEX), two major regulators of stress response. In addition, we studied plasma adrenocorticotropic hormone (ACTH) and corticosterone levels after CRF treatment. Plasma ACTH and corticosterone were increased at 2 h after i.p. injection of rat/human CRF (40 μg/kg/0.2 ml of saline) and returned to basal levels at 6 h after the injection. Intraperitoneal injection of r/h CRF resulted in a dramatic increase in specific [^{125}I]IL-1α binding in the pituitary at 2 and 6 h after the injection although it did not affect [^{125}I]IL-1α binding in the hippocampus, spleen and testis at any time after the injection. [^{125}I]IL-1α binding was unchanged at 2 h following DEX treatment (1 mg/kg/0.2 ml of 4% ethanol-saline) in the pituitary and the hippocampus. In contrast, DEX inhibited CRF-induced upregulation of IL-1 receptors in the pituitary at 2 h after the injection. These data demonstrate complex interactions between CRF and DEX on IL-1 receptors during stress.

548.14

ELECTROMAGNETIC FIELDS ALTER MELATONIN SYNTHESIS AND IMMUNE FUNCTION IN MAN. E.M. DeMet*, A. Chicz-DeMet, F. Chiappelli, J.R. Aniline, S.G. Matta, A.R. Shoho, and M.A. Kung. Dept. Psychiatry & Human Behavior, UC Irvine, CA 92717, Psychiatry Service, VAMC Long Beach, CA 90822, and School of Dentistry, Lab. Human Oral & Molec. Immunol., UC Los Angeles, CA 90095.

Epidemiological surveys have linked electromagnetic field (EMF) exposure to cancer, mental illness, birth defects, and Alzheimer's disease. Animal studies indicate that EMF inhibits pineal function, but it is unclear if a similar effect occurs in man. The present paired double-blind study examined EMF effects on salivary cortisol and melatonin levels in healthy volunteers. Changes in immune function were also measured as phytohemagglutin-stimulated interleukin-2 (IL-2) levels in T-lymphocytes. The natural earth's magnetic field was electrically balanced during sleep. No current was applied under control conditions. The applied EMF did not alter cortisol levels, but resulted in a 70% decrease in the nocturnal melatonin peak. A two-fold increase in mitogen stimulated IL-2 was also found which was not correlated with melatonin or cortisol changes. The results confirm that alterations in the earth's EMF inhibit melatonin synthesis in man. The EMF condition acts as an immuno-stressor although cortisol and melatonin may not be involved in this action.

548.15

CORTICOSTERONE AND METAPROTERENOL (BETA-ADRENERGIC AGONIST) INCREASE BREAST CANCER METASTASIS IN F344 RATS: POSSIBLE MEDIATION BY NATURAL KILLER CELLS S. Ben-Eliyahu*, S. Haim, G.G. Page, E. Rozen, G. Shakhar, I. Shtinebach. Dept. of Psychology, Tel Aviv University, Tel Aviv 69978, Israel.

Several hormonal and immunological mechanisms have been implicated in mediating adverse effects of stress on immunity and tumor development. Specifically, corticosteroids have been shown to suppress natural killer (NK) cell activity in vitro, and similar effect of adrenaline were reported by some but not other investigators. Nevertheless, the in vivo effects of these hormones on NK activity and the biological significance of such suppression on tumor development is controversial. Therefore, we studied the effects of corticosterone (CORT) and metaproterenol (MP, a bet-adrenergic agonist) in an in vivo system that is highly sensitive to NK cell activity in the context of host anti-metastatic activity. Rats were injected s.c. with either vehicle, 1, 3, or 9 mg/kg of CORT, or 1, 3 , or 9 mg/kg of MP. One hour later all rats were injected intravenously with 10^5 radiolabeled MADB106 tumor cells, and 16 hr later rats were sacrificed and their lungs removed to assess tumor retention . These tumor cells metastasize only to the lungs and their lung clearance and the number of consequent lung metastases are controlled by large granular lymphocytes (LGL)/NK cells: Selective depletion of LGL/NK cells causes approximately one-hundred fold increase in these two measures, and augmentation of LGL/NK activity reduces them. Our findings indicated that both CORT and MP significantly and markedly increase lung tumor retention in a dose-dependent manner. These findings suggest that both drugs suppress LGL/NK activity in vivo. Because these drugs are known to affect adhesion and redistribution of lymphocytes, these findings should be interpreted in consideration of these process which are studied now in our laboratory.

548.16

AUTOIMMUNE MICE CAN LEARN AVOIDANCE CONDITIONING L.M. Schrott* and L.S. Crnic. Depts. of Psychiatry and Pediatrics, Univ. of Colorado School of Medicine, Denver, CO 80262

Prior studies have demonstrated deficits in two-way active avoidance conditioning paradigms in autoimmune mice which are related to the degree of autoimmunity. To explore possible mechanisms of this deficit, autoimmune female NZB x NZW F1 hybrid (B/W) mice were tested in a one-way active avoidance conditioning paradigm in which there was no conflict situation and a low number of trials to minimize fatigue. B/W mice were able to learn the one-way paradigm as well as mice from the NZW control strain. No strain differences in the days to criterion or avoidance time were found. NZW mice did have more avoidances on the first test day ($p<.02$), but the strains were similar on the remaining days. Since B/W mice could learn one-way avoidance, a separate group was tested in two-way avoidance which had the parameters altered to avoid fatigue, clarify contingencies, and increase cue salience. B/W mice were also able to learn this task and did not differ from NZW mice in the days to criterion or number of avoidances. B/W mice had faster avoidance times ($p<.009$), as well as faster escape times on the first 2 days of testing ($p<.04$). There was some evidence of poorer retention in the B/W mice, as their performance was worse on the first 10 trials of each day than on the last 10 trials ($p<.002$). Thus, the two-way active avoidance deficit may be more the result of performance factors than actual cognitive problems. Recent evidence suggests that B/W display heightened anxiety and this may interfere with the learning complex tasks. Supported by MH49043, MH10643 and HD04024.

548.17

INCREASED ANXIETY BEHAVIORS IN AUTOIMMUNE MICE
L.S. Crnic* and L.M. Schrott. Depts. of Pediatrics and Psychiatry, Univ. of Colorado School of Medicine, Denver, CO 80262

The human autoimmune disorder SLE is accompanied by psychiatric symptoms including an increased incidence of anxiety. We compared the performance of autoimmune NZB x NZW F1 hybrid (B/W) mice to non-autoimmune NZW control mice on 3 measures of anxiety. B/W mice displayed an anxiety profile in the elevated plus maze as evidenced by avoidance of the open arms ($p<.0001$ for both percent entries and time). Separate groups of mice were water deprived and tested in the open field drink test. B/W mice made fewer approaches to the water bottle which was suspended in the middle of the field ($p<.02$) and spent less time drinking ($p<.003$) than controls. They also had an increase in freezing behavior ($p<.0007$). In a task designed to assess exploration of a novel object, B/W mice also displayed increased anxiety. They spent less time in the center portion of an open field ($p<.004$), had fewer contacts with the novel object ($p<.001$), and a lower contact duration ($p<.05$). Since these anxiety behaviors may be modulated by over-expression of cytokines that accompany the autoimmune disease, the NZW controls were injected with the cytokine IFN-α and tested on the elevated plus maze. The IFN treated mice displayed an anxiety profile similar to that seen in the autoimmune mice with less time spent in open arms ($p<.05$). Thus, autoimmune mice and mice exposed to the cytokine IFN-α display heightened anxiety which is characterized by avoidance of open and exposed places even when the motivation to explore these areas is great. This increased anxiety may interfere with learning in paradigms where aversive stimuli or conflict are present. Supported by MH49043, MH10643, and HD04024.

548.18

Acquisition and Retention of Conditioned Immunosuppression of IgM Production is Mediated by the Insular Cortex. V. Ramírez-Amaya*, B. Alvarez-Borda and F. Bermúdez-Rattoni. *Instituto de Fisiología Celular. UNAM, México, D.F. 04510.*

Conditioned Immunosuppression is obtained by the pairing of a gustative stimulus with an immunosuppressive drug, in this case cyclophosphamide, on subsequent presentation of the gustative stimulus alone the animal shows an attenuated immune response. In previous studies it was found that insular cortex lesions disrupt the acquisition of conditioned immunosuppression, as measured by the production of antibodies to sheep red blood cells. In the present work we evaluated the effects of NMDA induced lesions in the insular cortex and the amygdala of male Wistar rats, on conditioned immunosuppression. This was measured by the production of IgM to ovalbumin. It was found that insular cortex lesions clearly disrupt both the acquisition and retention of conditioned immunosuppression, as shown by higher titer of IgM antibodies to ovalbumin compared to conditioned controls. On the other hand, amygdala lesions partially disrupt the acquisition but not retention of conditioned immunosuppression. Additionally, it was observed that both lesions did not affect the normal immune response. These results suggest that the amygdala could be important for the acquisition and that the insular cortex is essential for both acquisition and retention of the conditioned immunosuppression. This research was supported by DGAPA IN201993.

548.19

FEMALE CHEMOSIGNALS MODULATE THE NUMBER OF BRAIN MAST CELLS IN MALE HAMSTERS. C.M. Novak* and A.A. Nunez. Dept. of Psychology & Neuroscience Program, Michigan State University, East Lansing, MI, 58824-1117.

Photoperiod and age modulate the number of mast cells found in the brains of male Syrian hamsters; more mast cells are found in the brains of older animals and animals housed in a long photoperiod. Thus, reproductive status correlates with increased brain mast cell numbers. In this experiment, the influence of sexual experience or exposure to female chemosignals on brain mast cell number was investigated. Adult male hamsters were given 2 hours of exposure to (1) a receptive female hamster in a test area, (2) female hamster vaginal secretions (FHVS) in the same test area, or (3) the test area alone. Animals were perfused immediately after exposure to each condition; their brains were sectioned and stained with toluidine blue. Peri-hippocampal and thalamic mast cell numbers were counted. Significantly more mast cells were found in the brains of animals exposed to the FHVS ($p<0.05$). The animals that mated did not have more brain mast cells than control animals. It is possible that the stress associated with the presence of female chemosignals in the absence of mating is responsible for the rise in mast cell number seen here. (Supported by NSF grant IBN9209473 to A.A.N.)

CARDIOVASCULAR REGULATION: DETERMINANTS OF SYMPATHETIC NERVE RESPONSE

549.1

THE EFFECTS OF PREGNANCY AND A PROGESTERONE METABOLITE [3α -OH-DIHYDROXYPROGESTERONE (DHP)] ON BAROREFLEX CONTROL OF RENAL SYMPATHETIC NERVE ACTIVITY (RSNA). S. Masilamani & C. Heesch*. Dept. of Physiology, The Ohio State Univ., Columbus, OH 43210.

Previous experiments in anesthetized rats suggest that pregnancy (P) results in an attenuated ability to reflexly increase RSNA. Studies have shown that 3α-OH-DHP potentiates central GABA-ergic mechanisms and P rats have elevated levels of 3α-OH-DHP. The present study obtained baroreflex (BX) curves in conscious rats by recording reflex changes in RSNA due to increases and decreases in mean arterial pressure (MAP) (iv phenylephrine & nitroprusside infusions): in P rats (control), and in virgin (V) rats 1) before (control); 2) 15 min (exp 1); and 3) 30 min (exp 2) after infusion (iv) of 3α-OH-DHP. BX measurements are shown below. (* = P< 0.05 vs V control).

	Baseline MAP (mmHg)	Midpoint (mmHg)	Max NA (mmHg)	NA (% increase from baseline)	Max Slope
V(control)	124 ± 6	121 ± 11	235 ± 35	135 ± 35	-3.56 ± 0.82
V (exp 1)	136 ± 7	124 ± 9	208 ± 34 *	106 ± 34 *	-2.68 ± 0.62
V (exp 2)	138 ± 9	125 ± 7	214 ± 35 *	114 ± 35 *	-2.24 ± 1.26
P (control)	103 ± 4 *	131 ± 8	121 ± 8 *	21 ± 8 *	-2.42 ± 0.47

The expected gestational hypotension was seen (decreased MAP) and the ability to increase NA (max NA & % increase from baseline NA) was virtually eliminated in P rats. In V rats, within 15 min of 3α-OH-DHP administration there was a diminished ability to increase NA (max NA & % increase from baseline NA) with 3α-OH-DHP (exp 1 & 2). These results demonstrate that pregnancy is associated with an inability to increase NA above baseline levels and that 3α-OH-DHP may in part participate in this diminished ability of P rats to increase NA. Supported by NIH grants RO1-HL 36245 and F32 HL 09183.

549.2

SUSTAINED ACTIVATION OF ARTERIAL BARORECEPTORS ALTERS THE BASIC PATTERN OF SYMPATHETIC NERVE DISCHARGE IN SPONTANEOUSLY HYPERTENSIVE (SH) RATS. M.J. Kenney*, D.E. Claassen and T. Hirai. Dept. of Anat. and Physiol., Kansas State University, Manhattan, KS 66506

Sustained increases in arterial pressure (AP) can induce prolonged inhibition of sympathetic nerve discharge (SND) which outlasts the period of stimulation. The aim of the current study was to determine if sustained increases in AP alters the basic pattern of SND. For this purpose we used autospectral and coherence analyses to 1) characterize the frequency components in basal SND (renal, n=4; splanchnic, n=6; lumbar, n=3) and 2) determine the frequency-domain relationships between SND and AP in chloralose-anesthetized, baroreceptor-innervated SH rats before and after sustained (30 min) phenylephrine (PE)-induced increases (50 mmHg) in AP. During control before PE infusion, 40 ± 3% of total power was contained in the frequency band which corresponded to the frequency of the heart rate (6-9 Hz) and the coherence value relating AP to SND was 0.90 ± 0.01. After the sustained increase in AP, despite the return of AP to control levels, only 20 ± 2% of total power was contained in the 6-9 Hz frequency band and the coherence value was reduced to 0.49 ± 0.09. The intracerebroventricular administration of PE did not alter the basic pattern of SND (n=4). These results indicate that sustained activation of arterial baroreceptors can alter the frequency components of SND in SH rats. Supported by NIH HL48564.

549.3

COHERENCE OF EEG, RESPIRATION, AND HEART RATE VARIABILITY
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Magnitude squared coherence (MSC) provides a method to evaluate the extent of a linear relationship among signals as a function of frequency. We hypothesize that, since physiologic mechanisms which govern EEG, respiration, and heart rate variations are organized at the level of the brainstem, there should be a high coherence among these signals at the respiratory frequency. We measured EEG (using standard 10-20 electrode locations), respiration (using pneumatic bellows around the chest), and heart rate variability (using ECG lead II), from normal volunteers, 22-26 years old. Subjects were supine, breathing at a self-paced regular rate with eyes closed.

Because breathing was at a regular (self-paced) rate, the power spectrum of the respiration signal routinely produced a clear peak at the respiratory frequency (0.1-0.3 Hz). A high coherence (0.8-1.0) was routinely observed between EEG and heart rate variability, EEG and respiration, and respiration and heart rate variability, at the respiratory frequency. Low coherence values (< 0.3) were computed at other frequencies. Coherence with EEG was widely distributed on the scalp. Preliminary data indicate that physiologic and behavioral manipulations can affect the level of coherence among EEG, respiration, and heart rate variability.

These data demonstrate that EEG, respiration, and heart rate variations are highly correlated at respiratory frequencies in normal subjects.

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549.4

NON-RESPIRATORY SLOW COHERENT RHYTHMS (0.2-2 Hz) IN MULTIPLE SYMPATHETIC NERVE DISCHARGES.
Q.P. Yu, W. -X. Huang, M.I. Cohen* and P.M. Gootman. Dept. of Physiol., Albert Einstein Col. Med., Bronx NY 10461.

Previous study has shown that sympathetic (symp.) nerve discharges have both fast rhythms (2-6 Hz or "3-Hz rhythm" and 7-13 Hz or "10-Hz rhythm"), and slower rhythms (<2 Hz) that are presumably respiratory-related. The present study examined the possible existence of symp. non-respiratory components in these rhythms. In 31 midcollicular decerebrate, vagotomized, paralyzed cats, simultaneous recordings were taken from phrenic and 2-4 symp. nerves (cervical, splanchnic, inferior cardiac). Partial coherence analyses were used to distinguish slower symp. rhythms that were not respiratory related. All ordinary coherences (symp.-phrenic, symp.-symp., symp.-(inspiratory-tag)). had components in the range of 0.2-2 Hz, in addition to the 3 or 10 Hz peaks in symp.-symp. coherences. After partialization with the phrenic or inspiratory-tag signal, slower components in the symp.-symp. coherences were reduced but not eliminated. Further, these slower components still remained during hypocapnic apnea (abolitition of normal phrenic rhythm). These results reveal for the first time that different symp. nerve discharges have coherent 0.2-2 Hz rhythms that are not related to respiration. (Supported by N.I.H. Grant HL-27300.)

549.5

CENTRAL ADRENERGIC NEURONS PLAY A ROLE IN EXPRESSION OF THE 10-HZ RHYTHM IN SYMPATHETIC NERVE DISCHARGE (SND).
S. Zhong*, H.S. Orer, S.M. Barman and G.L. Gebber. Dept. Pharmacol./Tox., Michigan State Univ., E. Lansing, MI 48824.

We tested the hypothesis that central adrenergic neurons are important for expression of the 10-Hz rhythm in SND of urethane-anesthetized, baroreceptor-denervated cats. In 15 cats in which the 10-Hz rhythm in inferior cardiac nerve activity was weak or absent, iv injection of the α2-adrenoceptor antagonists idazoxan (100 μg/kg) or rauwolscine (200 μg/kg) enhanced or induced the 10-Hz rhythm without affecting power at frequencies $\leq$ 6 Hz (demonstrated with autospectral analysis). Amphetamine (1-3 mg/kg iv) also enhanced or induced the 10-Hz rhythm without marked changes in power at frequencies $\leq$ 6 Hz (n = 5). In six cats in which the 10-Hz rhythm was prominent, prazosin (300 μg/kg iv), an α1-adrenoceptor antagonist eliminated the 10-Hz rhythm without changing power in SND at frequencies $\leq$ 6 Hz. These results support the view that central adrenergic neurons have a facilitatory effect on the 10-Hz rhythm. We propose that this effect is mediated through α1-adrenoceptors on their postsynaptic targets. Facilitation of the 10-Hz rhythm by idazoxan and rauwolscine may reflect enhanced activity of central adrenergic neurons perhaps by block of their α2-adreno-autoreceptors. Amphetamine is presumed to have released central catecholamines, thus facilitating the 10-Hz rhythm in SND. (Supported by NIH grants HL-33266 and HL-13187.)

549.6

SELECTIVE DEPRESSION OF THE 10-HZ RHYTHM IN SYMPATHETIC NERVE DISCHARGE (SND) BY CLONIDINE.
H.S. Orer*, S. Zhong, S.M. Barman, and G.L. Gebber. Dept. Pharmacol./Tox., Michigan State Univ., E. Lansing, MI 48824.

The current study on urethane-anesthetized, baroreceptor-denervated cats is the first to show that clonidine selectively depresses the 10-Hz rhythm in SND when it co-exists with irregular 2- to 6-Hz oscillations. As revealed by autospectral analysis, power in the 10-Hz band of inferior cardiac nerve activity was dose-dependently (3-30 μg/kg, iv) reduced whereas power at frequencies $\leq$ 6 Hz was not changed. Following a cumulative dose of 30 μg/kg iv, 10-Hz power was reduced to 18 $\pm$ 3% of control while power at frequencies $\leq$ 6 Hz was 97 $\pm$ 8% of control (n = 6). These effects were reversed by iv idazoxan or rauwolscine which are α2-adrenoceptor antagonists. Inhibition of the 10-Hz rhythm by clonidine was accompanied by a reduction in mean blood pressure from 87 $\pm$ 7 to 72 $\pm$ 7 mm Hg. The effects of iv clonidine on SND were mimicked by microinjection of the drug into the caudal ventrolateral medulla. In contrast, microinjection into the rostral ventrolateral medulla depressed both frequency components. The power in SND at frequencies $\leq$ 6 Hz was significantly depressed by iv clonidine in 5 cats in which the 10-Hz rhythm was absent. These results imply that the 10-Hz rhythm generator exerts an inhibitory influence on the circuits responsible for 2- to 6-Hz oscillations. Depression of the 10-Hz rhythm by clonidine would release this inhibition, thereby counterbalancing the direct depressant effect of the drug on the low frequency components in SND (Supported by NIH grants HL-33266 and HL-13187.)

549.7

SPINAL NETWORK CONTRIBUTE TO THE GENESIS OF LOW FREQUENCY RHYTHM PRESENT IN RR INTERVAL VARIABILITY OF ACUTE SPINAL CATS.
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Spectral analysis of RR interval and arterial pressure (AP) variabilities detects two major components at low (LF; 0.04-0.15 Hz) and high (HF; 0.25 Hz) frequency. HF is related to respiration and considered a marker of vagal modulation while LF is a marker of sympathetic modulation. In decerebrate cats, spectral analysis of cardiac sympathetic efferent discharge (SND), in the range between 0 and 0.5 Hz, showed an LF oscillation, highly correlated with the LF component detectable in RR interval variability. Moreover in sino-aortic denervated cats, we have recently observed an LF component in the discharge variability of single medullary neurons, belonging to areas involved in the regulation of sympathetic outflow. To evaluate whether an LF rhythm might also originate from the spinal cord independently of brainstem, we recorded SND, ECG and AP in 18 decerebrate cats, 3 hrs after spinal section at C1 level and bilateral cervical vagotomy. In a subgroup of 5 cats recordings were also performed after a subsequent C7-T7 dorsal root section (DRS). Autoregressive spectral analysis of RR and SND variabilities revealed a major LF oscillation (0.07 $\pm$ 0.02 and 0.09 $\pm$ 0.01 Hz; 57 $\pm$ 6 and 33 $\pm$ 3 nu, respectively) and a smaller HF ventilatory-related oscillation (0.32 $\pm$ 0.01 and 0.31 $\pm$ 0.01 Hz; 27 $\pm$ 4 and 22 $\pm$ 2 nu, respectively). DRS did not significantly modify the spectral profile of both signals. These data seem to suggest that spinal structures might contribute to the genesis of the LF oscillation detectable in RR interval variability, independently of peripheral modulation of afferent sympathetic cardiovascular fibers.

549.8

THE DISCHARGE PATTERN IN RENAL SYMPATHETIC NERVE ACTIVITY IN CONSCIOUS RATS.
T. Kunitake, A. Shimokawa, M. Saita, K. Katoh, T. Hanamori and H. Kannan*. Department of Physiolgy, Miyazaki Medical College, Miyazaki, 889-16, Japan

We investigated the change of discharge pattern in renal sympathetic nerve activity (RSNA) in conscious rats. Power spectrum analysis, employing a discrete Fast Fourier Transform algorithm, was used to quantify periodicities present in synchronized RSNA. In conscious rats with intact baroreceptors, RSNA was characterized by four frequency components occurring at about 0.5, 1.5, 6 and 12 Hz, which corresponded to the low-frequency fluctuation of heart rate, respiration, frequency of heart beat and its harmonics, respectively. After infusion of sodium nitroprusside (SN), the power for the component at 6 Hz which was substantially shifted with the frequency of heart beat, was significantly increased, while that for the three other components was attenuated. In sinoaortic denervated (SAD) conscious rats, all four components were abolished. We also performed the non-sequential analysis of both the peak to peak interval of RSNA and the relationship between RSNA and cardiac cycle. After detecting the peak in integrated RSNA by using the differential method, the peak to peak interval was measured. The time lag of each peak from the preceding R wave of electrocardiogram was also measured. The scatter diagram of peak to peak interval against time after R wave was made. In conscious rats with intact baroreceptors, three discrete areas that included almost all points were demonstrated on the scatter diagram. After infusion of SN, these three discrete areas persisted while their centers were slightly shifted. In SAD conscious rats, no discrete area appeared, those points were dispersed on the axis of time after the R wave. These findings suggest that 1) four frequency components exist in the synchronized RSNA of conscious rats, 2) the variation of RSNA at a frequency of heart beat is dominant during unloading of baroreceptor, and 3) arterial baroreceptors are responsible for generating the periodicity.

549.9

MUSCLE REFLEX INCREASES DISCHARGE OF SYMPATHETIC EFFERENTS. I.M. Hill, C.M. Adreani, C.L. Stebbins*, M.P. Kaufman. Division of Cardiovascular Medicine, University of California, Davis, CA 95616.

Two neural mechanisms contribute to exercise-induced increases in cardiovascular and ventilatory function. The first, central command, proposes a parallel activation of central locomotor and medullary circuits controlling cardiovascular and ventilatory function. The second, the muscle reflex, proposes contraction-activated groups III and IV fibers increase cardiovascular and ventilatory function. In humans, whole nerve recordings of sympathetic discharge suggest that central command increases sympathetic outflow to skin but not to skeletal muscle and that the muscle reflex increases sympathetic outflow to skeletal muscle but not to skin. We, therefore, tested the hypothesis that the muscle reflex, but not central command, increases the discharge of single sympathetic postganglionic efferents innervating the triceps surae muscles of unanesthetized, decerebrate cats. Hexamethonium (2-20 mg/kg; iv) abolished the spontaneous and evoked activity, verifying that nerve recordings were sympathetic. The discharge of 13 efferents was increased by contraction (0.6 ± 0.2 to 1.0 ± 0.3 imp/sec; $P < 0.05$) but was not increased by stimulation of the mesencephalic locomotor region (MLR) (0.6 ± 0.2 to 0.8 ± 0.2 imp/sec; $P > 0.05$). In 9 efferents, MLR stimulation before phentolamine (2 mg/kg; iv) did not increase efferent discharge (0.5 ± 0.6 to 1.0 ± 0.6 imp/sec; $P > 0.05$) whereas MLR stimulation after phentolamine did (1.2 ± 0.6 to 3.2 ± 0.6 imp/sec; $P < 0.05$). We conclude that the muscle reflex stimulates sympathetic post-ganglionic efferents innervating the vasculature of skeletal muscle. Furthermore, baroreceptors buffer MLR-induced increases in the discharge of these efferents.

549.10

EXCITATORY AMINO ACID CONCENTRATIONS IN THE CAT SPINAL DORSAL HORN INCREASE DURING STATIC MUSCLE CONTRACTION. G.A. Hand, G.L. Kramer, F. Petty, G.A. Ordway, and L.B. Wilson*. The Moss Heart Center and Depts. of Physiology and Psychiatry, UT Southwestern Medical Center, Dallas, TX, 75235.

In anesthetized cats, static hindlimb muscle contraction reflexly increases mean arterial pressure (MAP) and heart rate (HR). Evidence suggests that excitatory amino acids (EAAs) are involved in the spinal transmission of this reflex. We tested the hypothesis that static contraction of the triceps surae muscle increases the extracellular concentration of glutamate (GLU) and aspartate (ASP) in the L7 dorsal horn of the spinal cord. With the exception of the L7 dorsal root, the L5 through S2 spinal roots were cut ipsilateral to the contracting muscle. After the insertion of the microdialysis probes and a 3-hour recovery period, a 2-minute static contraction was evoked. MAP and HR increased by 53±8 mmHg and 20±4 bpm. The concentration of GLU increased 324±59 to 857±80 nM, while ASP increased 199±57 to 499±113 nM. GLU and ASP rose by similar amounts in 2 subsequent contractions. In these latter contractions, MAP and HR also increased significantly. By contrast, in cats whose L7 dorsal roots were cut after the first contraction, neither MAP, HR, GLU, nor ASP were significantly increased over baseline levels. These data demonstrate that static contraction of the hindlimb increases the extracellular concentration of GLU and ASP in the dorsal horn. Further, this study suggests that these two EAAs are involved in the spinal transmission of sensory information from the hindlimb muscle.

549.11

INDUCTION AND INHIBITION OF MEMBRANE POTENTIAL OSCILLATIONS IN RAT SYMPATHETIC PREGANGLIONIC NEURONES (SPN) BY NEUROTRANSMITTERS S.D. Logan*, M.F. Nolan, C. Cammack, I.C. Gibson and D. Spanswick. Department of Biomedical Sciences, Marischal College, Aberdeen, AB9 1AS, UK.

We have previously described spontaneous membrane potential oscillations in a population of spinal neurones which were presumed to be SPN. These oscillations could give rise to bursting or beating patterns of spike discharge and we have suggested that they result from electrotonic coupling between neurones.

Using the whole-cell recording technique in young rat spinal cord slices 26% of sympathetic preganglionic neurones show spontaneous membrane potential oscillations. These oscillations consist of trains of biphasic waves which we have termed spikelets because of their similarity to truncated action potentials. Spikelets are composed of a brief depolarising transient followed by a longer hyperpolarising phase.

A further 35% of SPN could be made to oscillate by the application of excitatory neurotransmitters such as L-glutamate acting at both ionotropic and metabotropic receptors, by substance P (1-5μM) acting at NK_1 receptors and acetylcholine acting at nicotinic receptors. The oscillations are inhibited by GABA (250-400μM), muscimol (1-10μM) and baclofen (100μM), noradrenaline (5-20μM), carbachol (10-100μM) and by anaesthetics, α-chloralose (100μM) and alphaxalone/alphadalone (5-50μM).

The actions of neurotransmitters in inducing oscillations means that over 60% of the SPN in this study were capable of exhibiting rhythmogenesis. The ability of groups of electrotonically-coupled SPN to generate spontaneous discharges within the spinal cord provides a novel mechanism for the integration and synchronisation of information within the sympathetic nervous system.

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549.12

Preferential Uptake of the Binding Fragment of Tetanus Toxin (TTC) by GABAergic Terminals in the Intermediolateral Cell Column (IML). An Immuno-Electronmicroscopic Study. M.A. Ligorio* and J.B.Cabot. Dept. of Neurobiology & Behavior, SUNY at Stony Brook, NY 11794-5230.

Tetanus is an acute infection marked not only by tonic muscle spasms, but also by potentially fatal symptoms characteristic of a hyperactive sympathetic nervous system; including, tachycardia, hypertension, and an elevated metabolic rate. Using local tetanus as an experimental model, Paar and Wellhöner (*Naunyn-Schmiedeberg's Arch. Pharmacol.* 276, 437-445, 1973) correlated an increase in sympathetic preganglionic neuron (SPN) output with a loss of inhibition in spinal polysynaptic reflexes. Tetanus toxin (a 150 kD heterodimer with a proteolytic subunit) may mediate these effects by specifically blocking the release of inhibitory neurotransmitters from axon terminals in the IML. The direct transport of TTC into the IML has previously been demonstrated (Cabot et al., Neuroscience 40, 805-823, 1991), but whether or not TTC is sequestered by terminals containing inhibitory neurotransmitters remains in question.

To test this hypothesis TTC was injected into the right superior cervical ganglion of male Sprague Dawley rats. After 2 days rats were perfused, and the first three thoracic spinal cord segments were processed for electron microscopy. Pre-embed immunoperoxidase and post-embed immunogold histochemistry were combined to determine if TTC and GABA colocalized in axon terminals in the IML.

Several non-overlapping photomontages of the IML were analyzed. An average of 74% ± 2.8% (SE) of all putative GABAergic (GABA+) terminals also contained TTC (TTC+). Contrarily, all terminals that were **not** GABA+ only had a 51% ± 1.7% (SE) chance of being TTC+. Furthermore, TTC and GABA were colocalized in a subpopulation of terminals closely apposed to identified SPN processes. Corresponding TTC+ percentages of this subsample were statistically indistinguishable from the IML averages (75% ± 9.7% (SE) for GABA+ terminals; 47% ± 6.4 (SE) for non GABA+ terminals). Assuming the presence of TTC in a given terminal means the same terminal would be poisoned by the native toxin in an actual case of tetanus, these results suggest that the selective uptake of tetanus toxin by GABAergic terminals is an important basis for the observed disinhibition of SPNs during tetanus. Supported by HLBI Merit award HL24103 to JBC.

549.13

RESPONSES OF SYMPATHETIC PREGANGLIONIC NEURONES (SPNs) IN A NEONATAL RAT BRAINSTEM-SPINAL CORD PREPARATION TO APPLICATION OF ATP. Susan A. Deuchars* Royal Free Hospital School of Medicine, Rowland Hill St., London. NW3 2PF U.K.

ATP acting via P_2-purinoceptors can elicit various responses while metabolism of ATP to adenosine may cause secondary effects via P_1-purinoceptors (Ziganshin, Hoyle and Burnstock, Drug Dev. Res. 32, 134-146, 1994). Autoradiographic studies have indicated the presence of a relatively high density of P_{2x}-purinoceptors in the intermediate zone of the spinal cord (Bo and Burnstock, Neuroreport 5, 1601-1604, 1994). Therefore the effects of ATP (and its metabolite adenosine) on SPNs have been studied.

Neurones verified physiologically and histologically as SPNs were recorded using the whole cell patch technique (Deuchars, Morrison and Gilbey, J. Physiol. in press, 1995). Drugs were applied either by superfusion or iontophoresis in the vicinity of an SPN. A separate multibarrelled electrode was advanced close to the patch electrode until applications of glutamate caused a brisk response in an SPN. ATP was then applied and caused membrane potential changes in eight of eleven SPNs. These consisted of a slow depolarisation and a decrease in input resistance (n = 2) or a slow depolarisation followed by a hyperpolarisation lasting up to 300s accompanied by an increase in membrane resistance (n = 6). The later response may be elicited by adenosine derived from the metabolism of ATP since applications of adenosine caused only a slow hyperpolarisation. The receptors involved in mediating these responses are under investigation. Supported by the BHF and the Wellcome Trust

549.14

METABOTROPIC GLUTAMATE RECEPTOR AGONIST, L-AP4, REDUCES MEDULLARY-STIMULUS EVOKED EPSPs IN NEONATAL RAT SYMPATHETIC PREGANGLIONIC NEURONS. B. Bhattacharyya* and S.F. Morrison. Dept. of Physiology, Northwestern University Medical School, Chicago, IL 60611.

The excitatory input from neurons in the rostral ventrolateral medulla (RVLM) maintains the basal discharge of spinal sympathetic preganglionic neurons (SPNs), is mediated primarily by glutamate and is important for the maintenance of resting arterial pressure. We used the *in vitro*, neonatal rat brainstem-spinal cord preparation to examine the effect of the metabotropic glutamate receptor agonist, L-AP4, on the EPSPs evoked in upper thoracic SPNs by stimulation of the RVLM (twin pulses, 0.8 ms duration, 6 ms apart, 200 μA, 0.3 Hz). Whole-cell patch recordings of SPNs yielded resting membrane potentials with a mean of - 47 ± 2 mV and RVLM stimulus-evoked EPSPs with a mean peak amplitude of 11 ± 3 mV at a holding potential of - 72 ± 1 mV. Bath-application of L-AP4 (10μM) reduced the EPSP amplitude by 71 ± 5 % (n=7) after 5 minutes. Following a 30-minute wash, simultaneous application of the antagonist, MAP4 (200μM), inhibited the ability of L-AP4 (10μM) to depress the evoked EPSP (reduction in amplitude: 11 ± 6 %, n=4). These data indicate the importance of a (potentially) presynaptic metabotropic glutamate receptor in regulating the response of SPNs to their primary excitatory input from the RVLM. Supported by NIH HL-47196.

549.15

DO SYMPATHETIC PREGANGLIONIC NEURONS RECEIVE SYNAPSES FROM INTRASPINAL CATECHOLAMINE NEURONS?

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Sympathetic preganglionic neurons (SPN) receive synapses from nerve fibers containing a variety of transmitters, including monoamines. Catecholamine synapses are thought to arise from supraspinal neurons, but dopamine synapses, which could come from intraspinal neurons, also occur in the intermediolateral cell column. To investigate whether SPN receive intraspinal catecholamine synapses, we transected the spinal cords of rats at T5. After 7-8 days, we injected cholera toxin B (CTB) into the adrenal medulla. Fourteen days after transection, the rats were perfused. Control rats received CTB injections but not transections. Sections of spinal cord from below the transection and from control segments were reacted for either tyrosine hydroxylase (TH)- and CTB-immunoreactivity simultaneously or TH-immunoreactivity alone. TH-positive fibers was present in the intermediolateral cell column below the transections, but there were fewer than in controls. SPN in both spinal and control rats received synapses from TH-immunoreactive boutons. Since degenerating nerve terminals in the intermediolateral cell column have virtually disappeared by 7 days after transection, these results suggest that some, but not all, of the catecholamine synapses on SPN come from intraspinal neurons. However, it is possible that non-catecholaminergic neurons synapsing on SPN could begin to express TH in response to the injury.

549.16

NADPH-DIAPHORASE REACTIVE SYMPATHETIC PREGANGLIONIC NEURONS & POSSIBLE INTERACTIONS WITH CATECHOLAMINERGIC FIBERS IN HUMAN SPINAL CORD. L. L. Smithson, K. G. Smithson* and E. E. Benarroch. Departments of Neurology and Anesthesiology, Mayo Clinic, Rochester, MN 55905.

Sympathetic preganglionic neurons (SPN)s at T6 to T10 levels are critical for maintenance of postural normotension in humans. Depletion of these neurons produces severe orthostatic hypotension. SPNs are nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) reactive. We sought to determine the distribution of NADPH-d reactive SPNs in human spinal cord and their interactions with descending catecholaminergic fibers, as identified by tyrosine hydroxylase (TH) immunoreactivity. Thoracic spinal cords were obtained at autopsy from one woman and two men (age 62-93). Fixed coronal, horizontal, and sagittal 50 μm sections were obtained at T4, T8, and T10 levels. Single sections were reacted for NADPH-d, TH, or both, and examined under bright field microscopy. Abundant NADPH-d reactive SPNs were observed at all segments examined, forming clusters concentrated in the intermediolateral cell column (IML) but also present in the adjacent white and gray matter. NADPH-d SPN processes ramified both mediolaterally and rostrocaudally, extending to adjacent SPN cell clusters. TH-immunoreactive fibers formed a loose bundle primarily located in a lateral column, adjacent to the IML. TH fibers were abundantly distributed within the NADPH-d reactive neuropil; some TH-immunoreactive varicosities appeared to directly contact NADPH-d reactive SPNs. Thus, adjacent clusters of NADPH-d SPNs appear to receive direct innervation from descending catecholaminergic innervation (presumably from C1 and A5 groups). These features may be important for mechanisms of normal maintenance of orthostatic normotension in humans and their potential involvement in clinical autonomic disorders. Supported by NIH PO1 NS32352.

549.17

CALRETININ-IMMUNOREACTIVITY IDENTIFIES A SUBPOPULATION OF ADRENAL MEDULLARY PREGANGLIONIC NEURONS IN THE CAT THAT INNERVATE ONLY NORADRENERGIC CHROMAFFIN CELLS. S.L. Edwards, C.R.Anderson* and R.M. McAllen[v] Department of Anatomy and Cell Biology and [v]The Howard Florey Institute, University of Melbourne, Parkville, Australia, 3052.

Immunohistochemistry of sections of cat adrenal gland showed that calretinin-immunoreactive (IR) nerve terminals were abundant in the medulla but had a patchy distribution. In sections stained for phenylethanolamine-N-methyl transferase (PNMT)-IR, the patches of calretinin-IR terminals were found to coincide exclusively with cords of chromaffin cells lacking PNMT-IR. Synaptophysin immunohistochemistry was used to identify the entire population of nerve terminals in the adrenal medulla. In combination with calretinin-IR, it was apparent that most, but not all, synaptophysin-IR terminals within patches of calretinin-IR terminals contained calretinin. Retrograde-labelling from the adrenal medulla using cholera toxin subunit B labelled preganglionic neurons between T4 and L1. One third of the labelled preganglionic neurons were also immunoreactive for calretinin. Although there was a major overlap, there was a clear difference in the distribution of retrogradely labelled preganglionic neurons with calretinin-IR compared to those lacking it. The distribution of the former was skewed caudally with the maximum numbers in T9 while the distribution of the latter was skewed rostrally with the maximum numbers in T7. Thus calretinin-IR marks a population of preganglionic neurons innervating noradrenergic chromaffin cells in the adrenal medulla, although calretinin is not found exclusively in these neurons as calretinin-IR was found in neurons in the intermediolateral nucleus from T1 to L5. This demonstration of separate populations of preganglionic neurons innervating the two classes of chromaffin cells provides for the first time evidence of the organisation of preganglionic neurons underlying the differential release of adrenaline and noradrenaline by the adrenal medulla in response to different physiological challenges.

549.18

STELLATE GANGLIA MODULATE THE CARDIOVASCULAR (CV) RESPONSES TO CHEMICAL STIMULATION OF SENSORY RECEPTORS IN THE LEFT VENTRICLE OF PIGLETS. Shiwei Tong, Phyllis M. Gootman*, Isaac D.Frasier Susan Ingenito, and Norman Gootman. Dept. of Physiol. SUNY- Health Science Center at Brooklyn, Brooklyn, NY 11203.

CV responses elicited by left ventricular (**LV**) injection of veratrum alkaloids (**VVA**) in developing piglets included bradycardia, vasodilation and hypotension; the responses were abolished following bilateral vagotomy (Gootman, et al. Am. J. Physiol. 1986;251:H748.). However, it is unknown whether cardiac sympathetic innervation modulate these CV responses. The present study was carried out in Saffan-anesthetized, paralyzed, thoracotomized, artificially ventilated (100% O_2) pigs (3-4 wks old) to determine whether left stellate ganglionectomy (**LSG**) or right stellate ganglionectomy (**RSG**) altered responses to LV injections of VVA (20 μg/kg). Aortic pressure (**AoP**) and left ventricular pressure (**LVP**) were recorded on a Grass Model 7C polygraph and on VCR tape for later analyses. Either RSG or LSG was carried out in each animal studied. At 30 min after RSG or LSG, a saline-control (0.1 ml/kg) injection was made via a catheter in the LV; 15 min later, VVA (20 μg/kg, 0.1 ml/kg) was rapidly injected (within 2-3 sec). Injections of VVA elicited greater decreases in mean AoP (15 ± 4 mmHg) and LVP (10 ± 2 mmHg) in the LSG group than in the RSG group ($p<0.05$). Heart rates were slower in the RSG group as compared to LSG group. These results indicate a greater role for the left stellate ganglion in modulating ventricular inotropic variables. This study provides further support of a laterality difference in postnatal development of cardiac sympathetic innervation (Tong, et al., Lab. Animal Sci. 1995, in press and Gootman et al., J. Auton. Nerv. Syst. 1992;38:191.). (Supported by NIH grant HD-28931)

549.19

SERIAL SECTION ULTRASTRUCTURAL ANALYSIS OF SYNAPTIC INPUTS TO DENDRITES AND CELL BODIES OF DYE-FILLED NEURONS IN GUINEA-PIG SYMPATHETIC GANGLIA. I.L. Gibbins*, H. Rodgers and S. Matthew. Department of Anatomy & Histology, and Centre for Neuroscience, Flinders University, SA 5042, Australia.

Sympathetic ganglion cells receive multiple convergent synaptic inputs from spinal preganglionic neurons. Although most ganglion cells have well developed dendritic trees, little is known of the distribution of synapses along the dendrites. Therefore, we injected neurobiotin or Mini-ruby into single sympathetic ganglion cells to reveal the full extent of their dendritic trees and prepared the neurons for electron microscopy. The distribution of synapses onto the cell body and dendrites of six cells were reconstructed from runs of up to 400 serial sections. Altogether, more than 1000 μm of dendrites and most of three cell bodies were reconstructed in detail. Synapses were rare, with most of the neuronal surface being covered with Schwann cells. Synapses usually formed small clumps, most often around the origins of dendrites, but also up to 100 μm from the cell body. On average, there was about one synapse per 25 μm of dendritic length. Up to 25% of synapses were made onto the cell body or proximal dendrites within 10 μm of the cell body. Most of the synaptic boutons surrounding a cell body actually made synapses onto the dendrites of other neurons in the neighbourhood. A typical neuron with a dendritic tree of 800 - 1200 μm total length would be unlikely to receive more than 100 synapses. Since a neuron of this size would have up to 10 convergent inputs, each input must form only a small number of synapses with the target neuron. The low density of axodendritic synapses, combined with the short electrotonic lengths of the dendrites, suggests that the function of the dendrites has little to do with either providing physical space for synapses or modulating the electrical integration of postsynaptic potentials.

550.1

CNS NEURONS CAPABLE OF GLOBAL CONTROL OF THE SYMPATHETIC NERVOUS SYSTEM. A.S.P. Jansen[1], V. Karpitskiy, T.C. Mettenleiter[2], and A.D. Loewy[1]. Dept. Anatomy and Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110 and [2]Fed. Research Centre for Virus Diseases of Animals, Friedrich-Loeffler Institutes, D-17498 Insel Riems, Germany.

Two different genetically engineered forms of the Bartha strain of pseudorabies virus (PRV) were used as transneuronal tracers to identify a common set of CNS neurons that regulate two different sympathetic outflow systems - one affecting the heart and the other controlling the adrenal gland. Both PRV strains contained a unique cellular marker so that they could be used together to visualize dually infected neurons by a double color fluorescence immunohistochemical staining procedure. Experiments were done in which one virus was injected unilaterally into the stellate ganglion and the other into the ipsilateral adrenal gland (or vice versa). After 4 days survival, the rats were perfused. In the brainstem, double labeled neurons were localized in the rostral ventrolateral medulla, rostral ventromedial medulla and caudal raphe nuclei. In the pons, double labeled neurons were found in the region of the A5 cell group and subcoeruleus area. A restricted part of the caudal ventrolateral periaqueductal gray matter contained double labeled neurons. In the hypothalamus, double labeled neurons were found in the paraventricular and lateral hypothalamic nuclei. This study shows that several specific areas in the brain contain neurons that are potentially able to modulate the sympathetic outflow to both adrenal gland and stellate ganglion. These neurons may cause a widespread coordinated multisystem visceral response during life-threatening situations (e.g., fight-or-flight response) or coordinate global sympathetic changes seen in the sleep-wakefulness cycle.

550.2

LABELING OF DIFFERENTIAL NEURONAL POPULATIONS AFTER VIRAL INFECTION OF THE DIFFERENT PARTS OF THE RAT HEART. G.J. Ter Horst* and M.H.J. De Jongste. Dept. Biol. Psychiat. and Thoraxctr. Univ. and Acad. Hosp. Groningen. Oostersingel 59, Groningen, The Netherlands.

Our knowledge of the anatomy of cardio-vascular neuronal networks is limited. We have now characterized the locations in the brainstem and thoracic spinal cord of neurons that may modulate the functioning of the various parts of the rat heart, using the retrograde transneuronal viral labeling method. Also, the link of the neurons to the ortho- or parasympathetic heart innervation was established. To do this, Th1 transsections were preformed. *Method:* Pseudorabies virus solution was deposited in the right (RV;n=10) or the left ventricular (LV;n=14) myocardium, and in the right atrium (RA;n=10). Four days after the infection the animals were fixed and sections of the brain were immuno-cytochemically studied to reveal the PRV-infected neurons. Spinalized animals (n=85) underwent the same procedure. *Results:* Bilateral, the intermediolateral cell group was infected at the levels Th1-Th11both after RV and LV inoculation. Uni-lateral labeling in the left IML of Th1-Th6 was seen after infection of the RA. Parasympathetic preganglionic neurons were found both in the DMnX (18.2%) and the peri-ambiguus area. Also, the location of the preganglionic parasympathetic neurons was related to the site of the virus injection. Higher order neurons were found in the area postrema, the solitary tract nucleus, the spinal trigeminal nuclei, the lateral reticular formation, and in the nuclei of the ventral medulla oblongata. The labeling in the latter areas, in particular the raphe pallidus, the ventromedial and the rostro-ventrolateral reticular formation was reduced or absent after the spinalization, showing the relation of these regions to the orthosympathetic heart innervation. Different neuronal populations were infected in the rostroventrolateral medulla. *Conclusion:* Viral retrograde transneuronal labeling of the heart innervating neuronal networks shows a "cardiomyotopy" in the spinal cord and the brainstem.

550.3

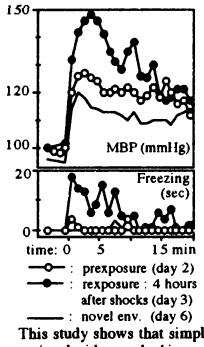
EARLY ENVIRONMENTAL INFLUENCES ON BLOOD PRESSURE RESPONSES TO STRESS IN BORDERLINE HYPERTENSIVE RATS. B.J. Sanders* and M.J. Gray. Department of Psychology, Drake University, Des Moines IA 50311.

Several converging lines of evidence suggest that the perinatal environment can affect cardiovascular regulation in the adult rodent. Our laboratory has used the borderline hypertensive rat (BHR) to study the interaction between genetic and environmental factors in the development of hypertension. In this study, we used a cross fostering paradigm to examine maternal influence on the cardiovascular functioning of adult male BHR. All BHR were bred by mating female spontaneously hypertensive with male Wistar-Kyoto rats. On the day of birth, litters of BHR pups were assigned to one of three conditions: cross-fostered to lactating WKY dams (BHR-CF); in-fostered to another lactating SHR dam (BHR-IF), or were allowed to be reared by their natural dam (BHR-NAT). Maternal observational data revealed that the WKY dams spent significantly more time engaged in passive as contrasted with arched nursing. Also, BHR-CF pups gained significantly more weight during the weaning period. At 8-10 weeks of age systolic (SBP) and diastolic (DBP) blood pressure, and heart rate (HR) was measured directly in subjects from each group. Resting SBP, DBP and HR did not differ between the groups. However, when exposed to 10 minutes of intermittent footshock stress, BHR-CF displayed significantly lower SBP and DBP throughout the stress session compared to the BHR-IF and BHR-NAT groups. These results suggest that cardiovascular functioning may be affected by early environmental influences, some of which may be related to maternal-pup interactions.

550.4

INCREASED BLOOD PRESSURE DURING CONTEXTUAL CONDITIONED FEAR : A STUDY USING TELEMETRIC PROBES. P. Carrive *, F. Westbrook and G. Paxinos, School of Psychology, University of New South Wales, Sydney 2052, Australia.

A rat which experiences footshock will rapidly acquire contextual fear to the environment in which it has been shocked. Contextual fear is observed upon rexposure and is characterized by a motionless posture, freezing, and signs of autonomic activation such as urination and defecation. The aim of this study was to determine the extent of the cardiovascular changes associated with this type of fear.



Systemic blood pressure, measured at the iliac bifurcation was monitored non invasively and continuously by telemetric probes implanted 1 week before training. Training consisted of 2 prexposures of 30 min to the shock chamber on days 1 and 2. On day 3, rats received 3 shocks in 30 min (1 sec, 1 mA). The fear response was assessed 4 hours and 24 hours later by rexposure to the shock chamber for 30 min. On day 5 or 6 rats were exposed to a novel and safe environment (clean transparent home cage). Average mean blood pressure (MBP) at rest in the home cage was 101 mmHg. Rexposure 4 or 24 hours after the shock session evoked freezing and a marked increase in MBP (+ 40 mmHg), which was significantly greater than during prexposure or exposure to novel environment after conditioning (+ 24 and + 25 mmHg, respectively). Peak effects usually occurred during the first 5 minutes.

This study shows that simple rexposure to an expected aversive environment is associated with a marked increase in systemic blood pressure in the rat.

Supported by the National Heart Foundation of Australia.

550.5

EFFECTS OF TEMPERATURE ON SCIATIC NERVE LASER DOPPLER FLOW. K.C. Dines, M.C.Jorge, A.P. Mizisin and M.W. Kalichman*. Department of Pathology, University of California, San Diego, La Jolla, CA 92093-0612.

One factor contributing to differences in nerve blood flow measurements, particularly in models of experimental diabetic neuropathy, may be differences in nerve and body temperature. Using laser Doppler flowmetry, the effects of nerve and body temperature were examined in mature female Sprague-Dawley rats (225-250g) under pentobarbital anesthesia (25mg/kg, i.p.). In the first of two studies, nerve laser Doppler flow was measured in the sciatic nerves of two groups of rats (n=6 per group) with normothermic core temperature (mean 37.2 °C) and nerve temperatures of either 30.5±0.4 °C (mean ± SEM) or 37.1±0.0 °C. In the second study, flow was measured in two groups of rats (n=6 per group) with mean nerve temperatures of 37.1 °C and a core temperature maintained either at 33.3±0.3 °C or 37.4±0.1 °C. Nerve temperature was regulated with an infrared heat lamp and core temperature was maintained with a heating pad. For all groups, Doppler flow, heart rate, blood pressure, and respiratory rate were monitored every 2 minutes for 20 minutes and averaged. In animals with core temperature maintained at 37°C, heating the sciatic nerve from room temperature to 37°C reduced nerve Doppler flux by 37.7±11.5%. This reduction was significantly greater ($P<0.05$) than that observed in nerves kept at room temperature (5.8±7.1%). Blood pressure tended to decrease with nerve warming, but other cardiovascular parameters were unchanged. In animals with nerve temperature at 37°C, maintaining core temperature at 37°C was associated with a reduction of 34.4±15.1% in Doppler flux from initial values. This reduction was not significantly different from that observed when core temperature was allowed to approximate room temperature (26.9±3.9%). Core hypothermia significantly depressed respiration rate and blood pressure, but not vascular resistance. These data are not consistent with the hypothesis that nerve blood flow measurements depend on core temperature, but suggest the possibility that hindlimb temperature has a role in regulation of nerve blood flow.

550.6

QUANTIFICATION OF CARDIAC NONDETERMINISM USING RECURRENCE PLOT STRATEGIES. Charles L. Webber, Jr.* and Joseph P. Zbilut. Departments of Physiology, Loyola University of Chicago, Stritch School of Medicine, Maywood, IL 60153 and Rush Medical College, Rush-Presbyterian-Saint Luke's Medical Center, Chicago, IL 60612.

Biodynamical systems can be classified as periodic, quasi-periodic, aperiodic, or chaotic. To account for dynamical noise, simulations of deterministic oscillatory behavior can be made more realistic by including additive or multiplicative noise. An additional approach is to view biodynamical systems as nondeterministic in nature. Such systems possess discontinuous, piece-wise deterministic components (dynamical trajectories) with interposing singularities (dynamical pauses). To quantitatively gauge the level of nondeterminism present in a common physiological dynamic, electrocardiograms were recorded from normal volunteers. Each cardiac cycle was manually partitioned into deterministic (PQRST interval) and stochastic (TP interval) components. Summation of both serial segments reconstructed the complete cardiac cycle (PP interval). Coefficients of variation (covar) were computed over hundreds of values for each component. The degree of nondeterminism present scaled directly as the ratio of PT-covar to PQRST-covar (ratios > 1). Recurrence plot analysis (RPA, *J. Appl. Physiol.* 76: 965-973, 1994) was next conducted on all intervals with the finding that deterministic structuring of the dynamic decreased as the level of nondeterminism increased. It is concluded that: 1) different subjects exhibit differing levels of nondeterminism in their cardiac dynamics; 2) resident nondeterminism can be gauged by RPA without laboriously partitioning each cycle into its principle components. Other systems may likewise exhibit nondeterministic structuring including even DNA information systems with interposing deterministic exons (coding DNA) and stochastic introns (junk DNA).

550.7

IDENTIFICATION OF BRAINSTEM AND DIENCEPHALIC CARDIORESPIRATORY AREAS ACTIVATED DURING EXERCISE IN AWAKE RATS. G.A. Iwamoto*, S.M. Wappel, G.M. Fox, K.A. Buetow and T.G. Waldrop. Depts. of Veterinary Biosciences, Physiology & Biophysics and Neuroscience Program, University of Illinois, Urbana IL 61801

Brain areas active during treadmill exercise (EX), compared to rest conditions, were identified using immunocytochemical labeling of the protein product of the proto-oncogene c-fos. Increased labeling with EX was observed in the "defence area" or "hypothalamic/subthalamic locomotor regions" including the posterior and lateral hypothalamic areas. Limited increases in labeling were also observed in the medial preoptic region. Increased labeling was also found in both colliculi, periaqueductal gray matter, cuneiform nucleus ("mesencephalic locomotor region") and in the region analogous to the A-5 catecholamine area and adjacent superior olive. Increased labeling with EX was also found in n. cuneatus and gracilis, medial portion of n. tractus solitarius, rostral and caudal ventrolateral medulla and medial structures equivalent to the raphe nuclei and adjacent ventral medulla. Conspicuous by an absence of labeling with EX were cells in thalamic areas associated with somatosensory function. Thus, areas in which labeling was increased during exercise closely correlate with brain areas which have been implicated in both autonomic and somatomotor control. These results from awake, exercising rats support those obtained previously in anesthetized animal preparations. (Supported by NIH HL06296 and NIH HL37400).

550.8

THE ROLE OF ADENOSINE AND ATP METABOLISM IN A HYPOTHALAMICALLY EVOKED CARDIOVASCULAR RESPONSE.
T. Thomas, J.H. St Lambert & K.M. Spyer*. Dept. of Physiology, Royal Free Hospital School of Medicine, Rowland Hill St, London NW3 2PF.

In α-chloralose anaesthetised rats, stimulation of restricted sites in the hypothalamus elicits a characteristic cardiovascular response that is an integral component of the defence reaction. Specifically there is a biphasic increase in arterial pressure and a tachycardia. We have illustrated that adenosine is important in these changes by acting on A_1 receptors within the brainstem (St Lambert et al., J. Physiol. 479.P, 15P, 1994). To determine whether this adenosine is released as a result of ATP metabolism the effects of α,ß-methylene ADP (α,ß-meADP), an ecto-5'-nucleotidase inhibitor, on the evoked cardiovascular response were examined. Microinjections of α,ß-meADP (500 μM, 50 nL) were made at 12 sites within nucleus tractus solitarii (NTS) and 9 sites in the rostral ventrolateral medulla (RVLM). Only the initial rise in arterial pressure was reduced (32.4%, $P<0.01$) by injection of α,ß-meADP at 6 sites within NTS; it had no effect when microinjected in the remaining 6 sites. On the other hand, α,ß-meADP reduced only the secondary increase in arterial pressure (17.8%, $P<0.05$) when injected into all sites tested within the RVLM. The tachycardia was unaffected by microinjection of α,ß-meADP into any region. These data suggest that adenosine which contributes to the cardiovascular changes associated with hypothalamic stimulation is derived from ATP metabolism. The different effects of α,ß-meADP in NTS and RVLM may be explained by a difference in adenosine A_1 receptor density within these regions (St Lambert et al., 1994).
Supported by MRC and BHF

550.9

ESTIMATION OF NICOTINIC RECEPTOR BINDING SITES IN THE CNS OF SPONTANEOUSLY HYPERTENSIVE (SHR) AND NORMOTENSIVE RATS BY AUTORADIOGRAPHY. M.Gattu, S.E.Urbanawiz, J.R.Pauly and J.J. Buccafusco*. Dept. Veterans Affairs Medical Center, and Dept. Pharmacology Toxicology, Medical College of Georgia, Augusta, GA 30912.

Central cholinergic neurons play an important role in central cardiovascular regulation and have been implicated in the pathogenesis of spontaneously hypertension. SHR respond with exaggerated pressor responses to pharmacologic stimulation of central cholinergic muscarinic receptors compared with normotensive Wistar Kyoto rats(WKY). Less is known regarding the role of central nicotinic receptors. We estimated the number of nicotinic receptor binding sites in the brains of 12 week old SHR and WKY by using standard autoradiographic techniques. Coronal sections were exposed to saturating concentrations of [^{3}H]-cytisine (α_4 subunit-containing sites) and [^{125}I]-α-bungarotoxin (α_7 sites). In general, [^{3}H]-cytisine binding was reduced (by up to 25%) in the brains of SHR *vs.* WKY. Significant reductions were observed in about 50% of the regions analyzed, including frontal cortex, caudate putamen, nucleus accumbens, medial habenula, superior colliculus and interpeduncular nucleus. SHR exhibited only regional reductions in [^{125}I]-α-bungarotoxin binding sites. These were relegated to the frontal cortex and anterior olfactory nucleus (interstrain differences 29 and 32%). However, no strain differences were observed in traditional cardiovascular regulatory areas such as locus coeruleus, rostral ventrolateral medulla, parabrachial nuclei, olivary nuclei and trigeminal nuclei. Further experiments will be necessary to determine whether these alterations in the levels of nicotinc receptors in SHR are reflected in functional differences between SHR and WKY. Supported by the Dept. VA Medical Center.

550.10

AUTORADIOGRAPHIC DEMONSTRATION OF A_1 RECEPTORS IN REGIONS OF THE RAT AND CAT CNS INVOLVED IN CARDIOVASCULAR CONTROL. J.H. St. Lambert, M. R. Dashwood, J. Muddle, C.D.Johnson* & K.M. Spyer. Dept. Physiology, Royal Free Hospital School of Medicine, Rowland Hill St. London, NW3 2PF.

In vivo studies in the rat have implicated central adenosine A_1 receptors in the cardiovascular changes associated with the hypothalamic defence response. Here *in vitro* autoradiography has been used to study the distribution of [^{3}H] DPCPX (an A_1 antagonist) binding sites in sections of the rat and cat forebrain and lower brainstem from images generated on radiation-sensitive film. Quantitative assessment of receptor density was performed and underlying tissue stained for histological examination. In both species there was a heterogenous distribution of A_1 binding sites in both the forebrain and the brainstem. Specifically there was a high density of A_1 binding sites in the hippocampus and thalamus. In addition low levels of binding were observed in the mamillary bodies and the hypothalamus. In the brainstem, the highest density of A_1 binding sites was observed in the ventral lateral medulla, hypoglossal nucleus and the caudal nucleus tractus solitarius. However, receptor density was low in areas of the rostral nucleus tractus solitarius and the trigeminal tract. These results illustrate that adenosine A_1 receptors are localised in specific CNS regions known to be involved in central cardiovascular control.
Supported by the MRC and BHF.

550.11

A WORKING HEART-BRAINSTEM PREPARATION (WH-BP) OF THE MATURE MOUSE FOR STUDYING REFLEX REGULATION OF CENTRAL CARDIO-RESPIRATORY ACTIVITY
J.F.R. Paton* Dept. Physiology, Univ. Bristol, Bristol, BS8 1TD, UK.

An *in vitro* wh-bp of the mouse is being developed to study medullary mechanisms controlling cardio-respiratory activity. Mice (3-4 weeks old) anaesthetised with ether were decerebrated, abdomenectomized and perfused via the descending aorta with carbogenated artificial cerebrospinal fluid containing an oncotic agent. Heart rate stabilized between 6-7Hz and generated an end-systolic left ventricular pressure of 100±5mmHg (mean±SD; n=21). Adaquate oxygenation of the brainstem was assessed by the *pattern* of central respiratory activity recorded from a phrenic nerve (PN). Both the oncotic and perfusion pressures of the perfusate were adjusted so that PN discharge included a ramp-inspiratory component (300-450ms duration), a rapid decline and post-inspiratory discharge (PI) which is comparable to that recorded from spontaneously breathing, anaesthetised, mice of the same age (Paton & Richter, 1995: *Pflugers Archiv.* **430**, 115-124). In the wh-bp PN discharge occurred regularly at 24±2.4 bursts/min and remained stable for >6 hours at 31^{0}C. An atropine-sensitive *sinus arrhythmia* (coincident with the onset of the PI phase) was indicative of a central respiratory modulation of cardiac vagal motor neurones. Further, activation of either left ventricular receptors (via intra-ventricular injection of veratridine; 2ug) or baroreceptors (by elevating perfusion pressure) enhanced and prolonged PI activity and evoked a vagally mediated bradycardia. The findings suggest that the wh-bp may be potentially useful for studying cardio-respiratory reflex afferent regulation of cardiac vagal motor outflow at a cellular level.
British Heart Foundation funded research.

550.12

THE EFFECT OF SPINAL CORD TRANSECTION ON THE NMDA RECEPTORS OF SYMPATHETIC PREGANGLIONIC NEURONS. N.R. Krenz* and L.C. Weaver. Neuroscience Program, The Robarts Res. Inst. and University of Western Ontario, London, Ontario, Canada N6A 5K8.

Spinal cord injury (SCI) at cervical and high thoracic levels often results in autonomic dysreflexia, which is characterized by increases in blood pressure in response to sensory stimulation. This enhanced excitability may be caused by overexpression of NMDA receptors on the sympathetic preganglionic neurons (SPNs). Intact male Wistar rats were compared to rats one and two weeks after spinal cord transection at the T_5 level. Antibodies to the NMDA-R1 receptor subunit, and binding of [^{3}H] MK-801, a specific NMDA receptor antagonist, were used to assess the density of NMDA receptors in the intermediolateral cell column which contains the SPNs. One week after transection, the density of NMDA receptors was significantly reduced in the segments immediately caudal to the injury. Two weeks after SCI, there was no difference in the density of NMDA receptors between control and spinal animals at any level of the cord. Since autonomic dysreflexia is well established in rats two weeks after spinal cord transection, NMDA receptor overexpression appears not to play a role in the development of abnormal blood pressure responses. Instead, the decrease and resurgence of NMDA receptor density parallels the somal and dendritic atrophy and recovery of SPNs observed after SCI, indicating that the changes in receptor density may be a function of membrane surface area. Supported by MRC Canada.

550.13

INTRASPINAL NEUROTRANSMITTERS POTENTIALLY INVOLVED IN AUTONOMIC DYSREFLEXIA AFTER CORD INJURY.

A.K. Cassam* and L.C. Weaver. Neuroscience Program, John P. Robarts Res. Inst. and University of Western Ontario., London, Ont., Canada, N6A 5K8.

Autonomic dysreflexia, a condition experienced after spinal cord injury, is characterized by large increases in arterial pressure in response to visceral stimulation. Intact intraspinal inputs to sympathetic preganglionic neurons (SPNs) caudal to the injury mediate reflexes responsible for autonomic dysreflexia. Immunoreactivity (IR) for Substance P (SP), Neuropeptide Y (NPY) and the noradrenaline synthesizing enzyme dopamine beta hydroxylase (DBH) was examined to determine the presence of these transmitters in intraspinal inputs to SPNs after cord injury. The spinal cords of anesthetized male wistar rats were transected between the 4th and 5th thoracic segments and the rats were allowed to survive for 14 days. SPNs were retrogradely labelled by i.p. injection of the tracer Fluoro Gold 7 days after cord transection. Fourteen days after cord transection NPY-, SP-, and DBH-IR was present in fibres around SPNs rostral to the injury, suggesting these inputs to the SPNs are of supraspinal origin. Caudal to the transection, NPY- and DBH-IR was almost completely lost around SPNs. Occasionally very faint levels of DBH-IR were noted in a few cells in the grey matter caudal to the injury. These findings indicate that, whereas both NPY and DBH inputs to SPNs are primarily of supraspinal origin, interneuronal spinal sources of catecholamines may also exist. Conversely, SP-IR was present in numerous fibres and terminals, but not in cell bodies caudal to the transection. This observation suggests that SP inputs to SPNs may originate in afferent, or ascending spinal pathways. We anticipate that intraspinal SP and catecholamines may play a role in autonomic dysreflexia after cord injury.

Supported by the Ontario Heart and Stroke Foundation.

550.14

SPECTRAL INDICES OF ORTHOSTATIC INTOLERANCE IN POSTURAL TACHYCARDIA SYNDROME (POTS). V. Novak, P. Novak, T.L. Opfer-Gehrking and P.A. Low*, Dept. of Neurology, Mayo Clinic, Rochester, MN 55905.

Postural tachycardia syndrome (POTS) is characterised by orthostatic tachycardia, dizziness, weakness, headache, gastrointestinal symptoms and blood pressure changes. Dynamic cardiovagal and adrenergic indices (respiratory oscillations in R-R intervals and nonrespiratory oscillations from 0.01 to 0.09 Hz in R-R intervals and blood pressure) were evaluated during head-up tilt (80^{o}) using modified Wigner distribution. Thirty patients (28 women and 2 men) presented with a mild form of postural tachycardia <120 bpm (POTS I) and thirty patients (24 women and 6 men) had more severe form with tachycardia >120 bpm (POTS II); fifteen controls participated in the study. All patients were symptomatic during the tilt.

POTS I group was characterised by reduced respiratory oscillations in R-R intervals at rest, normal sustained decrease of spectral powers during the tilt and rapid increment up to normal values after the tilt-down ($p<0.001$). Nonrespiratory oscillations in blood pressure revealed a transient elevation during the first minute of tilt, unlike the sustained increase in the control group.

POTS II group differed from POTS I and control group by an overall loss of variability in R-R intervals. Powers at respiratory and nonrespiratory frequencies in R-R intervals at were minimal at rest, diminished even further during the tilt, and increased after the tilt-down ($p<0.001$), but never reached control values. Elevation of nonrespiratory blood pressure oscillations was transient, similarly to the POTS I group.

CONCLUSION: Mild signs of orthostatic intolerance as seen in POTS I group are consistent with previously reported failure of sympathetic venomotor tone resulting in an excessive venous pooling. A new finding is that the more severe form (POTS II) had cardiac autonomic denervation.

550.15

BLOOD PRESSURE DIURNAL VARIATION IN HYPERTENSIVE PATIENTS WITH SUBCLINICAL CEREBRAL ABNORMALITIES

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Background and Purpose In a previous study of patients with well-treated hypertension of 10 or more year duration, anatomic imaging revealed brain atrophy. Positron emission tomography with 18FDG showed abnormal cerebral glucose metabolism. Although these patients had well-controlled office blood pressure readings, the pattern of their 24 hour ambulatory blood pressure was unknown.

Methods Nine hypertensive and six age-, sex- and race-matched normotensive volunteers had 24 hour ambulatory blood pressure and heart rate monitoring. Subjects were encouraged to pursue their routine activity on the day of monitoring. Every subject also had a volumetric magnetic resonance imaging of the head study.

Results The hypertensive patients had normal averaged 24-hour systolic and diastolic blood pressures (137/80 mm Hg). However, hourly systolic, diastolic and mean blood pressures showed no diurnal changes in the hypertensive group. Both groups had similar daytime blood pressures, but nighttime diastolic and mean blood pressures were higher in hypertensive patients. Hypertensive patients had larger cerebral lateral ventricles than normotensive controls.

Conclusion Treated hypertensive subjects with clinically silent brain morphometabolic abnormalities demonstrated well-controlled averaged 24 hour ambulatory blood pressure, but the pattern of diurnal blood pressure differed from normotensive controls.

550.16

CARDIOVASCULAR REGULATION IN MULTIPLE SCLEROSIS.

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Traditional assessments of autonomic nervous system function have depended on invasive and complex procedures. Vagal power, which is the respiratory component of heart rate variability (HRV) is an alternative and non-invasive measure for indexing autonomic nervous control of the heart. The present study was conducted in order to address two primary objectives. Previous studies have assessed cardiovascular regulation in MS in terms of heart rate (i.e.,interbeat intervals) or time-domain measures. Our prime objective was to examine parasympathetic and sympathetic activity during both paced and unpaced breathing in a population of clinically-definite MS patients evaluating both high frequency power (i.e., *frequency*-domain) and *time-domain* measures (i.e. heart rate). In the current study, 18 multiple sclerosis (MS) and 20 healthy subjects matched with respect to age, education and intelligence served as subjects. The MS group showed significantly lower vagal power (i.e., parasympathetic influence) during natural and paced breathing than healthy subjects. Importantly, time domain measures (i.e., heart rate) did not differ between the two groups The impact of lower parasympathetic activity, in regard to quality of life issues and vulnerability to adverse cardiac events needs to be further evaluated. The results of this study may have implications with respect to the feasibility of using HRV as both a diagnostic and prognostic tool for evaluating parasympathetic nervous system dysfunction in MS and in providing information for developing more effective treatment and rehabilitation.

CARDIOVASCULAR REGULATION: PHARMACOLOGY

551.1

SYMPATHOLYTIC EFFECT OF DESIPRAMINE: SITE AND MECHANISM OF ACTION IN ANESTHETIZED RATS. D.Huangfu*, Y. Hagiwara, and P.G.Guyenet, Dept. of Pharmacology, Univ. of Virginia, Charlottesville, VA 22908.

Intravenous desipramine (DMI), but not fluoxetine, dose dependently inhibited splanchnic sympathetic nerve discharge (sSND; -64±3% after 4 mg/kg iv DMI, 172 ng/ml plasma) in urethane anesthetized debuffered rats. Inhibition by DMI was reversed or prevented by microinjection of the α_2-adrenergic receptor (α_2-AR) antagonist 2-methoxyidazoxan (MOI) into the rostral ventrolateral medulla (RVLM; 1 nmol/side). However, sSND inhibition by baclofen i.v. ($GABA_B$ receptor agonist) was unaffected by MOI. MOI alone raise sSND 46±9%. Microinjection of 6-hydroxydopamine into the RVLM (2 µg/side, 10-13 days, to destroy noradrenergic terminals) did not change the effect of intravenous DMI or MOI on sSND. Slow firing presympathetic neurons of the RVLM were activated by iontophoresis of MOI and inhibited by iv DMI (-44±12%, effect reversed by α_2-AR antagonist given i.v.). Prior administration of 4 mg/kg DMI did not change the ability of i.v. clonidine (Clo) to inhibit residual sSND [ΔSND/$\log_2$(clonidine dose): -17.6±5.7 vs -16.5±3.9 SND units/$\log_2$(µg/kg), p=0.70].

Interpretations: 1) α_2-ARs in the RVLM are activated at rest by catecholamines probably released from C1 cells. This activation results in tonic SND inhibition. 2) DMI reduces sSND by increasing α_2-AR activation in the RVLM. 3) This effect of DMI is due neither to serotonin uptake inhibition nor to blockade of norepinephrine uptake by the RVLM noradrenergic fibers. 4) DMI and Clo have simple additive inhibitory effects on SND (Grant HL-28785 to PGG).

551.2

CALCIUM CHANNEL MODULATION BY PROSTAGLANDIN E2 IN CARDIOPULMONARY BARORECEPTOR NEURONS. Y. Liu*, H.A. Browne and M. Hay. Dept. of Physiology,Univ. TX Health Sci. Ctr., San Antonio, TX 78284.

Neurotransmission of cardiopulmonary baroreceptor (CPB) neurons is modulated by glutamatergic pathways and paracrines such as serotonin and prostaglandin E2 (PGE2). The purpose of the present study was to begin to characterize the biophysical mechanisms underlying PGE2 inhibition of the calcium current (ICa) in CPB neuron. The whole cell patch clamp was used to measure ICa in cultured CPB cells which were labeled and isolated from 3 weeks old rats. The pipette solution consisted of (in mM):124.0 CsCl, 1.0 MgCl2, 10.0 HEPES,1.0 CaCl2, 10.0 EGTA and 250 U/ml Nystatin. The bath solution consisted of (in mM): 139.0 TEACl, 5.0 4-AP, 2.0 CaCl2, 15.0 Glucose, 15.0 HEPES. Calcium currents were activated by 10 mV depolarizing step pulses from a -80 mV holding potential. 45 CPB neurons were tested. These neurons expressed a high threshold ICa activated near -30mV and peaked near 0 mV. Application of PGE2 (500 nM) reversibly reduced the peak ICa by 25.9±12.2% (n=9). Metabotropic glutamate receptor agonist t-ACPD at low dose alone (400 µM) had little effect on the peak ICa, but PGE2 plus t-ACPD at low dose inhibited the peak ICa in the same cells by 29.8±13.8% (n=10). Applying high dose t-ACPD (1.6 mM) significantly suppressed the peak ICa by 37.6 ± 10.0% (n=5), while PGE2 plus t-ACPD at high dose had no further inhibition on the peak ICa (37.9±6.7%). These results suggest that PGE2 inhibits the evoked calcium currents in CPB neurons and the lack of additive summation between PGE2 and t-ACPD suggests that these two ligands may share a common intracellular transduction mechanism. Supported by AHA #93G-1183, ALA and NIH #HL50304 to M. Hay.

551.3

DETERMINATION OF AGMATINE IN ABDOMINAL AORTA OF RATS AND GERBILS AFTER ISCHEMIA REPERFUSION INSULT (I.R.I.).G. Delbarre*, B. Delbarre and F. Calinon, Faculté de Médecine, 37032 Tours, France.

Agmatine has been recently identified as clonidine displacing substance (CDS) and purified from rat brain. Agmatine is locally synthesized from arginine by arginine decarboxylase. Until now, agmatine has been studied in the central nervous system but none in the vessels. This is why we have determined level of agmatine in abdominal aorta of rats and gerbils by HPLC/Electrochemical method according to Xu. et al. (Xu, X., et al., *J. Liq. Chrom.*, 9:10, 1986). 24 male adult animals were used : 12 wistar rats and 12 gerbils, (6 control and 6 ischemic, ischemia of abdominal aorta : 5 min. Reperfusion : 5 min.)

	RATS	GERBILS
Control (n=6)	0.058±0.020	0.028±0.012
I.R.I. (n=6)	1.150±0.214***	0.410±0.093**
% of increase	1883	1364

Unpaired Student t test versus control group (p≤0.01** ; p≤0.001 ***). Results of agmatine : mean±SEM (µg/mg of protein).

In the I.R.I. group, agmatine is significantly increased. Agmatine has been considered to be only precursor of putrescine and polyamines. Our study might show the possibility that agmatine may be a biological active molecule in its own right : in vessels, agmatine plays an important role in the vasomotricity. Because agmatine is synthesized from arginine, it is possible that drugs acting on level of agmatine are not only drugs correlated with its affinity for imidazole receptors.

551.4

BLOCKADE OF ICV AII MEDIATED HYPERTENSION BY α-ADRENERGIC RECEPTOR ANTAGONIST IS DEPENDENT ON DIETARY SODIUM CHLORIDE INTAKE. A.K.S. Camara, P.S. Clifford* and J.L. Osborn, Dept. of Physiol. Medical College of Wisconsin, Milwaukee, Wi 53226.

We have shown that chronic intracerebroventricular ICV AII hypertension in rats is mediated by brain AT_1 receptors coupled with central α-adrenergic neurotransmission. Experiments were conducted to determine the effect of periventricular α-adrenergic blockade on ICV AII-induced hypertension in rats raised on low (5.0 mEq/kg), normal (50 mEq/kg) and high (250 mEq/kg food) sodium chloride diets from weaning. Adult rats were instrumented with femoral catheters and brain cannulae which were implanted in the lateral ventricle and the anterior ventral third ventricle (AV3V). Blockade of pressor and plasma renin activity (PRA) responses to 100 ng ICV AII injection by 25, 12.5, 6.3, 3.1 and 1.6 µg of phentolamine was assessed in all three groups of rats. In control, ICV AII injection significantly increased ($P<0.05$) arterial pressure above control in all three groups of rats (low 17±3 mmHg; normal 15±1 mmHg; high 16±2 mmHg). The hypertension was abolished in all three groups of rats by pretreatment of the AV3V with 25, 12.5, and 6.3 µg phentolamine mesylate. However, microinjection of 3.1 µg phentolamine only blunted the pressor response to ICV AII by 53% in rats fed low but not normal or high sodium chloride diet from weaning. In contrast, 1.6 µg phentolamine did not block the pressor response to ICV AII in any group of rats. PRA was unaltered before and after AV3V pretreatment with phentolamine and ICV AII injection. These results demonstrate that periventricular AV3V α-adrenergic neurotransmission mediates ICV AII-induced hypertension which is not dependent on elevation of PRA. The interactions between the brain renin-angiotensin system and α-adrenergic receptors is enhanced by low dietary sodium chloride intake but not by normal or high sodium intake. Supported by NHLBI PO1-29587.

551.5

BRAINSTEM 5-HT_{1A} AND 5-HT_{1D} RECEPTORS MODULATE THE CARDIORESPIRATORY RESPONSES TO UPPER AIRWAY STIMULATION IN RABBITS. A.G. Ramage,* S. Dando & D. Jordan,† Departments of Pharmacology and Physiology†, Royal Free Hospital Medical School, Rowland Hill Street, London NW3 2PF, UK.

Previous studies have demonstrated that central application of buspirone, a partial agonist at 5-HT_{1A} receptors, potentiates the bradycardia evoked by upper airway stimulation in rabbits (Futuro-Neto et al., Brain Res., **629**, 349-354, 1993). The present study compared the effects of 5-HT_{1A} and 5-HT_{1D} receptors on the cardiorespiratory responses evoked by stimulating upper airway receptors.

Studies were carried out in urethane-anaesthetized, atenolol pretreated rabbits. Bi-directional tracheal cannulation allowed ventilation of the lungs and independent delivery of cigarette smoke to the upper airways. Recordings were made of baseline and reflexly-evoked changes in mean arterial blood pressure (BP), ECG, renal (RNA) and phrenic nerve activities. Buspirone (200µg kg^{-1} i.c., n=5) significantly decreased BP and RNA, increased phrenic rate and R-R interval, and potentiated the bradycardia evoked by a smoke challenge. These effects were abolished by pretreatment with WAY-100635 (100µg kg^{-1} i.v.), a 5-HT_{1A} receptor antagonist. In contrast, following pretreatment with WAY-100635, the 5-HT_{1D} receptor agonist sumatriptan (50µg kg^{-1} i.c.) significantly increased baseline BP, phrenic rate and RNA and attenuated the bradycardia, depressor response and apnoea evoked by smoke.

Theses data are consistent with the view that brainstem 5-HT_{1A} and 5-HT_{1D} receptors have modulatory roles in cardiorespiratory integration.

This work was supported by the British Heart Foundation and Wellcome Trust. We thank Wyeth Research U.K. for the gift of WAY-100635.

551.6

SUPPRESSIVE AND FACILITATING ACTIONS OF MORPHINE ON NOCIFENSIVE REFLEX RESPONSES. I.A. Belyantseva*, E.V. Lukoshkova, V.M. Khayutin, Dept. of Circulation Control & Biomechanics, National Cardiology Research Center, Moscow, 121552, Russia

The effects of morphine (M) on the pressor reflexes, heart rate (HR) respon-ses and reflex discharges in sympathetic (renal nerve - RN) and somatic (n. PBST) nerves to noxious stimuli were studied in chloralose-urethane anesthetized cats. Responses were elicited by single-shock or short-train electrical stimulation of the tibial and radial A+C-afferent fibres. M given either iv (5 mg/kg) or topically, to the dorsal surface of the spinal cord L4-S2 or C6-T1 segments (0.02-0.5%), suppressed pressor reflexes, C-responses in RN and afterdischarges in n. PBST, and enhanced HR-responses. Suppressive action of the topical M was local: being applied to L4-S2 segments, it suppressed only reflex responses induced by the tibial nerve stimulation; similar responses to radial nerve stimulation were unimpaired or even enhanced. On the contrary, facilitating action of M on the HR-response was common for the reactions elicited from either of these nerves, and only a smaller increase in HR-response to tibial nerve stimulation indicated to local suppressive action of M on this reaction. An opposite relationship between all reflex changes was observed when M was delivered to C6-T1 segments. The other effects of both topical and iv (0.2-5 mg/kg) M were reduction in sympathetic tone, arterial pressure and heart rate. The first two effects could be the cause of increase in pressor reflexes from remote input after topical M and from both, radial and tibial nerves after small (0.2-2 mg/kg). doses of iv M. All described effects were concentration- (dose-) dependent and naloxone reversible. Suppressive action of iv M decreased when the number of stimuli or the stimulation frequency was increased. The results are interpreted as showing that interneuronal circuits responsible for excitatory action of nociceptive afferent volleys on sympathetic neurons exist in the spinal cord dorsal horn, and some of these circuits are common for somato-sympathetic and somato-somatic reflexes.

551.7

THE ANGIOTENSIN II ANTAGONIST, LOSARTAN, DECREASES RENAL SYMPATHETIC NERVE ACTIVITY AND HEART RATE IN SODIUM DEPRIVED, BUT NOT SODIUM LOADED, RATS. L. Xu and V.L. Brooks*, Department of Physiology, Oregon Health Sciences University, Portland, OR 97201.

Angiotensin II (AII) may be involved in long term blood pressure (BP) regulation in part by supporting sympathetic nerve activity. To test this hypothesis, rats were placed on either a low salt (LS;<0.01% NaCl) diet, to increase AII levels, or a high salt (HS; 8% NaCl) diet, for 2 to 3 weeks. Experiments using conscious rats were conducted to determine if AII blockade with losartan (10 mg/kg, I.V.) suppresses renal sympathetic nerve activity (RSNA) and heart rate (HR) more in LS compared to HS rats. The experiment consisted of three periods, during which BP, RSNA, HR and central venous pressure (CVP) were recorded continuously: 30 min control, 40 min after losartan injection, and 30 min after BP was restored near to but not above control values by methoxamine infusion. Rats in the time control groups (n=5 for LS; n=6 for HS) received only vehicle (0.9% saline). In LS rats (n=6), 40 min after losartan, BP decreased from 115±7 to 81±14 mm Hg ($p<0.05$), RSNA increased from 100±7 to 163±29 % of control ($p<0.05$), but HR did not change significantly (from 485±43 to 495±57 b/min). After BP was restored to control levels (111± 8 mm Hg), both RSNA and HR were suppressed (RSNA: 46±17 % ; HR: 380±36 b/min; $p<0.05$). In contrast, there were no significant changes in BP (114±4 to 110±7 mm Hg), HR (460±9 to 475±15 b/min) and RSNA (101±3 to 109±17 %) in HS rats after losartan (n=4) or in time control rats. CVP did not change in any group. We conclude that endogenous AII supports RSNA and HR in conscious sodium deprived rats and this action can only be revealed when BP is maintained at basal levels. Supported by NIH HL 35872.

551.8

THE EFFECTS OF TAURINE AND HIGH SALT DIETS ON RENAL FUNCTION IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS (SPSHR). B.J. Jung, B. Eppler, S. Liu, and R. Dawson, Jr.* Dept. of Pharmacodynamics, Univ. of Florida, Gainesville, FL 32610

Taurine (TAU) has been shown to lower blood pressure in several experimental models of hypertension. The present study investigated the effects of different dietary levels of TAU on the development of hypertension in SPSHR on high salt diets. SPSHR/A3N were bred from NIH stock. Male SPSHR were randomly assigned to four dietary conditions (n=6-8 per group) when they were 6 weeks old: regular chow (CON), regular chow with high salt (CON/HS), TAU deficient chow with HS (NT/HS), regular chow with HS and 1.5% TAU in the drinking water (T/HS). All other groups received tap water to drink. The HS groups were maintained initially on chow with 1% NaCl for 84 days and then switched to chow containing 3% NaCl until the end of the study at 147 days. Blood pressure (BP) was monitored by the tail cuff method biweekly. Metabolic studies were performed after 55 days on the 1% NaCl diet and after 50 days on the 3% NaCl diet. Addition of 1% NaCl to the diet produced a significant acceleration in the development of hypertension, however by day 50 on the diets the BP in the CON group was not significantly different from the HS groups. Switching to 3% NaCl did not result in a further rise in BP. The T/HS group consistently had lower BP than the other HS groups but this difference was not statistically significant. The HS diet caused ventricular hypertrophy in the CON/HS and NT/HS groups but not the T/HS group when compared to the CON. TAU also protected against renal damage over time as indexed by urinary protein excretion. HS diets had no effect on urinary TAU excretion and TAU excretion was elevated about 40-fold in the T/HS group relative to the other groups. Urinary catecholamine (NE, EPI, DA) excretion did not differ among the groups, but urinary EPI excretion was significantly elevated at 135 days compared to 55 days. HS diets did not elevate urinary DA excretion which is an adaptive response exhibited by normotensive rats.

The results of this study suggest that 1.5% TAU does not significantly lower BP in SPSHR on high salt diets but did reduce ventricular hypertrophy and renal protein excretion. SPSHR do not exhibit a salt-induced increase in renal dopamine production which may contribute to the maintenance of elevated BP, particularly in light of the marked elevation in EPI excretion. (Supported by a grant from Taisho Pharmaceutical Co., LTD.)

551.9

GENDER DIFFERENCES IN RESPONSE TO MELATONIN. S. Doolen, D.N. Krause and S.P. Duckles*. Department of Pharmacology, College of Medicine, University of California, Irvine, CA, 92717.

Risk of cardiovascular disease appears to vary with gender and time of day. We have found the circadian hormone melatonin potentiates adrenergic vasoconstriction via a specific melatonin receptor in rat tail artery. Arterial ring segments from four month old male and female Fisher 344 rats were compared to investigate potential gender differences. Contractile responses to transmural nerve stimulation (TNS) were greater in male than in female rats. The circadian hormone melatonin potentiated TNS mediated vasoconstriction in both males and females. Male arteries were less sensitive to melatonin than females in estrous, metestrous, and diestrous. Sensitivity among these three cycles did not differ; however females in proestrous were less sensitive to melatonin than females in all other stages. Sensitivity to melatonin in proestrous arteries was similar to sensitivity in male arteries. Maximum potentiation was not significantly different in males compared to females in any stage of the estrous cycle. These data suggest that female sex steroids may modulate sensitivity to melatonin and influence sympathetic nerve-mediated vasoconstriction.

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551.10

CENTRAL AND SPINAL EXCITATORY AMINO ACID RECEPTORS MEDIATE SENSORI-AUTONOMIC PROCESSING IN SHR RATS. K.L. Youngblood, I.M. Khan, M.P. Printz*. Dept. of Pharmacology, U. C. San Diego, La Jolla, CA 92093-0636.

Sensory stimuli, such as tactile (airpuff) startle stimuli, elicit coordinated and concurrent behavioral and autonomic (ANS) responses. Inbred spontaneously hypertensive rats (SHR_{LJ}) exhibit exaggerated behavioral (motor) and autonomic (pressor and tachycardia) responses to airpuff startle stimuli relative to normotensive rats. Neural substrates and neurotransmitters involved in such sensori-autonomic processing have not yet been defined. We examined the role and subtype specificity of excitatory amino acid (EAA) receptors in sensori-motor-autonomic processing in the SHR. AP5 (NMDA receptor antagonist) or CNQX (non-NMDA receptor antagonist) were given ICV or intrathecally (IT) prior to delivery of the airpuff. AP5, ICV or IT, dose-dependently inhibited the motor response. ICV AP5 inhibited all ANS responses; however, IT AP5 unmasked a bradycardia response while inhibiting pressor and tachycardia responses. Further, with low dose of AP5 (12.6 nmol, ICV), a dissociation was evident between inhibition of motor and ANS responses - motor was inhibited to a greater degree than pressor or tachycardia. Only a high dose (126 nmol) of CNQX inhibited both motor and ANS responses to a similar fashion. Our data suggest that central and/or spinal EAA receptors of the NMDA subtype may be selectively involved in the efferent limb of the sensori-motor-autonomic processing pathway while AMPA/kainate receptors are not involved in this sensory-effector pathway and the apparent inhibitory effect of CNQX on sensori-autonomic processing may be a non-selective action.

551.11

GROWTH HORMONE REGULATES BRAIN ANGIOTENSIN II RECEPTORS IN VITRO BUT NOT IN VIVO B.D. Wyse* and C. Sernia,Neuroendocrinology Lab.,Dept of Physiology and Pharmacology, Univ. of QLD, Brisbane, Australia 4072.

Recently we reported that growth hormone (GH) upregulates peripheral angiotensin II (AT) receptors. Since the renin angiotensin system (RAS) has an important role in the regulation of cardiovascular function by the brain, we investigated the possible upregulation of brain AT by GH. AT receptor density and GH content in the brains of Dwarf (genetically GH-deficient) Lewis rats were compared with those of normal Lewis and Wistar rats. As expected, it was found that GH in the pituitary and plasma of dwarf rats (pit:1.4±0.8μg/mg protein;15±6ng/ml plasma); was lower than in the normal Lewis (pit: 25±7μg/mg protein;plasma:153.9±21.1ng/ml) and Wistar rats (pit:54.7±3.2ug/mg protein; plasma:152.2±72.7ng/ml). In addition, unexpected differences in the GH content of the hypothalamus of dwarf (0.08±0.03ng/mg protein), normal (2.03±.45ng/mg protein) and Wistar (2.29±.53ng/mg protein) rats were found. In spite of these brain GH differences, the density of hypothalamic AT receptors of the dwarf (74.4±16.4 fmol/mg protein), normal Lewis (79.6±20.1 fmol/mg protein) were the same. Thus *in vivo* GH upregulates AT receptors in the periphery and not the brain. This could represent true tissue specific regulation or alternatively, a dominance of non-GH regulatory mechanisms in the brain. To distinguish these two possibilities, astrocyte cultures were established from normal neonatal rat brains and the effect of 1ng GH/ml tested at confluence. There was an upregulation of receptors, reaching a maximum increase (158.3±18.2% of control) at 4h after the addition of GH. We conclude that GH upregulates brain astrocyte AT receptors *in vitro* and that this effect is being masked by non-GH regulatory mechanisms in the brain *in vivo*.

551.12

CARDIOVASCULAR EFFECTS ELICITED BY INTRACEREBROVENTRICULAR ADMINISTRATION OF CLONIDINE IN CONSCIOUS CATS. A. Ally*, G.A. Hand and J.H. Mitchell. Moss Heart Center, UT Southwestern Medical Center, Dallas, TX 75235, and Departments of Pharmacology and Biochemistry, University of New England College of Medicine, Biddeford, ME 04005.

The cardiovascular effects of an intracerebroventricular (i.c.v.) injection of clonidine were studied using conscious cats. Administration of clonidine (5-10 μg) i.c.v. decreased mean arterial pressure (MAP) and heart rate (HR) in a dose dependent manner. The highest dose of 10 μg of clonidine decreased MAP and HR by 39 ± 3 mmHg and 74 ± 20 bpm, respectively (n = 7). Pretreatment with yohimbine, the α_2-adrenergic antagonist (8 μg; i.c.v.) blocked the cardiovascular responses to subsequent i.c.v. injection of clonidine. Furthermore, preadministration of the imidazoline and H_2 histamine receptor antagonist, cimetidine (100 μg; i.c.v.) prevented the cardiovascular responses of the i.c.v. injection of clonidine (n = 4). By contrast, pretreatment with the specific I_1-imidazoline receptor blocker, efaroxan (100 μg - 500 μg; i.c.v.), did not inhibit the effects of i.c.v. administration clonidine (n = 3). These results demonstarte that the effects of centrally administered clonidine on MAP and HR are not mediated by the I_1 subtype of imidazoline receptors. Further, these cardiovascular responses to an i.c.v. injection of clonidine appear to result from activation of central α_2-adrenergic, H_2 histaminergic or the I_2 subtype of imidazoline receptors.

551.13

THE PRESSOR RESPONSE INDUCED BY CENTRAL INJECTION OF CARBACHOL IS BLOCKADE BY RAMIPRIL. **W.A. Saad, A.C. Luiz, L.A.A. Camargo, A. Renzi and J.V. Menani. Dept. of Physiol. Sci., School Odontol., Paulista State University - UNESP, 14801-903, Araraquara, SP, Brazil.**

We investigated the effects of ramipril, an angiotensin I-converting enzyme (ACE) inhibitor, on blood pressure induced by central injection of carbachol. Male Holtzman rats (250-300 g) were implanted with stainless steel cannula opening into the lateral ventricle (LV). Intracerebroventricular (ICV) injection of ramipril (1 μg/μl) significantly blockade the pressor response after ICV injection of carbachol. The value after injection of NaCl 0.15 M NaCl into the LV was 2 ± 2 mmHg. After injection of ramipril alone ICV this value was 5 ± 2 mmHg. The injection of carbachol (2 nmol/μl) produced an increase in arterial blood pressure (33 ± 6 mmHg). The injection of ramipril prior to carbachol blocked this effect (6 ± 3 mmHg). This results and anothers obtained in our laboratory strongly support the hypothesis that the carbachol may act via angiotensinergic receptors and that the ramipril can be transformed in ramiprilat, the active drug, by the brain.

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551.14

SODIUM DEPRIVATION INCREASES TYROSINE HYDROXYLASE mRNA IN RAT ADRENALS AND CELIAC GANGLIA. T.L. Silliman, L. Xu, J.A. Resko* and V.L. Brooks. Department of Physiology, Oregon Health Sciences University, Portland, OR 97201.

It is not clear whether chronic blood volume depletion produced by sodium deprivation increases sympathetic activity. Because long-term stimulation of the sympathetic nervous system is associated with elevated ganglionic tyrosine hydroxylase (TH) mRNA levels, we tested the hypothesis that sodium deprivation would increase TH mRNA in rats. TH mRNA was quantitated using a sequence-specific ribonuclease protection assay. Rats were placed on a low (LS; <0.01% NaCl), normal (CS; 1%NaCl) or high (HS; 8%NaCl) salt diet for 23 to 25 days. At the end of this period, body weight increases were blunted ($p<0.05$) in the LS (315 ± 19 to 346 ± 15 g;n=12) compared to the HS (252 ± 4 to 326 ± 10 g;n=6) and CS rats (313 ± 16 to 366 ± 12 g;n=13). Adrenal TH mRNA was elevated ($p<0.05$) in LS (1061 ± 81 fg/μg total RNA;n=9) compared to the HS (732 ± 90 fg/μg total RNA;n=6), but not to the CS rats (944 ± 69 fg/μg total RNA;n=9). In addition, TH mRNA was increased ($p\leq0.05$) in the celiac ganglia of LS rats (2572± 102 fg /μg total RNA;n=9) compared to the CS rats (2234±67 fg /μg total RNA;n=9) and HS rats (2328 ± 58 fg/μg total RNA;n=6). In contrast, TH mRNA in the superior cervical ganglia was not significantly altered by salt intake (LS, 5249 ± 500, n=6; HS, 4283 ± 223, n=3; CS, 4560 ± 256 fg/μg total RNA, n=6). In conclusion, salt deprivation increases TH mRNA in the adrenals and celiac ganglia. We speculate that the rise in TH mRNA levels is secondary to chronic blood volume depletion which increases sympathetic nerve activity. Supported by NIH HL35872.

551.15

EFFECT OF NOREPINEPHRINE (NE), VASOPRESSIN (AVP), AND OXYTOCIN (OT) ON MYOCARDIAL TISSUE STRUCTURE IN VITRO

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It has been shown that NE and AVP have a hypertrophic effect on the heart. We investigated the influence of AVP, NE, OT and dopamine (DA) on the structure of both cardiac muscle cells (CMC) and connective tissue cells (CTC). The relatioship between CMC and connective tissue may be important for maintenance of heart tissue homeostasis. This study was completed using 6 Wistar male rats (115-125g), during spring - summer period. Pieces of myocardial tissue (approximately 2.5 x 105 cells) were cultured in vials containing 199 Medium with 20% bovine fetal serum, 0.6 mg/ml each of adenosine, guanosine, uridine, cytidine, 1mg/ml glucose and 0.24 U/ml insulin for 3 days with constant rotation. There were 5 culture conditions: control, AVP (5 μIU/ml), OT (5 μIU/ml), DA (20 μg/ml) and NE (1 μg/ml). On day 3 tissue cultures were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer and embeded in Epon-Araldite resin. The average area of CMC was increased in rank order of AVP (39.26±0.5), OT (43.70±0.47), and NE (52.91±0.58). NE treatment also increased intercellular space and number of CTC, however, we did not find clear mitosis at this stage. Therefore, despite an increase in CMC area NE decreased the ratio of CMC number to interacellular space (0.029±0.001). CMC number / intercellular space was not changed in the DA, or control conditions (0.059±0.003) but was slowly decreased in the VP (0.044±0.0019) and was increased by OT (0.078±0.0024). These results imply that hormones can influence the relationship between CMC size, area of intercellular space and number of CTC. This relationship could be important for functioning of CMC in hypertrophic myocardium.

551.16

UNUSUAL ADRENOCEPTORS CONTRIBUTE TO SYMPATHETIC CONSTRICTION OF SMALL CUTANEOUS ARTERIES IN THE GUINEA-PIG EAR. J.L. Morris*. Department of Anatomy & Histology, and Centre for Neuroscience, Flinders University, S.A. 5042, Australia.

Local application of norepinephrine (NE) to small arteries in guinea-pig ears produces constrictions which are partly resistant to antagonism by the α-adrenoceptor antagonists prazosin, yohimbine and benextramine, but which are abolished by dihydroergotamine (Morris, Br. J. Pharmacol. 113:1105-1112, 1994). The present study set out to determine if these unusual adrenoceptors contribute to sympathetic vasoconstriction of the same arterial segments. Changes in internal diameter of arteries 80-150μm resting diameter in ears of guinea-pigs anaesthetized with ketamine were determined using on-line image analysis (DIAMTRAK). The cervical sympathetic nerve trunk was stimulated electrically with trains of 100 to 200 pulses delivered at 1-20Hz. Resultant constrictions were frequency-dependent, with the maximum constriction occurring at 5-10Hz. Prazosin (0.3-1μM) slightly reduced the magnitude of sympathetic constrictions, and consistently increased the latency of the responses. The remaining response was not reduced significantly by benextramine (1-10μM) or suramin (30μM), but was reduced dramatically by dihydroergotamine (1-10μM), sometimes leaving a small, slowly-developing constriction. Thus, sympathetic constriction of small cutaneous arteries can involve an initial phase mediated by α-adrenoceptors, a slightly slower phase mediated by non-α adrenoceptors, and a much slower response probably not mediated by norepinephrine.

551.17

SALT LOADING INCREASES CATECHOLAMINE SECRETION AND ARTERIAL PRESSURE IN mREN-2 TRANSGENIC RATS. P. Li*, M. F. Callahan, C. M. Ferrario, D. Ganten and M. Morris. Dept. Of Physiol. & Pharmacol. and Hypertension Center, Bowman Gray Sch. of Med. of Wake Forest Univ., Winston-Salem, NC 27257.

Experiments were performed to study catecholamine (CA) secretion in mRen-2 transgenic rats (TG) after salt loading. Male TG+ and TG- rats (12 wks age) were prepared with carotid arterial catheters. Mean arterial pressure (MAP) was recorded continuously. Blood samples of rats were taken before salt loading and after four days 2% NaCl water. Basal MAP was significantly elevated in TG+ (149±8.5 mmHg) as compared to TG- rats (92±3.5 mmHg). After salt loading, TG+ rats significantly increased their MAP to 175±10.9 mmHg while no change was observed in TG- rats. Basal levels of norepinephrine (NE) and epinephrine (EPI) were 217.9±15.1 pg/ml and 98.7±38.2 pg/ml in TG- rats, 249.4±50.7 pg/ml and 138.0±54.3 pg/ml in TG+ rats respectively. After salt loading TG+ rats increased both plasma NE and EPI approximately 3.7 fold. However, TG- rats increased plasma NE and EPI only 70% and 50% respectively. Thus TG+ rats showed an increase in MAP and CA secretion in response to high salt intake. This suggests that the increased secretion of CA may play a role in the pathogenesis of salt sensitivity in mRen-2 TG rats. (Supported by NIHLBI-HL51952 and AHA NC 93GS15)

PAIN MODULATION: ANATOMY AND PHYSIOLOGY—SPINAL CORD II

552.1

WIND-UP OF SPINAL CORD NEURONES IS INFLUENCED BY INFLAMMATION, SPINALIZATION, ENDOGENOUS OPIOIDS AND TYPE OF STIMULATION. J. F. Herrero , P. G*. de la Rubia and F. Cervero. Department of Physiology and Pharmacology, University of Alcalá de Henares, 28871 Madrid, Spain. SPON: Brain Research Association.

Frequency dependent facilitation of responses to C-fibre stimulation (wind-up) is used as a model to study central sensitisation. We have examined whether different experimental conditions influence this system. Spinal reflexes, recorded as single motor units, were elicited by percutaneous electrical stimulation in adult rats, under pentobarbitone anaesthesia. Experiments were performed in control and in carrageenan inflammatory conditions (monoarthritis), with the spinal cord intact or transected. Low and high frequency stimulation was alternated at 0.2, 0.5 and 2 ms pulse widths, using the voltage able to recruit C-fibre responses with the lowest pulse duration. Responses were challenged with i.v. naloxone 10, 100 and 1000μg/kg. Wind-up was observed at C-fibre latency in all conditions studied. Saturation of responses was reached earlier and at a significantly higher firing rate with longer pulse duration. During inflammation, but only in intact animals, the firing rate was increased 3 fold and the after-discharge responses 3, 4 and 5 fold for each pulse duration. The opioid antagonist naloxone induced a dose-dependent potentiation of these responses only in spinalized rats. Wind-up of A-fibre latency, however, was only observed during inflammation; similar firing rates were obtained with all pulse durations and no significant potentiation was found with naloxone. In conclusion, wind-up depends on pulse duration and spinal cord integrity, whereas inflammation alters only the firing rate. Naloxone is more effective in enhancing these responses in spinalized animals. Support: DGICYT APC-93-0102, PB-93-0491 and Química Farmacéutica BAYER S.A.

552.2

ANALGESIC EFFECTS OF TRANSCUTANEOUS ELECTRICAL NERVE STIMULATION ON RENAL PAIN IN CATS. T.S. Nam*, E.J. Baik, Y.U. Shin, Y. Jeong and K.S. Paik Dept. of Physiology Yonsei Univ. College of Med., Dept of Physiology Ajou Univ. College of Med. Dept of Int. Med. In Ha Univ. College of Med. Seoul, Korea

Most studies concerning the antinociceptive effects of transcutaneous electrical nerve stimulation(TENS) have dealt with somatic pain. Thus, we investigated the analgesic effects of TENS on renal pain as a model of visceral pain. The renal pain was induced by acute occlusion of the ureter or renal artery. Activities of single dorsal horn neurons evoked by the renal pain-producing stimuli were monitored before and after TENS. The main results are summarized as follows : 1) The more renal C afferent inputs the dorsal horn neurons had, the greater were the responses to the stimuli that elicited the renal pain. 2) The high threshold(HT) and wide dynamic range(WDR) cells exhibited a greater responses than low threshold(LT) cells to the renal pain-producing stimuli. 3) TENS reduced the C-fiber evoked responses of dorsal horn neurons to 38.9 ±8.4% of the control value. 4) By TENS, the responses evoked by acute occlusion of the ureter or renal artery were reduced to 37.5± 9.7% and 46.3±8.9% of the control value, respectively. 5) The responses elicited by squeezing the receptive fields of the skin were reduced to 40.7±7.9% of the control value. In conclusion, the dorsal horn neurons with renal inputs have the concomitant somatic inputs and TENS can alleviate the renal pain as well as somatic pain.

552.3

INTACT FEMALE RATS ARE MORE SUSCEPTIBLE TO THE DEVELOPMENT OF NEUROPATHIC PAIN THAN OVARIECTOMIZED FEMALE RATS. D.E.Coyle,* C.S. Sehlhorst, and M.M. Behbehani. Department of Anesthesia, University of Cincinnati College of Medicine., Cincinnati, OH 45267-0531.

As previously reported, this laboratory has determined that female rats are more prone to develop neuropathic pain than male rats using the partial sciatic nerve ligation model (Coyle et al. Neurosci. Let. 186:135-138, 1995). This finding correlates with the clinical observation that injury-related neuropathic pain is 2-3 times more prevalent in females than in males. In order to further characterize this gender difference, the role of ovarian hormones in predisposing female rats to the development of neuropathic pain was investigated. In a double blind randomized trial, 12 intact and 12 ovariectomized (ovexed) female rats underwent partial sciatic nerve ligation of the right rear leg. Sham operated (exposure of the nerve without ligation) and unoperated (incision through skin only) controls (ovexed and intact) were also included in the study groups. Animals were evaluated on three consecutive days for withdrawal from touch (Von Frey hairs 0.28-67g) starting on post-injury days 13, 20 and 27. The average of the minimum gram force needed to induce withdrawal was calculated and the distribution of the ovexed and intact groups compared. A significant difference was observed at post-injury day 20 and 27 between the two groups. The intact group resulted in 12/12 animals that were statistically more sensitive than controls compared to 6/12 for the ovexed group. On post-injury day 13 no statistical difference was seen between the two groups (8/12 for intact vs. 10/12 for ovexed). This study indicates that ovarian hormones, via some unknown mechanism, predispose female rats to develop neuropathic pain following injury.

552.4

TEMPORAL SUMMATION OF C-FIBRE AFFERENT INPUTS : COMPETITION BETWEEN FACILITATORY AND INHIBITORY EFFECTS ON A C-FIBRE REFLEX IN THE INTACT RAT. M. Gozariu, D. Le Bars* and J.C. Willer. Lab. de Neurophysiol. Hôp. Pitié-Salpétrière, 75013 Paris, * INSERM U-161, 2, rue d'Alésia 75014 Paris, France.

Long-lasting facilitatory effects due to temporal summation of nociceptive inputs were repeatedly described on spinal nociceptive reflexes in spinalised non-anaesthetised rats. Since noxious inputs also trigger powerful descending inhibitory controls, we investigated this question in intact, halothane-anaesthetised (0.9%) rats and compared these results with those obtained in other preparations.

Electromyographic responses elicited by single-square electrical shocks (2 ms, 0.16 Hz) applied to the sural nerve territory, were recorded from the ipsilateral biceps femoris. The signal was digitised, fullwave rectified and the C-fibre responses integrated in a 100-450 ms time window. The excitability of the C-fibre reflex recorded at 1.5 times the threshold (xT) was tested following electrical conditioning stimuli (2 ms, 1 Hz, 20 s) of the sural nerve territory. In intact rats, following facilitation during the conditioning period, the reflex was inhibited in an intensity-dependent manner. Conditioning at 10 xT resulted in a maximum inhibition of 79.9 $\pm$ 5.1 % during the 2nd min of post conditioning period, with a complete recovery within 20 min. Such an inhibition was completely blocked by decerebration at obex level and was replaced by a 20 min facilitation in non-anaesthetised rats (maximum during the 1st min of post conditioning period : 152.5 $\pm$ 14.8%). A similar facilitation was seen in non-anaesthetised spinalised (T_8-T_{10}) decerebrated rats (maximum during the 1st min of post conditioning period : 183 $\pm$ 25.9 %).

It is concluded that following high intensity repetitive stimulation of C-fibre afferents, powerful supra-spinally mediated inhibitory mechanisms counteract, in intact rats, the increase in excitability of the flexor reflex seen in spinal animals.

552.5

DORSAL ROOT REFLEXES RECORDED BILATERALLY FROM THE MEDIAL ARTICULAR NERVES IN UNILATERAL ADJUVANT ARTHRITIS. H. Rees*, K.A. Sluka, Y. Lu, K.N. Westlund and W.D. Willis. Marine Biomedical Inst., Univ. of Texas Medical Branch, Galveston, TX 77555-1069

Acute inflammation of the knee joint enhances the depolarization of primary afferents in the lumbar dorsal horn evoking dorsal root reflex (DRR) activity in afferent nerve fibers. The present experiments established that DRRs are present in later stages of the inflammatory process. Male rats (250-290g) were briefly anesthetized with Brevital (50 mg/kg i.p.) and one knee injected with either 75 µg or 250 µg incomplete Freunds adjuvant. Chronic unilateral inflammation developed over three days. Animals were tested daily for development of heat hyperalgesia using the paw withdrawal reflex. There was great variation in the time and degree of the development of heat hyperalgesia. The greatest effects were often recorded 6 h after the knee injection. The injected knee increased its circumference by an average of 16 mm, but little change was seen on the contralateral side. Four days following the initial injection, animals were reanesthetized with sodium pentobarbital (50 mg/kg i.p.), an intravenous cannula inserted and anesthesia maintained by i.v. infusion of sodium pentobarbital (3-6 mg/kg/h). Both medial articular nerves (MAN) were dissected free and cut distally. Recordings were made of afferent activity in each cut articular nerve. DRRs were recorded in the MAN supplying the inflamed knee following mechanical stimulation of the inflamed tissue. DRRs could not be recorded from the MAN supplying the inflamed knee following mechanical stimulation of the other knee. DRRs were also evoked in the contralateral MAN, although to a lesser extent, in response to stimulation of tissue around both knee joints. The results indicate that DRRs are present bilaterally in the chronic inflammatory state. This pathophysiological phenomenon may be an important factor in the severity of inflammatory disease. (Supported by the HFSP Fellowship and NIH grants NS11255, NS09743.)

552.6

THE ROLE OF G-PROTEINS AND PROTEIN KINASES IN ALLODYNIA INDUCED BY INTRADERMAL INJECTION OF CAPSAICIN IN THE RAT. W.D. Willis and K.A. Sluka*, Marine Biomedical Institute, Univeristy of Texas Medical Branch, Galveston, TX 77555-1069

Rats were tested for responses to von Frey filaments with bending forces in the innocuous range (10 and 90mN) as a measure of allodynia before and after intradermal injection of capsaicin. Additionally, the threshold to mechanical stimulation with von Frey filaments with bending forces from 0.1-900 mN was tested before and after capsaicin. Delivery of second messanger inhibitors to the spinal cord was through a microdialysis fiber implanted into the dorsal horn of the spinal cord. Intradermal injection of capsaicin (0.1%, 100µl) resulted in allodynia and a decrease in mechanical threshhold without heat hyperalgesia. The G-protein inhibitor, GDP-γ-S (0.01-1.0mM), raised the threshold to mechanical stimuli and reversed the allodynia.The general protein kinase inhibitor, H7(0.05-5.0mM), also reversed the allodynia by raising the threshhold to mechanical stimulation. Specific protein kinase inhibitors of protein kinase C (NPC15437, 0.1-10.0mM), protein kinase A (H-89, 0.001-0.1mM) or protein kinase G (KT4823, 0.100-0.1mM) were equally effective in reversing the allodynia and raising the threshhold to mechanical stimuli. Thus G-proteins and several protein kinases all appear to be involved in the maintenence of allodynia and mechanical hyperalgesia induced by intradermal injection of capsaicin in the rat.(Supported by NS09743, NS11255 and Kempner Fellowship to KAS).

552.7

THE ROLE OF G-PROTEIN AND PROTEIN KINASES IN THE SENSITIZATION OF7 SPINOTHALAMIC NEURONS INDUCED BY INTRADERMAL INJECTION OF CAPSAICIN IN THE PRIMATE. K.A. Sluka, H.Rees, M. Tsuruoka*, P.S. Chen and W.D. Willis, Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77555-1069

Following intradermal injection of capsaicin (3%, 100 µl) in the monkey, spinothalamic tract (STT) cells show increased 1) background discharges, 2) responses to innocuous brushing 3) responses to pressure applied to the skin. Inhibitors of second messenger systems were administered into the spinal dorsal horn by infusion through a microdialysis fiber. An inhibitor of G-proteins, GDP-γ-S (1.0mM), reversed the increased background discharges of the cell. GDP-γ-S also reversed the sensitization of the responses to brushing and pressure stimuli applied to the skin. Administration of a protein kinase inhibitor, H7 (5.0mM), had no effect on the increased background discharges of the STT cells but reversed the sensitization to brushing and pressure applied to the peripheral receptive field. The protein kinase C inhibitor, NPC15437 (10.0mM), reversed the increased background discharges of the cell and the sensitization of responses to brushing applied to the skin induced by intradermal capsaicin. Lastly, pretreatment prior to injection of capsaicin with the PKC inhibitor (NPC15437, 10mM) prevented the development of sensitization to brush stimuli applied to the receptive field without affecting background activity. Thus, G-proteins and PKC appear to be involved in the induction and maintenence of the sensitization of STT cells to mechanical stimuli. (Supported by NS09743, NS11255, Kempner Fellowship to KAS and Human Frontier Fellowship to HR).

552.8

RESPONSES OF SPINAL DORSAL HORN NEURONS TO THERMAL AND MECHANICAL STIMULI BEFORE AND DURING ZYMOSAN-INDUCED INFLAMMATION OF THE RAT HINDPAW. A. Randich*[1], S.T. Meller[2,3], and G.F. Gebhart[3]. [1] Dept. of Psychology, Univ. of Alabama at Birmingham, Birmingham, AL 35294, [2]OTC-HCTD Procter and Gamble Co., Cincinnati, OH, 45239, [3]Dept. of Pharmacology, Univ. of Iowa, Iowa City, IA 52242

Extracellular recordings were made of nociceptive specific (NS) and wide dynamic range (WDR) neurons in the spinal dorsal horn of the methohexital-anesthetized rat before and during zymosan-induced inflammation of the ipsilateral (left) hindpaw, Stimulus-response functions were generated for either mechanical (6.7 - 325.0 g) or thermal (36 - 50° C in 2° C increments) stimuli during a control period, and 1, 2, and 3 h after inflammation with zymosan.

The group mean thermal response threshold of all WDR neurons tested decreased significantly within 1 h of inflammation and remained at that level during the remaining 2 and 3 h test periods. In contrast, the group mean thermal response threshold of all NS neurons tested decreased in a slow and progressive manner across the entire 3 h period of testing. However, only 55 - 60% of these WDR and NS neurons showed a reduction in thermal response thresholds following inflammation, whereas 40% of both WDR and NS neurons did not change.

The group mean mechanical response threshold of all WDR neurons tested showed no change following inflammation of the hindpaw at any time. In contrast, the group mean mechanical response threshold of 80% of NS neurons showed a significant, slow and progressive reduction over the 3 h testing period. For both thermal and mechanical stimuli, the response thresholds of WDR and NS neurons were similar by 3 h.

552.9

DIFFERENTIAL EFFECTS OF NOXIOUS HINDPAW IMMERSION ON THE RESPONSES OF IPSILATERAL AND CONTRALATERAL DORSAL HORN NEURONS IN RATS. S. McGaraughty* and J.L. Henry. Dept. of Physiology, McGill University, Montreal, Que. H3G 1Y6.

A wealth of research demonstrates that noxious heterotopic stimulation inhibits dorsal horn neurons with both spinal and supraspinal components. However, there is conflicting evidence regarding the effects of noxious contralateral paw immersion. Single lumbar dorsal horn neurons were studied in intact rats that were either decerebrated or anesthetized with pentobarbital or ketamine and in decerebrate-spinalized rats. The afterdischarge (AD) response to an ipsilateral noxious pinch was increased by contralateral hindpaw immersion in 50°C water. This effect was seen in both intact and spinalized rats. The immersion had no effect on discharges during the pinch. Ipsilateral hindpaw immersion induced 2 types of responses - excitation or inhibition followed by an AD; this AD was inhibited by subsequent immersions. Neurons that were inhibited by ipsilateral immersion had both an excitatory and an inhibitory receptive field. Immersion, because of its wide stimulation area, may activate both the excitatory and inhibitory fields. Therefore, noxious immersion of a hindpaw may transiently activate inhibitory mechanisms which override the excitatory effects of the stimulus, and block contralateral inhibitory projections. *(Supported by MRC of Canada to JLH, and by Quebec's FCAR and the Royal Victoria Hospital Fellowship to SM.)*

552.10

SENSITIZATION OF DORSAL HORN NEURONS BY A SURGICAL INCISION IN THE RAT.

T.J. Brennan,* E. Vandermeulen, G.F. Gebhart; Depts. of Anesthesia and Pharmacology; Univ. of Iowa Hospitals and Clinics, Iowa City, IA 52242.

In previous studies, we developed an animal model for postoperative pain by placing an incision in the plantar region of the rat foot and measuring the decrease in withdrawal threshold to mechanical stimulation with von Frey filaments. Further studies on mechanical hyperalgesia were undertaken to examine the effect of a similar incision on the responses of rat dorsal horn neurons receiving input from the plantar region of the rat foot.

In halothane-anesthetized rats limited lumbar and cervical laminectomies were performed. Single lumbar dorsal horn neurons (n=43) were antidromically-activated from the cervical spinal cord, characterized as wide dynamic range (WDR), high threshold (HT), or low threshold (LT) and examined with von Frey filaments for receptive field size, threshold, and force-activity relations. All neurons were excited during the plantar incision made within the receptive field; however, 17 (15 WDR and 2 HT) of 39 neurons maintained an increased background activity 1 hour later. The average increase was from 2.9 imp/s to 5.2 imp/s. Receptive field size was expanded in 18 (17 WDR and 1 HT) of 39 indicating central sensitization. Background activity failed to remain elevated in 22 cells (13 WDR, 6 HT and 3 LT) and no receptive field expansion was observed in 21 (10 WDR, 8 HT and 3 LT). Most WDR neurons had thresholds for activation similar to the withdrawal thresholds after incisions observed in behavioral experiments.

In conclusion these studies demonstrate that central sensitization occurs following a surgical incision in rats. Sensitization of WDR neurons may result in mechanical hyperalgesia observed in behavioral studies.

552.11

ULTRASTRUCTURAL DISTRIBUTION OF NADPH-DIAPHORASE IN THE RAT DORSAL HORN AND ITS RELATIONSHIP WITH PEPTIDE- AND/OR TRANSMITTER AMINO ACID-IMMUNOLABELED NERVE PROFILES. A. Merighi* and P. Aimar. Dipartimento di Morfofisiologia Veterinaria, University of Turin, Turin, Italy.

Nitric oxide (NO) is a recently identified neuronal messenger which is linked to excitatory amino acid receptor activation within the central nervous system. NO is synthesized by specific populations of spinal neurons, among which are comprised a number of cells of laminae II and III of the dorsal horn, and appears to be implicated in nociception, particularly in the hyperalgesia which is observed in several models of persistent pain. A number of peptides and transmitter amino acids also play a role in pain perception and are specifically localized in nerve profiles within the superficial dorsal horn. We have used pre-embedding reduced nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase histochemistry of rat spinal cord Vibratome slices in combination with post-embedding immunogold labeling for calcitonin gene-related peptide (CGRP), substance P, glutamate and γ-amino butyric acid (GABA) to better characterize the synaptic relationship of these neuroactive molecules in the dorsal horn. NADPH-diaphorase positivity was observed in: i) nerve cell bodies in laminae II-III; ii) dendrites in laminae I-III which received synapses of the asymmetric type from axonal endings which were generally free of dense core vesicles. These dendrites were never seen in glomerular configurations; iii) unmyelinated axons in laminae II-III. In the immunolabeled preparations, there was no co-localization with peptide positivities, but CGRP-immunoreactive nerve endings were often visible in close spatial apposition with NADPH-diaphorase containing dendrites. On the other hand, co-localization with glutamate- and, to a lesser extent, GABA-immunoreactive nerve profiles was observed in a number of nerve profiles within lamina II. These results provide morphological evidence for possible interactions between NO-, peptide- and amino acid-containing nerve processes in the superficial dorsal horn.

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552.12

NALOXONE DOES NOT REVERSE TRAMADOL-INDUCED SUPPRESSION OF FORMALIN-EVOKED PAIN BEHAVIOR OR SPINAL CORD FOS EXPRESSION IN THE RAT. K.T. Marcus, H. Wang, L. Panyacosit and A.I. Basbaum*, Depts. Anatomy and Physiology and W.M. Keck Center for Integrative Neuroscience, UCSF, San Francisco, CA.

Tramadol is a centrally acting analgesic that produces its effect via both opioid and monoaminergic mechanisms (inhibition of noradrenaline uptake). To determine the mechanism through which tramadol is antinociceptive in a test of persistent pain, we examined the effect of oral tramadol on the behavior and fos-like immunoreactivity evoked in the formalin test. Thirty minutes prior to a hindpaw injection of 2.5% formalin (30 µl), the rats were anesthetized with halothane and given either tramadol (30, 60, 90, or 150 mg/kg) or saline vehicle. One hour after the formalin injection, the rats were anesthetized and perfused with fixative. We performed fos immunocytochemistry on 50 µm transverse sections of the lumbar spinal cord.

Although tramadol had little effect on Phase I behavior in the fomalin test, it produced a dose-dependent inhibition of pain behavior in phase 2. We compared the pattern of fos expression in control rats and in rats that received 90 mg/kg, the lowest dose that completely blocked Phase 2. Tramadol produced significant inhibition of fos expression in the superficial dorsal horn. We also attempted to reverse the effect of tramadol by combined administration of a dose of naloxone (2.0 mg/kg, i.p.) that completely antagonizes morphine analgesia in the formalin test. Naloxone was administered 5 minutes prior to tramadol and every 30 minutes after, to maintain adequate levels of this short-half life antagonist. Naloxone was without effect on the analgesia and fos suppression produced by tramadol. These results indicate that in the formalin test of persistent pain tramadol does not produce its antinociceptive effect via interaction with opioid receptors. Activation of bulbospinal monoaminergic systems that inhibit the firing of spinal cord nociresponsive neurons may be involved. This work was supported by DA08377.

552.13

ANTINOCICEPTION INDUCED BY MORPHINE IN THE CORTEX ACTS THROUGH DESCENDING INHIBITON ON SPINAL TRANSMISSION. *Serge Marchand*[1]*, *Earl Carstens*[2], *Adam Burkey*[3], and *Luc Jasmin*[3]. [1]Sciences de la Santé, Universtité du Québec, Québec, [2]Physiology and Behavior, University of California, Davis CA, [3]Neurosurgery & Cell biology, Georgetown University, Washington DC.

We previously reported that injection of morphine in the piriform cortex results in parallel inhibition of formalin induced pain behavior and *c-fos* expression in the lumbar spinal cord[1]. These results indicated that the cerebral cortex could be producing analgesia by an activation of the descending pain inhibitory system. In the present study, electrophysiological experiments were done to investigate the effect of morphine microinjected in this cortical analgesia site (CAS) on spinal neural responses to a nociceptive stimulus. In barbiturate-anesthetized rats, the dorsal horn neurons evoked activity to 48°C water (5 sec) was markedly suppressed during a electrical stimulation (200 µA pulse train) or following the infusion of morphine (0.13 to 0.26 nM) in the contralateral CAS. Locally injected naloxone (0.27 nM) reversed the effect of morphine. Morphine microinjections outside of the CAS had little or no effect on neuronal response to heat. We correlated morphine microinjection sites with morphine's effect on nociceptive behavior (formalin test) and lumbar *c-fos* expression using immunocytochemistry to delimit the area of morphine sensitivity. Morphine microinjection in a sphere of approximately 1.0 mm diameter, centered in the deep prepiriform cortex and including adjacent piriform and ventrolateral orbital cortices, the endopiriform nucleus and the claustrum, significantly suppressed formalin behavior and numbers of c-fos immunoreactive spinal cells. The present results provide further evidence that morphine in a CAS induces antinociception by an inhibitory effect on spinal nociceptive neurons.

[1] Wenniger et al., Neurosci Abs: 318.18 (1994).

552.14

NEONATAL CAPSAICIN TREATMENT REDUCES PERSISTENT, FORMALIN-EVOKED BEHAVIORAL AND CARDIOVASCULAR RESPONSES AND C-FOS EXPRESSION IN SPINAL CORD NEURONS. M.A. Peterson, B.K. Taylor, D.S. Rohde, C. Abbadie, W.R. McKay, S.M. Roychowdhury* and A.I. Basbaum. Depts. Anatomy, Physiology and W.M. Keck Foundation Center for Integrative Neuroscience, UCSF, San Francisco, CA 94143-0452.

To evaluate the contribution of small diameter primary afferents to the expression of both acute and persistent pain, we measured the behavioral, cardiovascular and spinal cord dorsal horn neuronal responses evoked by noxious stimulation in neonatally-capsaicin treated animals (100 mg/kg s.c. one day postpartum). At 3-4 months of age, we placed arterial catheters into the femoral artery for chronic measurement of blood pressure and heart rate. At time of testing, baseline blood pressure and heart rate was similar between capsaicin-treated and control animals. In control animals, we found that hindpaw injection of formalin (5%, 100 µl) produced acute (Phase 1) and persistent (Phase 2) nociceptive responses and ipsilateral activation of spinal cord dorsal horn neurons, as measured by fos-like immunoreactivity. Neonatal capsaicin treatment did not change formalin-evoked Phase 1 responses, but did significantly decrease Phase 2 behavioral and cardiovascular responses. Furthermore, neonatal capsaicin significantly reduced formalin-evoked fos expression in all laminae of the ipsilateral (but not contralateral) spinal cord. We conclude that myelinated afferents and/or small diameter afferents (not destroyed by the capsaicin pre-treatment) contribute to formalin-evoked Phase 1 responses. Our results strongly suggest that small diameter afferents contribute to the expression of persistent pain.

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552.15

SPINAL CORD SUBSTANCE P RECEPTOR IMMUNOREACTIVITY INCREASES IN BOTH INFLAMMATORY AND NERVE INJURY MODELS OF PERSISTENT PAIN. C. Abbadie*, J.L. Brown, P.W. Mantyh and A.I. Basbaum. Dept. of Anatomy, UCSF, San Francisco and VAMC, Minneapolis.

Numerous studies have implicated the primary afferent derived neuropeptide, substance P, (SP) which exerts its effects via the neurokinin-1/substance P receptor, (SPR) in the transmission of nociceptive messages at the level of the spinal cord. Immunocytochemical studies demonstrate that the SP receptor is concentrated in neurons of lamina I of the superficial dorsal horn. Since alterations in the number and distribution of the receptor may underlie persistent pain conditions, we have used immunocytochemistry to study the distribution of the receptor in two very different rat models of persistent pain: chronic inflammation, which is associated with increased levels of SP, and sciatic nerve section, which is associated with decreased levels of SP in the dorsal horn. Inflammation was produced by unilateral hindpaw injection of complete Freund's adjuvant.

We report that there is an upregulation of SPR immunoreactivity in the superficial laminae of the dorsal horn in both injury models. The increase was found at all time points studied (up to one week after induction of inflammation and up to two weeks after sciatic nerve section). The increase in SPR immunoreactivity was not only present in the medial part of the dorsal horn at segment L4, which is the region of input of the afferents from the hindpaw, but also in the lateral parts of the dorsal horn, and at segments rostral (L1) and caudal (S1) to the afferent input from the hindpaw. These results indicate that the regulation of the receptor is not predictable merely by the content of SP. The non-topographic upregulation of SPR in these different conditions may contribute to the central sensitization of dorsal horn nociceptors under conditions of persistent pain. To address the functional consequences of the increased SPR expression, we are evaluating the magnitude of peripheral stimulation evoked internalization of the SPR in the dorsal horn, a phenomenon that we recently described in normal rats.

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552.16

CHANGES IN LUMBAR DORSAL HORN FOS-LIKE IMMUNOREACTIVITY (FLI) DURING MORPHINE WITHDRAWAL: EFFECT OF SPINAL TRANSECTION, DORSAL RHIZOTOMY OR NEONATAL CAPSAICIN TREATMENT. D.S. Rohde*, D.S. Chang, W.R. McKay, A.I. Basbaum, W.M. Keck Center for Integrative Neuroscience and Depts. Anatomy, Physiology and Anesthesiology, UCSF and NYU School of Medicine.

Withdrawal from morphine evokes increased fos expression in the spinal cord, particularly in laminae I and II. To determine the origin of the increased fos expression, we selectively eliminated possible sources of input and monitored FLI after naltrexone-precipitated abstinence. Rats were implanted daily with 75 mg morphine or placebo pellets. On day 4, abstinence was precipitated. After one hour, the rats were anesthetized and perfused with a 10% formalin fixative. Transverse 50um frozen sections of the spinal cord were immunoreacted with a fos polyclonal antiserum using the ABC method.

In placebo-pelleted rats, neither transection nor unilateral dorsal rhizotomy (L4-S2) induced fos expression. However, compared to unoperated rats that withdrew from morphine, both transected and rhizotomized withdrawing animals showed significant increases in FLI in laminae I/II. FLI ipsilateral to the rhizotomy was also greater than on the contralateral side. Neonatal capsaicin, which eliminates C-fibers, did not alter FLI.

We hypothesize that the increase in FLI after spinal transection results from a loss of descending inhibitory control that is activated during withdrawal (perhaps from the locus ceruleus). The increase in withdrawal-induced FLI in rhizotomized rats may have resulted from a loss of inhibitory controls exerted by large diameter primary afferents or from injury-induced reorganization in the dorsal horn. The results from rats treated neonatally with capsaicin indicate that hyperactivity of opioid receptor-laden C-fibers are not a necessary contributor to the withdrawal-induced increase in FLI in laminae I and II. We conclude that the pattern of fos expression reflects compensatory responses in local circuits intrinsic to the dorsal horn. Supported by DA08763.

552.17

NOXIOUS STIMULATION EVOKES INCREASED FOS EXPRESSION IN SPINAL CORD NEURONS THAT CONTAIN GAD67 mRNA. H. Wang, P.W. Mantyh* and A.I. Basbaum. Depts. Anat. and Physiol. and W.M. Keck Fdn. Ctr. for Integr. Neurosci., UCSF, CA and Molec. Neurobiol. Lab., VA Med. Ctr. MN.

Hindpaw inflammation induces fos expression in large numbers of spinal cord neurons. Although many of these neurons can be double labelled by retrograde transport from supraspinal sites (Menétrey et al, 1989), the majority are presumed to be interneurons. Here we combined immunocytochemistry for the fos protein and in situ hybridization for GAD67 mRNA to identify presumed GABAergic interneurons that are activated by noxious stimulation.

Five rats (240-260 gm) were injected with 100 µl of 5% formalin into the hindpaw and were perfused with a 4% para one hour later. We performed in situ hybridization with digoxigenin to identify neurons that contain GAD67 mRNA (blue cytoplasmic staining) and followed this by fos immunohistochemistry using the ABC method, with DAB as chromogen (brown nuclear immunoreactivity). We used a camera lucida attachment to plot labelled neurons. Formalin increased the number of GAD-positive neurons in the ipsilateral cord. The numbers of labelled neurons in ipsi and contra laminae I/II were 27.5±1.6 and 19.8±2.8, respectively. The numbers in the ipsi and contra neck of the dorsal horn were 64.2±3.5 and 53±2.7. The percentage of fos-immunoreactive neurons that contain GAD message varied: 29.1±2.3% in laminae I/II; 41.1±5.2% in laminae III/IV; 56.4±1.6% in laminae V/VI; 77.3±6.0% in lamina VII and 89.5±4.9% in Lamina X. We did not find double labelling of fos-immunoreactive neurons in lamina VIII.

Our results confirm those of an earlier study of Todd et al (1994) that used combined GAD and fos immunocytochemistry. However, the present approach revealed significantly greater numbers of GAD positive neurons. Taken together these studies demonstrate that noxious stimulation increases both GAD message and protein, which indicates that activation of spinal cord inhibitory neural circuits is a significant component of the CNS/spinal cord response to noxious stimulation. This may serve to reduce the long term consequences of persistent noxious stimulation. Supported by NS 14627, NS 21445 and DA 08377.

552.18

UNILATERAL MICRODIALYSIS IN THE DORSAL HORN OF AN AWAKE, BEHAVING ANIMAL AS A MODEL TO STUDY PAIN MECHANISMS *IN VIVO*. L.E. Ross* and B.A. Donzanti. Department of Pharmacology, Zeneca Pharmaceuticals, 1800 Concord Pike, Wilmington, DE 19850-5437.

Few methods have been successfully developed to study the *in vivo* neurochemistry of pain physiology and its modulation. Reports of techniques in awake, unrestrained animals are even more rare. Skilling et al. (1988) have used horizontal microdialysis probes which transverse both sides of the dorsal horn. Our goal here was twofold: 1) refine the method by developing a less damaging preparation and 2) collect dialysates for the analysis of aspartate, glutamate, and substance P. Rats were anesthetized and mounted in a stereotaxic frame. An anchor was cemented to the skull to allow for later attachment of tubing for infusion of artificial CSF. An incision was made over the spinal cord and muscle tissue cleared away from vertebrae T13 and L1. Small holes were drilled horizontally through the spinous processes of these two vertebrae. Pieces of #2 stainless steel suture, 1.5-2.0 cm long were passed through these holes and twisted to serve as anchors for the microdialysis probe. A hole was drilled vertically through the posterior portion of vertebra T13, slightly to the right of the midline. A 4mm microdialysis probe, coated with epoxy for the top 2mm, was slowly lowered through the hole. The probe was lowered until the top of the dialysis membrane was level with the surface of the bone. A small piece of Gelfoam was placed around the probe and cranioplastic cement applied around the probe and the two anchoring wires. After the cement hardened the incision was closed with wound clips, and the rat returned to its home cage to recover. The next day the rat was infused with artificial CSF via the probe at a rate of 5 µl/min and dialysate samples taken for analysis by HPLC for glutamate and aspartate. We are optimizing an assay for substance P, and also will use this method for analysis of changes induced by a stimulus such as formalin footpad injections.

552.19

EXPRESSION OF CALCITONIN GENE-RELATED PEPTIDE IN PRIMARY AFFERENT NEURONS AFTER PERIPHERAL NERVE TRANSECTION. K. Miki*, T. Fukuoka, K. Noguchi
Dept. of Anatomy & Neuroscience, Hyogo College of Medicine, Nishinomiya, Hyogo 663, JAPAN

The expression of calcitonin gene-related peptide (CGRP) in primary afferent neurons has been shown to be down-regulated by peripheral nerve transection. However, we have recently shown that tachykinin peptide is induced in a subpopulation of DRG neurons by axotomy. This study was designed to reexamine the changes in CGRP mRNA in DRG neurons and CGRP immunoreactivity (CGRPir) in gracile nucleus and spinal dorsal horn after peripheral nerve transection.

Male Sprague Dawley rats (150-200g) received a unilateral injection of fluorogold into the gracile nucleus. The L-5 spinal nerve was transected 7d after fluorogold injection, and more 7d later, animals were sacrificed and L-5 DRGs were examined by *in situ* hybridization with radiolabeled CGRP probe. Control animals received a sham operation. The changes in CGRPir in gracile nucleus and spinal dorsal horn 1d - 28d after sciatic nerve transection were also examined using ABC method.

In control DRG, a small number of neurons labeled for fluorogold also expressed CGRP mRNA (3.7 % of fluorogold-labeled cells). In contrast, axotomized DRG neurons showed a dramatic increase in the number of double-labeled neurons (19.9 %). Gracile nucleus ipsilateral to the sciatic nerve transection exhibited an increased CGRPir compared to contralateral side. The increase in CGRPir was detectable a few days after axotomy and peaked at 2 weeks. However, in spinal cord, there was no lamina of dorsal horn showing an increased CGRPir. These results indicate that CGRP is *de novo* synthesized in a subpopulation of DRG neurons and transported to gracile nucleus, and may play a role together with tachykinin peptide in altered sensory transmission following peripheral nerve injury.

552.20

NOXIOUS STIMULI EVOKE FOS EXPRESSION EVEN IN THE SPINAL DORSAL HORN OF THE CHEMICALLY ANALGESIC RATS. T. Honda, N. Ozaki, Y. Tonosaki, K. Nishiyama and Y. Sugiura*. Dept. of Anatomy, Fukushima Med. Col., Fukushima, Japan, 960-12

It is well established that Fos protein is expressed by noxious stimuli in secondary neurons of the sensory pathway. In the present study, the Fos expression induced by various stimuli accompanied with or without injuries of the primary afferent fibers was examined in the spinal cord of the analgesic rats or the colchicine-treated rats.

To establish the analgesic rats, capsaicin or lidocaine hydrochloride were used. Capsaicin was systemically applied to newborn or adult rats. Lidocaine or colchicine were topically applied to the sciatic nerve of adult rats. As noxious stimuli, formalin injection into footpad or the sciatic nerve transection were performed. As painful stimuli without injuries of primary afferent fibers, electric stimulation of the sural nerve and bradykinin or histamine injection into footpad were used. Two to three hours after each stimulus, the spinal cord was removed and Fos expression was immunohistochemically examined.

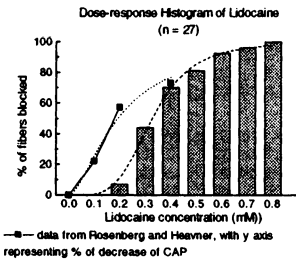
All capsaicin-treated rats became analgesic and showed obvious increase of threshold to noxious thermal stimulus. In the control rats after noxious stimuli, many Fos immunoreactive (Fos-ir) cells were found in laminae I, II, III, IV, occasionally V and X of the spinal cord. Similar density and distribution of Fos-ir cells was found in the capsaicin-treated rats. By electric stimulation of the sural nerve, Fos-ir cells were detected in laminae I, II, III, IV and X. Whilst capsaicin-treatment reduced Fos expression by electric stimulation in laminae I, II, III, and IV. Bradykinin or histamine induced Fos expression in laminae I, II, III and IV was reduced by capsaicin-treatment as well as lidocaine-treatment. Colchicine-treatment reduced formalin-induced Fos expression which was not reduced by capsaicin treatment in the spinal dorsal horn.

Our study showed that peripheral nerve injuries evoked Fos expression even in the spinal cord of chemically analgesic rats. This result strongly suggested the possibility of various signal-conduction mechanisms mediating the Fos expression in sensory processing.

553.1

EFFECT OF LIDOCAINE ON SCIATIC NERVE CONDUCTION IN RATS: AN *IN VIVO* SINGLE UNIT STUDY H. Huang, J.G. Thalhammer*, S.A. Raymond and G.R. Strichartz, Pain Research Group, Anesthesia Research Laboratories, Brigham & Women's Hospital, Harvard Medical School, Boston, MA 02115

Using an *in vivo* rat sciatic nerve preparation, with a fixed length of the nerve (22mm) exposed to lidocaine, we measured the tonic blocking concentration of lidocaine in functionally identified Aβ afferents, including rapidly adapting mechanoreceptors (RA), slowly adapting mechanoreceptors (SA) and muscle spindles (MS). A total of 27 units were studied and the blocking concentrations of lidocaine ranged from 0.2 mM to 0.8 mM. Mean blocking concentrations were: RA, 0.44 ± 0.15 mM, n =10; SA, 0.40 ± 0.15 mM, n=11; MS, 0.33 ± 0.09 mM, n=6 (NS, p=0.23). The dose-response curve from our study was compared with the data from Rosenberg and Heavner (*Acta anaesth. scand.* **24**: 314-320, 1980) in which inhibition of rat sciatic A-fiber compound action potential (CAP) was studied *in vitro* (■). The median value in our single-unit study was 0.4 mM, significantly higher than the 0.19 mM EC_{50} value from Rosenberg and Heavner. We postulate that the suppression of CAPs by low concentrations (<0.2mM) of lidocaine is not due to the abolition of impulse conduction in fibers, but instead to the slowing of impulse conduction and/or decrease in the impulse currents in single fibers. Furthermore, our results indicate that the variations in sensitivity to lidocaine among myelinated axons could not be linked to functional submodalities. (NIH USPHS GM 35647)



553.2

THE CONTRIBUTION OF PERIPHERAL SENSORY NEURONAL INPUT TOWARDS THE MAINTENANCE OF NEUROPATHIC PAIN. J.C. Hunter*, B. Martin, R. Lewis, L. Smith, D.J. Fontana and C. Lee. Dept. of Analgesia, Syntex Research, Palo Alto, CA 94303.

Current evidence suggests that while peripheral neural mechanisms contribute to its development, the persistence of neuropathic pain occurs predominantly through a prolonged state of central hyperexcitability and increased neural plasticity that can be maintained without further peripheral noxious input. The present study, however, investigated the possible contribution of ongoing afferent activity in the peripheral nerve to the persistence of pain following a peripheral nerve injury. QX-314, a quaternarized derivative of lidocaine and inhibitor of sensory neuronal sodium channels, was tested for its analgesic potential against the cold water (0°C) allodynic response in rats following a chronic constriction injury to the common sciatic nerve. Lidocaine (10-100 mg/kg, sc), but not QX-314 (10-100 mg/kg, sc), when administered as an acute 1hr pre-treatment in rats 5 days post-surgery, produced a dose-dependent anti-allodynic effect. In contrast, QX-314, when given by chronic administration (b.i.d. at 30mg/kg, sc), exerted an almost complete anti-allodynic effect after 5, but not 1 or 3 days. The allodynia returned within 48hrs of termination of the chronic dosing schedule. In vivo microdialysis (icv) studies with ^{14}C-QX-314 showed that no drug entered the CNS during chronic dosing. Similarly, behavioral studies found no evidence of the locomotor effects associated with lidocaine and other local anesthetics that can readily penetrate the blood brain barrier. In conclusion, established neuropathic pain can be blocked by the sustained, but not acute, action of a peripheral local anesthetic.

553.3

AMELIORATION OF MECHANO-SENSITIVITY IN TWO RAT MODELS OF NEUROPATHIC PAIN BY CLINICALLY EFFECTIVE ANALGESICS. M.F. Jett, J. Ravenscroft, D. Waligora, C. Kermeen, R.M. Eglen* and J.C. Hunter. Dept. of Analgesia, Syntex Research, Palo Alto, CA 94303.

Increased mechano-sensitivity to noxious (hyperalgesia) and innocuous (allodynia) stimuli is a hallmark of neuropathic pain in humans. Drugs that are clinically effective (lidocaine, mexiletine, desipramine and amitriptyline), less effective (morphine), or ineffective (indomethacin) in the treatment of neuropathic pain were evaluated for efficacy in reducing mechano-sensitivity using the spinal nerve injury (SNL) and chronic constriction injury (CCI) models of neuropathic pain in rats. These models differ in that the SNL surgery provokes a pain response with a significant sympathetic component. Vehicle or drug was administered subcutaneously 1 h prior to evaluation of the hyperalgesia response to pin prick and the allodynia response using von Frey filaments. Lidocaine (10-60 mg/kg), mexiletine (10-100 mg/kg), desipramine (10-30 mg/kg) and amitriptyline (3-30 mg/kg) attenuated hyperalgesia and allodynia in the SNL and CCI models. The doses employed did not produce significant behavioral effects. Neither morphine (1-3 mg/kg at 30 min prior to testing) nor indomethacin (5 mg/kg) significantly reduced mechano-sensitivity in either of the models. In conclusion, drugs which are effective in reducing human neuropathic pain ameliorate mechanical hyperalgesia and allodynia in SNL and CCI models of neuropathic pain in rats.

553.4

PRE-EMPTIVE EFFECTS OF INTRATHECAL LIDOCAINE ON FORMALIN-INDUCED NOCICEPTION AND C-*FOS* EXPRESSION. K. Yashpal[1]*, P.A. Mason[2], J.E. McKenna[3], S. Sharma[4], J.L. Henry[4] and T.J. Coderre[1]. [1]Pain Mechanisms Lab, Clinical Res. Instit. of Montreal, [2]Operational Technologies Corp., San Antonio, [3]Dept. of Psychol., UBC Kelowna, [4]Dept. of Physiol., McGill Univ.

Pretreatment with intrathecal (i.t.) lidocaine produces a prolonged antinociception during the late phase of the formalin test. We now examine whether this treatment produces a similar reduction in the formalin-induced expression of c-*fos* in spinal cord. Formalin pain behavior, in response to a 50 µl injection of 2.5% formalin into the hindpaw, was assessed in rats given a lumbar i.t. injection of 50 µl of 2.0% lidocaine, either 5 min before or 5 min after formalin injection, and compared with nociception in rats that received 50 µl of i.t. saline 5 min before formalin. Two hours after the formalin injection, the rats were sacrificed, and spinal cords were removed and processed for c-*fos* (Ab-1) immunocytochemistry or immunoprecipitation.

Pain behaviors in the late phase of the formalin test were significantly reduced in rats given lidocaine 5 min before the hindpaw formalin injection, but were unaffected in rats given lidocaine 5 min after formalin. Fos protein-like immunoreactivity in response to formalin injection was restricted to the spinal cord ipsilateral to the injected hindpaw. Fos positive cells were found to be maximal in the lumbar (L2-L6) spinal cord, predominantly in the superficial layer (lamina I), as well as deeper layers (lamina V-VI). The number of Fos positive cells was significantly reduced in rats that received lidocaine before formalin, but was only slightly reduced in rats that received lidocaine after formalin. Kinetic studies on formalin-induced c-*fos* expression using monoclonal antibody (Ab-1) and immunoprecipitation exhibited a maximal induction at 2 hrs, with a greater enhancement in the dorsal, as opposed to the ventral, horn. C-*fos* expression was significantly suppressed in rats which received i.t. lidocaine before the formalin injection, confirming lidocaine's pre-emptive effect in the formalin test.

553.5

INHIBITION OF NEUROPATHIC NEURAL ACTIVITY BY LIDOCAINE AND QX-314. J. Omana-Zapata*, K.R. Bley, J.C. Hunter and D.E. Clarke, Departments of Mechanistic Pharmacology & Analgesia, Roche Biosciences, Palo Alto, CA 94305.

Neuropathic pain is correlated with persistent high-frequency nerve activity originating from peripheral nerve injury sites and the dorsal root ganglia (DRG), and is associated with long lasting changes in the responsiveness of dorsal horn (DH) neurons in the spinal cord. In order to test the sensitivity of nerve injury-induced activity to local anesthetics, we have intravenously administered lidocaine and QX-314, the quaternary derivative of lidocaine, during extracellular potential recordings from the DRG and the DH. Experiments were performed on anesthetized adult male rats whose common sciatic nerve had been transected 5 to 8 days previously. In order to monitor effects on the heart and central nervous system, EKGs and EEGs were recorded simultaneously. Lidocaine or QX-314, given i.v. at 8 min intervals, produced a dose-dependent and significant reduction of ongoing activity in the DRG and DH. ID_{50}s (in mg/kg) for lidocaine (3.0 at DRG and 3.3 in the DH) were lower than for QX-314 (16.8 at DRG and 37.2 in the DH). The lower potency of QX-314 might be attributed to a delayed onset of effect due to slow tissue penetration. In contrast, the duration of decreased nerve activity following QX-314 infusion was much longer than for lidocaine, suggesting a persistent block of Na^+ channels. Consistent with a common mechanism of action, heart rate was rapidly decreased by either lidocaine (ID_{50} ~ 27) or QX-314 (ID_{50} ~ 51). These results suggest that QX-314 can acutely block neuronal and heart Na^+ channels, contrary to previous notions that it is only active following intracellular application (also see Bley *et al.*, this meeting). Whereas QX-314 does not readily penetrate the blood-brain barrier, the decrease of DH activity that followed QX-314 infusion may be secondary to the effects on the peripheral nerve or the DRG, or might be attributed to the surgical disruption of the spinal blood-brain barrier required for electrode placement.

553.6

STRYCHNINE- AND BICUCULINE-EVOKED INCREASES IN DORSAL HORN CELL ACTIVITY ARE MODULATED BY LIDOCAINE. S. Puig* and L.S. Sorkin, Dept. of Anesthesiology, UCSD, La Jolla, CA. 92093-0818

Blockade of GABA and strychnine (STR) sensitive glycine receptors within the spinal cord results in a profound allodynia/hyperalgesia. This study examined the relative efficacy of lidocaine (LID), a local anesthetic on this enhanced neuronal activity.

Dialysis probes were placed in the lumbar dorsal horn of α-chloralose anesthetized cats and extracellular recordings made from cells in close proximity. Physiological parameters were monitored and maintained. Artificial CSF was perfused through the probes and baseline responses to a range of mechanical stimuli measured. The perfusate was changed to STR (1.0 mM) or bicuculline (BIC) (0.5, 1.0 mM). An iv infusion of LID resulting in plasma levels of 1-6 mg/ml was then given. Receptive field properties were redetermined under each condition and for several LID concentrations (plasma LID concentration confirmed by gas chromatography).

Increasing plasma levels of lidocaine did not significantly reduce the STR enhanced response to low threshold stimuli until close to levels that produced conduction blockade. However, in the same cells, responses to noxious stimulation were reduced to pre-STR levels or below. BIC-enhanced responses to noxious intensities of stimulation were also preferentially modulated.

These results indicate that at the spinal level, systemic local anesthetics are better able to reduce transmission of afferent input mediated by fine myelinated or unmyelinated fibers than to reduce allodynia which is mediated by large myelinated fibers. Supported by NIH grant NS11255.

553.7

NOCICEPTIVE AND LOW-THRESHOLD RESPONSES IN THE RAT'S THALAMUS ARE DIFFERENTIALLY AFFECTED BY HALOTHANE ANESTHESIA. C. Vahle-Hinz*. Physiologisches Institut, Universitäts-Krankenhaus Eppendorf, D-20246 Hamburg, Germany.

The influence of varying depths of halothane anesthesia on neuronal activity and response properties was studied in single-unit recordings in the region of the ventrobasal complex. Adult rats were respired and anesthetized with 1.5% halothane and O_2/N_2O (30:70) for the preparations, and end-tidal CO_2 and heart rate were monitored continuously and kept in the physiological range. Non-noxious mechanical stimulation of fur and skin as well as noxious mechanical and thermal stimuli were applied to characterize the neuronal responses under 0.5% halothane. The halothane level then was varied between 0.5% and 1.5%. A dose-dependent reduction or suppression of the ongoing and evoked activity was seen in low-threshold mechanosensitive (LT) and wide-dynamic range nociceptive (WDR) neurons. At higher halothane levels, the LT responses changed from tonic to phasic response type, and the sensitivity as well as the receptive field sizes of the neurons were reduced. In WDR neurons, the responses to noxious stimulation changed from a sustained discharge to an on-/off-response, while the afterdischarges first were reduced in frequency and duration and then were abolished. The LT responses in both kinds of neuron were more robust than the nociceptive responses in WDR neurons and were still present when all nociceptive activity was suppressed. Thus, sensory processing of information about non-noxious and noxious events may be modulated differentially at the thalamic level.

553.8

EFFECTS OF PRE- AND POST-TREATMENT WITH INTRAVENOUS ANESTHETIC AGENTS ON NOCICEPTIVE BEHAVIOURS IN THE RAT FORMALIN TEST. L.Gilron[1] and T.J. Coderre[2]*. [1]Department of Anaesthesia, McGill University, and [2]Pain Mechanisms Laboratory, Clinical Research Institute of Montreal, 110 Pine Ave. W., Montreal, PQ, CANADA H2W 1R7.

The rat formalin test has been used as a model of injury-induced central sensitization and as an animal model to study the efficacy of pre-emptive analgesia. In this study, we evaluated the nociceptive behaviour of rats from 15 to 60 min after subcutaneous hindpaw injection of 50 µl of 1.5% formalin. In each trial, anesthetics and their respective vehicles were administered by tail-vein injection either 1 to 10 min prior to or 5 min following formalin injection.

Nociceptive scores were unaffected by pentobarbital (20 mg/kg) or propofol (10 mg/kg). Alfentanil at an anesthetic dose of 170 µg/kg produced profound analgesia which dissipated approximately 35 min after formalin injection. The timing of alfentanil administration had no influence on its analgesic efficacy. Alphaxalone pretreatment (1.5 mg/kg) produced analgesia compared to vehicle, and resulted in significantly lower scores than post-treatment from 15 to 30 min after formalin injection. Ketamine (10 mg/kg) produced analgesia compared to vehicle when given either pre- or post-formalin. From 20 to 50 min after formalin injection, ketamine pretreatment resulted in significantly lower scores than post-treatment.

Our results show no antinociceptive effects with pentobarbital or propofol in this model, and although alfentanil did produce profound analgesia, the timing of administration did not play a role. These data do demonstrate pre-emptive analgesia with both IV alphaxalone and IV ketamine. Further studies are being conducted in our laboratory to elucidate the mechanisms of these effects.

PAIN MODULATION: PHARMACOLOGY—NEUROPEPTIDES

554.1

A MATHEMATICAL MODEL TO EVALUATE THE EFFECTS OF NEUROTRANSMITTER RESPONSES TO PROLONGED NOXIOUS STIMULATION. E.Reiner* and B.J.Winterson. Dept. of Physiology, University of New England College of Osteopathy, Biddeford, ME 04005

Osteopathy has long recognized somatic dysfunction, the alterations in tissue texture, range of motion and tenderness, as a body's response to prolonged changes in circulation, extracellular fluid distribution, connective tissue and neural mechanisms. Two classes of neurotransmitters (NT) may be involved; the tachykinins, e.g. Substance P (SP) and Neurokinins A (NKA), and the excitatory amino acid, glutamate (Glu). One method used to analyze these manifestations uses a prolonged noxious stimulus to induce a persistent flexion reflex as an analog to somatic dysfunction.

The specific roles of SP, NKA and Glu on the development of the persistent hindlimb flexion reflex can be evaluated using a matrix model. Noxious stimulation is delivered for 3, 30 and 300 seconds in anesthetized spinalized rats. One set is the control, while the second, third and fourth sets are given the NT receptor (NMDA, N-methyl-D-aspartate, NK_1, and NK_2) antagonists, APV (2 amino-5-phosphonovaleric acid), CP-96,345 and SR-48,968, respectively. A matrix has been hypothesized as a mathematical model for the effects of neurotransmitter antagonists on the flexion reflex for a given stimulatory duration. The equations are expressed in terms of the flexion reflex against time, where the reflex is monitored as force, P_i.

$$P_i = \sum_{j=1}^{3} C_{ij} t_j + p_i(0); \qquad \text{for } i=1,2,3$$

Where $p_i(0)$ is control results, i is duration and, j is antagonist.

This mathematical approach represents a novel methodology to evaluate multiple neurotransmitter and/or pharmacological effects that are overlapping but not strictly coincident. This method may have value for studying other multiple factor neuronal systems.

(Burroughs-Wellcome Fellow)

554.2

THE INTERACTION BETWEEN NEUROKININ$_1$ AND METABOTROPIC GLUTAMATE RECEPTOR ANTAGONISTS IN BEHAVIOURAL NOCICEPTIVE TESTS IN NORMAL AND CARRAGEENAN-TREATED RATS. M.R. Young, S.M. Fleetwood-Walker, P.J Birch[1], C. Bountra[2] and J. Bowers[2], Department of Preclinical Veterinary Sciences, University of Edinburgh, U.K., [1]Department of Molecular Science (Greenford), [2]Pharmacology I (Ware), Glaxo Group Research, U.K. SPON: European Brain and Behaviour Society.

Intrathecal metabotropic glutamate receptor antagonists (S)-4-carboxy-3-hydroxy-phenylglycine [(S)-CHPG, (48nmol)] and L-1-amino-3-phosphopropionic acid (L-AP3, 60nmol), increase paw withdrawal latencies (PWLs) to both noxious mechanical and noxious thermal stimulation, in conscious rats. Such elevations were more profound after carrageenan-induced inflammation in the paw. However, in the same study the NK_1 receptor antagonist, GR82334 (3nmol), failed to alter PWLs (Young *et al* (1995) Br. J. Pharm. **114**:316P). We have addressed the effect of combined mGlu and NK_1 receptor antagonists in such behavioural nociceptive tests. Lister-hooded rats (30-70g), with either normal or carrageenan-inflamed paws, received a co-injection of GR82334 and either (S)-CHPG or L-AP3 (n=16 per group), at doses equivalent to those above (in 10µl). Now, the increases in PWL due to mGluR antagonists were occluded when GR82334 was given simultaneously, except the (S)-CHPG-induced increase in PWL to noxious thermal stimulation in the non-inflamed paw. Statistical significance of effects (% change from pre-drug levels) was determined compared to mGluR antagonists alone, ** - $p<0.01$; * - $p<0.05$ (Mann-Whitney U-test) w.r.t. the co-injection in the inflamed state:

		L-AP3	Co-injection	(S)-CHPG	Co-injection
MECHANICAL	1 min	123±47	19±8*	130±31	54±19**
THERMAL	1 min	97±6	9±9**	71±10	11±7**

These results indicate that in the state of carrageenan-induced hyperalgesia, NK_1 receptor antagonism appears to exert an adverse effect on the analgesia normally elicited by mGluR antagonists.

554.3

THE SPINAL RELEASE OF MET-ENKEPHALIN IS TONICALLY STIMULATED BY CHOLECYSTOKININ IN THE RAT. F. Cesselin, S. Bourgoin, A. Mauborgne, M. Hamon* and J. J. Benoliel. INSERM U 288, 91, Boulevard de l'Hôpital, 75634 Paris cedex 13, France.

Numerous data suggest that cholecystokinin (CCK) acts as an « anti-opioid » peptide. Thus, the blockade of CCK receptors can potentiate morphine-induced analgesia and prevent tolerance to the analgesic effects of opioids. We showed that opioids, through the stimulation of a subtype of ∂ receptors, can increase the release of CCK from central areas in the rat (Benoliel et al., J. Neurochem., 58 [1992] 916-922; Brain Res., 653 [1994] 81-91). This suggests that opioids could activate central CCK-ergic neurons, which act as a component of a homeostatic system tending to reduce the effects of opioids. Whether CCK (through CCK-A or CCK-B receptors) could in turn modulate the activity of a category of opioidergic neurons, i.e. the spinal met-enkephalinergic (ME) neurons, was presently investigated. For this purpose, slices of the dorsal half of the lumbar enlargement of the rat spinal cord were superfused with an artificial CSF (1 ml/4 min), and exposed to various CCK receptor ligands for assessing their possible effects on the spontaneous and K^+ (30 mM)-evoked release of ME-like material (MELM, determined using a specific radioimmunoassay). Compounds A 71378 and BC 264 were used as selective agonists at CCK-A and CCK-B receptors, respectively, and compounds L 364718 and L 365260, as selective antagonists at these receptors. The spontaneous outflow of MELM was affected by none of these drugs. By contrast, a significant increase in K^+-evoked MELM overflow occurred upon stimulation of CCK-A receptors by A 71378 (+17% at 1 µM, +40% at 10 µM), whereas their blockade by L 364718 resulted in a significant decrease in the peptide overflow (-53% at 1 µM, -70% at 10 µM). The K^+-induced release of MELM was less affected by ligands of CCK-B receptors (+25% with 10 µM BC 264, -15% with 10 µM L 365260). These results suggest that CCK acts preferentially at CCK-A receptors for exerting a tonic excitatory influence on spinal MELM release. Thus, CCK and ME may form a couple of mutually « antagonistic » peptides which reciprocally modulate their release in the rat spinal cord.

554.4

CAPSAICIN- AND SUBSTANCE P (SP) NH_2-TERMINUS-INDUCED ANTINOCICEPTION PRECEDES THEIR INHIBITION OF SP RELEASE FROM MOUSE SPINAL CORD. V.M. Goettl* and A.A. Larson. Graduate Program in Neuroscience, Univ. of Minnesota, St. Paul, MN 55108, U.S.A.

SP appears to be released in the spinal cord in response to a nociceptive stimulus. Capsaicin also elicits release of SP producing an initial hyperalgesia followed by antinociception. This antinociception has been attributed to the ability of capsaicin to decrease the content or release of SP associated with primary afferent C-fibers. Recently, we reported that the NH_2 terminus of SP may mediate the antinociceptive effect of capsaicin. To investigate the relationship between capsaicin-induced antinociception and changes in the content, release and action of SP from the spinal cord, we studied the effects of pretreatment with capsaicin and SP(1-7), an NH_2-terminal metabolite of SP, in mice. Twenty-four hr after the intrathecal (i.t.) injection of capsaicin or SP(1-7), mice are antinociceptive in the acetic acid writhing, the hot plate and the formalin (second phase) assays. However, 24 hr after 0.8 µg capsaicin or 30 nmol of SP(1-7) i.t., there was no change in SP release from perfused mouse spinal cord tissue in response to 3 µM capsaicin (1 min) compared to vehicle injected mice. In contrast, there were significant decreases at 48 hr ($p<0.02$) in the amount of SP released (pg SP/mg tissue/3 min) from mice injected with capsaicin (1.73 ± 0.32) and SP(1-7) (2.49 ± 0.31) vs. vehicle (4.48 ± 0.92). There was no change in total spinal cord content of SP at either time. This indicates that, like capsaicin, SP(1-7) inhibits SP release. However, their antinociceptive effects do not appear to be mediated by changes in SP release or content. (Supported by NIDA T32 DA07234 and DA04090.)

554.5

INFLUENCES OF VARIOUS NEUROPEPTIDES ON EXCITATORY AMINO ACIDS RELEASE IN TRIGEMINAL SPINAL SENSORY NUCLEUS. J.H.LEE*, J.S.HAM, K.P.PARK and J.S. KIM (Dept. of Oral Physiol., Coll. of Dentistry, SEOUL NAT'L. UNIV., KOREA)

Many neurotransmitters, such as excitatory amino acids(EAA), substance P(SP), calcitonin gene related peptide (CGRP), play a role in pain transmission in trigeminal spinal sensory nucleus. This study was performed to elucidate the role of SP, CGRP and the role of GABAergic neurons on the release of aspartate(Asp) and glutamate(Glu) from trigeminal spinal sensory nucleus caudalis.

Push-pull perfusion(40μl/min) was performed in trigeminal sensory nucleus of rabbits (adults either sex) and perfusate was analyzed using HPLC-FD employed o-phthaldialdehyde derivatization method. The release of Asp and Glu was evoked by noxious electrical stimulation of inferior alveolar nerve with or without perfusion of SP, CGRP, GABAergic agonists and antagonists to the trigeminal sensory nucleus.

The release of EAA was increased by electrical stimulation of inferior alveolar nerve. SP and CGRP increased the release of Asp and Glu from trigeminal spinal sensory nucleus. Muscimol,$GABA_A$ agonist, significantly reduced the effects of electrical stimulation. This inhibitory effect of muscimol was antagonized by bicuculline and the relase of EAA was potentiated by addition of bicuculline alone in caudal area of trigeminal sensory nucleus.

These results suggest that the EAA could be a neurotransmitter of orofacial pain transmission, and the release of EAA could be modulated by various neuropeptides.

554.6

NOVEL NK-1 RECEPTOR ANTAGONISTS LY303870 AND LY306740 BLOCK THE RESPONSES OF CAT DORSAL HORN NEURONS TO SUBSTANCE P AND TO PERIPHERAL NOXIOUS STIMULI.

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In this study, we have tested the effects of novel substance P (NK-1) receptor antagonists LY303870 and LY306740, as well as LY306155, the enantiomer of LY303870, on the responses of nociceptive spinal dorsal horn neurons to iontophoretically applied substance P and to peripheral noxious stimuli. Extracellular recordings were obtained using multibarrel electrodes from L_4-L_7 segments of the spinal cord in cats anesthetized with α-chloralose and spinalized at the L_1 level. The antagonists were given i.v. (0.5-4.0 mg/kg). Responses to substance P were significantly inhibited by LY303870 (mean % inhibition: 1 mg, 36; 2 mg, 93; 4 mg, 95; n=5-6) and by LY306740 (1.5 mg, 49; 2 mg, 81; n=5) but not by LY306155 (4 mg, 17; n=4). Responses to peripheral noxious thermal stimulation were inhibited by LY303870 (2 mg, 57; 4 mg, 69; n=6) and LY306740 (1.5 mg, 39; 2 mg, 52; n=4). Interestingly, noxious thermal responses were also inhibited by LY306155 but only at a higher dose (2 mg, 24; 4 mg, 44; n=4). Responses to noxious pinch stimuli were inhibited by LY303870 (1 mg, 26; 2 mg, 51; n=4) and LY306740 (1.5 mg, 43; 2 mg, 53; n=4). LY306155 lacked consistent effects on pinch responses. LY303870 selectively inhibited the late component of the response to stimulation of the superficial peroneal nerve with a train of high intensity electrical stimuli (1 mg, 26; 2 mg, 62; n=3). These data suggest that LY303870 and LY306740 pass from the circulation into the spinal cord where they antagonize dorsal horn neuron responses to substance P and to nociceptive inputs. *(Supported by Eli Lilly and Company, Indianapolis, and the Canadian MRC).*

554.7

SPINAL CCK_B RECEPTORS MEDIATE NEUROTENSIN HYPERALGESIA FROM THE NUCLEUS RAPHE MAGNUS (RMg).

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The neuropeptide neurotensin produces a hyperalgesic/antianalgesic effect on the spinal nociceptive tail-flick reflex when injected into the medullary RMg of rats. Cholecystokinin octapeptide (CCK), on the other hand, has been implicated as an antianalgesic mediator in the spinal cord. Thus, the present study was designed to determine a potential involvement of spinal CCK in mediating neurotensin hyperalgesia from the RMg. Microinjection of neurotensin (30 pmol) into the RMg of awake rats produced a facilitation of the tail-flick reflex that was completely inhibited by intrathecal (i.t.) administration of the non-selective CCK receptor antagonist proglumide (100 ng). I.t. administration of the selective CCK_B receptor antagonist L-365260 dose-dependently inhibited neurotensin hyperalgesia from the RMg (ID_{50} = 0.42 ng) at doses approximately 1000 fold less than that observed with the selective CCK_A receptor antagonist L-364718 (ID_{50}= 646 ng). Injection of CCK alone i.t. (30-100 ng) produced a hyperalgesic effect on the tail-flick reflex which was inhibited by i.t. L-365260 (ID_{50} = 0.59 ng) at doses approximately 1000 fold less than that observed with i.t. L-364718 (ID_{50} = 630 ng). These data indicate that spinal CCK hyperalgesia involves the CCK_B receptor subtype, which is consistent with the suggestion that spinal CCK_B receptors mediate neurotensin hyperalgesia from the RMg.

554.8

NK1 RECEPTOR ON LAMINA 1 NEURONS OF THE SPINAL CORD: DISTRIBUTION, RELATION TO SUBSTANCE P AND RESPONSE TO PERIPHERAL STIMULATION. J.A. Palmer, R. Munglani[1], G.D. Smith[2], P.J. Birch*[2] and S.P. Hunt. Neurobiology Division, MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK. [1]Dept. of Anaesthesia, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, UK. [2]Dept. of Molecular Science, Glaxo Research & Development Ltd, Greenford Road, Greenford, Middlesex, UB6 0HE, UK.

We have examined the distribution of the NK1 receptor in the dorsal horn of the spinal cord using immunohistochemistry with a specific antibody raised to a 15 amino acid C terminal sequence of the native protein (Vigna et al., 1994). Immunoreactivity was particularly intense on the large neurons of lamina 1 which in most cases were also positive for calbindin 28K-like immunoreactivity. In horizontal sections of the spinal cord the receptor protein was found to be present over the entire extent of these neurons while colocalization studies with antibodies directed against the NK1 ligand, substance P (SP), revealed that SP positive terminals were only found at certain sites on the cell surface; either the distal or proximal dendrites or less frequently over the cell body. In pentabarbital anaesthetised rats, section or crush of the sciatic nerve resulted, within 5 minutes, in the rapid internalization of NK1 immunoreactivity which recovered within 20min. This internalization was seen throughout the neuron and not restricted to areas of direct synaptic contact by SP. This strongly suggested that SP could diffuse from the presynaptic site of release and affect NK1 receptor 'at a distance' by volume transmission. We also examined these neurons 14d following adjuvant-induced inflammation of the hind paw but found no change in receptor number, cellular localization or distribution within lamina 1. However, in hyperalgesia produced at 28d following loose ligation of the sciatic nerve, we were able to detect a marked increase in NK1 receptor immunoreactivity within lamina 1.

Vigna et al., (1994) *J. Neurosci.* 14 834-845

554.9

GLUTAMATE INHIBITS SUBSTANCE P RELEASE FROM TRIGEMINAL NUCLEUS CAUDALIS CAPSAICIN-SENSITIVE PRIMARY AFFERENTS.

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Substance P (SP) and glutamate (GLU) are present in primary afferent C-fibers. These two substances play important roles in producing persistent inflammatory-evoked and neuropathic pain. Although it is clear that both of these substances are released by C-fibers, little is known about the modulatory effects of GLU on the release of SP from C-fibers. In the present study, we have examined whether GLU modulates SP release from trigeminal nucleus caudalis slices evoked by the C-fiber selective stimulant capsaicin (1 μM). Immunoreactive SP was measured in perfusates. A very low concentration of GLU (10 nM) inhibited capsaicin-evoked SP release without affecting basal release. The competitive metabotropic GLU receptor antagonist (±)-alpha-methyl-4-carboxyphenylglycine (5μM (±)-MCPG) reversed GLU inhibition of capsaicin-evoked SP release to a facilitation. The non-competitive NMDA receptor antagonist, dizocilpine (MK-801, 0.3 μM) did not block GLU inhibitory effect. These results suggests the existence of both inhibitory and excitatory (masked) presynaptic GLU receptors on SP-containing primary afferents. (Supported by TW00305 & DE07509).

554.10

DYNORPHIN A_{1-17} ENHANCES SUBSTANCE P RELEASE FROM TRIGEMINAL NUCLEUS CAUDALIS CAPSAICIN-SENSITIVE PRIMARY AFFERENTS.

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The outcomes of several recent studies suggest that the endogenous opioid peptide Dynorphin A_{1-17} (DYN) produces an anti-analgesic action in the spinal cord and may contribute to the development of persistent pain in chronic inflammatory conditions. The spinal mechanisms involved in DYN actions are not completely understood. Since substance P (SP) is a pro-nociceptive neuromodulator released by primary afferent C-fibers, we have examined whether DYN modulates SP release from trigeminal nucleus caudalis slices evoked by the C-fiber selective stimulant capsaicin (1 μM). Immunoreactive SP was measured in perfusates. A very low concentration of DYN (0.1 nM) strongly facilitated capsaicin-evoked SP release without affecting basal release. The competitive μ-opioid receptor antagonist beta-funaltrexamine (20 nM) and the non-selective opioid-receptor antagonist naloxone (100 nM) blocked the facilitatory effect of DYN. In contrast, the κ-opioid receptor antagonist, nor-binaltorphimine (3 nM) did not alter DYN's facilitatory effect. The effect of the μ_1-receptor opioid receptor antagonist, naloxonazine (1 nM) is currently under investigation and will be discussed. These data suggest that DYN increases nociceptive transmission at the spinal level by increasing SP release from primary afferents following the stimulation of an opioid receptor which is sensitive to beta-funaltrexamine. It is possible that this receptor is the proposed μ/δ-receptor complex. (Supported by TW00305 & DE07509).

554.11

PERIPHERAL MODULATION OF CAPSAICIN-INDUCED NEUROPEPTIDE RELEASE BY NICOTINE. S. Kilo, K.M. Hargreaves* and C.M. Flores, Laboratory of Neuropharmacol.ogy, Univ. of Minn., Minneapolis, MN 55455

There have been conflicting reports on the ability of nicotine to stimulate neuropeptide secretion from peripheral primary afferent terminals. The purpose of the present study was to assess whether nicotine could alter capsaicin-evoked calcitonin gene-related peptide (CGRP) secretion in a newly developed rat skin superfusion model and whether such effects are receptor mediated.

Non-glabrous skin from the rat hind paw was dissected and placed in superfusion chambers bathed in normal Kreb's solution and connected by tubing to a fraction collector. After several baseline fractions, drugs were added to the buffer followed by a washout period. Fractions were subjected to radioimmunoassay using a polyclonal antiserum against CGRP.

Capsaicin alone evoked a dose-dependent, transient increase in immunoreactive CGRP (iCGRP) which peaked approximately 10 min after stimulation before returning toward baseline values. Nicotine by itself showed no effect on iCGRP release. However, when coadministered with capsaicin, nicotine displayed a dose-dependent, biphasic modulation of iCGRP release. Thus, treatment of the tissue with ≤3 mM nicotine hydrogen bitatrate resulted in potentiation of capsaicin-evoked iCGRP release. In contrast, when chambers were perfused with higher concentrations of nicotine, there was an inhibition of the iCGRP response to capsaicin. These effects were reversed by pretreatment of the tissue with the non-competitive nicotinic receptor antagonist mecamylamine. Interestingly, not only did mecamylamine by itself evoke an increase in iCGRP with a similar time course as capsaicin, but also mecamylamine potentiated capsaicin-evoked release. These data indicate that nicotine can modulate the capsaicin-evoked release of iCGRP from peripheral terminals of primary afferent neurons. The ability of mecamylamine to reverse these effects and the dose-dependence of the response indicate that nicotine may be acting either directly or indirectly through a neuronal nicotinic receptor. This work was supported by grant #0490-01 from the Smokeless Tobacco Research Council.

554.12

NEURONAL NICOTINIC RECEPTOR EXPRESSION IN THE TRIGEMINAL GANGLION OF THE RAT C.M. Flores*, S. Kilo, R.M. DeCamp and K.M. Hargreaves. Lab. of Neuropharmacol., Univ. of Minn., Minneapolis, MN 55455.

A growing body of evidence indicates that primary afferent sensory neurons express nicotinic receptor subunit mRNAs, exhibit electrophysiological responses to nicotinic agonists and are capable of releasing neuropeptides from capsaicin-sensitive peripheral terminals following application of nicotine. The purpose of the present studies was to determine in the rat trigeminal ganglion (TGG) whether there exist high affinity neuronal nicotinic receptors, which neuronal nicotinic receptor subunit mRNAs are expressed and whether any of these are colocalized with the neuropeptides substance P (SP) or calcitonin gene-related peptide (CGRP) in individually identified neurons.

High affinity binding sites were measured in membrane homogenates using ^{3}H-cytisine in a vacuum filtration assay. Excess unlabeled nicotine (100 μM) was used to define non-specific binding. *In situ* hybridization with antisense ^{35}S-labeled riboprobes complimentary to the mRNA encoding the α2, α3, α4, α5, β2 or β4 subunit was performed on 20 μm frozen sections of adult, male rat TGG. For each subunit, sense probes were used as controls. For colocalization analyses, hybridized tissue sections were subsequently subjected to immunocytochemistry using polyclonal antisera directed against either SP or CGRP.

Low but detectable specific binding of ^{3}H-cytisine in TGG was obtained. Compared with sense controls, in most of the neurons of the TGG specific hybridization was detected for each of the α and β subunits tested with the exception of α3, which was intensely localized to an undefined subset of predominantly large, type A neurons. Colocalization studies indicated that the cellular expression of neuronal nicotinic receptor subunit mRNA included but was not restricted to neurons which contained SP and CGRP. Taken together, these findings provide a molecular and biochemical basis for the ability of nicotine to directly modulate peptide release from sensory neurons.

This work was supported by grant #0490-01 from the Smokeless Tobacco Research Council.

554.13

CYCLIC AMP MODULATES PEPTIDE RELEASE INDUCED BY ACTIVATION OF PROTEIN KINASE C IN SENSORY NEURONS. L.A. Barber* and M.R. Vasko. Dept. of Pharmacol. and Toxicol., Indiana U. School of Medicine, Indianapolis, IN 46202

We have demonstrated that protein kinase C (PKC) activators augment resting and evoked release of neuropeptides from rat sensory neurons in culture. In addition, increasing intracellular cAMP sensitizes sensory neurons, enhancing peptide release. Because activation of PKC generates cAMP in other cell types, we examined whether PKC-mediated alterations in peptide release are modulated by an increase in cAMP.

Sensory neurons were dissociated from fetal rat dorsal root ganglia (E15-17) and grown in culture for 9-11 days. Basal release was determined by exposing neuronal cultures to HEPES buffer, whereas evoked release was obtained in buffer containing 50 nM capsaicin. Calcitonin gene-related peptide (CGRP) and cAMP content were measured by radioimmunoassay.

Exposing neurons to 10 or 100 nM phorbol 12,13-dibutyrate (PDBu) increases cellular cAMP by 50% and 200%, respectively. In contrast, the inactive analog, 4-a PDBu (100 nM) has no effect on cAMP. Treating cells with 1 nM PDBu does not alter resting release of CGRP, yet increases capsaicin-stimulated release 2-fold. Ten and 100 nM PDBu produce a 10- and 40-fold increase in peptide release above resting values, respectively. Pretreating neurons with 5 mM 9-(tetrahydro-2-furyl)adenine, an adenylyl cyclase inhibitor, significantly attenuates PDBu-induced peptide release.

These results suggest that a component of the PKC-induced peptide release from sensory neurons is dependent on the cAMP transduction pathway. This "cross-talk" among transduction cascades may be important in regulating peptide release from sensory neurons. (Supported by Amer. Heart Assn. Ind. Affil. and PHS NS 34159)

554.14

THE EFFECTS OF SELECTIVE NK_1 ANTAGONISTS ON SPINAL RESPONSES TO NOXIOUS PINCH IN THE ANAESTHETIZED RAT

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The effects of selective NK_1 receptor antagonists have been examined on responses of dorsal horn neurones to noxious mechanical stimuli. The non peptide NK_1 antagonists L-733,060 (rNK_1 receptors IC_{50} 700 nM; hNK_1 receptors IC_{50} 0.87 nM), CP-99,994 and their NK_1 inactive enantiomers (L-733,061 and CP-100,263), and morphine were administered i.v. Extracellular recordings of action potentials were made from single neurones in male, Sprague-Dawley rats (320-480g) anaesthetized with α-chloralose. Noxious pinch stimuli were applied to the hind paw at three intensities (pinch 1, 2-3N/28mm^2; pinch 2, 1.5-2N/28mm^2; pinch 3, 0.8-1N/28mm^2) that evoked distinct firing rates (53±10spikes/s, 33±5sp/s and 15±3sp/s respectively). Antagonist effects are expressed as percentages of the mean of three pre-drug control responses after subtraction of background activity. Significant changes (t-test) are indicated by * ($p<0.05$). CP-99,994 (n=5) and CP-100,263 (n=4) had no effect at 1 or 10 mg/kg. L-733,060 and morphine reduced responses to noxious pinch similarly; lower intensity responses were depressed more than higher intensity responses.

drug	dose	pinch 1	pinch 2	pinch 3	n
L-733,060	1 mg/kg	85±8%*	88±13%	63±8%*	5
L-733,061	1 mg/kg	107±5%	106±9%	104±8%	5
L-733,060	10 mg/kg	75±4%	64±7%*	43±10%*	5
L-733,061	10 mg/kg	90±4%	85±6%	85±8%	5
morphine	1 mg/kg	81±7%	71±10%	55±13%*	6

The inactivity of L-733,061 at 1 mg/kg suggests that these effects were not due to ion channel blockade. The data indicate that spinal responses to noxious mechanical stimuli are at least, in part, mediated by NK_1 receptors. NK_1 antagonists may therefore be useful in the control of pain.

554.15

INVOLVEMENT OF CGRP AND SUBSTANCE P IN NEUROGENIC VASODILATION IN RAT HIND PAW. S.L. Shepheard, D.A. Cook, R.G. Hill,* & R.J. Hargreaves. Merck, Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Essex, CM20 2QR, U.K.

Vasodilation produced in the skin of the hind paw of the rat in response to electrical stimulation of the saphenous nerve has been shown to involve CGRP released from sensory nerve terminals. Another vasodilator, substance P, is known to be co-localised with CGRP in sensory nerve fibres. The possible interactions of these two substances in the production of neurogenic vasodilation in the rat hind paw were studied in pentobarbitone anaesthetised rats. Changes in blood flux through the skin of the hind paw were measured using a laser doppler fluxmeter. Following an injection of vehicle, the left saphenous nerve was stimulated (5 Hz, 2 ms, 10 V for 10 s) and then repeat stimulations were made at 15 min intervals after injections of the CGRP antagonist hαCGRP$_{(8-37)}$ (0.1, 0.3 & 1 mg kg^{-1}) or the NK_1 receptor antagonist RP-67580 (0.1, 0.3 & 1 mg kg^{-1}). In another series of experiments, three stimulations were made at 15 min intervals after injection of firstly vehicle, then hαCGRP$_{(8-37)}$ (1 mg kg^{-1}) alone, then hαCGRP$_{(8-37)}$ (1 mg kg^{-1}) plus RP-67580 (1 mg kg^{-1}). All drugs were administered intravenously.

Stimulation of the saphenous nerve after vehicle produced an immediate increase in hind paw skin blood flux which reached a maximum by 30-60 s and returned to baseline by 15 min. hαCRGP$_{(8-37)}$ (1 mg kg^{-1}) markedly reduced this response so that only a 'fast component' remained, i.e. a transient increase in flux reaching the same max. as that seen after vehicle but returning to baseline within 1-2 min. RP-67580 had no obvious effects on the flux response when given alone but when co-administered with hαCRGP$_{(8-37)}$ the response to electrical stimulation was almost completely abolished, i.e. RP-67580 blocked the 'fast component' revealed by administration of hαCRGP$_{(8-37)}$. These results suggest that the neurogenic vasodilation produced in the hind paw skin of the rat has two components; a 'slow component' mediated by CGRP and a 'fast component' involving substance P.

554.16

THE EFFECTS OF PITUITARY ADENYLATE CYCLASE ACTIVATING PEPTIDE (PACAP) ON NOCICEPTIVE BEHAVIOR EVOKED BY INJECTION OF FORMALIN.

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Our recent studies have suggested that PACAP may be involved in nociceptive transmission. The present study investigated the effect of intrathecal PACAP on the nociceptive behavior in the formalin test. In anesthetized male Sprague-Dawley rats (280-320g), a catheter (PE-10) was inserted through the atlanto-occipital membrane into the subarachnoid space at the level of the rostral edge of the lumbar enlargement. Seven to ten days after surgery, animals with normal motor function were selected for use in the study. PACAP-27 was administered intrathecally. Twenty minutes later, formalin solution (50 ml of 5%) was injected subcutaneously into the dorsal surface of the right hind paw. Intrathecal PACAP-27 (0.6 pmol) significantly suppressed the second phase flinching response to formalin injection. Suppression was dose-dependent. The data suggest that intrathecal injection of PACAP produces a dose-dependent depression of prolonged nociceptive behavior evoked by injection of formalin.

555.1

ACTIVATION OF PERIPHERAL α_1-ADRENOCEPTORS CONTRIBUTES TO PAIN INDUCED BY NORADRENALINE PLUS α-METHYL-5-HT OR BY FORMALIN, Y. Hong* and F.V. Abbott, Dept. of Psychiatry, McGill Univ., Montreal, PQ, Canada H3A 1A1.

Peripheral adrenergic mechanisms contribute to chronic pain, hyperalgesia to pressure, and formalin-induced pain. Here we examined the effects of local injections of adrenergic receptor antagonists on pain induced by intraplantar injection of α-methyl-5-HT (α-m-5-HT) plus noradrenaline (NA) or PGE_2, which induce behavioural responses similar to the second phase of the formalin test (Neurosci. 63, 1994, 827), and also on pain induced by formalin.

All agents were injected S.C. into the plantar surface of rats' paws, and the lifting and licking response recorded. Pain produced by α-m-5-HT (10 μg) plus NA (10 μg) was blocked by pretreatment with the nonspecific α antagonist, phentolamine (10 μg), but not by the nonspecific β antagonist, timolol (10 μg). Neither of these agents altered the pain produced by α-m-5-HT plus PGE_2 (0.1 μg). Pain induced by formalin (1%, 50 μl) was biphasic, and adrenergic antagonists did not alter the first phase. The second phase was attenuated by 40% by phentolamine injected either 5 min before formalin or at the beginning of the second phase. Timolol had no effect on either phase. Pain produced by α-m-5-HT plus NA or by formalin were also attenuated by the α_1 antagonist, prazosin (40 μg), but not by the α_2 antagonist, idazoxan (40 μg). Thus, activation of α_1 adrenergic activation contributes to pain associated with inflammation in some circumstances, but pain induced by α-m-5-HT and PGE_2 is independent of adrenergic function, indicating that adrenergic function is not necessary for induction of pain by inflammatory mediators. Moreover, α_1 adrenergic blockade, unlike 5-HT_{2A} blockade (Abbott et al, this meeting), attenuates pain when given after development of pain, implying that adrenergic mechanisms contribute to ongoing maintenance of pain.

555.2

ACTIVATION OF $5HT_2$ RECEPTORS POTENTATES THE SPONTANEOUS INHIBITORY POSTSYNAPTIC CURRENTS (sIPSCs) IN TRIGEMINAL NEURONS. K. Sugiyama, B. Haber*, and L.-Y. M. Huang. Marine Biomedical Institute, Department of Physiology and Biophysics, The University of Texas Medical Branch, Galveston, Texas 76555-1069.

Serotonin (5HT) has been shown to inhibit the transmission of nociceptive signals in the medullary and spinal dorsal horns. One of the possible mechanisms by which 5HT exerts the analgesic action is thought to increase the release of inhibitory transmitters GABA and glycine. To test this possibility, we examined the actions of 5HT on dorsal horn neurons in the spinal trigeminal subnucleus caudalis using the whole cell patch recording technique. All the recordings were made from trigeminal neurons in thick medulla slices prepared from 15-20 day-old rats. 5HT (30-100 μM) increased sIPSCs in 52% (29/56) of the neurons. The frequency of sIPSCs increased from 0.5±0.4 Hz to 18.4±8.6 Hz and the amplitude of sIPSCs increased from 25±12 pA to 66±42 pA (n=4). The 5HT-induced sIPSCs were completely abolished by bicuculline and strychnine. Various 5HT agonists and antagonists were used to determine the 5HT receptor subtype that mediates this 5HT action. The potentiation of sIPSCs could be mimicked by the $5HT_2$ agonist α-methyl-5HT (3-20 μM) and be blocked by the $5HT_2$ antagonist ketanserin (1-3 μM). The $5HT_3$ antagonist ICS205-905 (0.2-1 μM) failed to inhibit the effect of 5HT on sIPSCs. In addition, we also found that the potentiation of sIPSCs was completely abolished in the presence of tetrodotoxin (0.3 μM). These results suggest that 5HT activates $5HT_2$ receptors on interneurons and promotes the release of GABA or glycine, thus reducing the excitability of medullary dorsal horn neurons. (Supported by NIH grants NS11255, NS30045, NS23061).

555.3

THE ROLE OF 5-$HT_{2A/2C}$ RECEPTORS IN SPINAL NOCICEPTIVE TRANSMISSION AT UPPER THORACIC SEGMENTS IN RATS. A. Kjørsvik, A. Tjølsen, B. Srebro* and K. Hole. Department of Physiology, University of Bergen, N-5009 Bergen, Norway.

Aim of investigation: The role of 5-$HT_{2A/2C}$ receptors in regulation of nociception was investigated by using spinal administration of the 5-$HT_{2A/2C}$ receptor agonist (±)DOI·HCl. The 5-$HT_{2A/2C}$ receptor concentration in rats is highest at the upper thoracic segments in the spinal cord, and the injections were performed at this level.

Methods: DOI was given through intrathecal catheters. The intrathecal catheterization involved a catheter-through-needle technique in the lumbar region (L5-L6) of halothane-anesthetized rats. DOI was dissolved in 0.9% NaCl and injected in a volume of 15 μl. Experiments were performed 1-2 days after implantation of catheters. The increasing temperature hot-plate test was performed two min after drug injection. The temperature was increased from 42 °C at a rate of 3 °C/min and a cut-off temperature of 50 °C was set to avoid tissue damage. The second time the animal licked one of the forepaws terminated the testing procedure. The temperature at this point was considered the response temperature.

Results: DOI (0.001 mM - 10 mM) injected at the upper thoracic level induced a dose-dependent decrease in the response temperature. At 100 mM DOI a considerable motor depression (hind limb abduction) was observed, and the animals seemed sedated. The rats were unaffected by the increasing temperature and showed no licking response.

Conclusion: Upper thoracic 5-$HT_{2A/2C}$ receptors appear to be involved in enhancement of nociception. High doses of DOI seemed to mask the pain-like behaviour.

555.4

THE ROLE OF THE SEROTONIN-1B RECEPTOR IN GENETIC SENSITIVITY TO OPIATE ANALGESIA IN MICE. J.S. Mogil*, B. Sadowski, and J.K. Belknap. Oregon Health Sciences University, Portland, OR 97201.

Morphine analgesic magnitude is a trait subject to large individual variability. A number of genetic mouse models exist that display highly divergent morphine analgesia; these include the DBA/2 and C57BL/6 inbred strains, the HAR and LAR lines selectively bred for high and low opiate analgesia, and the HA and LA lines selectively bred for high and low stress-induced analgesia. In an attempt to identify genes determining morphine analgesic magnitude in mice, we have recently performed quantitative trait locus (QTL) mapping experiments using mice derived from the DBA/2 and C57BL/6 strains (see Belknap and Crabbe, *Ann. N.Y. Acad. Sci.*, 1992). These studies have revealed two QTLs that together account for over 50% of the total genetic variance in this phenotype. One QTL, located 40 cM from the centromere on chromosome 9, shows tight linkage with the *Htr1b* gene. The *Htr1b* gene, which encodes the serotonin-1B ($5HT_{1B}$) receptor subtype, is thus implicated as a candidate gene for the QTL. Serotonin receptors are known to be important in pain modulation, but the specific role of the $5HT_{1B}$ receptor has remained unknown due to the lack of a specific antagonist. In the present studies, analgesic dose-response relationships (0-75 mg/kg, i.p.) of the specific $5HT_{1B}$ agonist, CGS-12066A (CGS), were investigated in DBA/2, C57BL/6, HAR, LAR, HA, and LA mice. The CGS potency ratios for "high strain" (DBA/2, HAR, and HA) ED_{50}s relative to "low strain" (C57BL/6, LAR, and LA) ED_{50}s were found to be similar to those seen for morphine. The mixed $5HT_{1A}/5HT_{1B}$ antagonist, (-)-pindolol (0-10 mg/kg, i.p.), was found to dose-dependently block CGS analgesia in all strains. Intriguingly, the opiate receptor antagonist, naloxone (10 mg/kg, i.p.), antagonized CGS analgesia only in high strains. These findings support a role for the $5HT_{1B}$ receptor in the mediation of opiate analgesic magnitude. Supported by VA Merit Review to JKB.

555.5

SENSITIZATION BY SEROTONIN OF FORMALIN OR PGE_2 INDUCED PAIN: PROPHYLACTIC BUT NOT THERAPEUTIC EFFECTS OF 5-HT_{2A} RECEPTOR ANTAGONISTS, F.V. Abbott*, Y. Hong and P. Blier, Dept. of Psychiatry, McGill Univ., Montreal, PQ, Canada H3A 1A1.

We have shown that 5-HT potentiates behavioural pain responses to other inflammatory mediators, and that 5-HT_{2A} antagonists dose-dependently block pain induced by either 5-HT plus another inflammatory mediator or by formalin (Neurosci. 63, 1994, 827; Neurosci. Abst. 20, 1994, 962). The present study examined the temporal characteristics of serotonergic sensitization of inflammatory pain.

Pain was induced by intraplantar injection of the 5-HT_2 receptor agonist, α-methyl-5-HT (α-m-5-HT), plus PGE_2, or 50 μl of 1% formalin. Simultaneous injection of α-M-5-HT (10 μg) plus PGE_2 (0.1 μg) produced lifting and licking of the injected paw for a total of 10 min during 30 min of observation. When α-m-5-HT was injected before PGE_2, sensitization gradually decreased as the interval between α-m-5-HT and PGE_2 was increased to 30 min. When PGE_2 was injected prior to α-m-5-HT, sensitization persisted for more than 60 min. Pain induced by the combination of α-m-5-HT and PGE_2 was blocked by intraplantar injection of ketanserin (5-$HT_{2A/2C}$ antagonist; 5 μg) or spiperone (5-HT_{2A} antagonist; 0.3 μg) 5 min before the algogens. This analgesic effect was attenuated by about 60% when the algogens preceded the antagonist by 2 min. Formalin produced a biphasic pain response, and the second phase was selectively blocked by ketanserin (50 μg) and spiperone (3 μg) injected 5 min before formalin. When the antagonists were injected at the beginning of the second phase, the analgesic effect was attenuated by about 75%. Thus, 5-HT produces an irreversible sensitization of tissue to other inflammatory mediators that wanes over 30 min. The data imply that 5-HT_{2A} antagonists prevent the evolution of pain in injured tissue, but they cannot reduce already-present pain.

555.6

DIFFERENTIAL PHARMACOLOGY OF RAPHE MAGNUS STIMULATION PRODUCED ANTINOCICEPTION FOR RAT FOOT WITHDRAWAL RESPONSE EVOKED BY DIFFERENT RATES OF NOXIOUS RADIANT HEATING Y. Lu, H. K. Proudfit, and D. C. Yeomans*. Dept. of Pharmacology, University of Illinois at Chicago, 60612

Electrical stimulation of neurons in the Nucleus Raphe Magnus (NRM) inhibits transmission of nociceptive information within the spinal cord. This antinociception is at least partially mediated by spinal 5-HT, α_2-adrenergic and opiate receptors as indicated by the attenuation of this effect by intrathecal application of antagonists to these receptors. The purpose of this study was to determine: (1) if NRM stimulation preferentially attenuates foot withdrawal responses (FWR) evoked by high or low rates of skin heating; and (2) if 5-HT, α_2, or opiate receptor antagonists preferentially attenuate the effect of NRM stimulation on high heating rate (HHR) or low heating rate (LHR) responses. To do this, we determined the NRM stimulation current thresholds to produce antinociception for FWR using high and low rates of noxious radiant heat. The α_2 antagonist yohimbine, the 5-HT antagonist methysergide, or the opiate antagonist naltrexone was then injected intrathecally. Fifteen minutes later, the current threshold for antinociception was remeasured. We found that NRM stimulation increases foot withdrawal latencies for both HHR and LHR, but that somewhat higher currents were necessary for HHR responses. Intrathecal applicatation of yohimbine preferentially attenuated the antinociceptive effects of NRM stimulation on HHR responses, methysergide preferentially blocked the effect on LHR responses, and naltrexone was approximately equally effective at blocking the effects on HHR and LHR responses. We conclude that NRM stimulation differentially attenuates nociceptive responses evoked by either HHR or LHR, and that these antinociceptive effects are mediated by different neurotransmitters. Supported by PHS Grant no. DA08256.

555.7

CHANGES IN ALPHA 2-ADRENERGIC RECEPTOR SUBTYPE GENE IN THE SPINAL CORD OF RATS IN AN EXPERIMENTAL MODEL OF NEUROPATHIC PAIN. J.W. Leiphart, C.V. Dills, H.A. Duncan, H.H. Engelhard*, and R.M. Levy. Departments of Neurosurgery and Physiology, Northwestern University, Chicago, IL 60611.

Tying four loose ligatures around the sciatic nerve of a rat leads to neuropathic pain signs indicating spontaneous pain, allodynia and hyperpathia (Bennett and Xie, 1988). We have demonstrated that the intrathecal administration of tizanidine, an α_2-adrenergic agonist, directly to the lumbar region of the spinal cord alleviated pressure hyperpathia in neuropathic rats while preserving normal nociceptive sensation of pressure and heat. Further characterization of the pharmacology of intrathecally administered tizanidine as an analgesic for neuropathic pain indicated that its analgesic effect was primarily mediated through the B subtype of α_2-adrenergic receptor. This was surprising since α_{2A} is the predominant α_2-adrenergic receptor subtype in the spinal cord whereas α_{2B} is only present in trace amounts.

In this study, we used the cDNA clones for the α_{2A} (RG-20) and α_{2B} (RNG) adrenergic receptors to determine if an increase in spinal α_{2B} receptor may underlie the unique specificity and pharmacology observed in intrathecally administered tizanidine. Sciatic nerve ligation and sham surgery were performed on male Sprague-Dawley rats for comparison with unoperated rats. Seven days after the surgery, the rats were tested for signs of allodynia to cold (4°C) and pressure hyperalgesia. The rats were sacrificed, lumbar spinal cords were removed (L2-S1) and divided into left and right halves. A Northern blot of the spinal cord RNA was performed and probed with RG-20 and RNG cDNA. Preliminary data demonstrate bilateral decreases in RG-20 mRNA in both the ligated rat (40.8% ± 1.8) and the sham operated rat (22.3% ± 3.3) compared to the unoperated rat. Bilateral increases in RNG mRNA were observed in both the ligated rat (22.6% ± 4.5) and the sham operated rat (21.0% ± 11.4) compared to the unoperated rat. Our results suggest that there is a change in relative proportions of spinal α_2-adrenergic receptor subtype genes in neuropathic and post-surgical pain.

555.8

EXPRESSION OF α1-ADRENERGIC RECEPTOR mRNAs IN RAT MODELS OF NEUROPATHIC PAIN. A.L. Oaklander*, A.J. Belzberg Dept. Neurosurgery, Johns Hopkins Sch. Med., Baltimore, MD, 21287-7509

In certain patients with neuropathic pain, sympatholysis by intravenous α-adrenoceptor antagonist, anesthetic blockade of appropriate sympathetic ganglia or surgical sympathectomy reduces pain and hyperalgesia. Rats with a spinal-nerve-ligation model of neuropathic pain also display adrenergic sensitivity, suggesting similar underlying pathophysiology to the patients'. We postulate that in these neuropathic pain states there is a change in the expression of α-adrenoceptors by nociceptors or associated cells and that the release of norepinephrine from sympathetic terminals can then directly or indirectly activate nociceptors.

In these experiments, the expression of α1-adrenoceptor subtypes was examined. We used reverse-transcription Polymerase Chain Reaction to measure expression of all known α1-adrenoceptor subtypes in dorsal root ganglia (DRG) of rats with the L5-L6 spinal-nerve-ligation model (19 days-postoperative) and in the hyperalgesic skin and DRG of rats with the chronic-constriction-injury model (15 days-postoperative). mRNA was isolated from the dermis, epidermis and DRG and reverse transcribed to produce cDNA. Primer pairs specific for rat α1B, α1C, and α1A/D adrenoceptor subtypes were developed and used to amplify the cDNA .

There was no substantial upregulation of α1-adrenoceptor mRNA subtype expression in experimental versus control tissues. Assuming that expression of α1-adrenoceptors is at least partly transcriptionally regulated, possible explanations for our finding are that other α-adrenergic receptor types are involved or that α1-adrenoceptors may be involved at other time points or in other tissues than those studied.

555.9

SPINAL ALPHA 2- NORADRENERGIC RECEPTORS DIFFER IN SUBSTRAINS OF SPRAGUE-DAWLEY (S-D) RAT. B.A Graham*[1], D.L. Hammond[1], and H.K. Proudfit[2]. [1]Dept. of Anesthes. & Crit. Care, Univ. of Chicago, Chicago IL 60637, [2]Dept. of Pharmacol., Univ. of Illinois at Chicago, Chicago, IL 60612.

The origin of noradrenergic innervation to the dorsal horn differs in S-D rats obtained from the Sasco and Harlan vendors (Brain Res. 591:44-53, 1992). This study examined whether there were differences in the subtype of α_2-noradrenergic receptors that mediate antinociception in these substrains. Male S-D rats from both vendors were fitted with intrathecal catheters and equiantinociceptive doses of either ST-91 (3 μg) or dexmedetomidine (DEX; 2 μg)) were intrathecally administered in the absence or the presence of doses of idazoxan ranging from 0.03 to 30 μg i.t.. TFLs were redetermined 10, 20, 30, 45, and 60 min later. In vehicle-treated rats, these doses of ST-91 and DEX increased TFL to approximately 10 sec. No differences were observed between Sasco and Harlan rats in the ID_{50} of idazoxan (2 μg) for antagonism of the anitnociceptive effects of DEX. However, the ID_{50} of idazoxan against ST-91 in Sasco rats was 0.1 μg, whereas in Harlan rats it was 2 μg. These findings suggest that there are functional differences in the subtypes of spinal α_2 noradrenergic receptors that mediate antinociception among substrains of S-D rats. This work was supported by grant DA03980 from the National Institute on Drug Abuse.

555.10

S 18616: A POTENT, HIGH EFFICACY AND SELECTIVE SPIROIMIDAZOLINE AGONIST AT α_2-ADRENERGIC RECEPTORS (ARs). M.J. Millan, K. Bervoets*, S. Girardon, A. Gobert, A. Newman-Tancredi, V. Audinot, J. M. Rivet, J.-M. Lacoste and A. Cordi, Institut de Recherches de Servier, 125 Chemin de Ronde, 78290 Croissy, Paris, France.

We compared the novel α_2-AR ligand, S 18616 ((S)-[7,8](2-chlorobenzo)-2-amino-1-aza-3-oxa-[4,5]spirodeca-1,7-diene) to the α_2-AR agonists, dexmedetomidine (DMT) and clonidine. Affinities (K_is) were determined at α_1- and α_2-ARs in rat frontal cortex with [^{3}H]-prazosin (0.1 nM) and [^{3}H]-RX 821002 (0.2 nM), respectively. *In vivo* methods used male Wistar rats (200 - 220 g) and NMRI mice (20 - 25 g). Drugs were given s.c.. Doses are in μg/kg.

	Formalin	Acetic acid	STFs	Loss RR	NAD TO	α_2	α_1
	ID_{50}	ID_{50}	ID_{50}	ED_{50}	ID_{25}	nM	nM
S 18616	1	1	0.5	90	2.7	0.2	120
DMT	6	4	0.9	10	2.6	2.2	NT
Clonidine	30	20	50	> 2500	4.0	46	560

Data are for inhibition of the late phase of hind-paw licking induced by formalin in mice; inhibition of writhing induced by abdominal acetic acid in mice; inhibition of spontaneous tail-flicks (STFs) elicited by the 5-HT_{1A} agonist, 8-OH-DPAT (0.63 mg/kg, s.c.), in rats; induction of a loss of righting reflex (RR) in rats and inhibition of NAD turnover (TO) in rats. ED/ID = Effective/Inhibitory Dose. NT = not tested.

Upon i.t. administration onto lumbar cord, S 18616 (0.001) also inhibited STFs It was active in the mouse hot-plate test upon s.c. (ED_{50} = 66) and p.o. (100) administration. S 18616 (160) reduced NAD levels by > 90 % in frontal cortex dialysates of freely-moving rats. In conclusion, S 18616 is a potent, orally-active, selective and efficacious ligand of pre- and post-synaptic α_2-ARs.

555.11

CHARACTERIZATION OF THE ANTINOCICEPTIVE PROPERTIES OF CIMETIDINE AND A STRUCTURAL ANALOG. B. Y. Li[a], J. W. Nalwalk[a], L. A. Barker[b], P. Cumming[c], M. E. Parsons[d] and L. B. Hough[a,*]. [a]Dept. of Pharmacol. and Neurosci., Albany Med. College, Albany, NY 12208; [b]Dept of Pharmacol., LSU Med. Ctr., New Orleans, LA; [c]Montreal Neurological Inst., Montreal, Quebec, Canada; [d]Biosci. Div., Univ. of Hertsfordshire, Hatfield, Hertsfordshire, UK.

Previous work has established that histamine in the brain is a mediator of analgesia and may play a role in morphine analgesic mechanisms. Although H_2 antagonists have been shown to *block* histamine analgesia, the H_2 antagonist cimetidine alone has been reported to *induce* analgesia. In the present study, the antinociceptive and pharmacological properties of cimetidine and a novel chemical congener were characterized. On both the hot plate and the tail flick nociceptive tests, cimetidine and the congener induced complete, dose-related analgesic responses when injected into the rat lateral ventricle. The congener showed strong similarities to cimetidine in analgesic efficacy, slope of dose-response curves, and chemical structure, suggesting that these compounds share a common mechanism. In contrast, histamine induced sub-maximal antinociceptive effects, and the H_3 antagonist thioperamide, a known histamine-releasing drug, had little or no analgesic effects. Compared with cimetidine, the congener showed very weak activity on H_2 receptors *in vitro* (isolated guinea-pig atrium) and *in vivo* (rat gastric secretion). In addition, the analog (100 μM) had neither agonist nor antagonist action on guinea pig ileum H_1 receptors. Weak antagonism on H_3 receptors by the congener was also exhibited on guinea pig ileum bioassay and brain autoradiography. The compound (up to 10 μM) also had no effect on unpurified rat brain histamine N-methyltransferase activity. These results support the hypothesis that cimetidine induces analgesia by a novel brain mechanism unrelated to H_1, H_2, or H_3 receptors (supported by DA-03186).

555.12

EVIDENCE THAT BLOCKADE OF DOPAMINE RECEPTORS POTENTIATES VAGINOCERVICAL STIMULATION PRODUCED ANALGESIA IN RATS. S. T. Cunningham* and B. R. Komisaruk. Institute of Animal Behavior, Rutgers, The State University of New Jersey, Newark, NJ 07102.

Previous work from this laboratory has demonstrated that probing the vaginocervical region of the rat induces analgesia (e.g. it elevates vocalization threshold to electroshock of the tail and increases tail-flick latency to radiant heat). Several studies have implicated monoaminergic pathways in the antinociceptive effects of vaginocervical stimulation (VS). Crowley et al. (Brain Res., 137, 1977) found that dopamine (DA) receptor stimulation diminished VS-induced analgesia whereas DA D2 receptor blockade potentiated this effect. In the present experiment, we investigated the involvement of D1 receptors in VS-produced analgesia. Ovariectomized rats (N=10) were administered intraperitoneally the D1 antagonist SCH 23390 and distilled water (0, 0.0005, 0.005 and 0.05 mg/kg/1 ml distilled water) in a counterbalanced order. Ten minutes post-injection, all animals received two baseline trials followed by VS (100g and 300g) administered during two consecutive trials in (1) measures of vocalization to tail shock and (2) latency to flick tail in response to radiant heat. Experimenters were blind to drug manipulation throughout testing procedures. Results indicate that SCH 23390 dose-dependently potentiated vocalization thresholds to electroshock to the tail during VS, with no significant effects on tail-flick latency. In addition, this potentiation was most pronounced (347%) at 300 g force of VS. Taken together, these data suggest that blockade of D1 receptors markedly facilitates VS-produced analgesia. In addition, the D1 receptor modulates nociceptive pathways involved in vocalization to electroshock of the tail. Support: 5S06GM08223-11 (BRK).

555.13

ANTAGONISTS OF DA RECEPTOR ENHANCE ACUPUNCTURE ANALGESIA. C. B. Zhu, Y. Q. Wang, G. Wu, S. F. Xu, X. D. Cao and G-C. Wu* Dept of Neurobio, Shanghai Med. Univ., Shanghai, 200032, P. R. China

Both l- tetrahydropalmatine (l- THP) and droperidol (Dropl), antagonists of DA receptor, are being used as drugs with potentiating effect on acupuncture anesthesia in clinics. In the present experiments, the effects of l-THP and Dropl on acupuncture analgesia (AA) were observed in rabbit and rat models. It was found that the pain thresholds were significantly increased in group l-THP + AA or Dropl + AA . SKF 38393, the agonist of D1 receptor, could block the analgesia induced by acupuncture or l-THP(icv or microinjected into N. Accumbens). By using HPLC-ECD, it was shown that l-THP brought forth an elevation of DA in CSF with the increase of pain threshold. There was a negative correlation between changes of DA content and changes of pain threshold. Acupuncture combined with l- THP caused a decrease in DA content significantly. Changes of different types of opioid receptor in rat brain was observed during AA potentiated by Dropl by using receptor autoradiograpic technique. The results showed that the mu and delta binding sites were increased in many nuclei of the brain. Concerning the binding sites of 3H- U69593, an agonist of K receptor, however, no obvious changes were observed. Non-radioactive in situ hybridization histochemistry technique (digoxigenin) was used to observe the expression of preproenkephalin(PPE) mRNA and POMC mRNA in rat brain following the combination of Dropl with AA. Acupunctuer could enhance the expression of PPE and POMC mRNA and Dropl could potentiate this action. These results suggests that antagonists of DA receptor may be achieved by blocking the DA receptor and diminishing the inhibition on opioidergic system.

555.14

POTENTIATION OF ANALGESIA IN DIABETIC RATS BY THE MODULATION OF THE ENDOGENOUS DOPAMINERGIC SYSTEM. L.P. Rutledge, J. M.Ngong, P.R. Cosby and M.G. Kolta*. College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL 32307.

The present study examines the modulating role of dopamine (**DA**) receptors on the antinociceptive response in diabetic rats with or without insulin therapy and in non-diabetic controls (n=12/group). Diabetes was induced by streptozotocin (**STZ**, 45 mg/kg, IV). Insulin replacement was initiated after 6 wks of diabetes (2-3 IU/ 12hrs/ rat, SQ) for 7 wks. Hot plate (**HP**) latency test was measured before diabetes induction and every three wks thereafter and at day 5, 9 and 14 of treatment with **DA-ergic** agents. Four rats from each group were injected with either bromocriptine (**BROM**, 3 mg/kg/12hrs/SQ), haloperidol (**HALO**, 2mg/ kg /12hrs, SQ) or vehicle. The results show that **HALO** significantly potentiated the analgesic response in **STZ**-diabetic rats and significantly increased such response in the other two groups when compared with the vehicle group. **BROM** significantly decreased the **HP** response only in the **STZ**-diabetic rats as compared to **STZ**-vehicle rats. Since **DA** modulation is known to reciprocally alter Met-enkephalin (**ME**) level, therefore it is concluded from the present study that the elevated pain threshold in **STZ**-diabetic rats may be mediated, in part, by the endogenous **ME** system. (Supported by NIH/MBRS grant GM 08111 and NIH/RCMI RR 03020)

RETINA III

556.1

A NOVEL TYPE OF BISTRATIFIED GABAERGIC AMACRINE CELL IN THE RABBIT RETINA. L. L. Wright and D. I. Vaney*. Vision, Touch and Hearing Research Centre, The University of Queensland, Brisbane 4072, Australia.

GABA-containing amacrine cells in the mammalian retina comprise numerous distinct types, most of which have extensively overlapping dendritic trees. This study utilised Neurobiotin tracer-coupling combined with immunocytochemistry to characterise the dendritic morphology, cellular array and neurotransmitter content of a new type of GABAergic amacrine cell whose morphology differs significantly from previously characterised types. Following pentobarbitone overdose and enucleation, identified neurons in superfused DAPI-labelled retina were injected intracellularly with Neurobiotin under direct microscopic control, and then the wholemount preparations were fixed in paraformaldehyde and processed for GABA or glycine immunocytochemistry using newly developed antisera. The tracer-coupled neurons and the GABA/glycine immunoreactive neurons were visualised with Texas Red and FITC, respectively, and imaged by confocal microscopy. The bistratified cells show strong homotypic tracer-coupling and are immunopositive for GABA but immunonegative for glycine. They comprise 1.3% of the amacrine cells in the rabbit retina, with a cell density ranging from 100 cells/mm^2 in the peripheral retina to 350 cells/mm^2 in the visual streak. The soma gives rise to one or two stout primary dendrites which descend to sublamina *b* of the inner plexiform layer, forming a spiny dendritic tree about 150 µm wide in peripheral retina. Arising from the distal dendrites are fine varicose processes that ascend abruptly to arborise in sublamina *a*. Interestingly, the dendritic tree in sublamina *b* exhibits a high degree of territoriality, not seen in either the sublamina *a* arborisation or the dendritic trees of other types of GABAergic amacrine cells.

556.2

C38 ANTIGEN MOLECULE LOCALIZES IN RETINAL GANGLION CELLS. T. Wakabayashi[1], J. Kosaka*[1], M. Mochii[2], G. Eguchi[2] and Y. Fukuda[1]. [1]Dept. of Physiology, Osaka Univ. Med. Sch., Suita, Osaka, 565, JAPAN. [2]Div. of Morphogenesis, Natl. Inst. for Basic Biology, Okazaki, Aichi, 444, JAPAN.

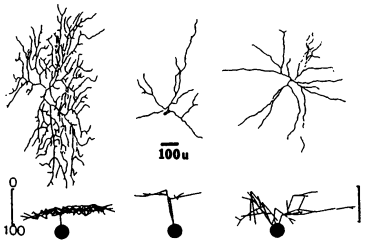
We report here a new molecule which localizes in retinal ganglion cells (RGCs) in mammals. Monoclonal antibodies against RGCs were developed using an immunosuppressive method. One of these antibodies, C38 stained the somas located in the ganglion cell layer of rat and cat retinas. We verified in flatmount preparations of the rat retinas, that over 90% of retrogradely labeled cells overlapped with cells stained with C38. In optic nerve transected rat, C38 labeled only survived RGCs without erroneous labeling of glial cells which usually occurs after retrograde labeling. From these observations, we conclude that C38 is a specific marker for RGCs in flatmount preparation.

C38 recognized the antigen of deduced molecular weight of 24kDa on Western blotting. The cDNA of C38 antigen has been cloned and sequenced. By *in situ* hybridization, we were able to verify that C38 antigen was expressed in RGCs.

556.3

MORPHOLOGY OF CAT RETINAL GANGLION CELLS THAT PROJECT TO SUPRACHIASMATIC NUCLEUS (SCN) M. Pu,[1] P. Sterling[1], and G. Pickard*[2]. Departments of Neuroscience[1] and Psychiatry[2], University of Pennsylvania, Philadelphia PA 19104

The circadian rhythms governed by the SCN are entrained by signals from retina, but which types of ganglion cell carry these signals is unknown. To identify these ganglion cells we injected rhodamine latex beads into the SCN. Following a two day survival, we placed the retina *in vitro*, identified retrogradely labeled ganglion cells by fluorescence microscopy, and injected them with Lucifer yellow and neurobiotin to visualize their full dendritic arbors. At eccentricities of 4-10mm, the dendritic trees were 600-1200µm diameter (vs 500-900µm for alpha cells in the same region). Three morphological types were observed: dense-branching, ON(40-68% of IPL); sparse-branching, OFF (2-26%); sparse-bistratified, ON/OFF(6-93%).



(Supported by MH47501 and EY00828)

556.4

RETINAL LOCATIONS OF GANGLION CELLS WHICH PROJECT TO FROG ANTERIOR THALAMUS AND OPTIC TECTUM. E. Kicliter* and M. Gonzalez. Dept. of Anatomy and Inst. of Neurobiol., Univ. of Puerto Rico, Med. Sci. Campus, San Juan, PR 00901.

The anterior thalamus of *Rana pipiens* receives retinal projections from both eyes. The retinal ganglion cells which terminate here are uniquely ON-type, responding to the onset of a light flash. Lesion of this region results in a loss of the *blue preference*, a positive phototaxis most easily elicited with short wavelength light. The present experiments were performed to study the retinal loci of ganglion cells which project to the anterior thalamus and compare them with those of cells which project to the optic tectum. A retrograde tracer, the B fragment of cholera toxin (CTB, 1%, 0.1-3.0 µl), was injected into either the retinal recipient neuropils of the anterior thalamus (N=5) or the tectum (N=6) of anesthetized frogs. After survival periods of 3-30 days the frogs were given a lethal dose of anesthesia and perfused transcardially with 4% paraformaldehyde. The toxin was visualized by immunocytochemical techniques in the retinas which were prepared as wholemounts. Locations of labeled cells were plotted on maps of the retinas, using a Neurolucida image analysis system. Ganglion cells labeled after anterior thalamic lesions were distributed bilaterally. In both eyes labeled cells were located in an intermediate position, with few labeled cells near the optic nerve head or in the periphery of the retina. The largest concentration of labeled cells was in the superior nasal retina. In the retinas of subjects with thalamic injections a bimodal distribution of soma sizes was observed. Smaller cells generally ranged from 6-12 µm in diameter while larger cells generally ranged from 14-20 µm. Distributions of large and small cells were overlapping. After tectal injections labeled cells were observed throughout the retina. In subjects which had received tectal injections the distribution was unimodal and soma diameters ranged from 8-23 µm. Supported, in part, by NIH Grants MH-48190 and NS-07464 and ONR Grant N00014-89-J3070.

556.5

MORPHOLOGY OF RETINAL GANGLION CELLS MEDIATING THE PUPILLARY LIGHT REFLEX IN PIGEONS. H.Y. Zhang*, J.B. Williams and P.D.R. Gamlin. Department of Physiological Optics, University of Alabama at Birmingham, Birmingham, AL 35294.

Area pretectalis (AP), a retinorecipient pretectal nucleus, mediates the pupillary light reflex (PLR) in the pigeon (Gamlin et al., JCN, 226:523, 1984). To investigate the morphology of pupillomotor retinal ganglion cells (RGCs), we injected 0.8 µl of either 10% fluorescein-dextran or Mini Ruby stereotaxically into AP in anesthetized pigeons. After a 10-14 day survival period, animals were deeply anesthetized and perfused with 4% paraformaldehyde in 0.1M phosphate buffer. Retinae were removed and retrogradely-labeled RGCs were injected using glass micropipettes filled with a 4-5% solution of either Lucifer Yellow or Mini Ruby.

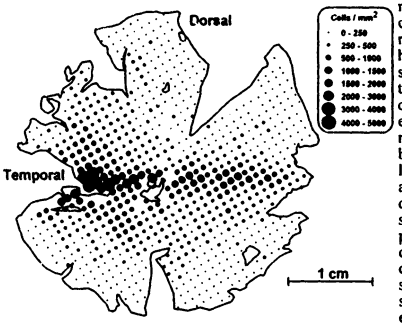
As previously reported, injections of AP result in retrograde labeling of relatively few RGCs that are distributed evenly across the retina except for an apparent paucity in the Red Field and extreme ventral retina. All AP-projecting RGCs possess very broad dendritic fields ranging from 300 to 600 µm in diameter. In the peripheral retina, ganglion cells have extensively branched dendritic fields and soma sizes ranging from approximately 15-25 µm. In the central and near peripheral retina, many ganglion cells have less extensively branched dendritic arbors and soma sizes ranging from approximately 8-20 µm.

To our knowledge, this is the first report in any vertebrate class of the detailed morphology of pupillomotor RGCs. Our results demonstrate that the broad dendritic fields of pupillomotor RGCs ensure that relatively few ganglion cells can provide the retinal coverage necessary for mediating for the PLR. (Supported by EY09380 and P30 EY03039).

556.6

RETINAL GANGLION CELL DISTRIBUTION IN THE SPOTTED HYENA, *CROCUTA CROCUTA*. J.B. Calderone*, B.E. Reese & G.H. Jacobs. Neuroscience Research Institute & Department of Psychology, University of California, Santa Barbara, CA 93106.

The spotted hyena is a member of the Superfamily Feloidea; its nearest relatives are civets, mongooses and cats. We have studied the retinal topography of this unique animal. A retina from an adult hyena was flat-mounted, stained with cresyl violet, and examined using light microscopy. Presumed retinal ganglion cells were counted at 1 mm intervals across the retina. We attempted to distinguish gangiion cells from other retinal cells (i.e., glial and displaced amacrine cells) based on their location, size, and appearance of a nucleus and substantial cytoplasm. From these measurements it was calculated that the hyena retina contains approximately 260,000 ganglion cells. A prominent area centralis was found in the temporal retina, where ganglion cell density reaches about 4200 cells/mm^2. The hyena retina also has a horizontal visual streak similar to that found in the retina of the domestic cat. An estimate of the spatial resolution was made based on the axial length of the hyena eye and peak ganglion cell density. The maximum spatial resolution predicted from this calculation is about 9 c/deg. This figure is similar to the maximum spatial resolution estimated for the cat.



556.7

TUBULAR EYES OF DEEPSEA FISHES: PHOTORECEPTOR AND RETINAL GANGLION CELL TOPOGRAPHY. S. P. Collin*, R. V.Hoskins and J. C. Partridge. Dept. of Zoology, University of Western Australia, Nedlands 6009 WA, Australia and School of Biological Sciences, University of Bristol, Bristol, United Kingdom.

The mesopelagic ichthyofauna of many of the worlds' deep oceans boast a number of species with asymmetric or tubular eyes. The retina consists of main and accessory components which are thought to mediate form vision and movement perception within dorsal and lateral visual space, respectively. To investigate the topography of both photoreceptor and retinal ganglion cell arrays, ten different species were collected from depths between 200 and 900 meters. Each specimen was anaesthetised by immersion with an overdose of tricaine methane sulphonate (1:2,000), fixed and wholemounted. Peak photoreceptor densities range from 97.6 X 10^3 rods per mm^2 in the main retina to 39.6 X 10^3 rods per mm^2 arranged in a tightly packed hexagonal array. Peak ganglion cell densities range from 28.1 X 10^3 (*Stylephorus chordatus*) to 63.6 X 10^3 cells per mm^2 (*Opisthoproctus grimaldi*) in the central region of the main retina and between 0.8 X 10^3 (*Argyropelecus gigas*) and 10 X 10^3 cells per mm^2 (*Scopelarchus michaelsarsi*) throughout most of the accessory retina. Analysis of ganglion cell soma size shows a heterogeneous population of cells with larger cells in the accessory retina of all species. A cohort of giant alpha-like ganglion cells located in the temporal transitional zone between the main and accessory retinae of *Scopelarchus michaelsarsi* is analysed and hypothesized to represent a specialized area within the binocular visual field.

556.8

MORPHOLOGICAL ANALYSIS OF THE FASCICULAR PATTERN OF THE MONKEY OPTIC NERVE ACCORDING TO THE FASCICULAR SIZES J. Naito*. Lab. of Animal Morphology and Function, Sch. of Agriculture, Nagoya Univ., Chikusa-ku Nagoya 464-01, Japan.

Fascicular pattern made of astrocytes' processes were analyzed in the monkey optic nerve using an image analyzer. Sectional areas of fascicles increased sharply in the distal optic nerve, and further increased gradually in the remaining part of the optic nerve. This change is mainly responsible for 'fusion' of small fascicles. Most of the fascicles were small in the distal optic nerve. Leaving the eyeball, they decreased sharply in number. In contrast, the fascicular numbers of other groups, middle-sized, large, and largest fascicles increased gradually in the distal optic nerve and become constant in the proximal optic nerve. More small and middle-sized fascicles were distributed in the central area of the optic nerve than larger fascicles. The largest fascicles were mainly distributed in the circumference of the optic nerve. Progress of the decrease in the fascicular number accurately correspond to that of the dispersion of the optic nerve fibers. The size-distribution of fascicles seems to be related roughly to the retinotopic fiber order.

MOTOR CORTEX: HUMAN STUDIES I

557.1

MOTOR CORTEX EXCITABILITY DURING REACTION TIME. P. Romaiguère, T. Hasbroucq, C-A. Possamaï and P. Salin*. NBM, LNC, and LNCF, CNRS, 13402 Marseille cedex 20. France.

Changes in the motor cortex excitability before a voluntary movement were measured using transcranial magnetic stimulation (TMS) during a choice reaction time task. Because of its involvement in the execution of a movement, the motor cortex can be expected to be most likely activated in the latest part of the reaction time (RT). Three times of stimulation were thus chosen: T1, concomitant with the response signal; T3, just before the response; T2, at the mid-point between T1 and T3. The six subjects were over-trained, so that their RTs were stabilized and T3 could be determined for each of them with the best possible precision. T3 was chosen as late as possible but still ensuring that the evoked response never occured after the onset of voluntary EMG. Changes in the evoked response size can be due to excitability changes occuring either at the cortical level or at the spinal level. To separate these changes, a second experimental session was performed, with H-reflexes evoked at T1, T2 and T3, instead of TMS responses.

The results showed that the latest the TMS, the greatest the response, while no significant change could be demonstrated in H-reflex sizes at the same times. On the other hand, TMS induced a lengthening of RTs, such that the latest the stimulation, the longest the RT; while electrical stimulations used to evoke H-reflexes induced a shortening of RTs, such that the earliest the stimulation, the shortest the RT.

We feel that these results can be replaced within the issue of continuous vs. discrete transmission of sensorimotor information.

557.2

ETHANOL SUPPRESSES HUMAN MOTOR CORTEX EXCITABILITY: A TRANSCRANIAL MAGNETIC STIMULATION STUDY. U. Ziemann*, S. Lönnecker, W. Paulus. Dept. of Clinical Neurophysiology, University of Göttingen, D-37075 Göttingen, Germany

Transcranial magnetic stimulation (TMS) was used in six healthy volunteers to investigate the effect of ethanol on central motor system excitability. The threshold intensity for the motor evoked potential (MEP) in the resting and active abductor digiti minimi muscle, the variation of MEP amplitude with increasing stimulus intensity, the duration of the TMS induced cortical silent period (CSP), and the intracortical inhibition and facilitation after paired magnetic stimulation (interstimulus intervals 1-30 ms) were studied. In addition, the maximum M wave (Mmax) after supra-maximal electrical ulnar nerve stimulation and the peripheral silent period (PSP) were obtained. Baseline values were compared with data obtained on a mean ethanol blood concentration of 0.8 ml/l.

Resting and active motor thresholds, Mmax, MEP/Mmax ratios at various TMS intensities and the duration of the PSP were not affected by ethanol. The mean duration of the CSP was prolonged from 199 to 234 msec. The mean intracortical inhibition was enhanced, and the mean intracortical facilitation was abolished at all interstimulus intervals tested.

Our results show a suppression of motor system excitability at the cortical level by ethanol. They are compatible with data from animal experiments which have shown an attenuation of NMDA receptor-mediated synaptic currents and a potentiation of GABA currents as the main modes of action of ethanol in the central nervous system.

557.3

MODIFICATION OF POST EXERCISE MEP DEPRESSION

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Division of Restorative Neurology and Human Neurobiology
Baylor College of Medicine, Houston, Texas 77030

It has been reported that surface EMG recorded motor evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS) of the motor cortex in human subjects are depressed following maximal voluntary contraction (MVC). Two means of modifying this post-exercise effect were examined. First, 4 subjects maintained tibialis anterior MVCs until force decreased by 50% in separate sessions, one of which included TMS delivered at 15 second intervals. Mean peak MEP amplitudes were depressed by 56% following MVC but with TMS delivered during MVC, this depression was not seen. Second, the effect of MVC duration was examined in 5 subjects during separate sessions of 5, 15 and 30 s MVCs repeated 3 times each. TMS was delivered at 5s, 30s and 1 through 5 minutes following each MVC. Depression of 1-5 minute post-exercise MEPs developed progressively over three repetitions of 5s MVCs. Following 15 or 30 second MVCs, MEPs were significantly depressed as early as 30 seconds after the first MVC and no further depression occurred with repetition. However, MEPs collected at just 5 seconds post-MVC were of significantly higher amplitude than subsequent responses. Modulation of post-exercise MEP depression, described here, results from changes in motor cortex excitability that are likely brought about by dynamic intracortical mechanisms.

This work was supported by V.L. Smith Foundation for Restorative Neurology, Houston, Tx USA.

557.4

CORTICOMOTOR REPRESENTATION OF THE STERNOMASTOID MUSCLE STUDIED WITH TRANSCRANIAL MAGNETIC STIMULATION

M. L. Thompson, G. W. Thickbroom, R.J.Lockwood* and F. L. Mastaglia.
Australian Neuromuscular Reasearch Institute and University Department of Medicine, QEII Medical Centre, Nedlands, WA 6009.

We have investigated the corticomotor representation of the sternomastoid (SM) muscle in fifteen healthy subjects using transcranial magnetic stimulation. Motor evoked potentials (MEPs) were recorded from the contralateral SM muscle via surface electrodes in all subjects, and with dual monopolar shielded needles inserted into the muscle near the motor point in one subject. A Magstim 200 with 5cm diameter figure 8 coil was used to deliver threshold-adjusted stimuli at multiple scalp sites over the motor cortex during a low-level voluntary contraction of the SM muscle. The corticomotor representation of the SM muscle was estimated by constructing maps of MEP amplitude as a function of scalp site stimulated. In 8 subjects, a response could not be evoked even with 100% stimulator output. In 5 subjects, stimulation of two separate areas of cortex gave rise to a MEP on the contralateral side, one medial and the other lateral to the hand area. In the remaining 2 subjects, the main body of the cortical representation was restricted to the region lateral to the hand area. Intramuscular recordings from the SM and platysma muscles demonstrated that the cortical representation of the SM was located in the area medial to the hand representation, and that of the platysma in the area lateral to the hand area. These findings indicate that the representation of the SM muscle forms part of the trunk representation, contrary to the conventional understanding that it is represented close to the face area (1), and that surface MEP recordings of the SM muscle include a significant contribution from the platysma muscle.

1. Penfield, W. and Rasmussen, T. (1952) The cerebral cortex of man. New York: Macmillan.

557.5

LOCALIZATION OF THE CENTRAL SULCUS BY RELATIVE CORTICAL THICKNESS OF THE PRE AND POST CENTRAL GYRI ON TURBO IR MR IMAGING OF THE BRAIN J.R. Meyer, M.D., S. Roychowdhury, M.D., C. Callahan, R.T., D. Gitelman, M.D., M-M. Mesulam, M.D., T.C. Hain* and E. J. Russell, M.D., FACR, Dept of Radiology and Center for Behavioral & Cognitive Neurology, Northwestern University, Chicago, IL 60611

To determine whether relative cortical thickness measurements of the pre-central (PCG) and post-central (POCG) gyri can differentiate the central sulcus from adjacent cortical sulci. Turbo IR MR imaging (TR 2010 msec., TE 18 msec., TI 300 msec., 512 matrix, 5mm.) was performed of the entire brain with axial images parallel to the anterior commissure - posterior commissure line.

Cortical thickness was measured in each hemisphere using a jewelers eye piece with .1mm gradations. Three measurements were obtained perpendicular to the central(CS), precentral(PCS), and superior frontal sulci(SFS), as determined by other established anatomical methods. The ratios of cortical thickness on both sides of the CS, PCS, and SFS were calculated and compared. The mean cortical thickness (MCT) ratio of PCG/POCG was 1.65rh (right hemisphere=rh), 1.53lh (left hemisphere=lh). The MCT ratios across the PCS and SFS were 1.01rh, 1.01lh and 1.03rh, .987lh, respectively. Statistical analysis using unpaired t-test yielded significant differences ($p < .0001$) for PCG/POCG vs MCT ratios across the PCS and SFS.

Cortical thickness measurements across the CS provides a new method for localizing the pre and post central gyri. The higher mean ratio of cortical thickness across the CS correlates with known cytoarchitectonic relationships.

557.6

THREE DIMENSIONAL ANATOMY OF THE HUMAN CENTRAL SULCUS AND TOPOGRAPHY OF THE HAND SENSORY-MOTOR MAPS. Sastre-Janer F.A., Belin P., Mangin J.F., Frouin V., Dormont D., Masure M.C., Marsault C., Samson Y*., Regis J. CEA-SHFJ, Orsay. Salpêtrière, Paris. La Timone, Marseille.

We analyzed the 3D morphology of the central sulcus (CS) in 17 normal subjects (10 left- and 7 right- handers), using contiguous horizontal T1-weighted 1.4 or 2 mm thick images obtained in the AC-PC plane, with a .5T or a 1.5T MR unit. A dedicated software was used to draw both CS on each horizontal section, and to visualize 3D wire-frame images of CS. The morphology was complex but two distinct elements were sharply interconnected, and could always be identified. The lower element extends in Talairach's space from z: +17.7±5 to +49±3.5mm, and, viewed from above, is orthogonal to the brain surface. The upper element has a more oblique sigmoidal shape. It extends from +40.9±3.1 to +67.1±2mm. It is initially connected internally, posteriorly, and orthogonal to the lower element, and reaches the surface only at high z levels. These measurements were not affected by the MR unit, the side of the CS, the gender or handiness of the subjects.

The level of the middle of the superior root (z: +54±2mm) is similar to that of previously reported spot of PET activation during fingertips vibrotactile stimulation (+55.9±.4mm, Meyer et al, 91), and our preliminary PET results suggest that vibration spots may be localized vertically in the superior root, from 64±1.3mm, near the surface of the brain, to 41.8±3.8mm in the deep of the CS close to inferior limit of its superior root.

Taking in account the 3D anatomy of the cerebral sulci may be helpful to obtain accurate matching between structure and function in the cerebral cortex, and relevant morphometrical measurements of cortical regions.

557.7

NONINVASIVE LOCALIZATION OF SOMATOSENSORY CORTEX IN NEUROSURGICAL PATIENTS - A COMPARISON OF STRUCTURAL (MRI) AND FUNCTIONAL (MEG) APPROACHES WITH VALIDATION BY INTRAOPERATIVE MONITORING. J. Lewine*, N. Baldwin, J. Sanders, J. Anson, A. Halliday, W Orrison. New Mexico Institute of Neuroimaging and the Departments of Radiology, Psychology and Neurosurgery, The University of New Mexico, Albuquerque, NM.

Structural magnetic resonance imaging (MRI) and functional magneto-encephalographic (MEG) methods were used in an attempt to identify the post-central gyrus and central sulcus in 40 neurosurgical patients with mass lesions in the frontal or parietal lobe. MRI data were assessed by multiple 2D and 3D methods for identification of the central sulcus. MEG data were obtained in response to electrical stimulation of the median nerve, and dipole modelling was then used to localize the cortical source of the 20 msec component of the evoked magnetic signal, which is believed to be generated in primary somatosensory cortex. Two patients did not undergo surgery because neuroimaging data suggested that surgery would have compromised motor function. MRI alone provided confident identifications of the central sulcus in only 23 of the remaining 38 patients. In contrast, intraoperative electrocorticography confirmed the correctness of MEG-based localizations of the central sulcus in 36 of these 38 patients. The MEG data of the remaining 2 patients were obscured by artifacts that precluded central sulcus identification. For 10 patients, intraoperative stereotaxic procedures were used to specify the spatial coordinates of the hand representation of sensorimotor cortex as defined by corticography. Preoperative MEG localizations of sensorimotor cortex predicted these intraoperative localizations to within 2-8 mm. It is noteworthy that for 6 patients who had initially been determined to be very poor surgical candidates MEG demonstrated that the patient's lesion had actually displaced functional sensorimotor cortex and surgical resection was accomplished without compromise of motor function.

557.8

MORPHOLOGICAL AND FUNCTIONAL EVIDENCE FOR TWO DIFFERENT SUBREGIONS WITHIN THE HUMAN PRIMARY MOTOR CORTEX.

S. Geyer[1], K. Zilles*[1], A. Dabringhaus[1], T. Schormann[1] and P.E. Roland[2].
[1]Brain Research Institute, Heinrich Heine University, Düsseldorf, Germany and [2]Division of Human Brain Research, Dept. of Neuroscience, Karolinska Institute, Stockholm, Sweden.

In this study we describe two structurally different subregions within the primary motor cortex of man (Brodmann's area 4), which, as can be shown by positron emission tomography (PET), are functionally separate entities.

Two subregions can be defined within the primary motor cortex (an anterior one [4a] and a posterior one [4p]) due to different cell density, especially in cortical layer III and different concentrations of the muscarinic M_2 and serotonergic 5-HT_2 binding sites across all cortical layers. These two subregions were delineated in 5 normal postmortem brains which were subsequently adapted to the reference brain of a computerized brain atlas to compensate for distortions due to tissue processing and to permit the analysis of intersubject variability.

Several groups of subjects performed different types of voluntary movements of the right upper extremity during which their regional cerebral blood flow (rCBF) was measured with ^{15}O-butanol and PET. Cluster images showing significantly activated cortical fields were transferred to the same reference brain for direct comparison with the morphological data.

Fast simple movements activate both subregions. Movements guided by somatosensory information tend to recruit area 4p, whereas simple or learned responses to visual input irrespective of the speed of the response activate area 4a (Roland PE et al., Human Brain Mapping, 1995, in press).

The results suggest that the two subregions of the primary motor cortex also have different functions. Supported by the DFG (SFB 194/A6).

557.9

QUANTITATIVE COMPARISON OF CORTICAL ACTIVATION IN CENTRAL AND POST-CENTRAL SULCI DURING MOTOR, TACTILE AND HAPTIC TASKS, REVEALED BY FUNCTIONAL MRI. J.L. Anton, E. Guigon, H. Benali, M. Di Paola, J. Bittoun, Y. Burnod, M. Dufossé* INSERM CREARE, UPMC, 75005 Paris, France; INSERM U66, CHU Pitié Salpêtrière, Paris, France.

We investigated the activation of the sensorimotor cortical areas during three right-hand-fingers tasks, which share information processing components : motor sequence, tactile stimulation, haptic task. Frontal MRI images were acquired around the Rolando sulcus in right-handed subjects, using a standard 1.5T Signa (GEMS) with a standard surface coil : 1/ Anatomical images were used to detect the somatomotor hand area, 2/ Angiography images allowed to detect the main veins, 3/ Functional images (gradient echo sequence) were acquired during the different behavioral conditions. The functional images were first processed to compensate the inter-frame motion due to small movements of the head and the brain. These images were then analyzed by a new statistical method based on conditioned analysis and orthogonal statistical maps, which allow to estimate the relevant part of the signal variation, free of the noise and of the unrelated signal. The highest values of the activation maps were correlated with the main veins, giving thus a rough approximation of the spatial location of the neural activity. The middle range values were correlated with the cortical ribbon on the anatomical images, revealing two main compact zones of activation, one in the central sulcus and the other one in the post-central sulcus. Quantification and comparison between tasks and subjects showed that the extent of the activated zone in the central sulcus was similar in the three tasks. This zone probably corresponds to the finger region in the Primary Motor Area (MI) and the Primary Somatosensory Area (SI). Conversely the other zone of activation in the post-central sulcus was revealed mainly for the tactile and haptic tasks, with a larger extent for the haptic task. This second region could play a role in the integration of tactile information present in both tasks and could be modulated by the active aspect of the haptic task.

557.10

RELATION OF SCALP-MEP-MAPS TO UNDERLYING CORTICAL PROFILE IN RIGHT- AND LEFT-HANDERS. J. Netz, V. Hömberg*. Neurological Therapy Centre, Institute at the Heinrich-Heine-University, Hohensandweg 37, D-40591 Düsseldorf, Germany

In 10 normal subjects (5 right-handed, 5 left-handed) the cortical motor area of the right and left thenar was mapped by focal transcranial magnetic stimulation at a 2,5 % spacing of the respective circumferences of the 10/20 EEG-electrode positioning system. The maps were projected to the cortex profile represented in a 3D-MRI-flash-scan (128 slices) by a software developed especially for this purpose using different landmarks of the outer skull for adjustment. In 15 out of 20 cases there was a distinct single maximum but 5 maps from 4 left-handed subjects showed two distinct maxima. All maps covered at least in parts the precentral gyrus but the proportions of the maps extending onto the region anterior to the precentral gyrus ranged from 0-80 % and the proportion covering the postcentral gyrus ranged from 0-50 %.

In conclusion the cortical maps of left-handed subjects seem to differ in their spatial distribution from those of right-handed subjects. The variance of MEP-map locations in reference to the central sulcus as well as in reference to the scalp is considerable. The knowledge of the cortical profile is of limited help in predicting the exact location of MEP-maps. A further consequence of this result is, that it is problematic to average topographic data from different subjects.

557.11

ACTIVATION OF THE MOTOR CORTEX IN MONOZYGOTIC TWINS DETERMINED BY FUNCTIONAL MRI AT 4 TESLA. J. Ashe*, S-G Kim, G. Tagaris, K. Uğurbil, A.P. Georgopoulos. Brain Sciences Center, VAMC, and Center for Magnetic Resonance Research, University of Minnesota, Minneapolis, MN 55417.

There is an hemispheric asymmetry in the activation detected by functional magnetic resonance imaging (MRI) within the motor cortex during hand movements (Kim et al., Science 261: 615, 1993). The right motor cortex is activated almost exclusively during movements of the contralateral hand. The left motor cortex is also activated predominantly during contralateral movements but there is significant activation during ipsilateral movements as well. The determinants of this asymmetry are not known. Among the possible explanations are (i) that the asymmetric functional activation is genetically determined, or (ii) that it is related to hand use. We have examined this issue by studying the functional activation of the motor cortex in pairs of monozygotic (MZ) twins with different handedness during performance of hand movements. Six pairs of MZ twins performed visually paced finger-thumb opposition movements with the right and left hand while their precentral gyrus was scanned using axial multislice BOLD functional imaging with a 4 Tesla system. The order of left/right movements was randomized. We tested the null hypothesis that functional activation is genetically determined and thus should be similar within twin pairs. We found that both the volume and the pattern of functional activation in the precentral gyrus differed significantly between subjects within pairs. This finding rejects the null hypothesis above and indicates that motor cortical activation during hand movements is determined by other factors (e.g. hand use) that may interact with genetic factors. (Supported by NIH grants NS32437, NS32919 and RR088079.)

557.12

LOCALIZATION OF FUNCTIONAL REGIONS OF HUMAN MESIAL CORTEX. T. Allison*, G. McCarthy, M. Luby, A. Puce, and D.D. Spencer. Neuropsychology Lab., V. A. Medical Center, West Haven, CT, 06516 and Section of Neurological Surgery and Dept. of Neurology, Yale Univ. Sch. of Medicine, New Haven, CT, 06520.

We describe methods of localizing some functional regions of the mesial wall, based on 47 patients studied intraoperatively or following chronic implantation of subdural electrodes. Somatosensory evoked potentials were recorded to stimulation of posterior tibial, pudendal, median, and trigeminal nerves. Bipolar cortical stimulation was performed, and in a few cases movement-related potentials were recorded.

The genitalia are represented near the cingulate sulcus, anterior to the foot sensory area occupying the posterior paracentral lobule. These areas are posterior and inferior to the foot motor representation. In agreement with some previous studies, there is a rough somatotopic organization within the supplementary motor area (SMA), with the face represented anterior to the hand. However, there was no clear evidence of the "pre-SMA" region identified in monkeys. Complex movements involving more that one extremity were elicited by stimulation of much of the paracentral lobule. The cingulate and marginal sulci form the inferior and posterior borders of the sensorimotor areas and the SMA. The region comprising the supplementary sensory area is unclear, but may involve much of the precuneus. Movement-related potentials did not provide additional localizing information, although in some recordings focal readiness potentials were recorded from the SMA.

557.13

IMPORTANCE OF EKG GATING IN FUNCTIONAL MAGNETIC RESONANCE IMAGING (fMRI) OF HUMAN MOTOR CORTEX.
K. Kuppusamy, W. Lin*, E.M. Haacke. Mallinckrodt Institute of Radiology, Washington Univ., St. Louis, MO 63110.

We delineate the importance of EKG gating in fMRI of human motor cortex. Transaxial echo planar images covering the primary motor strip were obtained from 14 subjects (without EKG gating) and 7 subjects (with EKG gating) on a SIEMENS 1.5T VISION system using TR/TE/FA/TH=3000/66/90°/3-5mm, Number of slices=10, FOV=200mm, matrix size=64X64 (sinc interpolated to 128X128) and 60 time course images/slice. TR will be slightly altered with EKG gating depending on subject's heart rate. The time course had a repeated sequence of 5 resting and 5 activation state scans. The paradigm was "sequential finger tapping" of digits 2-5 onto digit 1 of right hand. The functional images were analyzed using cross-correlation, subtracted t-test and variance methods. The variance image was obtained using sum of the variances of the resting state images and activated state images of the entire time course data. This variance image is a primary indicator of physiological and systematic noise. The signal change increased from $1.1 \pm 0.1\%$ (without EKG gating) to $3.1 \pm 1.0\%$ (with EKG gating) in the contralateral primary motor cortex region. There was a considerable reduction of variance of 10–30% with EKG gating, indicating the reduction of physiological noise. This reduction of variance leads to increase in mean value of cross-correlation and hence an increase in the number of activated pixels in the contralateral primary motor cortex region. There is a very little change in total scan time with EKG gating. Our findings indicate that it is important to have EKG gating while performing any fMRI experiment.

557.14

COMPARISON OF FUNCTIONAL MAGNETIC RESONANCE IMAGING (fMRI) WITH POSITRON EMISSION TOMOGRAPHY (PET) USING A QUANTITATIVE FORCE-RELATED PARADIGM.
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The purpose was to compare anatomical location and amplitude of signal changes in PET and fMRI using an identical force-related paradigm with each technique. 6 male subjects - 5 were identical in both studies - were asked to press repetitively a morse-key at 1Hz with their right index finger. Data were collected during 6 different conditions: at rest, at 5% of their maximum voluntary contraction (MVC), at 10%, 20%, 40% and 60%. Regional cerebral blood flow (rCBF) was determined using the $H_2{}^{15}O$ bolus injection technique. fMRI data were collected with a conventional 1.5-tesla clinical MR system in one transverse section through the primary motor cortex (M1). Both data sets were subjected to the same statistical analysis (categorical and parametric analysis). Statistical parametric maps were superimposed on corresponding T1 weighted MR images for anatomical localization. Both techniques identified M1 and the posterior supplementary motor cortex in 6 out of 6 subjects. While fMRI foci were at the border of grey matter and sulci, PET foci were within the tissue 0 to 8 mm distant from the fMRI focus. While the fMRI signal increased by 4.8%, rCBF increased by 12.3% at a comparative peak force. rCBF and signal increased logarithmically in a constant ratio, suggesting that change in signal is proportional to stimulus intensity and rCBF.

557.15

THE DECISION TO MOVE MAY BEGIN IN THE PREFRONTAL CORTEX. G.H. Yue*, M. Xue, T. Ng, and R.M. Enoka. Depts Biomedical Engineering and Radiology, The Cleveland Clinic Foundation, Cleveland, OH 44195

Before a voluntary movement begins, an intent to do so must arise in the brain. This intent informs the lower hierarchical motor centers of the proposed action and a muscle contraction follows. Despite numerous studies on primates and humans, it has not been possible to clearly identify the brain area(s) responsible for initiating the command to generate a motor action. The purpose of this study was to identify the brain regions involved in making the decision to move by using functional magnetic resonance brain imaging. Healthy human subjects performed various combinations of real and imagined pinches involving the index finger and thumb. These experiments were performed on a Siemens 1.5 T Vision system using a circular polarized head coil. Ten 6 mm-thick, continuous oblique-angle slices were imaged. The images were acquired with an interleaved multislice Gradient Echo pulse sequence (TR/TE = 400/40 ms, FOV = 220-230 mm, matrix size =128 x 128). Images during rest and activity were compared with *t* test on a pixel-by-pixel basis and pixels showing significant activation were determined. The results demonstrated that tasks involved with a heightened intent to move were associated with a strong, consistent activation of the prefrontal cortex, indicating its role in generating the intent to move. Low-intent motor tasks displayed only motor cortex activity, presumably because the activity in the prefrontal cortex had already dissipated.

557.16

READINESS POTENTIAL VS MOVEMENT PREPARATION

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The goal of the study was to define the relationships between movement preparation and the readiness potential, under conditions in which the choice of movement is recalled from memory. There were either two or four possible finger movement sequences in each experiment, and these were memorized before the experiment began. Based upon a visually presented letter, the subject chose one of the previously memorized sequences of movements, and performed them. Three basic relationships were identified. 1) The reaction time and 2) the duration increased with either the number of previously memorized sequences, or with the complexity of the sequence. 3) The start of the readiness potential was time locked to the stimulus, regardless of the number of memorized sequences or the complexity of the sequences. These results indicate that the latency of the start of the readiness potential does not depend upon the time it takes to recall the sequence from memory, and thus cannot represent the cognitive activity of preparing for movement. The readiness potential may, however, represent a priming of motor or premotor cortex.

557.17

ACTIVATION OF SUPPLEMENTARY MOTOR AREA DURING SIMPLE, COMPLEX, AND BILATERAL LIMB MOVEMENTS IN HUMANS. M. Xue, T. Ng, G.H. Yue, Y.G. Comair, R.M. Enoka*, H.O. Lüders, and M.T. Modic. The Cleveland Clinic Foundation, Cleveland, OH 44195

The supplementary motor area (SMA) in humans is thought to contribute to the programming of complex sequence of limb movements, to the coordination of independent bilateral tasks, and to the execution of simple motor actions. It has been suggested that the extent of SMA activity during a simple movement may depend on the magnitude of the subject's intent. The purpose of this study was to identify the regions of the SMA that are active during motor tasks of varying intent and complexity using functional magnetic resonance brain imaging. Healthy human subjects performed various combinations of real and imagined unilateral and bilateral finger movements. These experiments were performed on a Siemens 1.5 T Vision system using a circular polarized head coil. Ten 6 mm-thick, continuous oblique-angle slices were imaged. The images were acquired with an interleaved multislice Gradient Echo pulse sequence (TR/TE = 400/40 ms, FOV = 220-230 mm, matrix size =128 x 128). Images during rest and activity were compared with *t* test on a pixel-by-pixel basis and pixels showing significant activity were determined. We found that independent bilateral movements of the hands were associated with the strongest SMA activity followed by a complex finger-tapping sequence. Simply pressing two fingers together continuously exhibited least SMA activation. These results suggest that the magnitude of human SMA activation depends largely on task complexity and less on intent.

557.18

DISTINCT CEREBRAL ACTIVITY IN IMAGINATION AND EXECUTION OF MOVEMENT. M.-P. Deiber, V. Ibañez, R. Raman[1], N. Sadato*, C. Toro, L. Cohen, M. Hallett. Human Motor Control Section, [1]Neuroimaging Branch, NINDS, NIH, Bethesda, MD 20892.

Regional cerebral blood flow measured by [O-15] water and PET was obtained during performance of tasks in which 10 right-handed normal volunteers responded to a first (warning) signal by imagining moving one of two right fingers (index or little finger) in one of two dimensions (abduction/elevation). According to the task, they either executed their movement or held it at the second (imperative) signal. Each trial lasted about 8 seconds, and the delay period between the warning and imperative signals varied between 2.75 and 5 seconds. Two situations were tested, one in which complete visual advance information as to finger and direction was given in the warning signal (full condition), and the other one in which the subject freely selected any of the four possible movements (free condition) (Deiber et al., Soc. Neurosci. Abstr., 20 (1), p. 443, 1994). Statistical parametric maps were generated by making planned comparisons of task and rest conditions.

When subjects imagined the movement but held their response, in both the full and the free conditions, there was an activation of the left parietal area 39/40, the left frontal area 9 and the left anterior supplementary motor area (pre-SMA). In the free condition, the cingulate cortex, right premotor area 6 and right parietal area 39/40 were also activated. When movements were actually executed after having been imagined, the right cerebellum was additionally activated in both full and free conditions. Right cerebellar activity remained when the imagination component was cancelled out by the direct comparison of tasks with imagination and execution versus tasks with imagination only. These results suggest that imagination of movement involves contralateral associative parieto-frontal areas and pre-SMA. They further suggest that the cerebellum plays a major role in processes linked to motor execution, and not to movement imagination.

MOTOR CORTEX: HUMAN STUDIES II

558.1

DO GRASPING AND MATCHING OF OBJECTS INVOLVE SEPARATE VISUAL PATHWAYS ? FUNCTIONAL ANATOMY WITH PET.

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In order to determine the neural substrate involved in grasping and in form identification respectively, task related changes in regional cerebral blood flow (rCBF) were measured in eight healthy volunteers using positron emission tomography (PET) and $H_2{}^{15}O$ i.v. bolus injection. Subjects were scanned during the performance of three tasks using identical stimuli, i.e polyhedrical objects of different sizes and orientations, presented at a rate of 0.3 Hz. The tasks were the following: (1) pointing with the right hand to object's center, (2) grasping objects accurately and (3) matching the form of the actual object to that of the previous one irrespective of size or orientation. Statistical analysis was performed with the 94' version of Statistical Parametric Mapping (SPM).

- During the matching task, relative to grasping task, rCBF increase was located in the left prefrontal cortex and in the right inferior temporal cortex.
- During the grasping task, relative to the matching task, rCBF increased controlaterally to the grasping hand in the inferior parietal cortex, in the occipito-parietal cortex, in the sensori-motor cortex and in the lateral premotor cortex. In addition, rCBF increased in the cerebellum as well as in the median cingulate cortex.
- During the grasping task, relative to pointing task, rCBF increase was observed in the controlateral inferior parietal cortex.

Object shape seems to be processed by different structures according to the goal of the task. These results are discussed within the framework of separate visual pathways for perception and action.

558.2

CORTICAL FOCI RELATED TO HAND REACHING AND GRASPING IN HUMANS EXAMINED BY REGIONAL CEREBRAL BLOOD FLOW

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To identify cortical foci related to reaching and grasping movements, we measured regional cerebral blood flow by PET in six right-handed normal volunteer (with written informed consent). Five cylinders with different diameters were fixed on a board in front of the subjects. In the saccade task, subjects were ask to look at a LED, embedded in the center of the each cylinder, which was lit in the random order. In the reaching task and grasping task, subjects were ask to touch the LED or grasp the cylinder with their right hand. After the anatomical standardization of the PET images by computerized brain atlas system (Roland et al. 1994), percent change of relative rCBF between two globally normalized images (reaching and saccade, also grasping and reaching), and three-dimen-sional t-images were calculated to find fields of activation.

In the reaching task, fields of activation were observed bilaterally in premotor areas(PMA), inter parietal sulcus areas (IPSA) and prefrontal areas, as well as in the left sensori-motor area. In the grasping task, bilateral PMA, the right IPSA, and bilateral visual areas showed significant increases in rCBF. The results indicate that human PMA and IPSA may be the key structures for both reaching and grasping movements.

558.3

FUNCTIONAL NEUROANATOMY OF GOAL-DIRECTED HAND AIMING MOVEMENTS: A PET STUDY. C.J. Winstein*, P.S. Pohl, & S.T. Grafton. Motor Behavior Lab & PET Imaging Center, University of Southern California, Los Angeles, CA 90033.

The purpose of this study was to examine the effects of task complexity on the functional neuroanatomy of rapid goal-directed aiming movements. We used the PET brain imaging technique with 6 right handed young adults (M = 23 $\pm$ 3.8 yrs) while they performed reciprocal aiming in three conditions of task difficulty [Fitts' (1954) Index of Difficulty] (2.32, 4.32, 6.32) each with two target width/amplitude combinations (Type) resulting in six conditions. Six movement scans (90 s) were acquired in counterbalanced order. The path of the stylus was recorded by video (120 Hz) using 2-D kinematic techniques. Number of cycles (target to target), and kinematic features (average movement time, peak velocity, and % acceleration time) were analyzed together with the magnitude of regional cerebral blood flow (rCBF). ID, Type, cycles, and kinematic features were used as linear contrast weights in planned comparisons of rCBF means across the six movement conditions to identify regional brain activity proportionate to these effects ($p < .005$). When the aiming task was easy (low ID, high cycles, high peak velocity), rCBF was greatest in the ipsilateral anterior cerebellum, contralateral posterior visual association (movement perception), and ipsilateral ventral premotor areas. Surprisingly, rCBF in the motor cortex was only mildly increased ($p < .05$). With a high ID (low cycles, low velocity), rCBF was greatest in bilateral visual association, contralateral ventral and dorsal parietal, premotor, and rostral SMA areas. Difficult closed-loop aiming relies more on the dorsal parietal "stream" as well as premotor and rostral SMA cortex.

558.4

FUNCTIONAL MRI OF CORTICAL MOTOR AREAS DURING SEQUENTIAL TYPING MOVEMENTS. A.M. Gordon*, D. Flament, J.-H. Lee, K. Ugurbil, S.-G. Kim and T.J. Ebner. Depts. of Physiology, Neurosurgery and Center for Magnetic Resonance Research, Univ. of Minnesota, Minneapolis, MN 55455

In the present study we examined the activation of cortical motor areas using functional MRI (4T, echo planar imaging) during the execution of a well-learned sequence of finger movements. We chose typing since it is a highly skilled task which is goal directed (i.e., requires a series of keys to be struck in a specific spatio-temporal order). Experienced typists performed several typing tasks, which varied in the spatial and temporal requirements, on a standard computer keyboard situated on their lap. They performed a repetitive movement with the right index finger (typed "J"), a repetitive sequence with the same finger (typed "JUYH"), a repetitive sequence with all four fingers of each hand (typed "JFIELSPQ"), and typed sentences which required the use of all fingers. The number of keypresses was recorded to assess motor performance. Twenty-four contiguous coronal slices (5mm each) were collected through the temporal, parietal and part of the frontal lobes. During the performance of the repetitive movements with the right index finger ("J"), a consistent activation was observed primarily in the contralateral primary motor cortex (M1). During the performance of the other tasks, which involved sequencing of movements of either the right index finger or all of the fingers, activation was also observed in the premotor cortex (PM) and supplementary motor area (SMA). In addition, for the latter tasks, parietal areas 5, 7 and 40 were activated. The results suggest a hierarchy of cortical structures involved in producing a series of finger movements, that is dependent on the degree of sequencing (PM and SMA) as well as the spatial demands of the task (parietal cortex).

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558.5

A NEW APPROACH TO ASSESS CORTICAL PROCESSING OF FAST REPETITIVE FINGER MOVEMENTS: STEADY-STATE MOVEMENT-RELATED CORTICAL POTENTIALS AND THEIR GENERATORS. C. Gerloff, C. Toro, L. Leocani and M. Hallett*.

Human Motor Control Section, Medical Neurology Branch, NINDS, National Institutes of Health, Bethesda, MD 20892, U.S.A.

We investigated movement-related cortical potentials (MRCPs) and their electric sources during fast repetitive metronome- and self-paced finger extensions.

MRCPs were recorded from 6 volunteers using a 32-electrode cap (sample rate 250 Hz, upper cutoff 50 Hz, time constant DC). Subjects performed steady-state metronome-paced simultaneous extensions of their fingers II-V at 2 Hz and comparable self-paced movements. EMG-locked averaging was done offline using 500 ms time windows, each corresponding to one full cycle of the continuous 2 Hz movement. Per subject and task, 266 (mean, SD 40) movements were averaged. Brain electric source analysis (BESA) was used to model the underlying generators.

A distinct cortical potential was found in all subjects, with a parietal negative peak preceding EMG onset by about 75 ms, and a frontal negative peak following EMG onset by about 105 ms, both more pronounced contralateral to the moving side. During self-paced movements, an additional negative peak was seen at about 170 ms before EMG onset, with maximum over frontocentral midline electrodes. For both conditions, dipole modeling revealed 2 pairs of tangential sources in contra- and ipsilateral sensorimotor cortex, one of them oriented parietally, generating the earlier parietal peak, the second pointing frontally, generating the frontal peak. For the self-paced condition, contribution of an additional source in the frontocentral midline varied across subjects. The models accounted for over 91% of grand average data and for over 89% (mean, SD 3.9%) of individual data.

Steady-state MRCPs reflect rapid changes of cortical activation during repetitive movements. We propose that their main generators are located in the pre- and post-central gyri, and show phasic, alternating activity during maintainance of this type of motor behavior.

558.6

AN EEG-BASED BRAIN-COMPUTER INTERFACE (BCI): IMPROVING PERFORMANCE BY INCREASING SPATIAL RESOLUTION. D.J. McFarland*, L. McCane, S. David, and J.R. Wolpaw, Wadsworth Labs, NY State Dept Health and SUNY, Albany, NY 12201.

Humans can learn to control the amplitudes of specific EEG components and use them to move a cursor in one or two dimensions to a target on a video screen (Electroenceph clin Neurophysiol 78:252-259, 1991 and 90:444-449, 1994). We are trying to improve the rapidity and accuracy of cursor control by using EEG reference methods that increase spatial resolution. The present study compares four reference methods: a standard monopolar ear reference, a common average reference (CAR), and two LaPlacian derivations (nearest neighbor and next-nearest neighbor). The CAR and LaPlacians are spatial filters that accentuate EEG components of high spatial frequencies.

We analyzed 64-channel EEG data that had been gathered while four well-trained subjects controlled cursor movement. For each reference method, we defined EEG dependence on target location by computing scalp topographies of r^2 (the coefficient of determination) for the frequency bands that had been used to control cursor movement online.

In all subjects, the best results (i.e., highest r^2 values for the scalp locations used for cursor control online) were given by the CAR method (0.36($\pm$0.08SD). Values were lower for the next-nearest neighbor LaPlacian method (0.29($\pm$0.09)) and much lower for the monopolar and nearest neighbor LaPlacian methods (0.19($\pm$0.09) and 0.18($\pm$0.11) respectively).

While these results suggest that the CAR method is the best choice for a brain-computer interface (BCI) system, it is important to note that these subjects had been trained primarily with the CAR. Had they been trained with another reference, the outcome might have been different. Nevertheless, the results indicate that spatial resolution is an important factor in BCI design. (Supported by NIH grant HD30146).

558.7

AN EEG-BASED BRAIN-COMPUTER INTERFACE: ALTERNATIVE METHODS FOR CONTROLLING TWO-DIMENSIONAL CURSOR MOVEMENT. L. McCane*, D.J. McFarland, T.M. Vaughan, and J.R. Wolpaw, Wadsworth Center for Laboratories and Research, NY St Dept of Health and State Univ of NY, Albany, NY 12201.

Humans can learn to control the amplitudes of specific EEG components and use them to move a cursor in one or two dimensions to a target on a video screen (Electroenceph clin Neurophysiol 78:252-259, 1991 and 90:444-449, 1994). We are trying to improve the rapidity and accuracy of two-dimensional cursor control by incorporating EEG from additional or alternative scalp locations into the algorithm that controls cursor movement.

EEG is recorded from 64 scalp locations while subjects control cursor movement. Offline analysis of these data seeks to find scalp locations and/or frequency bands that show strong correlations with target location and thus may be expected to improve online control. These parameters are then tested online.

In studies to date, two algorithms have provided significant two-dimensional cursor control. In both algorithms, vertical movement is controlled by the amplitude(s) in an alpha (i.e., 8-12 Hz) or beta (i.e., 20-25 Hz) frequency band over one (or both) sensorimotor cortices. In one of these algorithms, horizontal movement is controlled by the difference between these amplitudes over sensorimotor cortex. In the other, horizontal movement is controlled by the difference between amplitudes in an alpha or beta frequency band over right and left parietooccipital cortices.

Current studies are comparing these two algorithms to determine which is superior and are also seeking to identify further alternatives. It is likely that the optimum algorithm will be different for different subjects. (Supported by NIH grant HD30146.)

558.8

CHANGES IN MOTOR CORTICAL AND SUBCORTICAL ACTIVITY, DURING THE ACQUISITION OF MOTOR SKILL, INVESTIGATED USING FUNCTIONAL MRI (4T, ECHO PLANAR IMAGING). D. Flament*, J.H. Lee, K. Ugurbil and T.J. Ebner. Dept. Neurosurgery and Center for Magnetic Resonance Research, Univ. Minnesota, Minneapolis, MN 55455.

In an earlier study we showed that cerebellar activity is inversely related to performance, decreasing during the learning of a novel motor task. In this study we investigated whether motor cortical and subcortical areas also exhibit learning-dependent changes in activity. The reference task consisted of center-out movements to 8 targets, where a joystick was used to superimpose a cross-hair cursor onto the targets. With practice, subjects were able to do these tracking movements with a high degree of accuracy. The number of movements made and the path length of the trajectories to each target were calculated, and used to generate a performance index. The learning paradigm was a modification of this task, where the relationship between movement of the cursor and joystick was reversed. Initially, the number of errors increased, fewer accurate movements were made, and path lengths increased (performance index decreased). In 5 of 8 subjects tested, the performance gradually improved and approached or reached the performance level of the control task. We imaged 24, 5mm slices through the temporal, parietal, and part of the frontal lobes. On average, as learning progressed, the area of activation in M1, SMA and PM increased. The caudate nucleus had no change in the area of activation while the lentiform nucleus had an increased area. These findings confirm that the changes in cerebellar functional activation measured previously are not due to global hemodynamic alterations. The increased activation observed in M1, SMA and PM suggest that these areas may be directly related to the process by which skill is acquired when learning a novel motor task.

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558.9

VISUAL ORIENTATION DISCRIMINATION: EFFECT OF TRAINING ON BRAIN ACTIVITY, A PET STUDY. C.Schiltz, S.Dubois, J.M. Bodart, C. Michel[1], G. Orban[2], and A. Rouçoux*. Lab. of Neurophysiology, U.C.L., Brussels; [1]PET Unit, U.C.L., Louvain-la-Neuve; [2]Lab. Neuro -en Psychofysiologie, KULeuven, Leuven, Belgium.

The aim of the present experiment was to investigate the neural mechanisms involved in visual perceptual learning. An orientation discrimination task was used as it is known to engage early visual areas (Dupont et al, PNAS, 90:10927-10931,1993). Four male right-handed subjects were scanned before and after training (10,000 trials) in orientation identification. Three conditions were tested: Identification with right and left hand button press as response, Passive viewing and Fixation only. In each scanning (ECAT 961 HR) session, these conditions were repeated four times in random order. Statistical analysis on the rCBF brain images was performed by Statistical Parametric Mapping (Friston et al., 1994). Substracting fixation only from identification before training revealed activations in calcarine, lingual and fusiform cortices, primary motor cortex bilaterally, cerebellum, anterior cingulate cortex and putamen. After training, the same substraction yielded much reduced occipital activation leaving only a V1-V2 focus. There was also a decrease in primary motor activation combined with an increased activation in SMA, putamen and anterior thalamus. Similar observations were made when substracting Passive Viewing from Discrimination. Before training, temporal, parietal and frontal regions were deactivated during discrimination. Most deactivations disappeared with training: this is probably related to the decrease in the level of difficulty after training, since the orientation difference (15 deg.) was kept constant.

558.10

OCULOMANUAL DYSMETRIA IN PATIENTS WITH POSTERIOR PARIETAL LESIONS. K.R.Kessler[1], S.H.Brown*[2], H.-J.Freund,[1]. Neurologische Klinik, Univ. Duesseldorf, FRG[1], Kinesiology, Univ. Michigan, Ann Arbor, MI[2].

We recently reported the effects of altered visual feedback on the initiation of coupled eye and arm movements associated with generalized parietal lobe dysfunction. These studies have been extended to patients with discrete lesions of the posterior parietal cortex which is thought to play an important role in sensorimotor integration. Using a step-tracking task, horizontal saccadic and elbow movements were examined under full visual feedback of both handle and target position and random blanking of either handle or target position. No signs of major visual field defects, optic ataxia or spatial neglect were clinically apparent at the time of testing. Eye movements were recorded using an infrared technique and arm movements were recorded by potentiometers mounted below the elbow. The contralateral arm was tested in all cases and, in one patient, both arms were tested.

The pattern of saccadic eye movement was direction dependent with multisaccades occurring in movements made away from the side of the lesion. In some cases, eye movements often resembled cerebellar saccadic dysmetria. Removal of visual information regarding target position led to an increase in the number of secondary and tertiary saccades in both movement directions. Target blanking also resulted in uncoupling of oculomanual initiation in that eye onset did not occur until after onset of arm movement. Interestingly, similar abnormalities in eye-arm tracking (delayed onset times, prolonged movement durations) occurred in the ipsilateral arm although the impairment was not as severe as in the contralateral arm.

These findings support the view that posterior parietal cortex is involved in transforming external coordinates into ocular and limb motor commands. The similarity in dysmetric eye movement patterns to that observed in cerebellar disease may reflect the functional significance of parieto-pontine-cerebellar projections in the control of eye-arm movements. (Supported by the DFG, SFB 194,A4)

558.11

MENTAL SIMULATION OF MOVEMENT IN PATIENTS WITH POSTERIOR PARIETAL CORTEX LESIONS A. Sirigu, J.-R. Duhamel, B. Pillon, L. Cohen, B. Dubois, Y. Agid* I.N.S.E.R.M. Unité 289, F-75013 Paris, France

The posterior parietal region has long been known to be involved in skilled arm and hand movements. Although the nature of this contribution is not fully understood, a recurring hypothesis is that the parietal cortex maintains internal models of learned limb postures and movement patterns. We recently proposed that the study of mentally simulated actions, or motor imagery, in neurological populations could provide insights into the role of different cerebral areas in movement representation (Sirigu et al., NeuroReport, vol.6, 1995).

Patients with parietal lobe lesions were compared to normal subjects and to a patient with motor cortex damage on mental simulation of finger, arm, and leg movements sequences. The goal of these tasks was to determine if the time employed to simulate a movement accurately predicts the duration of the actual movement. Normals showed consistent correlations between actual and mental movement times. This was also true for the patient with motor cortex damage in whom motor impairments of the contralesional limb could be measured just as accurately in the imagery condition as in the actual movement condition. Parietal lesions also slowed the execution of finger movement sequences, but their duration was not correlated with mental movement times: simulated movements systematically underestimated or overestimated actual movement times. One patient with a left parietal lobe lesion showed minimal slowing of actual finger movements, yet was severely impaired for all simulated hand movements. In all cases, actual and imagined movements for other body parts were well correlated, ruling out a global mental imagery impairment. While the patient with motor cortex lesions reported subjectively experiencing her motor deficit during imagery, parietal patients consistently reported difficulties in generating an image of their own hand in movement. We conclude that the posterior parietal cortex plays a critical role in activating internal representations of limb configurations and movements during mentally simulated actions.

558.12

VISUAL AND MOTOR INTERACTION: SPATIOTEMPORAL PATTERNS OF INTER- AND INTRAHEMISPHERIC CORTICAL ACTIVATION C.D. Saron*, H.G. Vaughan, Jr., G.V. Simpson, and J.J. Foxe, Depts. of Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, NY 10461.

Event-related potentials from 63 scalp sites were elicited from 5 right-handed males (23-35 yrs.) in response to checkerboard patterns presented to the left or right lower visual field quadrants in a simple reaction time (RT) task. The purpose of this experiment is to characterize the time course, regional pattern of activation and probable routes of communication between several processing stages in this basic sensorimotor task. We broadly define these stages as activation of striate, extrastriate, visuomotor, premotor and motor output regions. By examining the effects of unilateral visual stimulation and ipsimanual vs. contramanual response requirements, the timing and locations of interhemispheric transfer of both visual and motor information can be assessed. Control conditions that use stimuli without a response requirement and responses (finger lifts) without a stimulus, provide templates for subtraction from, and comparison with, the RT task conditions. Stimulus- and response-synchronized averaging optimizes measurement of early (striate and initial extrastriate) and late (premotor and motor output) activity respectively. Selection of trials for averaging from the 2nd and 3rd quartiles of each individual subject / RT condition combination sharpens the motor activity visible in the stimulus-triggered averages and the visual activity obtained in the response-triggered averages. These data are further analyzed using scalp current density (SCD) mapping and spatiotemporal dipole source modeling with correlation to individual subject brain MRIs. Between-site timing differences are measured using SCD maxima and cross-lag correlations on surface Laplacian and modeled source waveforms. The data reveal consistent initial occipital activation contralateral to the field of input followed by a delayed ipsilateral occipital response. Multiple episodes of this posterior interhemispheric transfer begin at approximately 80, 130, 230 and 400 ms post-stimulus. Activation of motor cortex contralateral to the responding hand appears to flow from ipsilateral posterior regions independent of input field. Combined SCD mapping and spatiotemporal dipole analysis disclose characteristic individual patterns of visuomotor cortical activation and interhemispheric transfer that appear to account for variations in RT. These data provide evidence for both visual and motor routes of interhemispheric interaction with inter- and intra-hemispheric activity modulated by motor demand after 80 ms post-stimulus.

558.13

REDUCED SYNCHRONIZATION OF BIMANUAL LIMB MOVEMENT INITIATION TIMES IN AN ANTERIOR CALLOSOTOMY PATIENT FOLLOWING SURGERY. **J.C. Eliassen* and M.S. Gazzaniga. Center for Neuroscience, Univ. of Calif., Davis, CA 95616.**

The role of the anterior callosum in the synchronization of bilateral movement initiation was studied by observing pre- and post-operative performance. The patient, a 41 y.o. left-handed female with ~80 seizures/month, underwent an anterior callosotomy. Seizures fell to 10% of pre-op levels at the first post-op visit and were increasing at the second. Medication stayed constant during the experiment: 1 pre-op and 2 post-op visits (@ 1 mo. and 3 mos.). The subject sat facing 2 electronic timers, each stopped by a separate button placed in front of the patient. The paradigm consisted of 7 blocks of 40 trials each: 1 simple left-handed reaction time (RT) block, 1 simple right RT block, 1 dual R/L RT block, and 4 blocks where the subject was instructed not to react as quickly as possible but instead to stop the clocks simultaneously to within 1 millisecond (timer accuracy). Of the 4 simultaneous stop blocks, the subject used her hands on 2, and her index fingers on 2. She was provided with full visual feedback of limbs and performance times on 2 of the blocks, 1 hand block and 1 fingertip block. Following surgery the variance of her simple and dual reaction times for each limb individually was reduced indicating overall improvement. In contrast, the variability of stop time differences between the limbs in the simultaneous blocks increased to statistically significant levels. Additionally, the correlation between right and left hand stop times in the dual reaction time block dropped after surgery: before surgery r = .9984, first post-op visit r = .7851, second post-op visit r = .9629. These data indicate a difficulty initiating movements of the limbs simultaneously following section of the anterior callosum. The increased correlation at the third visit suggests compensatory mechanisms are developing. Further visits will illuminate the time course of these processes. NIH/NINDS P01 NS17778-13 and McDonnell-Pew Foundation.

558.14

FUNCTIONS OF THE CORPUS CALLOSUM IN INTERHEMISPHERIC INTERACTION: TRANSFER AND INHIBITORY MODULATION. B. Preilowski. (SPON: European Brain and Behaviour Society) Tübingen Univ., Weissenau Field Station, D-88214 Ravensburg, Germany, and Keck Center, UCSF, San Francisco, CA. 94143.

Pathological conditions as diverse as schizophrenia and dyslexia have been related to aberrations in the quality of interhemispheric interactions due to structural as well as functional abnormalities of the corpus callosum. In support of this view deficits in bimanual coordination and intermanual transfer are often cited.

Partially and completely commissurotomized patients as well as patients with pathological lesions of the corpus callosum of various etiology show different degrees of dysfunctions in coordinating both hands in novel tasks. This varies from extreme deficits with uncontrollable, independent movements and intermanual blocking to forms of coordination under visual control with evidence of intermanual coupling. Bimanual skills acquired before surgical or pathological lesions are still demonstrable and independent of the degree of deficits shown in the novel tasks. These skills also persist in patients with unilateral apraxia and alien hand syndrome. In rhesus monkeys a lack of intermanual transfer of learning despite intact commissures and evidence for such transfer despite callosotomy has been found.

These data raise doubt about simplistic models of callosal functions. Instead, it is suggested that the role of interhemispheric inhibition, the possibility of regional and state dependent changes in callosal functions and the interaction of callosal and subcortical mechanisms be given more detailed consideration. (Supported by Tübingen Univ., the James S. McDonnell Foundation and The Pew Charitable Trusts)

559.1

DIFFERENTIAL REGULATION OF CREB PHOSPHORYLATION BY CALCINEURIN IN DEVELOPING STRIOSOMES AND MATRIX OF ORGANOTYPIC STRIATAL CULTURES. F.-C. Liu* and A.M. Graybiel. Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

Ca^{2+}/calmodulin-dependent protein phosphatase-2B (PP-2B or calcineurin) is one of a set of striatum-enriched signaling molecules. In the present study, we tested whether calcineurin could modulate signaling cascades in striatal neurons through dephosphorylation of the cAMP response element-binding protein (CREB), a transcription factor known to be regulated by cAMP and calcium signals. We have previously shown that activation of L-type voltage-dependent calcium channels by BAY K 8644 can induce immunodetectable Ser^{133} phosphorylated CREB-like protein (PCREB) in organotypic cultures of neonatal striatum (Liu et al., 1994). We report here that in 1-day striatal slice cultures, processed 30 min after stimulation, the PCREB induced by BAY K 8644 is primarily in the striatal matrix: double immunostains showed that PCREB was not co-localized in DARPP-32-positive neurons of striosomes, except far medially. Pretreating such striatal cultures with the calcineurin inhibitor, FK506 (10 μM), strikingly increased PCREB expression in striosomes: PCREB was now co-localized in DARPP-32-positive neurons of striosomes including the striatal lateral streak. This effect was not observed in slices pretreated with the FK506 control, rapamycin (10 μM), which does not activate calcineurin. Immunostaining for calcineurin in the 1-day striatal cultures showed that calcineurin was expressed at high levels in developing striosomes relative to the matrix, except medially.

Our study suggests that activation of calcineurin by L-type voltage-dependent calcium channels could function as a Ca^{2+} signal filter to inactivate phosphorylation of CREB-like protein primarily in the developing striosomal system, and hence may differentially regulate PCREB-mediated gene expression in striatal compartments during development. We thank Drs. D.D. Ginty and M.E. Greenberg for PCREB antiserum and Dr. I. Bekersky of Fujisawa Pharmaceuticals-USA for FK506. Supported by NIH 1 R01 HD28341, The Council for Tobacco Research-USA, and the Science Partnership at MIT.

559.2

PSA-NCAM IN THE DEVELOPING STRIATUM: EFECTS OF PERIPHERAL INJECTIONS OF MK-801. A.K. Butler*, G. Rougon, and M-F Chesselet. Inst. of Neurol. Sci., and Dept of Pharmacol., U. of Penn. Phila, PA19104 and U. Aix-Marseille, Luminy, France.

Polysialic acids associated with the Neural Cell Adhesion Molecule (PSA-NCAM) play a critical role in the development of the nervous system. In the visual system of Xenopus Laevi, glutamatergic stimulation triggers a loss of synaptic plasticity and of PSA-NCAM. Little is known about the molecular mechanisms involved in the regulation of PSA-NCAM expression in mammalian brain. Previous studies in our laboratory have shown that loss of PSA-NCAM immunoreactivity in the striatum occurs after the formation of corticostriatal synapses, which use glutamate as a neurotransmitter. To determine whether glutamate is involved in the loss of striatal PSA-NCAM, Sprague Dawley rat pups were given the non-competitive NMDA antagonist MK-801 (0.25 mg/kg once daily, ip) from postnatal day 14 (P14), before corticostriatal synapse formation, until P24, after the number of corticostriatal synapses has reached the adult level. Rats were sacrificed on P25. Control animals received saline injections or were not injected. Levels of PSA-NCAM, NCAM, and synaptophysin were examined with immunohistochemistry. All MK-801 injected animals showed a loss of PSA -NCAM expression in the cortex and corpus callosum compared to controls, and 75% of these rats also had a loss of PSA-NCAM expression in the striatum. No differences were seen in the expression of NCAM or synaptophysin. In contrast, no loss of PSA-NCAM expression was observed when rats were treated for 3 days (P14 until P16) or 5 days (P14 until P18) with the same dose of MK-801. The results suggest that NMDA receptors play a role in regulating PSA-NCAM expression in the striatum after the formation of corticostriatal synapses and that PSA is down-regulated independently of NCAM and synaptophysin. Supp. by PHS grant NS-29230 and NIMH grant 1 F31 MH10794-01.

559.3

DEVELOPMENT OF INHIBITORY SYNAPSES IN RAT NEOSTRIATUM N.A. Sharpe*, and J.M. Tepper, Aidekman Research Center, Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ USA 07102.

Excitatory input to the rat striatum increases significantly over development. Most of this increase occurs during the 3rd postnatal week due to an increase in the density of asymmetric axospinous synapses (*Soc. Neurosci. Abst., 18*:697, 1992). In the present study we examined the postnatal development of symmetric (S) striatal synapses in the rat.

The density of S synapses in adult striatum (10.8±3.7 synapses/100 μm^2) was not significantly different from that of P21 (10.8±3.6/100 μm^2), P15 (10.9±5.1/100 μm^2) or P10 (9.1±3.4/100 μm^2). However, the postsynaptic targets of S synapses changed significantly. The density of S axodendritic synapses decreased over development ($F=3.8$, $df=3,39$, $p<0.05$): P10 (5.6±4.4/100 μm^2), P15 (10.1±5.1 /100 μm^2) P21 (7.0±4.1/100 μm^2), adults (3.8±3.7/100 μm^2). Axospinous (necks and heads) synapses showed the opposite relationship: adults (3.2±3.7 /100 μm^2), P21 (3.2±3.6/100 μm^2), P15 (0.8±2.3/100 μm^2). At P10 no axospinous S synapses were noted. At P15 axospinous S synapses were rare, and usually terminated onto immature spines. Axospinous S synapses in adult and P21 rats typically terminated onto spine necks. Symmetric synapses terminating onto spine heads, were proximal to asymmetric synapses. Symmetric synapses in the adult were evenly distributed between axodendritic (35%) and axospinous (29%) postsynaptic targets. At P21, P15 and P10 the majority of S synapses were axodendritic (65%, 93%, and 62%, respectively). The distribution of S axospinous synapses decreased over development (30%, 7% and <1% respectively).

Thus, the number of inhibitory inputs is similar whereas their postsynaptic targets change over development. Since inhibitory input in neonates primarily terminates onto dendrites, the efficacy of each input may be greater than that of the S axospinous synapses noted in adults. Thus, these data support previous anatomic and electrophysiologic studies, indicating that intrinsic inhibitory circuits develop earlier than excitatory inputs in the neonatal striatum. Supported by NS30679 and Rutgers Biomedical Research Support Grant.

559.4

EXPRESSION OF DOPAMINE RECEPTOR PROTEINS IN STRIATONIGRAL PROJECTION NEURONS. E.S. Ince*, B.J. Ciliax, A.I. Levey. Dept. of Neurology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

Dopamine has profound modulatory effects in the mammalian neostriatum, mediated by a heterogeneous group of dopamine receptors. A critical and unresolved question in the literature is whether D1 and D2 dopamine receptors, which together account for the vast majority of subtypes in the striatum, are expressed in the same or different striatal projection neurons. Various methods have yielded conflicting results on whether the D1 and D2 receptors are segregated to separate projection pathways or if they are co-localized in the same projection neurons. Most studies, however, have either indirectly measured the presence of the receptor proteins by mRNA analysis (e.g. *in situ* hybridization or polymerase chain reaction) or are not selective for the molecular subtypes (e.g. ligand binding). In order to address the question of segregation or co-localization of the actual receptor proteins, we have used combined immunocyto-chemistry and neuroanatomical tracing methods to determine if D1 and D2 proteins are expressed in striatonigral neurons in rats.

A cholera toxin-colloidal gold (Ct-Au) tracer was injected unilaterally into the substantia nigra pars reticulata and retrogradely transported to the perikarya of striatonigral neurons. Adjacent striatal sections were processed for D1 or D2 immunocytochemistry and visualized with 3,3'-diaminobenzidine tetrahydrochloride. Sections were then incubated in a silver intensification solution to visualize the Ct-Au tracer. Quantification of the neuronal perikarya indicates that 78% of the retrogradely labeled striatonigral cells were labeled with the D1 receptor antibody (n=214), but only 2% of the labeled striatonigral neurons were labeled with the D2 receptor antibody (n=340). These results indicate that at a receptor protein level, there is segregation of D1 and D2, with D1 predominantly expressed by striatonigral neurons and D2 presumably expressed mainly in striatopallidal neurons. Supported by the APDA.

559.5

LOCALIZATION OF m4 RECEPTOR PROTEIN IN RAT NEOSTRIATUM. T.M. Thomas*, H. Yi, C.-A. Gutekunst, A.I. Levey, S.M. Hersch. Department of Neurology, Emory University School of Medicine, Atlanta, GA 30322

Muscarinic acetylcholine receptors play an important role in the modulation of striatal circuits. To further understand the role of m4 in the striatum, we performed immunoelectron microscopy on dorsal rat striatum using an m4 receptor subtype-specific antibody (Levey et al., 1991). Visualization of the antibody was achieved using either 3,3'-diaminobenzidine tetrahydrochloride or silver intensified immunogold. The immunoperoxidase technique showed m4 to be localized in the dendrites and spines of medium spiny neurons. m4 immunoreactive dendrites accounted for 53% of all dendrites counted and m4 immunoreactive spines accounted for 49% of all spines counted. Previous cell count data have shown that 44% of all striatal neurons express m4 (Hersch et al., 1994). Our data indicate that the neurons expressing m4 distribute the protein throughout their dendritic trees. m4 immunoreactive spines were found postsynaptic to asymmetric synapses. Using immunogold, m4 receptors were found to be localized in the membrane peripheral to the asymmetric active zones, suggesting that parasynaptic acetylcholine may be modulatory at these synapses. To characterize which glutamatergic synapses may be modulated by m4, we performed aspiration lesions of motor cortex. Degenerating motor corticostriatal terminals were found to synapse with m4 immunoreactive spines, suggesting that m4 receptors directly modulate motor cortical input to striatum.

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559.6

SEROTONIN AGONIST-INDUCED *c-fos* EXPRESSION IN THE RAT STRIATUM. D.F. Cook* D. Wirtshafter. Dept. Psychology, Univ. Ill. at Chicago. Chicago, IL 60607-7143.

The current studies examined Fos-like-immunoreactivity (FLI) in the striatum following systemic injections of equimolar doses of several serotonin agonists with different profiles of receptor subtype selectivity. The selective 5HT-2 agonist DOI (6.6 mg/kg) produced a modest increase in FLI which could be markedly attenuated by pretreatment with the 5HT-2 antagonist ritanserin. DOI induced staining was most prominent in the medial, paraventricular, region of the striatum. In contrast, the 5HT-1a/5HT-1b agonist RU-24969 (5.1 mg/kg) produced a very robust increase in FLI which was unaffected by ritanserin pretreatment. RU-24969 induced FLI maximally in the dorsal half of the striatum, although patchy staining was present in the ventral half of this structure. The mixed 5HT-1/5HT-2 agonist mCPP (5 mg/kg) resulted in an intermediate amount of staining which was not significantly altered by ritanserin pretreatment.

In order to examine the role of dopamine in these responses, we examined the effects of these drugs in animals with unilateral 6-OHDA lesions. These lesions markedly reduced staining in the medial striatum following injections of RU-24969 or mCPP, but had no effect on DOI induced staining. The D1 antagonist SCH-23390 also attenuated 5HT-1 induced staining. These results suggest that striatal Fos expression can be increased by stimulation of both 5HT-1 and 5HT-2 receptors, but that these effects are produced through different mechanisms.

559.7

DIFFERENTIAL EFFECTS OF SCOPOLAMINE AND QUINPIROLE ON D1 AGONIST INDUCED STRIATAL c-*fos* EXPRESSION IN THE RAT. D. Wirtshafter*K.E. Asin[2] and A. Nikkel[2]. Dept. Psychology, Univ. Ill. at Chicago, Chicago, IL 60607 and [2]Neuroscience Research, D-47U, Abbott Laboratories, Abbott Park, Il 60664.

We have reported previously that the full D1 dopamine agonist A77636 induces substantial Fos-like-immunoreactivity (FLI) in the normosensitive striatum. We here examined the ability of the antimuscarinic drug scopolamine (SCOP) to modify A77636 induced FLI. SCOP (3 mg/kg) induced little FLI by itself, but potentiated the response to A77636 (2.9 mg/kg) in the lateral, but not medial, striatum. No effects were seen in rats pretreated with SCOP methylbromide, which doesn't cross the blood brain barrier. In animals given A77636 alone or in combination with SCOP, labeling was distributed in a locally homogeneous fashion; in contrast we have shown that combined injections of A77636 and the D2 agonist quinpirole (QUIN) result in a patchy pattern of staining (Brain Res. Bull.35:85-91,1994). To investigate these differences, we examined the effects of pretreatment with SCOP (6 mg/kg) and/or QUIN (2.5 mg/kg) on A77636 induced FLI using a 2 X 2 factorial design. Both drugs potentiated A77636 induced FLI in the lateral striatum, but statistical analysis showed that only QUIN pretreatment resulted in a "patchy" staining pattern . Additionally, QUIN, but not SCOP, actually reduced labeling in the medial striatum. These effects of QUIN were not modified by coinjection of SCOP. These results suggest that the effects of QUIN on striatal FLI are not mediated entirely through cholinergic mechanisms.

559.8

CO-LOCALIZATION OF SOMATOSTATIN, NEUROPEPTIDE Y, NEURONAL NITRIC OXIDE SYNTHASE AND NADPH DIAPHORASE IN STRIATAL NEURONS. G. Figueredo-Cardenas*, M. Morelloţ, G. Sancesariotţ, G. Bernardiţ and A. Reiner. Dept. Anat. & Neurobiol., Univ. Tennessee, Memphis, TN 38163; †Clin. of Neurol., Univ. of Rome Tor Vergata.

The neuropeptides somatostatin (SS), neuropeptide Y (NPY), and the enzymes neuronal nitric oxide synthase (NOS1) and NADPH diapohorase (NADPHd) are extensively colocalized in striatal interneurons, which has led to the widely held view that all four substances are completely co-localized within a single class of striatal interneurons. We have explored the validity of this assumption in rat striatum using triple label immunofluorescence for SS, NPY and NOS1 combined with NADPHd diaphorase histochemistry. Both conventional epi-illumination fluorescence microscopy and confocal laser scanning microscopy (CLSM) were used. Following image capture of the multiple-label fluorescence data on CLSM, sections were labeled for NADPHd for comparison to the SS, NPY and NOS1 labeling. All neurons containing NOS in rat striatum were found to be NADPHd+, consistent with the report that neuronal NADPHd activity is accounted for by NOS1. We found similar results with epi-illumination fluorescence microscopy and CLSM for SS, NPY and NOS1 localization. Of those striatal neurons containing any combination of these, 60% contained all three, 25.5% contained SS and NOS, 8% contained NOS only, 6.5% contained SS only and none contained NPY alone or with NOS. These results indicate that while there is a large population of striatal neurons in which SS, NPY, NOS1 and NADPHd are co-localized, there appear to be smaller populations of neurons in which SS or NOS alone or together are found.
Supported by NS-19620, NS-28721 (AR) and the Italian CNR (MM).

559.9

LOCALIZATION OF THE NMDA RECEPTOR NR2A/2B SUBUNITS ON SPECIFIC STRIATAL NEURON TYPES IN RATS. A. Reiner* and Q. Chen. Dept. Anat & Neurobiol., Univ. of Tennessee - Memphis, Memphis, TN 38163.

NMDA receptors are heteromers of diverse NMDA receptor-specific subunits, namely NR1 and NR2A-2D. The presence of NR2 subunits in the receptor significantly potentiates NMDA channel responses to agonists, and the type of NR2 subunit affects the physiological and pharmacological properties of the receptor channel. Knowledge of the precise NR2 subunits present in the NMDA receptors of specific striatal neurons is critical for understanding the selective vulnerability of striatal neurons to NMDA excitotoxins and in putative NMDA receptor-mediated diseases, such as Huntington's disease. We therefore used an antiserum recognizing both NR2A and 2B subunits (the only NR2 subunits present in striatum) (from Chemicon) to study the expression of NR2A/B subunits on specific striatal neuron types with immunofluorescence double-labeling methods and confocal laser scanning microscopy. Somatostatin (SS), cholinergic, and parvalbumin (PARV) interneurons, enkephalin (ENK) projection neurons and calbindin enriched matrix neurons were identified with specific antibodies for their defining peptide/protein; substance P (SP) striatonigral neurons were retrogradely labeled by fluorogold injection into substantial nigra. Our results showed that the majority of striatal cholinergic, ENK, SP, and calbindin containing matrix neurons expressed NR2A/B subunits. 56% of PARV and none of striatal SS interneurons possessed NR2A/B. Absence of NR2A/B subunits from NMDA receptors on SS striatal interneurons may confer resistance to NMDA receptor-mediated excitotoxicity on these neurons. NS-19620, NS-28721 (AR) and UT, Neuroscience of Excellence (QC).

559.10

COORDINATED EXPRESSION OF m1-m5 MUSCARINIC ACETYLCHOLINE RECEPTORS IN MEDIUM SPINY NEURONS OF THE RAT NEOSTRIATUM. D.J.Surmeier*, Zhen Yan, Helen Carter-Russell,Dept. of Anatomy & Neurobiology, College of Medicine, U. of Tennessee, Memphis, TN 38163.

Previous electrophysiological studies revealed the presence of parallel muscarinic signaling pathways in most medium spiny neostriatal neurons (Howe and Surmeier, J. Neurosci., 1995). These results suggest that muscarinic receptors with differential coupling to intracellular signaling pathways are frequently co-localized. Molecular cloning studies have identified five muscarinic receptors (m1-m5). Based upon their preferential coupling to second messenger systems, these subtypes can be grouped into two broad categories: m1-class (m1, m3, m5) and m4-class (m2, m4). Previous studies have suggested that m1 and m4 mRNA are frequently co-expressed by neostriatal neurons.

Acutely-isolated adult neostriatal neurons were patch-clamped and their pharmacological properties determined. The cellular contents were aspirated and the polyadenylated mRNA reverse transcribed. This cDNA was then amplified using techniques we have previously described yielding enough template for up to 50 PCR reactions. PCR primers specific for each muscarinic receptor were then used to determine how expression was coordinated.

In preliminary studies, m1 and m4 mRNA were co-localized in over three quarters of all medium-sized neurons. In addition, m3 and m5 mRNA were frequently co-expressed with m1 mRNA; m2 mRNA was never found in medium-sized neurons. These results confirm the inference from previous electrophysiological studies that medium spiny neurons have multiple, parallel muscarinic signaling pathways.
This work was supported by N.I.H. grant NS 26473 to D.J.S. and S.T. Kitai.

559.11

COORDINATED EXPRESSION OF DOPAMINE RECEPTORS (D1-D5) IN SINGLE NEOSTRIATAL NEURONS. H.R. Carter-Russell*, W.J. Song, D.J. Surmeier. Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, TN 38163.

There has been a continuing debate about whether D1- and D2-class dopamine receptors are co-localized in medium spiny neurons of the neostriatum. Physiological and biochemical studies have consistently found evidence for co-localization yet anatomical techniques have not. One of the shortcomings of these earlier studies is that they have assumed that neostriatal neurons express little or no D4 or D5 (D1b) mRNA.

We have developed a procedure which enables amplification of the mRNA from single cells followed by specific PCR analysis to investigate the cellular complement of dopamine receptor mRNA's. The initial amplification provides sufficient material to perform approximately 50 PCR reactions. PCR based cellular profiles for D1-D5 receptor mRNA's were constructed in neurons that had been physiologically characterized and retrogradely labeled.

Preliminary experiments (n=22) suggest that the expression of dopamine receptor mRNA is heterogeneous among medium spiny neurons. D1a mRNA was found only in retrogradely labeled striatonigral neurons. In contrast D1b mRNA was found in nearly half of all unlabeled (presumably striatopallidal) medium-sized neurons. D2 receptor mRNA was seen in less than half the striatonigral cells and most of that was the long splice variant. D4 receptor mRNA was present in many of those neurons lacking D2 mRNA. Both long and short isoforms of the D2 receptor mRNA were found in many of the unlabeled cells, but D4 mRNA was less abundant in this group. D3 mRNA was rarely seen in neurons in which RNA polymerase II had been inhibited following tissue slicing.
Supported by NIH grant NS26473 to D.J.S. and S.T. Kitai.

559.12

STRIATAL REGIONALIZATION AS DEMONSTRATED BY DIFFERENTIAL DISPLAY. W.J. Rushlow*, N.S. Khoo, N. Rajakumar, B.A. Flumerfelt and C.C.G. Naus. Dept. of Anatomy, University of Western Ontario, London, Ontario, Canada, N6A 5C1.

A variety of studies have demonstrated that the caudate-putamen of the rat is a highly heterogenous structure. There are gradients of neurochemicals across the striatum, differences in the topographical orientation of functionally specific projections and compartmentalization of the caudate-putamen into patch and matrix compartments. The purpose of the current study was to determine regional differences in gene expression in the caudate-putamen of the rat. This was accomplished by isolating mRNA from different regions of the rat striatum and using a reverse transcriptase-polymerized chain reaction technique referred to as differential display. The differential display products were run on a polyacrylamide gel in order to select candidate genes that are apparently expressed in one region but not another. These bands were excised and sequenced to determine if they are novel. The differential display technique yielded a large number of potentially differentially expressed novel genes. Several of these clones are currently being investigated. The results suggest that the striatum of the rat can be regionally subdivided based on gene expression. [This work was supported by the Medical Research Council of Canada and Huntington's Society of Canada.]

559.13

REGIONALLY EXPRESSED cDNA'S IN THE RAT BRAIN. B.A. Flumerfelt*, W.J. Rushlow, N.S. Khoo, N. Rajakumar and C.C.G. Naus. Dept. of Anatomy, University of Western Ontario, London, Ontario, Canada, N6A 5C1.

Differential display is a powerful new technique that can be used to examine the differences between two groups of cells or regions. It is a reverse-transcriptase-polymerized chain reaction (RT-PCR) based technique that selects for poly-A adenylated cDNA's. Separation of the RT-PCR products on a polyacrylamide gel permits for direct comparison between the different samples. In the current study this technique has been applied to the striatum of the rat in order to identify genes that are regionally expressed. Using this technique, a number of candidate genes have been identified and partially sequenced. In situ hybridization studies are currently in progress to determine the anatomical distribution of these candidate genes in both the striatum and the remainder of the brain. One such candidate gene is highly expressed in the lining of the ventricles and the hippocampus but is also present in the medial portion of the caudate-putamen. These results suggest that there are a number of differences between different regions of the rat caudate-putamen. [This work supported by the Medical Research Council of Canada and the Huntington's Society of Canada.]

559.14

EFFECTS OF CHRONIC NEUROLEPTIC TREATMENT ON GENE EXPRESSION IN THE RAT STRIATUM AS REVEALED BY DIFFERENTIAL DISPLAY. N. Rajakumar*, W.J. Rushlow, C.C.G. Naus, J. Stoessl and B.A. Flumerfelt. Depts. of Anatomy and Clinical Neurological Sciences, University of Western Ontario, London, Ontario, Canada, N6A 5C1.

Typical neuroleptics are a common treatment for a variety of psychotic disorders. Unfortunately, chronic neuroleptic treatment may lead to movement disorders similar to those observed with Parkinson's patients. These side effects are thought to be caused by an alteration in dopamine neurotransmission in the striatum. The goal of the current study was to determine what effect chronic treatment with the typical neuroleptic fluphenazine has on the caudate-putamen of the rat at the molecular level. In order to accomplish this, the differential display technique was used. Comparison of vehicle versus chronically fluphenazine treated rats yielded numerous candidate genes that may be fundamentally altered by the drug. Specifically, we found four candidate genes that appear to be turned off following chronic fluphenazine treatment and nine candidate genes that appear to be turned on. These results suggest that the typical neuroleptic fluphenazine causes significant alterations in striatal gene expression. [This work supported by the Medical Research Council of Canada, Ontario Mental Health and Huntington's Society of Canada.]

559.15

TEMPORAL CHANGES IN BASAL GANGLIA NEUROPEPTIDE mRNAs IN RESPONSE TO SEROTONIN REDUCTION BY *P*-CHLOROAMPHETAMINE. P.J. Gresch* & P.D. Walker, Department of Anatomy & Cell Biology, Wayne State University School of Medicine, Detroit, MI. 48201

Reduced serotonin (5-HT) neurotransmission leads to a decrease in preprotachykinin (PPT) and preproenkephalin (PPE) mRNAs in the rat striatum (STR). In this study, we examined the long-term effects of lowered 5-HT on PPT and PPE mRNA levels in striatal subregions following *p*-chloroamphetamine (PCA) treatment. Adult male Sprague-Dawley rats received single ip injections of saline or PCA (10mg/kg) and were sacrificed 7 or 21 days later. Dorsomedial (DM), dorsolateral (DL), ventromedial (VM), and ventrolateral (VL) striatal subregions were assayed for monoamine and metabolite levels by HPLC-EC and mRNA levels by Northern analysis.

	7d after PCA			21d after PCA		
	5-HT	PPT mRNA	PPE mRNA	5-HT	PPT mRNA	PPE mRNA
DM-STR	-39.2	-33.1	-11.2	-24.1	58.1	-22.2
DL-STR	-60.1	23.3	5.1	-22.4	174.2	153.5
VM-STR	-47.8	-74.2*	-63.3*	-6.5	-15.0	-10.6
VL-STR	-67.0	-25.1	-25.0	-48.0	-70.6*	-40.2*

Results are expressed as % change from saline controls (n=6; *$p< 0.05$)

No significant mRNA changes were observed in the DM- or DL-STR. Although PPT and PPE mRNAs in the VM-STR were significantly reduced 7d after PCA, this decrease was not observed at 21d. However, lowered PPT and PPE mRNA levels were detected in the VL-STR 21d after PCA. These results demonstrate a temporal dissociation of neuropeptide mRNA levels in medial vs. lateral subregions of the ventral striatum in response to 5-HT reduction. Supported by NIH NS 30550

559.16

EM STUDY OF NOS+ STRIATAL TERMINALS IN RAT. M. Morelloţ, E.J. Karle*, G. Sancesariotţ, R. Massatţ, G. Bernardiţ and A. Reiner. Dept. of Anat. & Neurobiol., Univ. of Tenn., Memphis, TN, 38163; †Clin. Neurol., II Universita di Roma "Tor Vergata", Rome, ITALY, 00173.

Somatostatin, neuropeptide Y and neuronal nitric oxide synthase (NOS) have been shown to be largely contained within the same population of striatal interneurons. To help elucidate the role of these neurons in the striatum, we have used EM immunohistochemistry to study synaptic contacts of NOS+ neurons within the rat striatum. In one series of single-labeled sections, neurons were immunolabeled with diaminobenzidine (DAB) using antibodies against NOS. In a separate series of double-labeled sections, striatal parvalbumin-containing (PV+) neurons were immunolabeled with DAB, while NOS+ neurons were labeled with silver-intensified immunogold (SIG). Our single-label results showed that NOS+ perikarya and dendrites receive diverse synaptic inputs from unlabeled terminals and rarely from other NOS+ neurons. Double-label material revealed that NOS+ perikarya and dendrites receive synaptic input from PV+ terminals, as well as the unlabeled terminals and rare NOS+ terminals. In single-labeled material, the NOS+ terminals were observed to form appositions and synapses primarily with dendritic spine necks and distal dendritic shafts of unlabeled neurons, seldomly with perikarya or proximal dendritic shafts. NOS+ terminals were not observed to contact PV+ neurons in double-labeled material. Our findings suggest that NOS+ terminals frequently contact spiny projection neurons in the striatum, and only rarely contact two of the major types of striatal interneurons (i.e. NOS+ and PV+ types). These results suggest that NOS+ striatal interneurons may exert their major influence on striatal projection neurons. Supported by NS-19620 & NS-28721 (A.R.) and the Italian CNR (M.M.)

559.17

SEROTONERGIC INFLUENCE ON STRIATAL NEURONS CONTAINING NEUROPEPTIDE Y, SUBSTANCE P AND MET-ENKEPHALIN. Compan V., Dusticier N., Nieoullon* A. and Daszuta A.
Cellular and Functional Neurobiology Unit, CNRS, Marseille, France.

The aim of the study was to perform a regional analysis of the serotonergic (5-HT) influence on various subsets of striatal populations such as interneurons containing neuropeptide Y (NPY) and neurons projecting either to the substantia nigra (SN) and containing substance P (SP), or to the globus pallidus (GP) containing Met-Enkephalin (M-Enk). Using immunocytochemistry and taking into account the plastic nature of the 5-HT systems, we compared the effects of uncomplete and complete neurotoxic lesion obtained by injecting different volumes of 5,7-dihydroxytrytamine into the anterior raphe nuclei in rats.

Within 3 weeks after the complete lesion occurred indicated by undetectable level of 5-HT, we found a general increase in the immunoreactivity of the various peptides which was selectively expressed in different striatal regions for NPY and SP, and more homogeneously distributed in the whole structure in the case of M-Enk. Increases in the SP immunoreactivity was also differentially expressed in the SN pars compacta as compared to the pars reticulata. In contrast, uncomplete lesion of the 5-HT neurons leading to about 80% decrease in 5-HT tissue levels were found to induce various responses (increase, decrease or no change) depending on the peptidergic population examined. These results indicate that the high degree of plasticity of 5-HT neurons has to be taken into consideration before to conclude for functional effects of neurotoxic lesion. Opposite changes in the responses obtained after partial and complete lesion may also be related to more or less complex 5-HT/peptides neuronal interactions. Moreover, the regional analysis points to the needs for considering the heterogeneity of the striatal organization as a main prerequisite for understanding function.

559.18

NALTREXONE REVERSES UNILATERAL 6-OHDA LESION-INDUCED DOPAMINE D_1 RECEPTOR SUPERSENSITIVITY AND ENHANCES THE LESION EFFECT ON DYNORPHIN mRNA PRODUCTION IN RAT STRIATUM. N.D. Guttenberg, B. Drukarch, H. J. Groenewegen* and P. Voorn. Depts. of Anatomy and Neurology, Research Institute. Neurosciences, Vrije Universiteit, Amsterdam, the Netherlands.

6-OHDA lesions cause an increase in the striatal synthesis of (prepro)enkephalin (Enk). This increase results in enhanced stimulation of the mu opioid receptor subtype, for which Enk is the endogenous ligand in the striatum and globus palidus. Previous studies have demonstrated interaction between the mu opioid and dopamine (DA) D_1 receptors. The present study examines the effects of antagonizing the increased mu receptor stimulation after 6-OHDA lesions on DA D_1 receptor sensitivity and on lesion-induced changes in Enk, substance P (SP) and dynorphin (Dyn) mRNA levels in the striatum. Unilaterally 6-OHDA-lesioned rats were treated for 7 days with the selective mu opioid antagonist naltrexone (NAL) via subcutaneously implanted ALZET osmotic mini-pumps (mean pumping rate 10µl/hr, 10mg/kg). DA D_1 receptor-stimulated adenylyl cyclase activity was measured in superfused striatal slices of non-lesioned and lesioned striatum by calculating the conversion of [^{3}H]ATP to [^{3}H]cAMP. mRNA levels were measured by quantitative *in situ* hybridization. NAL treatment reversed 6-OHDA lesion-induced DA D_1 receptor supersensitivity to control levels. Densitometrical analysis of hybridized sections showed that NAL treatment had no significant effect on lesion-induced changes in Enk or SP mRNA levels. However, for Dyn the difference between lesioned and non-lesioned striatum and the ratio of lesioned to non-lesioned striatum had increased (up 48% and up 44%, resp.). These results confirm the interaction between mu and D_1 receptors and suggest a possible strategy for interfering with changes in DA receptor sensitivity related to [the pharmacotherapy of] Parkinson's disease. In addition, our results suggest a direct regulatory influence of the mu receptor on Dyn synthesis.

559.19

ROLE OF NMDA RECEPTORS IN SENSITIZATION TO DOPAMINE D1 RECEPTORS IN DOPAMINE DENERVATED RATS: STUDY OF C-FOS EXPRESSION. G. Di Chiara*, S. Fenu, M. Morelli. Dpt. Toxicology, Univ. of Cagliari, 09100 Cagliari, Italy.

In this study we examined the effect of blockade of N-methyl-D-aspartate (NMDA) receptors on the long-term effects induced by administration of dopamine agonists, in rats with a 6-hydroxydopamine (6-OHDA) lesion of the dopaminergic nigro-striatal pathway. MK 801 potentiated the turning behavior and decreased c-fos expression induced by L-dopa or apomorphine in the 6-OHDA lesioned striatum. At the same time MK 801 blocked the sensitization (priming) induced by L-dopa or apomorphine. In these rats, in fact, administration of SKF 38393, three days after priming, did not induce any turning behavior as compared to rats primed with apomorphine or L-dopa alone; this effect was also accompanied by a reduced activation of c-fos by SKF 38393. At variance, in rats primed with SKF 38393 instead of apomorphine or L-dopa, MK 801 did not influence the activation of c-fos during the induction of priming. In these rats the turning behavior and the striatal c-fos activation induced by the subsequent administration of SKF 38393 was not reduced. The results suggest that repression of c-fos expression by MK 801 during the induction of priming might be part of the mechanisms responsible for the blockade of priming. Therefore early-genes might mediate the long-lasting changes taking place in the process of behavioral sensitization.

559.20

FULL DOPAMINE RECEPTOR AGONIST SKF-82958 INDUCES c-*fos* AND *zif/268* mRNA EXPRESSION IN THE INTACT RAT STRIATUM AND MUSCARINIC CHOLINERGIC RECEPTOR ANTAGONIST SCOPOLAMINE AUGMENTS THIS INDUCTION. J.Q. Wang* and J.F. McGinty. Dept. of Anatomy and Cell Biology, East Carolina Univ. Sch. of Med., Greenville, North Carolina 27858-4354, USA

It is generally accepted that the partial dopamine D_1 receptor agonist, SKF-38393, does not induce immediate early gene (IEG) expression in striatal projection neurons unless dopamine receptors are sensitized by 6-hydroxydopamine lesions or reserpine treatment. In contrast, this study demonstrates, with quantitative *in situ* hybridization, that the full D_1 receptor agonist, SKF-82958, induced robust expression of c-*fos* and *zif/268* mRNAs in the intact rat striatum and cerebral cortex (particularly in cingulate, upper limb region of sensory cortex and piriform cortex) 45 min after acute injection. The induction of these 2 striatal IEGs is characterized by 1) a substantially more intense induction in the nucleus accumbens and olfactory tubercle than in the dorsal striatum; 2) induction in both patch-like and matrix compartments; 3) dose-dependent (0.02, 0.1, 0.5 and 2.0 mg/kg, s.c.) induction correlating well with dose-dependent increases in locomotor activity; and 4) blockade by the D_1 receptor antagonist, SCH-23390 (0.1 mg/kg, s.c.). The muscarinic cholinergic receptor antagonist, scopolamine (5 mg/kg, s.c.), which itself did not alter striatal gene expression, profoundly augmented the behaviors and expression of the 2 IEGs in the striatum induced by 0.1, 0.5 and 2.0 mg/kg SKF-82958, with greater augmentation at the lower doses. However, scopolamine attenuated basal, and SKF-82958-stimulated, expression of c-*fos* and *zif/268* mRNA in the cortex. Scopolamine also enabled SKF-38393 (5 mg/kg, s.c.) to induce locomotor stimulation and c-*fos* and *zif/268* mRNA expression in the intact rat striatum when SKF-38393 alone caused no such changes. These data demonstrate an ability of full D_1 receptor stimulation by SKF-82958 to induce IEG expression in intact rats. Furthermore, intrinsic cholinergic transmission may provide an activity-dependent inhibitory control on striatal D_1 receptor stimulation (Supported by DA03982).

BASAL GANGLIA: MOVEMENT DISORDERS AND EXPERIMENTAL MODELS

560.1

NEUROCHEMICAL CHANGES IN THE PRIMATE BASAL GANGLIA FOLLOWING UNILATERAL INTRACAROTID MPTP. S.B. Evans* and S.N. Haber. Dept. of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester NY 14642.

MPTP causes a selective population of midbrain dopaminergic neurons to die. The susceptible cells are in the ventral tier of the substantia nigra (SN) and project to the sensorimotor striatum. These cells contain higher levels of message for the dopamine uptake site (DAT) and the D2 receptor (D2R) than the more resistant dorsal tier cells. Furthermore, these cells are calbindin D28-k (CABP) negative. The pattern of cell loss after MPTP treatment mimics that of Parkinson's Disease. In this study, two monkeys were given intracarotid MPTP at 0.4 mg/kg and then allowed to survive for two years. The brains were then removed, sectioned, processed for *In-situ* hybridization and immunohistochemistry, and examined by optical density analysis using NIH Image 1.52. We found that CABP expression increased significantly in the caudal putamen and GPe on the lesioned side. The pattern of CABP expression in the SN also changed from one of distinct CABP-positive and negative areas in the nonlesioned SN, to a homogenous, filled-in pattern in the lesioned SN. Enkephalin mRNA levels increased at all levels of the caudate and putamen on the lesioned side. Substance P mRNA showed decreases on the lesioned side that were not significant in all cases. DAT and D2R mRNA both showed marked decreases in the SN on the lesioned side. These results provide evidence for nigrostriatal dopamine playing a dynamic modulatory role in the striatum, for the heterogeneity of the nigrostriatal projection and for the plasticity of the primate basal ganglia. (Supported by NS22511, MH45537, and the Lucille P. Markey Charitable Trust.)

560.2

REGULATION OF THE GLUR-1 GLUTAMATE RECEPTOR SUBUNIT IN THE DOPAMINE-DEPLETED PRIMATE STRIATUM J Timothy Greenamyre*, Richard HP Porter, Monica Garcia-Osuna & Nicole L Marmarosh University of Rochester, Rochester, NY 14642.

After dopaminergic denervation of striatum, there is electrophysiologic and biochemical evidence for polysynaptic activation of glutamatergic subthalamofugal and corticostriate pathways. In rats, we previously showed that there are regulatory changes in both NMDA and AMPA receptor binding in the dopamine-depleted basal ganglia. We are now beginning to study the molecular regulation of glutamate receptors in the basal ganglia of primates. Two elderly female rhesus monkeys were infused via the right carotid artery with MPTP, causing a stable hemiparkinsonian syndrome. Two years later, the monkeys were euthanized and perfused for anatomical studies. In some studies, brain sections were double-labeled immunocytochemically for tyrosine hydroxylase (TH) and GluR1, using DAB as the chromogen for TH and an [^{125}I]-secondary antibody to localize GluR1 autoradiographically. In other studies, calbindin and GluR1 were labeled using a similar protocol. In addition, mRNA for the "flip" and "flop" splice variants of the GluR1 gene was assessed by *in situ* hybridization in free-floating, fixed sections that were later processed for TH ICC. MPTP caused a profound ipsilateral loss of TH and a striking *increase* in GluR1 protein in the caudate and putamen. The increase in GluR1 protein was most pronounced in patches that corresponded to calbindin-poor patches; however, there was also an increase in the matrix. Similar changes were not found in rats with 6-OHDA lesions. These results support the ideas that *(i)* the glutamatergic system undergoes regulatory changes after dopamine depletion; and *(ii)* it is not always possible to extrapolate results from 6-OHDA treated rats to MPTP treated primates. *(Supported by a Mallinckrodt Scholar Award, USPHS grant NS33779 and the National Parkinson Foundation Center of Excellence at the University of Rochester.)*

560.3

THE ROLE OF OPIOID PEPTIDES IN THE NEURAL MECHANISMS UNDERLYING L-DOPA-INDUCED DYSKINESIAS IN PARKINSON'S DISEASE. J.M. Brotchie*, B. Henry, S. Duty, Division of Neuroscience, School of Biological Sciences, University of Manchester, M13 9PT.UK

Long-term L-DOPA therapy in Parkinson's disease is plagued by debilitating dyskinetic side-effects. The neural mechanisms that underlie these dyskinesias probably involve abnormalities in GABA and EAA transmission within the basal ganglia. Striatal output neurons projecting to the external segment of the globus pallidus utilise GABA and enkephalin as co-transmitters, whilst outputs projecting to the substantia nigra and internal globus pallidus utilise GABA and dynorphin. We have recently shown that enkephalin and dynorphin serve to modulate GABA and EAA release within the basal ganglia. The aim of this study was to assess enkephalin and dynorphin transmission in striatal outputs in animal models of L-DOPA-induced dyskinesias.

We have defined the regional pattern of changes in the levels of mRNA encoding the enkephalin precursor pre-proenkephalin A and the dynorphin precursor pre-proenkephalin B, using *in situ* and Northern hybridisation with ^{35}S-and ^{32}P-labelled oligoprobes. We find that the expression of opioid peptides is significantly elevated, though shows topographical variations, in animal models of L-DOPA-induced dyskinesias. Anti-parkinsonian therapies that are associated with a lower incidence of dyskinesias e.g. lisuride or bromocriptine treatment, are not associated with such changes in opioid peptide expression. Furthermore, blockade of opioid receptors can block hyperkinetic symptoms in animal models of L-DOPA induced dyskinesias.

These findings suggest a potential role of increased enkephalin and dynorphin transmission in mediating the symptoms of dopamine-agonist-induced dyskinesias.

560.4

EFFECTS OF HIGH FREQUENCY STIMULATION OF THE GPI VERSUS MPTP-INDUCED PARKINSONISM IN RHESUS MONKEYS : AN ELECTROPHYSIOLOGICAL STUDY. T.Boraud*, B.Bioulac, and Ch.Gross. CNRS URA 1200, Université de Bordeaux 2, 33076 Bordeaux Cedex France.

Parkinsonian motor symptoms are associated with an over activity of the internal part of the Globus Pallidus (GPi). Inhibition of this structure using surgical lesions (Leitinen et al., 1992), or high frequency stimulation (HFS) (Boraud et al., submitted) reduce these symptoms in parkinsonian subjects. In this study, we wanted to evaluate effect of HFS of the GPi upon neurons of the Basal Ganglia. Extracellular, unit recordings were performed upon the external part of the Globus Pallidus (GPe) and the GPi in two Macacca Mulatta under three experimental conditions : 1°) in normal animals, 2°) after MPTP injection, 3°) before and just after stimulation of the GPi. After MPTP, monkeys exhibit a stable akineto-rigid form of hemiparkinsonian syndrom and were unable to move spontaneously their parkinsonian arms. Firing rate of neurons of the GPe was decreased, with appearance of irregular bursts. In GPi, firing rate was increased with a more regular pattern. This results are similar to those obtained by other teams (Filion and Tremblay, 1991). We have implanted a bipolar stimulating electrode (Medtronic®) in the antero-medial part of the GPi using ventriculographic landmark. We recorded each cell before and just after the stimulation (130Hz, 0.6ms PW, 5 seconds train) in order to avoid artefact disturbance. During stimulation, rigidity decreased in the parkinsonian side, and monkeys were able to move their parkinsonian arms. Stimulation did not induced significant effect on the GPe. On the GPi, it induced a long lasting (20 to 130 seconds) significant decrease in firing rate ($p<0.001$) of 95% of GPi cells inside the stimulated area, and burst almost disappeared. Effect of HFS seems to be a direct unspecific depolarisation block on neurons of the GPi as already suggested for HFS of STN (Burbaud et al., 1995).

560.5

THE MOTOR THALAMUS IN THE PARKINSONIAN PRIMATE: ENHANCED BURST AND OSCILLATORY ACTIVITIES. Y. Kaneoke*, J.L. Vitek. Dept. of Neurology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

We have previously reported an abnormal pattern of neuronal activity in the motor thalamus of parkinsonian primates. To characterize the neuronal discharge patterns in the parkinsonian state we have developed algorithms to distinguish the altered patterns of neuronal activities. We define *burst* as a period in which neuronal discharge rate is significantly higher than other periods in a spike train, while *oscillation* is defined as neuronal activity in which there is significant periodicity in its autocorrelogram. One pigtail monkey developed hemiparkinsonism following injection of 2 mg MPTP (intracarotid). The monkey was trained to sit quietly on the chair and spontaneous neuronal activities were recorded throughout the pallidal (VLo) and the cerebellar (VPLo) receiving areas of the thalamus. Burst periods were identified in 59.2% (103/174) of VLo and 36.0% (104/289) of VPLo neuronal spike trains ($p<0.001$, Chi-square test). The rate for VLo was significantly higher ($p<0.001$, Chi-square test) than the rate for normal monkeys' motor thalamus neurons (32.7% in 49 neurons). Intraburst interspike interval analysis revealed a pattern similar to that found in sleep (gradual increase in interspike interval) in 46.6% of VLo neurons while 27.9% of VPLo neurons revealed such patterns ($p<0.001$, Chi-square test). Oscillation was found in 19.0% of VLo and 27.0% of VPLo spike trains ($p<0.001$, Chi-square test). Both were significantly ($p<0.001$, Chi-square test) higher than the rate for normal monkeys (14.3%). Although the monkey did not manifest tremor, oscillation between 3-8 Hz were found in 33.3% of VLo and 19.2% of VPLo neurons. These observation suggest that the motor symptoms in parkinsonism may result from dysfunction in both pallido- and cerebello- thalamocortical circuits. Furthermore, given the relative difference in the pattern of neuronal activity and termination sites within the cortical motor area, these regions may have different roles in the development of parkinsonian motor symptoms.

560.6

EFFECTS OF SUBTHALAMIC NUCLEUS LESIONS IN A RODENT MODEL OF TARDIVE DYSKINESIA. **A. J. Stoessl*. Clin. Neurol. Sci., Univ. of Western Ontario & Robarts Research Institute, London, ON, Canada N6A 5A5.**

Current models of basal ganglia function suggest that dyskinesias result from *hypofunction* of the subthalamic nucleus (STN). It has been suggested that this mechanism would also apply to the development of tardive dyskinesia (TD) in individuals exposed to chronic neuroleptics. This view is supported by observations of reduced glucose metabolism in the STN of monkeys with neuroleptic-induced dyskinesias, but is in contrast to the model of TD recently proposed by Trugman et al. (1994), in which activity is *increased* in both the STN and the striatonigral pathway. Based on Trugman's hypothesis, as well as the recent observation (Parry et al., 1994) that intra-STN infusion of apomorphine elicits D1-dependent vacuous chewing movements (VCMs) in rats, we examined the effects of STN lesions on VCMs induced by chronic neuroleptics in the rat.

Male Sprague-Dawley rats were treated for 24 weeks with fluphenazine decanoate (FLU; 25 mg/kg i.m.) or its vehicle q 3 weeks. Bilateral STN lesions were made by stereotactic infusion of quinolinic acid (100 nmol/μl/side). FLU resulted in increased VCMs, considered analogous to TD in humans. This response was attenuated to control levels by STN lesions, which also induced increased locomotion and sniffing in vehicle-treated animals. Lesion-induced increases in these responses were suppressed by FLU. Our data indicate that neuroleptic-induced VCMs in the rat are dependent upon (?D1-mediated) stimulation of the STN and suggest that current concepts of dyskinesia should be reassessed with respect to the role of this structure, in keeping with the model recently proposed by Trugman et al. (1994).

560.7

ORAL DYSKINESIA INDUCED BY KAINIC ACID LESIONS OF THE SUBTHALAMIC NUCLEUS IN THE RAT. R.J.A. Banks, P.M. Brun* and M-F. Chesselet. Univ. of Penn., Dept. of Pharmacology, Philadelphia, PA 19104.

The subthalamic nucleus (STh) has been implicated in the control of dyskinetic movements in humans, monkeys and rats. In the rat, microinjections of dopamine and serotonin agonists into the STh induce oral dyskinesia (characterised by vacuous chewing, jaw tremor and tongue protrusions). In the present study, we have assessed the effects of kainic acid-induced lesions of the STh on oral dyskinesia in the rat. Male Sprague-Dawley rats under equithesin anaesthesia were stereotaxically infused with 0.9% saline or kainic acid (100 ng/100 nl) into the STh. Behaviour of the animals was recorded for a period of 20 min at time points 3 hours, 4 days and 7 days after completion of the infusion. Microinfusion of kainic acid into the STh significantly increased the duration of locomotor activity and sniffing, and the frequency of oral dyskinetic movements (characterised by transient mouth opening and closing described as gaping) in rats at time points 3 hours and 4 days surgery. In contrast, the locomotor activity, sniffing and oral dyskinetic responses returned to control levels 7 days after completion of kainic acid infusion. Lesions of areas surrounding, but not including the STh showed no significant difference from saline treated animals at any time point, suggesting the response to be specific to the STh. These data confirm and extend previous studies suggesting a role of the STh in control of oral dyskinesia. In addition, the data provide the first example of oral dyskinesia induced by loss of STh neurones in rats (as kainic acid induces complete lesions 3 days after completion of infusion). The long duration of the behavioural response (4 days) suggest this model may provide a useful tool for investigating oral dyskinesia and the role of the basal ganglia in this response. Furthermore, the changes which occur during the transition between 4 days (oral dyskinesia) and 7 days (no oral dyskinesia) are currently under investigation using biochemical and molecular biological techniques in this laboratory.

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560.8

EFFECTS OF NMDA RECEPTOR BLOCKADE OR SUBTHALAMIC LESION ON REACTION-TIME PERFORMANCE IN A RAT MODEL OF PARKINSONISM. M. Amalric*, C. Baunez and A. Nicoullon Cellular and Functional Neurobiology Laboratory, CNRS, 13402 Marseille cedex 20 (France).

There is substantial evidence that excessive excitatory amino acid (EAA) activity in the striatum and in the subthalamic nucleus could account for the expression of the motor deficits resulting from alteration in dopamine function in the basal ganglia. The present study investigated the potential benefit of blocking EAA transmission at different levels in the basal ganglia in rats previously subjected to dysfunction of dopaminergic transmission and trained to perform a reaction time (RT) task. Disruption of dopamine activity induced by 6-OHDA injected in the striatum or by the D2 dopamine receptor antagonist, raclopride, impaired the performance of rats trained to release a lever as fast as possible after the occurrence of a visual stimulus. The major deficit observed was a significant lenghtening of RTs. The blockade of EAA activity with either systemic or intrastriatal injection of NMDA receptor antagonists was found to reverse the motor deficits induced by the dopaminergic lesion or by raclopride treatment. In contrast, the excitotoxic lesion of the subthalamic nucleus (STN) was found to only partly alleviate the movement initiation deficits induced by the dopaminergic lesion. In addition, the lesion of the STN was found to induce other deficits expressed by an increase of premature responding, preventing the animals to recover a level of performance comparable to pre-operative values. These results suggest that the blockade of EAA transmission at the NMDA receptor subtypes might be beneficial to prevent the akinetic symptoms produced by a dopaminergic denervation of the striatum. In contrast, beside alleviating parkinsonian symptoms in our model, the STN lesion produced additional deficits, leading to question the potential therapeutic benefit of subthalamotomy in Parkinsonism.

560.9

REGULATION OF mRNA LEVELS ENCODING FOR AMPA AND NMDA GLUTAMATE RECEPTOR SUBUNITS IN THE ADULT RAT STRIATUM AFTER 6-OHDA. J.-J. Soghomonian*, P. Salin and M. Tremblay, Centre de Recherche en Neurobiologie, Fac. Med., Univ. Laval, Quebec (QC.) G1K 7P4.

Levels of mRNAs encoding for subunits of AMPA and NMDA glutamate receptors were measured in various sectors of the rat dorsal striatum two weeks after unilateral injections of 6-hydroxydopamine (6-OHDA) in the substantia nigra. The relative mRNA levels were measured on X-ray films after *in situ* hybridization with oligonucleotides labeled at their 3' end with ^{35}S-dATP and complementary to the NMDAR1, GluR1 or GluR2 subunits. The extent of dopaminergic denervation was determined on adjacent sections after ^{3}H-mazindol binding. On the side ipsilateral to the lesion, mazindol binding was decreased on average by 68% with the highest decrease found in a dorsolateral striatal sector (-85%). When compared to the contralateral striatum, NMDAR1, GluR1 and GluR2 mRNA levels were higher (+14%, 18% and 21%, respectively) in a dorsomedial sector of the striatum ipsilateral to 6-OHDA. GluR1 mRNA labeling was also significantly higher (+15%) in a ventromedial striatal sector. When compared to the saline-injected rats, higher NMDAR1 or GluR1 labeling appeared to result from an increase ipsilateral to 6-OHDA. However, higher GluR2 labeling appeared to result from an increase ipsilateral and a decrease contralateral to 6-OHDA. Changes in mRNA levels encoding for glutamate receptors may reflect a compensatory response to changes in the activity of glutamatergic afferents to the striatum in this rat experimental model of Parkinson's disease. (Supported by the Parkinson Foundation of Canada, NSERC-0155607 and FRSQ).

560.10

DIFFERENTIAL REGULATION OF GAD65 AND GAD67 IN SUBPOPULATIONS OF STRIATAL NEURONS. N. Laprade* and J.-J. Soghomonian, Centre de Recherche en Neurobiologie, Hôpital de l'Enfant-Jésus, Québec, Qué. Canada, G1J 1Z4.

Levels of mRNAs encoding for the two isoforms of glutamate decarboxylase (GAD65 and GAD67) are differentially regulated by dopamine receptors in rat striatum. Indeed, dopamine receptor stimulation with apomorphine or SKF-38393 increases GAD65, but not GAD67, mRNA levels while dopamine receptor blockade with haloperidol or sulpiride increases GAD67, but not GAD65, mRNA levels (Laprade and Soghomonian, Soc. Neurosci. Abs., 24 (1994) p. 988). Striatal GABAergic projection neurons are divided in two subpopulations: striatopallidal and striatonigral. Preproenkephalin (PPE) is expressed preferentially in striatopallidal neurons. In order to determine if dopamine receptors regulate each GAD isoform in PPE positive neurons or not, double *in situ* hybridization on striatal sections was performed using radioactive GAD65 or GAD67 cRNA probes labeled with ^{35}S and a PPE cRNA probe labeled with digoxigenin. mRNA levels encoding for GAD65 were increased predominantly in neurons negative for PPE following stimulation of dopamine receptors. Quantification of silver grains at single cell level revealed that the increase in GAD65 mRNA levels when compared to control animals was of 29 % (448.5 +/- 44.9 vs 577.9 +/- 38.6) after apomorphine treatment and 23% (448.5 +/- 44.9 vs 552.4 +/- 37.9) after SKF-38393 treatment. On the other hand, mRNA levels encoding for GAD67 were increased mainly in neurons positive for PPE following blockade of dopamine receptors. The increase relative to controls animals was of 54% (160.9 +/- 33.2 vs 247.7 +/- 32.0) after haloperidol treatment and of 36% (160.9 +/- 33.2 vs 218.5 +/- 19.7) after sulpiride treatment. Thus, our results suggests that GAD65 and GAD67 mRNAs are differentially regulated in striatopallidal and striatonigral neurons. (Supported by the Parkinson Foundation of Canada, NSERC-0155607 and FRSQ).

560.11

ABNORMAL [F18]SPIPERONE BINDING IN PUTAMEN IN IDIOPATHIC DYSTONIA. J.S. Perlmutter, J. Giovanni, K.J. Black, L. McGee-Minnich, L.W. Tempel,* S. M. Moerlein. Departments of Radiology, Neurology & Psychiatry. Washington University School of Medicine, St. Louis, MO 63110

Meige syndrome refers to involuntary eyelid closure associated with lower facial spasms. The eyelid squeezing can be sufficiently severe to render an individual functionally blind. Hand cramp refers to involuntary spasms of hand, forearm and arm muscles with specific actions such as writing. Both are limited forms of idiopathic dystonia with unclear pathophysiology. We measured [F18]spiperone uptake with positron emission tomography in 6 patients with Meige syndrome (4 women; mean age = 50 ± 4 yrs), 3 with hand cramp (1 woman; mean age = 46 ± 7 yrs) and 6 normals (5 women; mean age = 50 ± 16 yrs). No patient had dystonia in other body parts and no subjects had other neurological or psychiatric abnormalities. Each subject also had a normal MRI of the brain. PET studies were done with the Siemens 953b in the 2d mode with 31 simultaneous slices (3.4 mm center-to-center slice separation). Reconstructed transverse resolution was 7 mm FWHM and axial resolution was about 4 mm. Three to 5 mCi of [F18]spiperone were injected i.v. and sequential PET scans were collected for 3 hrs. The last 30 mins of PET data were combined into a single frame and transformed into stereotactic space for subsequent data analysis. The locations of the peak concentrations of [F18] activity in left and right putamen were identified by a computerized search routine. The mean Talairach & Tournoux Stereotaxic Atlas (1988) coordinates are for the Meige subjects (±23.0, 4.3, 5.5), for the hand cramp subjects (± 25.0, 0.0, 9.3) and for the normals (± 25.2, -0.7, 4.3). These locations were significantly different by MANOVA ($p < .037$). Descriptively the locations for the hand cramp subjects were higher than the others. The Meige subjects' peaks were more medial and posterior than normals. These data are consistent with somatotopic functional organization of dopamine receptors in putamen, and are the first in vivo demonstration of a change in dopamine receptor binding in idiopathic dystonia.

560.12

THE RELATIONSHIP BETWEEN STRENGTH AND RATE OF FORCE DEVELOPMENT IN PARKINSON'S DISEASE. D. M. Corcos*, C. -M. Chen, N. P. Quinn and J. C. Rothwell. MRC Human Movement & Balance Unit and Department of Neurology, The National Hospital for Neurology & Neurosurgery, London WC1N 3BG and University of Illinois at Chicago, Chicago, IL, 60608.

This study was designed to determine the relationship between muscle strength, rate of force development, and the time to relax muscle activity or actively return force to initial resting levels. Nine patients with Parkinson's disease were tested in three movement tasks both on and off antiparkinsonian medication. In the first task, they performed maximal voluntary flexion and extension isometric contractions in order to determine their strength. In the second task, they were instructed to generate a 50% maximal voluntary contraction as fast as possible, hold the contraction for four seconds and then relax. In the third task, they were again instructed to generate a 50% maximal voluntary contraction as fast as possible, hold the contraction for four seconds and then return the force to baseline as quickly as possible. Patients were weaker off medication in both flexion and extension but this change was greater for extension. The cause of this weakness was due both to reduced agonist muscle activation and, in some patients, increased muscle coactivation. Patients took longer to generate force, relax and to return actively when off medication. Patients who became weakest when off medication also tended to develop force at reduced rates which was related to a reduction in the phasic EMG burst off medication. Removing medication resulted in an increase in all measures of contraction time but the most sensitive measure was an increase in relaxation time. These findings further our understanding of the motor deficits of Parkinson's disease in two ways. First, they suggest that one possible cause of the flexed postures adopted by patients with Parkinson's disease is a proportionately greater reduction in strength in muscle extensors. Second, the inability to relax suggests that one component of bradykinesia is related to muscle deactivation.

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560.13

THE STRIATUM AND CEREBRAL NETWORKS OF VERBAL AWARENESS IN NORMAL AND HYPERKINETIC CHILDREN

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ADHD is a common disorder of the developing nervous system, affecting 3–6% of all school children. Striatal dysfunction is a key pathogenetic mechanism in hyperactivity which may be induced experimentally by mutations in the dopamine D1 receptor gene. To explore the role of the striatum in verbal "awareness" (attention, perception and association) we measured regional cerebral blood flow (rCBF) by single photon emission tomography in 11 normal and 16 hyperactive children during presentation of white noise or animal names. The latter increased striatal flow. Factor analysis of co–variance of rCBF identified "attentional", "linguistic", and "associative" networks. The right striatum participated in all networks in normals, but not in the stymied "associative network" in ADHD where striatal flow was generally reduced. It is concluded that the striatum is anodal structure, allowing integration of functions in verbal awareness. In ADHD striatal dysfunction disturbs such a role.

REFLEX FUNCTION: ANIMAL STUDIES

561.1

STRETCH REFLEX MODULATION IN DECEREBRATE AND SPINAL CATS DURING TONIC AND PHASIC (LOCOMOTION) CONTRACTIONS. De Serres, S.J.*, Stein, R.B., Bennett, D.J., Department of Physiology and Division of Neuroscience, University of Alberta, Edmonton, Canada, T6G 2S2.

The stretch reflex was studied in the triceps surae muscle of decerebrate cats during postural tasks (tonic contractions) and, after spinalization, during locomotor activities (phasic contractions). Tonic activity was induced by stimulation of the contralateral common peroneal nerve (crossed extensor reflex) while the cats were given Naloxone and Clonidine, combined with perineal stimulation to induce locomotion. Short pulses 10 ms in duration (various amplitudes) were delivered by a motor to stretch the muscle at rest and during the contractions. With such short pulses we could cleanly separate in time the short-latency reflex force responses from the mechanical effects of the stretch. Similar recordings were also performed after deafferentation, allowing us to subtract the force changes due to the contractions. The reflex responses increase little when the amplitude is increased from 1 to 3 mm, indicating a saturation of the reflex loop. For 1 mm stretches the reflex responses are high at low levels of tonic muscle activation (decerebrate state), but gradually decrease with an increase in contraction levels. In the spinalized animals, the reflex is also high at rest (prior to locomotion), drops abruptly when locomotion starts, is small at low levels of activation (swing phase) and increases with higher levels of muscle contraction (stance phase). Thus, even in reduced preparations strong modulation of reflexes is seen which can change in opposite directions with increasing force levels during different tasks. (Research supported by MRC).

561.2

PLASTICITY OF THE EXTENSOR GROUP I PATHWAY CONTROLLING THE STANCE TO SWING TRANSITION IN THE CAT

P. J. Whelan*, G. W. Hiebert and K. G. Pearson. Dept. of Physiology, University of Alberta, Edmonton, AB, CANADA, T6G 2H7.

The brain possesses the capacity to reorganize itself after peripheral injury, often in a functionally meaningful way. Although much plasticity occurs at the cortical level, relatively little is known about the plasticity of spinal systems beyond the simple monosynaptic reflex. This study examines whether the efficacy of polysynaptic group I excitatory pathways to extensor motoneurones are modified after axotomy of a synergistic nerve. Previously, we have shown that stimulation of extensor nerves at group I strength can extend the stance phase and delay swing. Stimulation of the LG/S nerve prolongs stance for the duration of the stimulus train, whereas stimulation of the MG nerve moderately increases stance. We hypothesized that after axotomy of the LG/S nerve the efficacy of the MG group I input would increase. This idea was tested in 5 adult cats that had their left LG/S nerves axotomized for 3-28 days. On the experimental day we decerebrated the cats and stimulated the left (experimental) and right (control) LG/S and MG nerves during late stance. A significant increase in the efficacy of the left MG nerve occurred 5 days after axotomy of the LG/S nerve when compared to the control response. By contrast, the previously cut LG/S nerve showed a reduction in efficacy after 3 days compared to the control limb. Functionally, this plasticity may be an important mechanism by which the strength of the group I pathway is calibrated to different loads on the extensor muscles. On-line Poster: World Wide Web URL= http://www.ualberta.ca/~pwhelan/poster.html

561.3

THE SIZE PRINCIPLE OF MOTOR-UNIT RECRUITMENT CROSSES MUSCLE BOUNDARIES. A.J. Sokoloff* and T.C. Cope
Dept. Physiology, Emory University, Atlanta, GA 30322.

We are testing whether the size principle, evident in the orderly recruitment of motor units belonging to single muscles, applies to motor units belonging to different muscles. The medial and lateral gastrocnemius muscles (MG and LG, respectively) were activated in reflexes elicited in decerebrate cats (n=2) by controlled stretch of the Achilles tendon and by punctate stimulation of the heel pad. Recruitment sequence and axonal conduction velocity (an index of motor-unit size, and a good predictor of recruitment sequence) were determined for motor-unit pairs composed of one MG and one LG unit. Recruitment was tested in multiple reflex trials, each trial eliciting EMG activity in both muscles. For the majority of pairs in which units were distinguishable by size (CV difference >2m/s), the unit with slowest CV had the lowest recruitment threshold: 9/12 pairs in stretch reflexes, and 7/7 pairs in heel-pad reflexes. These preliminary findings demonstrate that motor units belonging to different muscles can be recruited in order by size, i.e. the size principle crosses muscle boundaries. Supported by NIH grant RO1 NS21023.

561.4

THE CUTANEOUS REGULATION OF ANKLE EXTENSOR GAMMA MOTONEURONE DISCHARGE IN THE CAT. P.H. Ellaway*, N.J. Davey, & M. Ljubisavljevic. Department of Physiology, Charing Cross and Westminster Medical School, London W6 8RF, United Kingdom.

Cutaneous afferents regulate the discharge of fusimotor neurones via oligosynaptic spinal pathways (*Davey & Ellaway 1989 J. Physiol. 411 97-114*). This work examines the effect of skin input from the heel on γ-motoneurones to muscle spindles of the gastrocnemius soleus (GS) muscles in the decerebrated, spinal (T9) cat.

We have stimulated discrete areas of skin within the sural nerve field using an electromagnetic vibrator pulsed at 10 Hz. Cross correlation and coherence analysis have been used to reveal the connectivity between sensory input and γ-motoneurones. Peristimulus time histograms (psth) showed that, although most γ-motoneurones were excited at short latency, a substantial minority were inhibited by skin stimulation. On the lateral, dorsal edge of the sural field, skin stimulation could evoke inhibition of the discharge of γ-motoneurones excited from elsewhere in the sural field. All recordings showed significant peaks of coherence (at 10 Hz) between stimuli and γ-discharge. Psth responses and coherence at 10 Hz were abolished or attenuated by section of the sural nerve. Both types of analysis revealed that excitatory and inhibitory influences had a topographic organization. The most powerful excitatory effects came from skin over the lateral aspect of the calcaneum. Inhibition had its strongest representation more lateral and dorsal to the calcaneum.

Sinusoidal stretch of GS muscles and recordings from muscle spindle afferents (*Ellaway et al 1994 J. Physiol. 479 30P*) suggested that the dominant effect of skin stimulation tends to be excitation of static γ-motoneurones which may be accompanied by either excitation or inhibition of dynamic γ-motoneurones.

We conclude that cutaneous input may have a role in the regulation of the different types of fusimotor action during movements involving stretch of the skin about the ankle joint. *Supported by the Medical Research Council, U.K.*

561.5

REGULATION OF STANCE PHASE DURATION BY FLEXOR MUSCLE AFFERENTS. G. W. Hiebert, P. J. Whelan, A. Prochazka, and K. G. Pearson*. Dept. of Physiology. Univ. of Alberta. Edmonton, Alberta, Canada, T6G 2H7.

One proposal concerning the regulation of stance-to-swing transition during walking is that swing is initiated when the leg is extended beyond a set position. We have tested the hypothesis that the stretch-sensitive afferents in flexor muscles provide one source of afferent input to terminate the stance phase in walking cats. Decerebrate cats were made to walk on a moving treadmill belt. The hip flexor iliopsoas (IP) and the ankle flexors tibialis anterior (TA) and extensor digitourm longus (EDL) were stretched, or the nerves to TA or EDL were electrically stimulated, at various phases of the step cycle. Changes in EMG amplitude, duration and timing were monitored. Stretch of flexor muscles during stance phase reduced the duration of extensor activity and resulted in the early activation of the flexor muscles. Vibration of IP and EDL muscles, or electrical stimulation of group I afferents from EDL nerve, produced a similar resetting of the step cycle indicating that group Ia afferents from flexors can terminate exensor activity. TA vibration and electrical stimulation of TA nerve at group I strengths did not reduce extensor duration. Activation of group II afferents from TA was required to produce an effect. Activation of the flexor muscle afferents during swing phase did not consistently alter the pattern of the locomotor activity.

It is proposed that when the flexor muscles are stretched as the stance phase progresses input from muscle spindle afferents acts to inhibit the center regulating extensor activity thus leading to the initiation of swing.

561.6

Ia AFFERENTS AND LOCOMOTION: RESETTING THE RECORD M.J. Angel*, P. Guertin & D.A. McCrea. Dept. Physiology, U. of Manitoba, Winnipeg, R3E 3J7, CANADA.

Stimulation of ankle extensor group I afferents during feline locomotion can either enhance ongoing extensor activity or reset the step cycle to extension. This extensor activation replaces the group I non-reciprocal reflex inhibition of synergists seen in non-locomoting preparations. Other labs have claimed that activation of Ia muscle spindle afferents is without strong effect on the locomotor pattern. This observation along with direct evidence for the involvement of Golgi tendon organ (Ib) afferents in resetting has resulted in the notion that it is mainly Ib reflexes that are reorganized during locomotion. This claim is refuted by recent data from our lab. We have shown that Achilles' tendon vibration activating only triceps surae and plantaris (TSPl) Ia afferents enhances locomotor extension as powerfully as electrical stimulation of individual ankle extensor nerves. Ia activation can also delay the onset of flexion and reset the step cycle. Intracellular recordings from extensor motoneurons show that selective activation of Ia afferents from TSPl evokes both powerful disynaptic and longer latency EPSPs in ankle, knee and hip extensor motoneurons during extension. We argue that Ia afferents also access the locomotor CPG resulting in prolongation of extension and a delay in the onset of flexion. To set the record straight, it appears that activation of Ia and Ib afferents results in similar reflex actions during fictive locomotion. These effects, perhaps via common interneurons, provide a positive length/force feedback system to aid in extension and forward propulsion during locomotion. *Supported by MRC & Rick Hansen Legacy Fund.*

561.7

ACTIONS FROM GROUP II MUSCLE AFFERENTS DURING FICTIVE LOCOMOTION ARE NOT MEDIATED THROUGH THE SHORT LATENCY FLEXION REFLEX PATHWAYS. M-C. Perreault* P. Guertin and D.A. McCrea. Dept. of Physiology, U of Manitoba, Winnipeg, R3E 3J7, Canada.

Hindlimb flexor group II afferents exert powerful effects during MLR-evoked fictive locomotion by resetting the step cycle to extension or prolonging extension (Perreault et al. 1995, J. Physiol. in press). These effects contrast with the depolarizing action of the flexor afferents onto flexor motoneurones evoked at rest and are distinct from those evoked by cutaneous and joint afferents. It was thus suggested that these group II actions were mediated through reflex pathways other than those responsible for the flexion reflex. In this study we use intracellular recording from pairs of motoneurones to assess the contribution of the different pathways activated by group II strength flexor nerve stimulation(5T, 20 shocks, 100Hz) during fictive locomotion. Preliminary results show that during flexion, such stimulation hyperpolarizes flexor MNs and frequently terminates the ongoing depolarization. This hyperpolarization occurs within 60ms and is not time-locked to each stimulus pulse. Concomitantly in extensor MNs, group II stimulation resets the step cycle and evokes IPSPs that ride on top of the LDP. The latencies of these stimulus-locked IPSPs are comparable to that of the short latency FRA IPSPs seen at rest but their amplitudes are much smaller. During extension, stimulation of Sart nerve prolongs extension evoking EPSPs in extensor MNs and small amplitude IPSPs in flexor MNs; in both cases the PSPs are evoked a latency of 4-5ms. In conclusion, it is becoming increasingly evident that during the flexion phase of the locomotor cycle, there is a clear separation of group II reflexes from those considered as part of the flexion reflex system. The reorganization of the group II pathways would then consist of a replacement of the group II FRA pathways with another set of pathways that have access to the CPG interneurones. During the extension phase, the possibility that the "alternate FRA" pathways are responsible for the effects remains open. *Supported by the MRC and the Rick Hansen Legacy Fund.*

561.8

COMBINED ACTIONS OF INTERNEURONS AND MOTONEURONS PRODUCE EXTENSION ENHANCEMENT DURING FICTIVE LOCOMOTION. D.A. McCrea*, P. Guertin, M.J. Angel and M-C. Perreault. Dept of Physiology, U of Manitoba, Canada.

Stimulation of extensor group I afferents can modulate the locomotor step cycle by increasing the duration and intensity of extensor activity throughout the ipsilateral hindlimb. While group I afferents partly exert their actions during locomotion through CPG circuitry, little is known about the possible contribution of intrinsic plateau-like potentials of motoneurons to their increased depolarization during extension enhancement. In the present study, intracellular recording during group I afferent evoked extension enhancement was used to compare the ability of group I stimulation to evoke plateau-like potentials of close synergist and other extensor motoneurons during MLR evoked fictive locomotion in cats. In close synergist motoneurons, ankle extensor group I stimulation evoked large monosynaptic and short latency excitation and often plateau-like potentials during extension enhancement. On the other hand in hip and knee extensor motoneurons, the smaller short latency depolarizations produced by ankle extensor afferents rarely evoked plateau-like potentials during extension enhancement. Thus the relative contribution of CPG evoked effects and intrinsic motoneuron properties to extension enhancement may depend upon of the amount of short-latency depolarization evoked by group I afferent stimulation in each motoneuron species. This suggests that group I afferents modulate motor output during fictive locomotion through a combination of intrinsic motoneuron properties and pre-motoneuronal pathways. Supported by the MRC and the Rick Hansen Legacy Fund.

561.9

SPATIAL ORGANIZATION OF CUTANEOUS POSTSYNAPTIC POTENTIALS IN MOTONEURONS OF ANKLE MUSCLES IN THE CAT. T.M. Hamm*, Z.-S. Han, M.L. McCurdy, and V. Turkin. Div. of Neurobiology, Barrow Neurological Inst., Phoenix, AZ 85013.

We have investigated the possibility that cutaneous stimulation of the paw might assist in the control of posture and locomotion by means of spatially organized pathways to motoneurons that innervate muscles with actions at the ankle, a group having diverse mechanical actions and patterns of use. Postsynaptic potentials were produced by brief taps and electrical stimulation applied to the surfaces of the paw and recorded in motoneurons of ankle muscles in decerebrate cats. The taps were applied to the dorsal and ventral surfaces of the paw, plantar cushion, and dorsal surface over the metatarsal-phalangeal joint with 1 cm discs attached to miniature air pistons. Forces of 2-3 N were applied in 30 ms pulses while recordings were made from motoneurons of the following pools: medial (MG) and lateral gastrocnemius (LG), tibialis anterior (TA) and extensor digitorum longus (EDL), flexor digitorum longus (FDL) and flexor hallucis longus (FHL), and peroneus longus (PL) and brevis (PB). Although variability was found in the postsynaptic responses, an overall pattern emerged for the responses to stimulation of most cutaneous sites in each of the motoneuron species. With the exception of peroneus brevis, in which excitation was the dominant pattern from all stimulation sites, the responses in each motoneuron species differed between stimulation sites. Moreover, comparison of responses to stimulation of each site in different motoneuron species revealed different patterns of postsynaptic potentials. Differences were evident in responses from stimulation of at least one site in comparison of LG and MG, PB and PL, FDL and FHL, and TA and EDL. Our results demonstrate a spatial organization of cutaneous pathways to motoneurons that differentiates between motor pools with different functions, suggesting the utility of these pathways in the control of locomotion and posture.

561.10

MODULATION BY CUTANEOUS AFFERENTS OF THE POSITIVE FEEDBACK OF MUSCLE CONTRACTION IN CAT PERONEAL MOTONEURONS J.-F. Perrier, D. Zytnicki*, B. Lamotte d'Incamps, N. Kouchtir, L. Jami. URA CNRS 1448, UFR des Saints-Pères, 75006 Paris, France.

In a previous work (1), it was established that afferent inputs generated during sustained isometric contraction of Peroneus Brevis (PB) elicit excitatory potentials in peroneal motoneurons. The aim of the present work was to investigate whether cutaneous inputs, known to occur during movements, could interfere with this positive feedback of muscle contraction. Motoneurons supplying peroneal muscles were recorded intracellularly in anesthetized cats in which a hindlimb had been denervated except for PB muscle and the skin territory supplied by Peroneus Superficialis (PS) nerve. Mechanical stimulation of the skin was elicited by ramp-and-hold indentations applied on PS territory with a servo-controlled length actuator. Complex excitatory and inhibitory responses were recorded in peroneal motoneurons mainly during the dynamic phases of the ramps. In most cases, ramps of 1-3 mm amplitudes evoked excitatory potentials in motoneurons. Inhibitory potentials and/or further excitation appeared during 4-5 mm ramps. These observations are consistent with the pattern of responses observed in peroneal motoneurons during graded electrical stimulations of PS nerve: excitatory potentials appeared for stimulations at 1.1-1.6T and inhibitory potentials appeared, in some but not all motoneurons, for stimulation above 2T.

When mechanical skin stimulation was applied during PB contraction, simple summation of effects elicited by cutaneous and muscular inputs occurred in most peroneal motoneurons. The net result was either an enhancement or a reduction of the positive contraction-feedback.

(1) Kouchtir et al. (1995). *J. Neurophysiol.* **73**, 974-982.

561.11

FACILITATION OF STARTLE BY ELECTRICAL STIMULATION OF AMYGDALA EFFERENTS: TIMING RELATIONSHIPS AND CIRCUITRY. **P. W. Frankland*, R. A. M. Brown and J. S. Yeomans. Dept. of Psychology, University of Toronto, Toronto, Canada M5S 1A1.**

Electrical stimulation of the caudal ventral amygdalofugal (VAF) pathway facilitates acoustic startle responses when the electrical and acoustic stimuli are presented close together in time (Rosen & Davis, 1990). Here we deliver a 0.1 ms pulse at currents below those required to evoke an unconditioned startle response together with stimulation of the acoustic startle pathway, either electrically (two 0.1 ms pulses) or acoustically (4 ms noise burst). Then we test which intervals between the startle-eliciting stimulus and the facilitation pulse evoke the strongest startle responses. Resulting curves were fitted with normal distributions to estimate the center and range of enhancement. For acoustic stimuli, the enhancement curves for stimulation of the VAF or lateral midbrain were centered on intervals of 3-4 ms. Stimulation of the ventral cochlear nucleus was enhanced by VAF stimulation at intervals centered on 1-2 ms. Stimulation of the medial longitudinal fasciculus in the medulla was enhanced by VAF stimulation at intervals centered on -1 to 0 ms. Therefore, 1) the transmission time from the VAF to the midbrain is less than 1 ms; 2) transmission times through the primary startle circuit are 2-3 ms from the speaker to the cochlear nucleus, and about 1 ms from the cochlear nucleus to the medulla; 3) the enhancement of startle by medulla stimulation suggests that integration can occur downstream from the RPC at the level of the spinal cord.

(Supported by NSERCC grant A7077 to J.S. Yeomans)

561.12

DOPAMINE D_2 RECEPTOR AGONISTS ALTER GAP MODULATION OF STARTLE AMPLITUDE. D. S. Leitner* and E. M. Girten. Department of Psychology, Saint Joseph's University, Philadelphia, PA 19131-1395.

Dopamine agonists have been demonstrated to disrupt the ability of innocuous sensory events (prestimuli) to reduce the amplitude of a subsequently elicited acoustic startle reflex (ASR). Pulses of pure tones or noise have typically been used as prestimuli. The present series of experiments used brief silent periods in otherwise continuous noise (gaps) to investigate how administration of D_1 and D_2 dopamine receptor agonists would affect gap's modulation of the rat's ASR amplitude.

The first and second experiments investigated the abilities of the D_2 receptor agonist (-)quinpirole hydrochloride (.1 to 1.6 mg/kg) and the D_1 receptor agonist (±)SKF-38393 (.1 to 5.0 mg/kg), respectively, to alter gap startle modulation. The third experiment investigated the effects of a single high dose of quinpirole (5.0 mg/kg) on gap startle modulation. The fourth experiment examined the ability of the dopamine receptor blocker haloperidol (.2 mg/kg) to reverse quinpirole's effects. The final experiment investigated whether simultaneous administration of quinpirole and SKF-38393 would produce a synergistic effect on gap startle modulation.

Quinpirole had a biphasic effect, with the lowest doses enhancing and the higher doses decreasing the ability of a gap to reduce startle amplitude. SKF-38393 had no reliable effect on gap detection at any dose used. At high doses quinpirole blocked gap inhibition of startle amplitude. The fourth experiment showed that haloperidol blocked the effects of quinpirole. The fifth experiment showed that ineffective doses of SKF-38393 interacted with quinpirole. These data suggest a commonality of mechanism supporting the abilities of gaps and pulses of pure tones and noise to modulate startle amplitude. All of these effects were very sensitive to the exact stimulus parameters used, suggesting differential neural processing of long and short gaps presented at long and short interstimulus intervals.

561.13

EMG RESPONSE OF PINNAE MUSCLES TO AZIMUTHAL VARIATIONS OF FREE-FIELD SOUNDS IN DECEREBRATE RATS. B. J. Frost and L. Li*. Visual and Auditory Neurosciences Lab., Psychology Dept., Queen's Univ., Kingston, Ontario, Canada, K7L 3N6

Electromyographic (EMG) recordings from the cervicoauricular muscle (CAM) group to free-field sounds were made in decerebrated and unparalyzed rats. The rat was pre-anesthetized deeply with sodium pentobarbital (60 mg/kg, i.p.). The brainstem was transected at the precollicular level. Very fine flexible EMG insulated wire electrodes were inserted into the CAM to record multi-unit activity. All the wounds were then sutured and anesthesia was discontinued. After the rat recovered from anesthesia, wide-band noise pulses (60 ms in duration with 5 ms rise and decay times) were presented and speaker positions were varied in azimuth. Sound-levels were set at 5 or 10 dB above empirically determined threshold for an EMG response to a sound from the extreme ipsilateral azimuth. Short latency transient EMGs were produced and in some cases varied in spike rate with sound source location. At least two major classes of the CAM EMG response were identified: omnidirectional and directionally sensitive. The omnidirectional CAM EMG exhibited a flat azimuth function or gradual decline only at extreme contralateral azimuths. For the directionally sensitive CAM EMG, the maximum activity was displayed at extreme ipsilateral azimuths, but as speaker was moved toward the extreme contralateral azimuth, the sound-evoked activity declined systematically. This study suggests that auditory spatial tuning curves can be measured in the pinnae motor system.

562.1

POWER DENSITY SPECTRUM OF SURFACE ELECTROMYOGRAM DURING DYNAMIC ISOMETRIC CONTRACTIONS. C. Maioli* and C. Orizio. Dip. di Scienze Biomediche, Univ. di Brescia, 25123 Italy.

It is well established that frequencies of the power density spectrum (PDS) of the surface electromyogram (SEMG) are related to the overall conduction velocity of the active motor units (MU) in the muscle, thus providing some insight on the underlying MU recruitment strategy. Accordingly, it has been reported that the high-frequency content of the PDS increases with force magnitude during static isometric contractions. By contrast, scanty information is available on the changes of the SEMG spectrum associated with dynamic variations of isometric force.

We recorded the bipolar SEMG (1 cm inter-electrode distance) from the biceps brachii of healthy males 22-40 years old. Subjects were required to match a visual target with a feedback signal proportional to the elbow flexion force. Three different time courses of the force target were used: 1) constant levels (3 s duration) from 10 to 80% of maximal voluntary contraction (MVC); 2) ramps from 0 to 80% MVC in 10 s; 3) steps from 10 to 40% MVC as a reaction-time response to an acoustic signal. Time-varying PDS was estimated by means of the application of an autoregressive model of order 20 to the SEMG signal sampled at 1024 Hz.

SEMG spectrum was found to be multi-modal with three distinct power peaks centered at about 40, 70 and 100 Hz. At constant force levels, the power ratio between the first and the second peak decreases with force magnitude, being the second peak almost absent at 10-20% MVC and as large as the first peak at 80% MVC. By contrast, during slow ramps the ratio of the first two peaks tends to be constant and close to 1 from 20-30% up to 80% MVC, in most subjects. In both motor tasks, the magnitude of the third peak was not consistently correlated with the level of force. Finally, in the dynamic phase of the force step trials the SEMG spectrum was characterized by the appearance of a sharp peak around 100 Hz. Its amplitude was usually larger than the amplitude of the third peak observed during the trials with constant or ramp force profiles.

562.2

DECOMPOSITION OF COMPOUND MUSCLE ACTION POTENTIALS (CMAP) FROM ABDUCTOR POLLICIS BREVIS MUSCLE (APB) WITH FEED-FORWARD NEURAL NETWORK. J. Fang, B. T. Shahani*, D. Graupe. Rehab. Med & Restorative Med. Sci., Univ. of Ill. at Chicago, Chicago, IL 60612

A feed-forward neural network was implemented to decompose the surface CMAP based on spatial and temporal summations of the templates of motor unit potentials. The motor unit templates for slow and fast types of motor units were collected by the low level voluntary contraction of the muscle, and near threshold stimulation of the median nerve at the wrist, respectively. The surface CMAPs from normal APB muscle corresponding to 100%, 50%, and 10% of the maximal stimulus intensities were obtained by electrical stimulation of median nerve at the wrist. The estimate of the number of motor units in the muscle was 157. Our study also showed that at 50% of maximal stimulus intensity, 30.9% of slow motor units had been recruited compared to 52.5% of fast motor units. Whereas, at 10% of maximal stimulus intensity, only 1% of slow motor units had been recruited compared to 24.2% of fast motor units. We believe that this technique is simple and non-invasive, and can be applied to electro-physiological examination of patients with neuromuscular disorders to obtain more information with regard to: 1) the number of motor units recruited; 2) the type of motor units and their distribution in the motor neuron pool; 3) the way different types of motor units are recruited; and 4) the conduction delay among individual motor units of different types.

562.3

AN EXPERIMENTAL SIMULATION METHOD FOR EVALUATION OF THE EFFECTS OF CENTRAL MOTOR UNIT ACTIVATION STRATEGIES ON EMG. M. Hulliger*, S.J. Day, W.M. Morrow and B. Kacmar. Dept. of Clinical Neurosciences, Univ. of Calgary, Alberta, Canada T2N 4N1.

The EMG is a complex interference signal resulting from the summation of trains of motor unit (MU) action potentials (MUAP). Its features are determined by peripheral (MUAP shape) and central (MU firing) parameters. However, during voluntary contractions the relative contributions of these parameters to EMG characteristics cannot be determined from EMG recordings alone. To complement and evaluate computer simulations of EMG (e.g. J. Neurophysiol. 70, 2470-88, 1993) an experimental EMG simulation technique has been developed, which is based on multi-channel independent stimulation of single MUs (SMUs) in a cat nerve-muscle preparation.

Currently up to 40-56 SMUs (master-set) of a hindlimb muscle are isolated in ventral roots. Successively, four subsets (10-14) of SMUs are stimulated independently using customized stimulator and electrode arrays and pre-edited computer-generated pulse trains (up to 56 individual patterns), with added Gaussian noise imitating physiological firing variability. Special software permits ready simulation of a range of central MU pool activation strategies, featuring e.g. varying degrees of recruitment, rate modulation and MU synchronization. Master-set EMG and force signals are obtained off-line by linearly summing raw EMG and force signals recorded during MU-subset stimulation.

Strictly simultaneous stimulation of substantially more than 10-15 MUs is unrealistic for various technical reasons. Therefore the above technique of successive stimulation of different subsets of MUs was adopted. This still permits activation of each MU with a unique pattern. Moreover, control experiments demonstrated that multi-MU EMG signals result from strictly linear summation of SMUAP trains, and that the intermediate forces (150-250 g, as generated by MU subset activation) also sum linearly.

The technique is used to evaluate EMG-force relations (Day et al., this volume) and effects of MU synchronization on EMG. It will also be applied in a study of the role of central MU control strategies on EMG characteristics during muscle fatigue. Supported by AHFMR and MRC.

562.4

THE EFFECTS OF VARIOUS ACTIVATION STRATEGIES ON THE ISOMETRIC EMG-FORCE RELATION: AN EXPERIMENTAL SIMULATION STUDY. S.J. Day, M. Hulliger, K.A. Scheepstra and R.G. Lee*. Dept. of Clinical Neurosciences, Univ. of Calgary, Alberta, Canada T2N 4N1.

While different motor unit (MU) pool activation strategies have been identified for various human muscles (J. Physiol. 308, 103-14, 1980), the relative effects of recruitment (Rc) and rate modulation (RM) on the EMG-force relation during asynchronous MU pool activation remain controversial. For single MUs force saturates with increasing rate of activation, whereas EMG magnitude increases linearly; in contrast, during increasing asynchronous stimulation of the MU pool, the EMG signal also demonstrates saturation, largely due to increasing signal cancellation (Soc. Neurosci. Abstr. 19, 405.03, 1993). Consequently, as motor drive increases, the relative degrees of saturation of EMG and force will determine the shape of the EMG-force relationship. In the present study a wide range of MU pool activation strategies were explored using a cat nerve-muscle preparation.

The L7 and S1 ventral roots were divided into filaments containing either 3-30 soleus MUs (multi-MU simulations) or a single MU. In either case, 10 filaments were activated independently and asynchronously at fixed length. Various 10 channel constellations of stimulation patterns imitated different central activation strategies, including pure Rc, pure rate RM and intermediate designs with varying contributions from each. For single-MU simulations raw EMG and force signals were summed for four 10 channel sets to simulate activation of a substantial proportion of the MU pool (Hulliger et al., this volume).

For both multi-MU and single-MU simulations pure Rc produced greater force increases than EMG (gain compression), while pure RM caused significantly larger increases in EMG than force (gain expansion). Combined activation strategies gave intermediate, mostly similar EMG-force relations, suggesting that for likely physiological MU activation strategies EMG-force characteristics are much less variable. Individual 10 channel constellations of single-MUs revealed substantially less EMG saturation than multiple 10 channel constellations, indicating that cancellation-related EMG saturation can be very pronounced, when sufficient numbers of MUs are activated at physiological rates. Supported by AHFMR and MRC.

562.5

SELECTIVE RECRUITMENT WITHIN TRICEPS SURAE MUSCLES OF THE DECEREBRATE CAT ALTERS KNEE TORQUE TRAJECTORIES. S.J. Bonasera*, J.H. Lawrence III†, C.M.J.I. Price, and T.R. Nichols. Department of Physiology, Emory University, Atlanta, GA 30322, and † Center for Biomedical Engineering, University of Kentucky, Lexington, KY 40506.

Differential activity of the two major heads of feline gastrocnemius (G) generate distinct ankle torque trajectories differing in the relative proportion of abduction to plantarflexion torques (Nichols et al., *Exp. Brain Res.* 97:366, 1993). These data suggested that the CNS both represented and exploited the complex biomechanical insertion of lateral gastrocnemius (LG) and medial gastrocnemius (MG) onto calcaneus to produce functionally different ankle movements.

The muscle origins of MG and LG at the knee also suggest that differential activation of G may evoke different knee torques. MG attaches at the femoral medial condyle, and thus may produce knee flexion and adduction; conversely, LG attaches at the femoral lateral condyle, and thus may produce knee flexion and abduction. To test this hypothesis, we used a three-dimensional force transducer to calculate knee joint torques in unanesthetized decerebrate cats in response to peripheral nerve stimulation evoking crossed-extension (XER) or caudal cutaneous sural nerve (SNR) reflexes. Both SNR and XER evoked strong flexion at the knee. In agreement with biomechanical predictions, torque trajectories evoked by SNR consistently adducted the knee, while torque trajectories evoked by XER consistently abducted the knee. This study further emphasizes the unique role of multi-articular muscles in the specification of whole limb mechanical properties, and demonstrates that functional roles of multi-articular muscles must be evaluated in the context of their mechanical actions at each spanned joint. (Supported by NIH Grant NS20855).

562.6

AGE AND PRACTICE EFFECTS ON MOTOR UNIT DISCHARGE RATE VARIABILITY C. Patten, J.R. Burke*, G. Kamen, D. Rowland. Motor Control Laboratory, Department of Physical Therapy, Boston University, Boston, MA 02215.

One possible explanation for the observation that older adults demonstrate greater motor performance variability than younger individuals lies in the underlying variability in motor unit discharge rates. The present study was conducted in an effort to measure discharge rate variability in young and older adults. Six young (21-30 yrs) and six older (65-82 yrs) subjects were studied on each of two days while performing 50% MVC and 100% MVC isometric contractions using fifth digit abduction. During the 50% MVC task subjects performed a ramp contraction for 15 seconds. Motor unit recordings were obtained from the abductor digiti minimi muscle using a four-wire needle electrode. Spike recognition software was used to obtain individual motor unit discharge occurrences. The results demonstrated that measures of discharge rate variability, including standard deviation (SD) and coefficient of variation (CV), were similar for both groups. For the 50% MVC condition maximal discharge rate standard deviation was 0.040 for the young subjects and 0.045 for the older adults ($p>.05$), and CV's demonstrated a similar effect (Y=2.12, O=2.16; $p>.05$). SD's were greater on day one than day two (0.070 day one vs. 0.015 day two; $p<.01$), and these results were mirrored by the results for CV (3.96 day one vs. 0.40 day two; $p<.01$). There were no statistically significant group x day interactions ($p>.05$). These results indicate that: 1) discharge rate variability appears to be similar in young and older adults, and 2) repeated practice reduces the variability of motor unit discharge rates.

Supported by AG 09662 and The Foundation for Physical Therapy Research

562.7

MOTOR UNIT SYNCHRONIZATION AND FORCE TREMOR ARE REDUCED IN SKILL- COMPARED TO STRENGTH-TRAINED SUBJECTS. J.G. Semmler and M.A. Nordstrom*. Department of Physiology, University of Adelaide, Adelaide 5005, Australia.

We have recently shown in right-handed subjects that motor unit (MU) synchronization in first dorsal interosseous (FDI) muscle is weaker in the dominant (skilled) hand than the non-dominant hand (Semmler & Nordstrom, Exp. Brain Res. in press, 1995). MU synchrony reveals information about corticomotoneuron (CM cell) activity and the distribution of their direct projections to motoneurons (Datta *et al.*, J. Physiol. 432:401-425, 1991). Our results suggest that preferential use of the hand may influence these features of cortical control of FDI. To assess the importance of muscle usage patterns on MU synchrony, we have studied 5 skill-trained (ST) musicians, 5 strength-trained (WT) weightlifters and 6 untrained (UT) subjects. Mean MU synchrony during index finger abduction (expressed as frequency of extra synchronous discharges above chance) was significantly lower in ST (0.22 ± 0.02 s^{-1}, 162 MU pairs) than UT subjects (0.32 ± 0.02 s^{-1}, 199 pairs, $P<0.05$) and WT subjects (0.44 ± 0.03 s^{-1}, 183 pairs, $P<0.01$). Differences in MU synchrony for UT and WT subjects were also significant ($P<0.01$). Duration of synchronous peaks was similar for each group. Force tremor was quantified during isometric abduction of FDI at 0.5 N and 3.5 N. Tremor RMS amplitude was significantly lower ($P<0.01$) in ST *vs.* WT subjects. However, there were no significant correlations between mean MU synchrony in a muscle and tremor amplitude (*cf.* Semmler & Nordstrom, Exp. Brain Res. in press, 1995). Therefore, differences in MU synchronization are not responsible for the differences in force tremor between groups. Weaker FDI MU synchrony in ST subjects may reflect a more restricted distribution of direct projections from CM cells to FDI motoneurons, or a reduced excitability of CM cells during the task. Our data suggest that skill- and strength-training are associated with opposite adaptations in these features of the cortical control of FDI.

562.8

INHIBITION OF THE LONG-LATENCY STRETCH REFLEX DURING RAPID STEP-TRACKING WRIST MOVEMENTS IN HUMANS AND MONKEYS. J. W. Mink*, R.J. Bateman, and W.T. Thach. Depts of Neurology and Anatomy & Neurobiology, and The McDonnell Center for Higher Brain Function, Washington Univ. Med. School. St. Louis, MO 63110.

The long latency stretch reflex (LLSR) involves a multi-synaptic, transcortical mechanism that is active to maintain posture against external perturbations. Previous studies have shown that the LLSR can be gated in response to instruction. We have previously hypothesized that posture holding mechanisms are inhibited by the basal ganglia inhibited during voluntary movement so as not to interfere with the desired movement (Mink and Thach, 1991). This study sought to determine if the LLSR in the wrist flexor muscles is inhibited during wrist movement.

Six healthy human volunteers and two rhesus monkeys performed a visually guided hold-step-hold wrist movement task. They performed the task by placing their hand in a wedge-shaped manipulandum that was controlled by flexing or extending the wrist in the horizontal plane. The task was performed with constant torque loads opposing or assisting the wrist movement. EMG was recorded from surface electrodes overlying the wrist flexor and extensor muscles. To elicit the LLSR, torque pulses (50 - 100 msec) or steps (1 sec) of several different magnitudes were superimposed on the static load in order to stretch the wrist flexor muscles.

In the maintained hold prior to movement, the LLSR occurred with a mean latency of 50 msec in humans and 26 msec in monkeys. The magnitude of the LLSR correlated with the magnitude of the preload and with the velocity of the imposed stretch. During rapid wrist movement, the LLSR was suppressed by 10 - 30% when the movement was made by contracting the loaded flexors and by 50- 80% when the movement was made by relaxing the loaded flexors. These results show that the LLSR is active during maintained wrist posture and is inhibited during voluntary wrist movement. (Supported by NIH R01-NS12777 and 5 T32 NS09027 19 and The McDonnell Center).

562.9

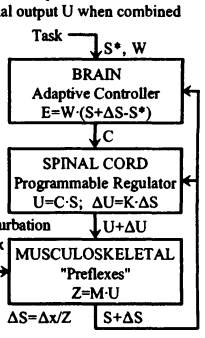
"PREFLEXES" - PROGRAMMABLE, HIGH-GAIN, ZERO-DELAY INTRINSIC RESPONSES OF PERTURBED MUSCULOSKELETAL SYSTEMS. I.E. Brown, S.H. Scott* and G.E. Loeb, Dept. Physiology, Queen's Univ., Kingston, ON K7L 3N6.

Motor physiologists have often viewed the ensemble of muscles and bones in a limb as undesirably complex and redundant for the performance of simple tasks such as reaching and walking. These complexities and redundancies can, however, be used by the nervous system to achieve much better performance in the face of external and internal perturbations than could be achieved by reflex control alone in mechanically simple systems. We hypothesize that the nervous system uses the "redundant" aspects of limb posture and muscle activation patterns to create a general mechanical impedance to perturbations, as originally proposed by Hogan (1984, IEEE AC-29:681). This generalized impedance typically includes "stiffness" terms related to the force-length relationships for muscle and connective tissues, "viscosity" terms related to the force-velocity relationships, and inertial terms related to skeletal posture. Using a new set of experimental data and equations that more broadly and accurately describe the mechanical properties of muscles and tendons, we have simulated the behavior of various model systems of muscles and skeletal linkages in response to typical perturbations. The results suggest that the nervous system could use these intrinsic properties (particularly the interaction between the force-velocity relationship and the series compliance of various muscle architectures) to achieve a broad and useful range of programmable responses to perturbations, in agreement with recent experimental evidence that it actually does so (Gottlieb, 1994, Exp. Br. Res. 97:545). In addition to high effective gain, these responses occur at zero time delay, hence the suggested term "preflexes." Thus, preflexes are particularly effective at reducing the instability inherent in relying exclusively on neurally mediated reflexes with high gains..

562.10

REALISTIC NEURAL CONTROL FOR REAL MUSCULOSKELETAL TASKS. G.E. Loeb*, Bio-Medical Engineering Unit, Queen's Univ., Kingston, ON K7L 3N6.

All motor tasks can be divided into a nominal trajectory (input motor signals U and output sensory signals S) and a set of perturbation trajectories (ΔU and ΔS) that would occur in response to a set of perturbations (Δx; e.g. external loads or internal computational noise) that the subject expects to encounter. The motor program controls $\Delta S(t)$ through two separate mechanisms: "preflexes" (see adjacent poster) that arise from the mechanical impedance (Z) presented by the skeletal posture and intrinsic properties of active muscles (M), and reflexes that arise from the spinal regulator gain (K) that generates ΔU in response to ΔS. The job of the descending control system is to find a set of commands (C) to the spinal interneurons that simultaneously produces both K and the desired nominal output U when combined with nominal sensory feedback S. The brain presumably uses adaptive control (e.g. neural networks) to learn to produce C according to performance criteria associated with a given task and based on responses to the set of perturbations that have been experienced previously. This learning requires computation of an error signal (E) from the weighted (W) summed differences between the sensory signals received ($S + \Delta S$) and the desired trajectory (S^*).



This schematization divides motor control into three very different but complementary functions and incorporates concisely and explicitly important aspects of the control problem (e.g. perturbations, impedance and performance criteria) that are often omitted or implicit.

562.11

DECORRELATION WITHIN AND CORRELATION BETWEEN ANTAGONIST MOTOR NUCLEI BY RECURRENT INHIBITION. M. G. Maltenfort*, C. J. Heckman, R. E. Druzinsky and W. Z. Rymer, Dept. of Physiology, Northwestern Univ. Med. Sch., Chicago, IL 60611

A functionally realistic computer model of motor pools with recurrent inhibition and force output was constructed. Synaptic connectivity was matched to physiology. Agonist-antagonist pairs were linked by inhibition between the two Renshaw cell (RC) populations. Stochastic inputs were used to simultaneously control stiffness and position.

Within a motoneuron pool, correlations between single units and population firing were both positive and negative along a range centered at zero. RC feedback narrows this range ($p < 0.01$), so that individual motoneurones are more likely to be uncorrelated. This decorrelation abolishes discrete peaks in the power spectra of force at the mean rate of motoneuron firing (15-35 Hz, depending on pool activity).

Simulations of the linked pools demonstrate that mutual inhibition between antagonist RCs can correlate unfused and partially fused motor unit twitches across the two pools. Coherence between pools is 0.4 -0.5 at the frequencies of motoneuron firing. Coherence of position vs. its control signal declined the same amount at the same frequencies, as synchronous units contracting with opposite actions canceled.

The net effect of both phenomena is to abolish 'microtremor' at the joint which could adversely interact with stretch receptors.

562.12

PRESYNAPTIC INHIBITION AND POSTSYNAPTIC GAIN RECRUITMENT MECHANISM DURING AN ISOMETRIC ANKLE JOINT RAMP CONTRACTION IN MAN - A SIMULATION STUDY. T. Sinkjaer* and R.J. H. Wilmink, Center for Sensory-Motor Interaction (SMI), Dept. of Medical Informatics and Image Analysis, Aalborg University, Fredrik Bajersvej 7D, DK-9220 Aalborg, DENMARK.

In this study we are applying a model which can describe some of the neural mechanisms underlying the facilitation of the H-reflex as it is observed during dynamic muscle contractions. Healthy subjects were asked to make a voluntarily isometric ramp-and-hold plantar-flexion around the ankle joint with a maximum force level of 15Nm, while getting visual force feedback. The ramp lasted for 0.5s followed by a 1s hold. During this we elicited H-reflexes with a 100ms interval before and during the active movement. The experiments showed an increase in the size of the H-reflex prior to the onset of the ramp-contraction and a slow decrease towards the end of the hold-contraction. The motoneuron model used to simulate the experimental observations was based on a dynamic pool with 200 alpha motoneurons. Each motoneuron had cell properties like excitability, firing rate dynamics and pre- and post-synaptic interactions. The output from the pool was translated into a soleus EMG signal from which the H-reflex was simulated. The pool itself was modelled as a continuum with a distributed excitability from the smallest (slowest) unit to the largest (fastest) unit (Slot, P., Sinkjær, T. Biological Cybernetics. 70:351-358, 1994). We simulated the experimental results using either a presynaptic inhibition or a postsynaptic gain recruitment mechanism. Both mechanisms were able to model the experimental outcome.

562.13

DO CONTRALATERAL INFLUENCES ONTO SOLEUS H REFLEXES REQUIRE COMPLEX SPINAL MECHANISMS? J.E. Misiaszek*, J. Cheng, J.D. Brooke and W.R. Staines. Human Biology Dept., University of Guelph, Guelph, ON, Canada, N1G 2W1.

Passive movements of a single leg phasically modulate soleus H reflexes of that leg. Reflexes of the opposite leg are also attenuated, however, phasing to the movement has not yet been demonstrated. Presently, H reflexes of the stationary leg were sampled from twelve subjects while the other leg was A) passively cycled at 20 and 40 rpm and B) stationary. Eight points across the cycle were tested. H reflex gain displayed a rate dependent reduction across the cycle ($p<0.01$). No phasing was detected . We hypothesized the lack of phasing could be attributed to sensory drive originating in biarticular muscles. Passive movements of the hip or knee were isolated in eight subjects to eliminate biphasic stretching of these muscles. Reflexes were sampled for four positions in the cycle (0, 25, 50 and 75% of the cycle) at 20 and 40 rpm and while stationary. Reflex gain in the leg contralateral to the movement was reduced across the cycle during hip movement at 40 rpm ($p<0.05$). Phasing was not evident. Similar results arose from knee movements. The ipsilateral influences of isolated joint movements displayed both a strong rate ($p<0.05$) and phase ($p<0.05$) effect. Phasic afferent discharging has been invoked to explain the ipsilateral influences, however, the results suggest a more complex system is required for the crossed effects. Supported by NSERC (Canada).

562.14

MOTOR UNIT ACTIVITY PATTERNS IN AN AMPLITUDE-SCALED ISOTONIC TASK IN THE THE PIGEON. R. Panteón and P. Zeigler.* Hunter College (CUNY), New York, NY 10021.

Eating and drinking in pigeons are stereotyped behaviors, differing primarily in jaw movement topography. Eating is an episodic response in which the jaw exhibits gape amplitudes scaled to the size of the food object. Drinking is rhythmic, with a relatively constant jaw opening amplitude. Both are produced by the same mechanical arrangement of bones and muscles, and common motoneuron pools. To examine motor control mechanisms of scaling and pattern generation, conditioning procedures were used to bring jaw-movements under experimental control in a fixed-head preparation. Displacements of the upper and lower beaks were monitored and correlated with EMG activity from lower jaw muscles. Jaw muscle activity was sampled at 10khz to isolate individual motor units and a spike train analysis carried out. Relations between variations in movement amplitude and motor unit properties (threshold, frequency) were examined to assess the manner in which these properties are modulated during amplitude scaling. The results suggest both the operation of the "size principle" and the existence of individual motor units reflecting different functional task groups. (Supported by NSF Grant BNS-88-10722, NIMH Grant MH-08366 and Research Scientist Award MH-00320 to H. P. Z.)

562.15

ACTIVITY OF SPINAL INTERNEURONS DURING "FICTIVE" BEHAVIORS IN THE FROG SPINAL CORD-HINDLIMB PREPARATION. Piron, L. and Schotland, J.* Dept Brain and Cog. Sci., M.I.T., Cambridge, MA 02139.

Spinal interneuronal networks have been implicated in the coordination of reflex behaviors and equilibrium postures (Bizzi et al. 1991). We sought to investigate the contribution of a wide range of spinal interneurons to the set of reflex behaviors that corresponds to microstimulation-induced equilibrium postures (Giszter et al. 1993) by using the *in vitro* frog spinal cord-hindlimb preparation.

The frog spinal cord and hindlimb were placed in separate chambers, connected only by the spinal nerves. We implanted electrode wires in four muscles that discriminate among the reflexes. We recorded interneuronal activity extracellularly using 10-15 MOhm NaCl-filled pipettes. Interneurons were identified by their response to stimulation of descending pathways via a suction electrode positioned on the lateral white matter at the rostral end of the hemisected spinal cord, and by their lack of antidromic response to stimulation of the main nerve trunk to the hindlimb. Reflex behaviors, including wipe, flexion withdrawal, and extension, were elicited by touching or pinching the skin of the hindlimb, which was fixed by pins at the hip.

Interneurons were recorded at depths ranging from 5-730 um from ten animals. As reported by Berkowitz and Stein (1994) in the turtle spinal cord, twelve interneurons were active during more than one different reflex behavior. In contrast to the turtle, however, interneurons in our paradigm exhibited a much broader range of activity patterns. Six interneurons were selectively activated during particular movement sequences. Eleven were activated during muscle contraction, even though no movement occurred. In some cases the activity closely matched the period of muscle contraction, while in others it could be considerably shorter, or could outlast the period of muscle activity. The activity of four interneurons could not be related to any obvious features of the experimental paradigm and six interneurons did not participate in the generation of any reflex behaviors.

MUSCLE: BIOMECHANICS

563.1

BIOMECHANICAL AND MUSCULAR LINKS BETWEEN THE SCAPULA AND HEAD-NECK SYSTEM. T. Liinamaa, R.J. Runciman and F.J.R. Richmond*. MRC Group in Sensory-Motor Physiology, Queen's University, Kingston, ON, Canada, K7L 3N6

Most studies of head movement in cats view the head and cervical column as if it were a self-contained motor system. However, biomechanical analyses suggest an important role for the scapula, both as a site at which forelimb loading forces are transmitted to the axial skeleton, and as an attachment for neck muscles. In the sitting cat, ground reaction forces transmitted through the forelimbs exert a parasagittally-directed torque on the scapula that tends to rotate its dorsal margin caudally. This force is presumably counterbalanced by the tonic contractions of muscles running rostrally to the head and neck. Electromyographic analyses of shoulder muscles are consistent with this premise. The rostralmost heads of rhomboideus that attach onto the skull (occipitoscapularis) and cervical vertebrae (rhomboideus minor) are active tonically in quietly sitting cats. These muscle regions are also richer in slow fibers than other parts of rhomboideus or of adjacent levator scapulae, which is active during horizontal turns. Results suggest that the biomechanical and neural mechanisms responsible for maintaining stable head posture may not be characterized completely unless consideration is given to the role of the scapula and forelimbs (and perhaps also the trunk) which convey ground-reaction forces to the head-neck complex. (Supported by MRC of Canada)

563.2

UNUSUAL MUSCLE SARCOMERE LENGTHENING AND SHORTENING WITH ELBOW JOINT FLEXION

R.L. Lieber* and J. Fridén Depts. of Orthopaedics and Bioengineering, UCSD, La Jolla, CA 92093 and Hand Surgery Unit, Göteborg University, Sweden.

Sarcomere length (SL) was measured in extensor carpi radialis brevis (ECRB) muscles during elbow flexion in 13 individuals undergoing surgical lengthening of the muscle for lateral epicondylitis (tennis elbow). As an adjunct to our previous study of the wrist (*J. Neurophysiol.* **71**: 874-881, 1994), we measured sarcomere length changes during elbow joint flexion at 30° intervals. Unexpectedly, sarcomere length demonstrated a biphasic response to continuous elbow flexion from 3.49±0.11 μm in the extended position increasing to 3.68±0.09 μm, then decreasing to 3.34±0.09 μm, then increasing to 3.81±0.12 μm and then decreasing again to 3.45±0.16μm. Such a response would be expected to have profound mechanical consequences with elbow flexion since the muscle would go through two active lengthening cycles which are known to cause muscle injury and high stresses. This unusual sarcomere behavior may underly some of the ECRB pathology as in tennis elbow. Anatomical dissections of cadaveric specimens failed to provide an obvious underlying structural basis for the observed results. Our provisional hypothesis is that the ECRB origin which overlies the lateral epicondyle is able to "flip" back and forth during elbow rotation, thus crossing the elbow axis of rotation.

This work was supported by NIH grant AR35192, the Department of Veterans Affairs and the Swedish Work Foundation.

563.3

IS MUSCLE A GOOD MECHANICAL DAMPER? D.C. Lin* and W.Z. Rymer, Depts of Biomedical Eng. and Physiology, Northwestern Univ., Chicago, IL 60611.

Force-velocity properties of muscle are often cited as having beneficial damping characteristics. Yet these properties are usually determined with steady-state activation and under unphysiological loading conditions. The purpose of this study is to determine how much damping is actually done by a muscle in a situation where damping is needed: specifically, we chose to simulate the action of the antagonist muscle in a rapid single-joint movement during which a brief burst of activation is used to generate the decelerating force acting on a load.

Experimental data were collected from the cat soleus muscle in an anesthetized preparation. Inertial loads were simulated using force feedback to a length servo. The protocol was to provide the load with an initial velocity to stretch the muscle and then apply an electrical stimulus for a brief period of time (100 ms). Electrical stimulation was applied at the ventral roots, making it possible to grade muscle force output. Muscle length and force were recorded. Both the initial velocity and magnitude of the inertial load were varied.

The most general method to quantify damping is to consider it as an energy dissipating process. Thus the loss of kinetic energy from the mass due to the opposing force generated by the muscle was computed. The data showed that the amount of energy dissipated by the muscle increased with both increasing inertia and initial velocity. However this result may have just been a consequence of the kinematics associated with varying the inertial load. In order to investigate this possibility, mechanical models of different linear actuators were tested under the identical loading conditions as the experiment. It was determined that only a minimal amount of viscoelasticity was necessary to replicate the energy dissipation characteristics of muscle. This result indicates muscle under these conditions acts similar to a force generator with limited mechanical properties rather than a passive mechanical element.

Robustness to variations in inertial load, which is improved by damping properties, was determined by examining the resulting kinematics (final velocity and maximum length excursion) at the end of the muscle activation period. The robustness of the muscle was comparable to an actuator with only modest damping properties, in agreement with the previous result.

This work supported by NS-28076, NS-19331 (WZR), and T32-HD07418 (DCL).

563.4

NONLINEAR SUMMATION OF FORCE IN CAT SOLEUS DURING DYNAMIC CHANGES IN STIMULUS RATE OR MUSCLE LENGTH T.G. Sandercock* and C.J. Heckman. Department of Physiology, Northwestern Univ. Sch. of Med., Chicago, IL 60611

Forces from different motor units within a muscle are often assumed to sum linearly. Yet, even in muscles with the simplest architecture, the fibers exert their force through tendons and aponeuroses that are crosslinked and therefore partially shared. This mechanical interaction may result in substantial nonlinear summation. The purpose of this study was to measure nonlinear summation in cat soleus during dynamic contractions most likely to produce nonlinear summation via shared series elastic elements. In 5 anaesthetized cats the soleus was attached to a servomechanism to control muscle length and record force. The ventral roots were divided into two bundles so each innervated approximately half of the soleus. Nonlinear summation was measured by subtracting the force when each part was stimulated alone from the force when both parts were stimulated together. Two experiments were performed: 1) During continuous stimulation of one part of muscle the stimulation of the other part was either abruptly started or terminated; and 2) During stimulation of one or both parts, the muscle was moved with quick ramp stretches or releases. Both experiments resulted in small but measurable nonlinear summation (less than 5% of maximum tetanic tension). To test the hypotheses that stretch of the common series elastic elements can account for the nonlinear summation, tendon stretch during stimulation of both parts of the muscle was estimated. The servomechanism was then programmed to mimic that tendon stretch during stimulation of a single part of the muscle. Most (70%) of the nonlinear summation could be accounted for by tendon stretch.

563.5

THE EFFECT OF VARIABLE FREQUENCY TRAIN VERSUS CONSTANT FREQUENCY TRAIN STIMULATION ON THE HUMAN PARALYZED SOLEUS MUSCLE. Y.J. Chang, R.K. Shields.* Physical Therapy Graduate Program, University of Iowa, Iowa City, IA 52242.

Muscle force generation depends not only on the frequency of stimulation but also on the pattern of stimulation (Stein and Parmiggiani 1979 Brain Res). Under fatigued conditions, doublets (6 ms ipi's) have been shown to enhance torque in paralyzed muscle (Shields and Shields 1994 Soc Neuro Abstr). Less certain are whether the enhanced torques are sustained using a variable frequency train and whether the "potentiated state of the muscle" influences this response. We compared the effect of a variable frequency train (VFT) to a constant frequency train (CFT) before, immediately after, and every 5 minutes for 20 minutes after a Burke-like fatigue protocol. Ten chronically paralyzed legs (> 1 year) had the soleus muscle activated via the tibial nerve with either 6 CFT trains (20 Hz, 10 pulses) or 6 VFT trains (1st ipi = 6 ms, train = 50 ms, 12 pulses). All CFT or VFT trains were separated by 1 s. Next, the soleus muscle was fatigued by activating the tibial nerve with a 20 Hz train every s for .333 s for two minutes. Following fatigue the same VFT or CFT protocol was repeated. The VFT or CFT was selected at random initially then all subjects returned 7 days later to receive the alternate protocol (CFT/VFT). The maximum torque (MT), mean torque (MNT), and rate of torque development (RTD) was normalized to a pre-fatigue 15 Hz contraction so that the CFT and VFT could be compared at each time period tested. The VFT caused a significant increase in RTD before and at all time periods after fatigue as compared to the CFT. The VFT MNT was not different than the CFT before fatigue but was significantly greater at all time periods after fatigue. The VFT MT was also not significantly different from the CFT before fatigue, but was significantly greater after fatigue. Although the magnitude of the difference was reduced when the muscle was further potentiated, all measurements (PT, MNT, RTD) for the VFT remained significantly greater than the CFT. Mechanisms contributing to these findings will be discussed.

563.6

EFFECTS OF MODE OF STIMULATION OF PARALYZED HUMAN MUSCLE ON FORCE AND FATIGUE CHARACTERISTICS. Y.M. Kots, S.J. Harkema, N.L. Banks, M.N. Bieschke, E. Eldred* B.H. Dobkin, V.R. Edgerton. Departments of Neurology and Physiological Sciences, University of California, Los Angeles, 90095.

The mode and level of current needed to stimulate human muscle is related to an array of factors such as safety, skin irritability, maximum evoked force, comfort level, muscle fatigue, as well as initiation of reflexes and co-activation of non-stimulated muscles. To address these issues a tendometer technique was developed to measure the isometric force generated by the tibialis anterior in response to sinusoidal currents at carrier frequencies (CF) of 1000, 2500 and 5000 Hz, with and without a 100-Hz full amplitude modulation, in 4 spinal cord injured (SCI) subjects and 4 control subjects. Current amplitude to muscle force ratios (A:F) were computed from the muscle's response to 3 sec stimulation elicited every 30 sec while increasing the current amplitude until a force plateau or the subject's tolerance level was reached. At a modulated CF of 2500 Hz the maximum force (MF) was similar to the MF produced with a 100-Hz rectangular pulse stimulation. At the lower CF, the A:F decreased and the MF increased. Modulation of the CF also increased the MF and decreased the A:F. This effect of modulation was most prominent at 2500 and 5000 Hz. Subjects perceived the modulation stimulation as more comfortable than the unmodulated one at each of the CF's. Muscle fatigue during 15 evoked contractions (4 or 5 sec duration, every 12 or 15 sec) was higher in SCI subjects than control subjects. In SCI subjects, the fatigue was lower at the higher CF's. As well, for equal CF's, the unmodulated CF produced a lower level of fatigue than the modulated CF. These data demonstrate that the identification of optimal modes of electrical muscle stimulation for subject's comfort as well as for determining maximum force and resistance to fatigue of a specific muscle group is feasible. (Supported by NIH grants NS 16333 and NRSA HD 07416).

563.7

FORCE-FREQUENCY AND FATIGUE PROPERTIES IN HUMAN LONG FINGER-FLEXOR MOTOR UNITS. A.J. Fuglevand*, V.G. Macefield, and B. Bigland-Ritchie. John B. Pierce Laboratory, 290 Congress Ave., New Haven, CT 06519.

Adaptation in motoneuron firing rate during fatigue may serve to optimize force output as the mechanical function of the muscle units change (Bigland-Ritchie et al. *J. Physiol.* 340: 335, 1983). The generality of this hypothesis rests in part on a fundamental question: how do the mechanical properties of different motor-unit types change during various fatigue tasks? To address this question, intraneural stimulation was used to elicit twitch and force-frequency responses before and after intermittent stimulation with a standard fatigue protocol (40 Hz train for 330 ms, 1 train per s, 2 min) in single motor units (n = 13) of human long finger-flexor muscles. Before fatigue, two distinct groups (Group 1, n = 8; Group 2, n = 5) of units could be identified based on the stimulus frequency needed for half-maximum force; Group 1 required (mean ± SD) 9.5 ± 0.7 Hz while Group 2 required 15.5 ± 1.1 Hz. Twitch contraction times between the two groups were not statistically different (Group 1 = 56.4 ± 15.5 ms; Group 2 = 46.4 ± 5.1 ms). For all units tested, one was fatigue-sensitive (fatigue index, FI < 0.25), 3 were fatigue-resistant (FI > 0.75) and the remainder were of intermediate fatiguability (0.25 ≤ FI ≤ 0.75). The fatigue index of Group 1 was not significantly different than that of Group 2 (0.62 ± 0.26 vs. 0.455 ± 0.27). After intermittent stimulation, the force-frequency curves in the five most fatiguable units (FI < 0.5) shifted rightward, exhibiting low-frequency fatigue which persisted after 10 min of recovery. These results suggest that force loss in fatigue-susceptible units would be exacerbated if firing rate declined during this type of task. Supported by USPHS NS 14576 & HL 30062.

563.8

CAT LATERAL RECTUS MOTOR UNITS EXHIBIT SUBMAXIMAL POWER IN RESPONSE TO A PULSE-STEP STIMULATION PARADIGM. K.E. Wilson, M.S. Shall, T. Ramos and S.J. Goldberg*. Depts. of Anat. and Phys. Ther., P.O. Box 980709, Med. Coll. Va.-VCU, Richmond, VA 23298-709.

Motor unit physiologists typically examine contractile characteristics such as tetanic tension and fusion frequency in response to motoneuron (MN) activation with a range of briefly applied (200 ms) monotonic stimulation frequencies. In this study we also used a 2 sec MN stimulation protocol which included a high frequency pulse lasting 25 ms followed by a constant frequency. The first 10 ms of stimulation (4 pulses) was at 350 Hz followed by 15 ms (3 pulses) at 250 Hz followed by 1975 ms at a constant frequency ranging from 40-200 Hz. This pulse-step stimulation paradigm mimics MN firing patterns observed during "real" saccades and fixation. The resultant range of tensions produced during the steady state phase of the pulse-step stimulation paradigm was compared to tensions evoked monotonically at similar frequencies.

We observed 63 twitch plus 6 nontwitch units. Both contraction speed and fatigue data were collected on 40 twitch units so they could be type classified as FF, FR, SF, and S (n=20, n=4, n=13, and n=3 respectively). All classes of twitch, as well as nontwitch, motor units demonstrated weaker maximum tetanic tensions in response to pulse-step stimulation as compared to monotonic stimulation at similar frequencies. And these tension differences were generally greater in FF motor units.

A wide range (~50 to ~200 Hz) of monotonic stimulation frequencies evoked linear tension increments in all units. But a much narrower range (~50 to ~120 Hz) of frequencies evoked linear tension increments following the pulse phase of our 2 sec stimulation paradigm. Indeed, no unit demonstrated a maximum tension greater than that attained during the pulse phase of stimulation. The pulse phase, then, created a tension "ceiling" that the units never exceeded. This tension was usually less than the maximum tension the units could reach during the strictly monotonic stimulation paradigm.

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563.9

CONTRACTILE PROPERTIES OF THE MG MUSCLE AND ITS TWO REGIONS IN THE ADULT RAT OF DIFFERENT AGES. S. Vanden Noven*, K.L. Seburn, and P.F. Gardiner. Sch. of Physical & Occup. Therapy, McGill Univ. (H3G 1Y5), & Sciences de l'Activite Physique, Univ. de Montreal, Montreal, QC.

To determine if the two regions (i.e., an oxidative/'deep' region and a low-oxidative/'superficial' region) of the medial gastrocnemius (MG) muscle with differing contractile properties are maintained in aging, contractile properties were compared in the Sprague-Dawley (Harlan) rat at three age periods: 2-3 mo (n=3), 6-7 mo (n=7) and 24-25 mo (50% survival age: n=4). These two regions have been shown to be supported, in part, by the two extramuscular nerve branches (NBr: proximal or distal; Vanden Noven et al., 1994). Isometric contractile properties were measured in situ, first with stimulation of the whole MG nerve followed by stimulation of either NBr. Tetanic force output, normalized to the ratio of muscle weight/body weight, was compared between the 3 groups. MG in animals at the 50% survival age showed the characteristics of 'aged' muscle: decreased mass, decreased force output and slowed contraction times. Furthermore, the region innervated by the proximal NBr of the MG nerve was more fatigue resistant than that innervated by the distal NBr. The differential contractile properties of the two muscle regions in the adult, identified by the extramuscular NBr innervation, are maintained or enhanced in animals at the 50% survival age (Funded: NSERC Canada).

563.10

CONTRACTILE PROPERTIES OF SKELETAL MUSCLE IN SHAKER RATS. J.S. Fisher, M. Brown, B.R. Clark, S.D. Minor* Program in Physical Therapy, Washington Univ. School of Med., St. Louis, MO 63108.

Gait deficits in the Shaker (SH) rat, a Sprague-Dawley mutant strain with progressive loss of cerebellar Purkinje cells, suggest possible muscle weakness. Accordingly, contractile characteristics of Shaker soleus (SOL), plantaris (PLA), peroneus longus (PER), and extensor digitorum longus (EDL) were compared to muscles of control Sprague-Dawley rats.

Muscle weight to body weight ratios for control and SH were not different, indicating that atrophy was not present. Peak tetanic tension (P_o), when expressed as the ratio of P_o to muscle mass, was comparable for controls and Shakers for all 4 muscles studied. Observed gait deficits, therefore, are not a result of distal muscle weakness. The possibility of hip and or trunk weakness cannot be discounted, however.

Time to peak twitch tension (TTP) was significantly faster in the Shaker SOL, PLA, and PER compared to controls. The decline in P_o in response to a 5 min fatigue protocol was greater (30 vs. 14%, $p<.05$) in SH SOL, but there was no difference in fatigue for the EDL. TTP and fatigue characteristics suggest a shift of muscle fiber type distribution from slow toward fast. Histological examination of SH soleus did not reveal a greater proportion of fast fibers than in controls. The faster SH TTP may be related to altered expression of fast and slow myosin or related to altered properties of the sarcoplasmic reticulum, characteristics that are undetectable with ATPase staining. The findings observed could be explained by altered neural drive secondary to abnormal cerebellar output. (Funding provided by NIH-AG00585 and the Program in Physical Therapy).

563.11

MECHANICAL PROPERTIES OF PHYSIOLOGICALLY RECRUITED TRICEPS SURAE MUSCLES OF THE CAT. Clotilde M. J. I. Price*, Timothy C. Cope and T. Richard Nichols. Dept. of Physiology, Emory University, Atlanta, GA 30329, USA.

Mechanical responses of active muscles result from the intrinsic properties of the muscle and the effects of proprioceptive feedback. This interaction is poorly understood for natural movements and especially for muscles of heterogeneous fiber type composition during natural recruitment. Following self-reinnervation of the triceps surae muscles, those muscles lack functional proprioceptive feedback (Cope et al., *J. Neurophysiol.* 71:817-820, 1994) but recover normal motor innervation. To evaluate the intrinsic mechanical properties of naturally recruited muscles and the contributions of component fiber types, velocity of stretch and contractile history, we studied the responses to lengthening of reinnervated whole muscles and type-identified single muscle fibers. When stretch was preceded by an isometric contraction, as in the case of a postural perturbation, the responses of whole muscles varied systematically with force in a manner consistent with the fiber type composition of motor units active at the onset of the stretch. However, at higher forces the short range stiffness did not appear to be velocity dependent as was expected from the single fiber data. When we preceded the stretch by a shortening, as occurs just before the stance phase of locomotion, stiffness was reduced and was no longer amplitude dependent. These results illustrate that the specialized dynamic properties of different fiber types are expressed at the corresponding force levels and that these properties adapt appropriately to the ongoing behavioral task. (Supported by NS 20855)

563.12

PRELIMINARY STUDIES OF MOTOR UNITS IN THE SUPERFICIAL MASSETER MUSCLE IN RATS. R. E. Druzinsky* and L.E. Wineski. Dept. of Physiology, Northwestern Univ., Chicago, IL 60611, and Dept. of Anatomy, Morehouse Sch. Med., Atlanta, GA 30310.

Little is known about the mechanical properties of individual motor units (MUs) of jaw adductor muscles. We have begun a program to study MUs in the superficial masseter muscle in rats, using neurophysiological, enzyme histochemical, and immunocytochemical methods. Preliminary data suggest that the superficial masseter is composed largely of type IIB fibers, with smaller populations of types I, IIA, and others which we presume to be IIX/IID. All fibers exhibit positive NADH reactions, suggesting that fatigue resistance is high, even in IIB MUs. Unlike the rabbit masseter, there are no positive cardiac alpha fibers in the rat SM.

We have developed a new experimental protocol which allows stable penetrations of axons of the trigeminal nerve in rats. The rat is mounted in a stereotaxic frame and a large craniotomy is made on one side of the skull. The tendon of origin of the superficial masseter muscle is freed from the zygomatic arch and clamped in series with a load cell, and the mandible is rigidly fixed using a clamp that grasps the mandibular body. Glass micropipette elecrodes filled with a solution of 3M KCl (12 - 20 MΩ) are driven into the nerve as it runs along the floor of the cranial cavity, while passing small current pulses (4-6 nA) through the electrode. Axons are identified as superficial masseter motor axons by the presence of visible contractions of the superficial masseter, by all or none twitch forces recorded at the tendon, and by unitary action potentials recorded from EMG electrodes inserted into the muscle.

Stable penetrations have been maintained for as long as 45 minutes. Preliminary studies have shown that twitch tensions of motor units with peak tensions as small as 159mg (1.6 mN) can easily be isolated in superfical masseter muscles that can generate 20 grams of twitch force. To our knowledge these are the first measurements of single MU forces measured directly from the tendon of a whole jaw adductor muscle.

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563.13

CHRONIC LEAD TREATMENT ALTERS STRUCTURE AND FUNCTION OF MOUSE FLEXOR MUSCLE. M.A. Fahim*, A.H. Al Dhaheri[1] and F. El-Sabban. Faculty of Medicine, U.A.E. University and Ministry of Health[1], P.O. Box 17666, Al Ain, United Arab Emirates.

The effect of chronic exposure to divalent cations on skeletal muscle was investigated. Two treatment levels of lead acetate (low 0.1 and high 1 mg/kg) were made by subcutaneous injections for 7 days. Comparative analyses of *in situ* muscle isometric contractile characteristics were studied in urethane-anesthetized (2 mg/g, i.p.) control and lead-exposed male mice. Control muscle twitch tension reached 1.81±0.2 g. Chronic lead treatments did not affect muscle contractile speed, but significantly reduced the twitch tension in both high and low doses. This effect was in a dose-dependent manner; 1.21± 0.07g for low dose and 0.9± 0.05g for high dose. Chronic lead treatments accelerated muscle fatigue after 200 stimuli in both doses equally. However, marked elevation in tetanic (25Hz) specific tension were observed in the high-dose treated animals, indicating changes in contractile apparatus function. The high dose of lead treatment induced ultrastructural changes in the intramuscular axons and neuromuscular junctions. The observed morphological changes could alter physiological responses attained; however, such a level of exposure to lead would be required to manifest these changes. These results suggest that lead exposure at a low concentration can compromise *in situ* skeletal muscle isometric contraction.

564.1

RECURRENT EXCITATORY CONNECTIONS IN CA1 MINI-SLICES MEDIATE CARBACHOL INDUCED OSCILLATIONS A. M. Borroni* & W.B Levy University of Virginia Health Sciences Center, Dept. of Neurosurgery Charlottesville, VA 22908.

A prominent feature of hippocampal activity is a 4-10 Hz oscillation that requires activation of muscarinic receptors. This oscillation is referred to as rhythmic slow activity (RSA), or theta rhythm, and is generally associated with exploratory behavior but can be seen under other circumstances (Reviewed in Bland & Colom, '93). The activation of muscarinic receptors in vitro induces oscillations of similar frequency to those seen in vivo. Such experiments (Konopacki et al, '87;'92) demonstrate that entorhinal and septal inputs are not necessary for the generation of oscillations in the hippocampus. Here we report a carbachol induced oscillation (2-3 Hz) in disinhibited CA1 mini-slices. These oscillations have a similar laminar profile to theta rhythms recorded in vivo and are atropine sensitive. Oscillations were inhibited by CNQX but not by APV or by high concentrations of bicuculline (> 80μM). In addition, baclofen and higher concentrations of carbachol, manipulations that decrease excitatory transmission through a presynaptic mechanism, also suppressed oscillatory activity. Cell firing recorded in stratum pyramidale (SP) was associated with the negative phase of the oscillation recorded in SP. These results suggest that carbachol induced oscillations in CA1 mini-slices arise from rhythmic activation of excitatory currents at the soma and/or proximal basilar dendrites. These currents are most likely due to glutamatergic connections between CA1 cells that are suppressable by carbachol and baclofen. In vivo, it is likely that this intrinsic CA1 system interacts with inputs from CA3 and the entorhinal cortex to produce oscillations of higher frequency. Supported by NIH NS 15488, MH00622 to WBL and NIH 5-T32-NS07199 to AMB.

564.2

COMPARISON OF INTRINSIC PROPERTIES IN DIFFERENT SUBTYPES OF CA1 INTERNEURONS USING WHOLE CELL RECORDINGS IN RAT HIPPOCAMPAL SLICES. J.-C. Lacaille*, F. Morin, and C. Beaulieu. Center for Research in Neurological Sciences and Departments of Physiology and Pathology, University of Montreal, Montréal, Canada, H3C 3J7.

Physiological classes of local circuit cells have been identified in the hippocampus of adult animals based on their distinctive intrinsic membrane properties. The aim of the present study was to compare the intrinsic properties of morphologically identified CA1 pyramidal cells (PC; n=9) and interneurons (INT; n=15) located in (1) str. oriens/alveus (O/A), (2) near str. pyramidale (PYR), and (3) at the str. radiatum and lacunosum-moleculare border (L-M), in hippocampal slices of young rats (14-22 days) using whole cell current-clamp recordings. Patch electrodes were filled with (in mM) 140 K-gluconate, 5 NaCl, 2 $MgCl_2$, 10 HEPES, 0.5 EGTA, 2 ATP-tris, 0.4 GTP-tris, and biocytin (0.1%). Significant differences were found between properties of INT and PC, but not between INT subtypes. Action potentials were smaller in amplitude (82 mV) and shorter in duration (1.9 ms) in INT than PC (95 mV, 3.0 ms, respectively). R_{in} was greater (236.1 MΩ) and τ_m faster (28.9 ms) in INT than PC (135.6 MΩ, 46.0 ms, respectively). Afterhyperpolarizations (AHPs) were biphasic but fast and medium duration components were larger in amplitude in INT (-13.2 and -5.4 mV, respectively) than in PC (-0.1 and 1.1 mV, respectively). Depolarizing current pulses evoked regular firing in all INT and burst firing in 50% of PC. During hyperpolarizing current pulses, responses in all cell types displayed inward rectification, followed by anodal break excitation. Thus, significant differences were observed with whole cell recordings between membrane properties of INT and PC. However, these membrane characteristics were similar across all INT subtypes. The maturation of distinctive membrane properties of interneurons in L-M may therefore not yet be completed in young animals.

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564.3

EXCITATORY AND INHIBITORY POSTSYNAPTIC CURRENTS EVOKED IN CA1 INTERNEURON SUBTYPES IN RAT HIPPOCAMPAL SLICES. F. Morin*, C. Beaulieu and J.-C. Lacaille. Center for Research in Neurological Sciences and Depts. of Physiology and Pathology, Univ. of Montreal, Montréal, Canada, H3C 3J7.

Excitatory and inhibitory synaptic mechanisms in hippocampal interneurons are mediated by glutamate and GABA. The aim of the present study was to characterize pharmacologically isolated non-NMDA, NMDA, and $GABA_A$ postsynaptic currents evoked in morphologically identified pyramidal cells (PC; n=16) and subtypes of interneurons (INT; n=47) in (1) str. oriens near the alveus (O/A), (2) near str. pyramidale (PYR), and (3) at the str. radiatum and lacunosum-moleculare border (L-M), using voltage clamp recordings in hippocampal slices of young rats. Patch electrodes were filled with (in mM) 120 CH_3O_3SCs, 20 QX-314, 8 NaCl, 1 $MgCl_2$, 10 HEPES, 1 EGTA, 2 ATP-tris, 0.4 GTP-tris, and biocytin (0.1%). Non-NMDA EPSCs were isolated in AP5 and bicuculline (BIC) and antagonized by CNQX. They were similar in all cell types, had linear I-V relations and reversed near 5 mV. Rise time and decay time constant of non-NMDA EPSCs were slower at depolarized membrane potentials. NMDA EPSCs, isolated in CNQX and BIC and antagonized by AP5, were generally similar in all cell types, except for a slower rise time in INT of O/A. NMDA EPSCs had a region of negative slope between -80 and -20 mV and reversed near 7 mV. They displayed faster rise time and slower decay time constant at depolarized potentials. $GABA_A$ IPSCs, isolated in AP5 and CNQX and antagonized by BIC, were similar in all cell types, with linear I-V relations and E_{rev} of -32 mV. Rise time of $GABA_A$ IPSCs was not voltage sensitive but decay time constant was slower at depolarized potentials. In conclusion, morphologically distinct classes of interneurons and pyramidal cells display similar non-NMDA and $GABA_A$ postsynaptic currents, however the kinetics of their NMDA currents may differ, being slower in interneurons of O/A.

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564.4

UNITARY IPSCs EVOKED IN CA1 PYRAMIDAL CELLS BY ACTIVATION OF SINGLE INTERNEURONS IN RAT HIPPOCAMPAL SLICES. M. Ouardouz* and J.-C. Lacaille, Centre de Recherche en Sciences Neurologiques et Département de Physiologie, Université de Montréal, Montréal (Qc), Canada, H3C 3J7.

Although GABAergic synaptic mechanisms are well understood in the hippocampus, little is known about the inhibition produced by individual interneurons. The aim of the present study was to characterize unitary IPSCs (uIPSCs) elicited in CA1 pyramidal cells by stimulation of single interneurons of different subtypes located in stratum (str.) oriens (OR-uIPSCs), near str. pyramidale (PYR-uIPSCs) and near str. lacunosum-moleculare (LM-uIPSCs). Specific cell types were visually identified in hippocampal slices of young rats (14-21 days) and whole cell voltage clamp recordings were obtained from pyramidal cells with K-gluconate or CH_3O_3SCs containing patch electrodes. Individual interneurons were stimulated (0.5-7 μA, 1ms) with a loose cell-attached patch electrode containing ACSF, in AP-5 and CNQX. At +20 mV, uIPSCs fluctuated in amplitude. Averaged LM-uIPSCs had significantly ($p<0.05$) smaller amplitude (mean ± sem: 39.9 ± 6.9 pA, n=14), slower rise time (12.0 ± 1.8 ms) and slower decay time constant (69.7 ± 11.8 ms) than OR-uIPSCs (76.3 ± 15.5 pA, 4.1 ± 0.8 ms, 54.4 ± 7.0 ms, respectively; n=14) and PYR-uIPSCs (108.6 ± 17.0 pA, 4.1 ± 0.8 ms, 43.4 ± 3.4 ms, respectively; n=16). Mean E_{rev} of averaged IPSCs was -53.1 ± 2.1 mV with K-gluconate (n=9) and -58.5 ± 2.6 mV with CH_3O_3SCs (n=13). uIPSCs were reversibly blocked by 25μM bicuculline to 2.8 ± 2.1% of control amplitude (n=19: 5 LM, 6 OR and 8 PYR). uIPSCs from all cell types were enhanced by the $GABA_B$ receptor antagonist CGP55845A (5 μM) to 141.6 ± 12.8 % of control amplitude (n = 10). In 9 cells examined, averaged uIPSCs did not show paired-pulse inhibition at intervals of 100 ms. These results suggest that specific subtypes of interneurons may generate Cl^--mediated $GABA_A$ IPSCs, which have different kinetics. These unitary IPSCs may be tonically modulated by $GABA_B$ receptors but may not show paired-pulse inhibition.

(Supported by MRC, FRSQ, FCAR, and Savoy Foundation)

564.5

IMAGING OF Ca^{2+} RESPONSES ELICITED BY GLUTAMATE IN CA1 INTERNEURONS OF RAT HIPPOCAMPAL SLICES. L. Carmant*, M. Ouardouz, R. Robitaille, J.-C. Lacaille. Center for Research in Neurological Sciences and Depts.of Physiology and Pediatrics, University of Montreal, Montreal (Qc), Canada H3C 3J7.

Glutamate excitation of hippocampal interneurons is mediated by ionotropic and metabotropic receptors heterogeneously expressed in different subtypes of interneurons. The aim of this study was to characterize Ca^{2+} responses evoked by glutamate in different populations of interneurons in rat hippocampal slices (P14-17). Interneurons were loaded with the fluorescent Ca^{2+} indicator fluo 3-AM by incubating slices for 60 minutes at 35°C in ACSF saturated with O_2 / CO_2 containing 10μM fluo 3-AM and 0.02% pluronic acid. Changes in fluorescence were observed using a BioRad 600 confocal microscope and expressed as percentage change from resting level. Local application of glutamate (500μM) evoked responses in 75% of cells in str. oriens (OR; n=21/28) and in 83% in str. radiatum/lacunosum-moleculare (LM; n=15/18). Three types of Ca^{2+} responses were observed. First, a transient Ca^{2+} rise of 59.1±7.7% was present in 76% of OR cells and 53% of LM cells. In 7 cells, the onset of this response was 12.0±4.8s and the duration was 24.7±3.6 s. Second, an oscillatory Ca^{2+} response was seen 24% of OR interneurons. It consisted of 3-4 peaks with an initial Ca^{2+} rise of 107.5±26.6% (onset 8.5±2.6s; total duration 174±31s; n=3). Third, a prolonged response was found in 47% of LM interneurons that consisted of an initial Ca^{2+} peak of 118.3±19.3% followed by a prolonged secondary peak (onset 26.1±13.9s; total duration 218±32s; n=2). Preliminary results show that CNQX and AP-5 reversibly blocked Ca^{2+} responses in 4 of 11 OR cells but responses were still present in 3 cells. Responses were reversibly blocked in 2 of 5 LM cells and no responses were seen in the others. In conclusion, Ca^{2+} responses in hippocampal interneurons may involve different mechanisms mediated by ionotropic and metabotropic glutamate receptors. These mechanisms may be selectively expressed in specific subtypes of interneurons. [Supported by the MRC, FRSQ, FCAR, Sloan Foundation and Savoy Foundation]

564.6

CHARACTERIZATION OF MOSSY FIBER-EVOKED RESPONSES IN THE CA3 SUBFIELD OF THE HIPPOCAMPUS, *in vivo* M.F. Yeckel* and T.W. Berger, *Dept. of Biomedical Engineering, and Program in Neuroscience, University of Southern California, Los Angeles, CA 90089-1451.*

The fundamental characteristics of mossy fiber innervation of CA3 pyramidal cells has been a topic of considerable debate. To date, most investigations have used sharp electrode and whole-cell recording techniques, and have focused on identifying biophysical properties of mossy fiber activation. Despite numerous studies examining changes in presumed mossy fiber-evoked field responses, a comprehensive characterization of mossy-evoked CA3 field responses has yet to be performed. Several properties of this pathway make characterization of mossy fiber-evoked responses in CA3 difficult: i) the projection is spatially restricted, and requires precise placement of the stimulating electrode, ii) the proximity of the somal region to synaptic innervation results in a considerable overlap between synaptically generated current sinks and current sinks generated by cellular discharge, and iii) due to convergence of several synaptic inputs, isolation of mossy fiber-evoked responses has proven to be problematic. In this study, we present a series of experiments in which we address these issues and characterize mossy fiber-evoked responses in the CA3c subfield, *in vivo*.

Stimulating electrodes were placed into the hilus of the dentate gyrus of halothane-anesthetized rabbits. Criteria used to identify mossy fiber-evoked monosynaptic responses of CA3 pyramidal cells, include: 1) short-latency, orthodromic activation of unitary discharges in s. pyramidale of CA3, while antidromically exciting the dentate, 2) current-source density analyses (CSD) identifying a short-latency current sink in s. lucidum of CA3 and, 3) the induction of NMDA-independent LTP. Relevant to the debate on mossy fiber excitation, CSD data show a narrow range along CA3 pyramidal dendrites (~100 μm) where field responses can accurately be interpretated as monosynaptically-evoked pop. EPSPs. In general, contamination by both CA3 pop. spikes, and disynaptic excitation of CA3 via recurrent collaterals, severly limits the evaluation of CA3 field responses. Supported by ONR, BMSR, NIH, Human Fron. Org., and NIMH.

564.7

USE OF A VOLTERRA FUNCTIONAL POWER SERIES TO CHARACTERIZE NONLINEARITIES IN RESPONSES OF NEURAL SYSTEMS TO PULSE TRAIN STIMULATION.

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Volterra (1930) developed a general functional power series (FPS) representation for dynamic nonlinear systems such that the kernels of the series have a simple and useful interpretation. For neural systems stimulated with a train of pulses, the Volterra series characterizes the dynamic nonlinearities of the system in terms of the synergistic affects of multiple pulses on the system response. The first order kernel specifies the response to a single stimulus pulse, the second order kernel specifies the change in the response due to the synergistic affects of pairs of pulses, etc. Thus, the Volterra series is a useful nonlinear system characterization tool and a useful predictive model.

In this work, we investigate the crosscorrelation technique for estimating the full waveform kernels of the Volterra series for neural systems. The procedure uses response data measured while stimulating the system with a Poisson random sequence of pulses. Krausz (1975) developed an orthogonal FPS model for this Poisson test input and showed how to estimate the kernels of the series using output-input crosscorrelations. We show that these kernels are different from the Volterra kernels in that they contain components involving any higher order nonlinearity in the system. However, the difference between the two sets of kernels only becomes significant if the system is stimulated at a high stimulus rate and the area under the higher order kernels is large. Thus, crosscorrelation is a useful method for full waveform kernel estimation. We describe practical techniques for estimating the Volterra kernels using crosscorrelation and we demonstrate these techniques using simulated data (for which the actual kernels are known) and experimental data from the rabbit hippocampus.

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564.8

ANALOG VLSI IMPLEMENTATION OF A NONLINEAR MODEL OF HIPPOCAMPUS.

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We have demonstrated that a nonlinear systems analytic approach can be used to develop an experimentally-based model in the form of the kernels of a functional power series of the dynamics of single neurons in the hippocampus. The kernels include the contribution of all possible sources of nonlinearities amd represent the cell's response to the present input in the context of a "sliding window" of the input history. We used the kernel models of dentate granule cells and inhibitory interneurons to implement a hardware representation of the dentate gyrus. A unique hybrid analog/digital design was used which includes the following: i) the input is a series of impulses that can be arbitrarily specified, with a sample of the recent history of input events stored in a set of shift registers; ii) the kernel functions are represented in analog, using capacitors charged to different voltage levels depending on the kernel value: the first order kernel, $h_1(\tau)$, is stored as a single value; the second order kernel, $h_2(\tau,\Delta)$, is stored as an array of values; the third order kernel, $h_3(\tau,\Delta_1,\Delta_2)$, is stored as a matrix of values; iii) the charges on the capacitors are "refreshed" periodically from an off-chip source; the design maintains total independence between the refresh functions and the computation functions; iv) with each input event, an output current is generated from the $h_1(\tau)$ memory cell; additional currents are generated by the subset of $h_2(\tau,\Delta)$ and $h_3(\tau,\Delta_1,\Delta_2)$ values addressed according to the pattern of previous input events stored in the shift registers; v) summation of the currents from the $h_1(\tau)$, $h_2(\tau,\Delta)$ and $h_3(\tau,\Delta_1,\Delta_2)$ sets of memory cells determines the "neuron" output. The above design has been incorporated into a 3x3 architecture for a locally interconnected network of 9 neurons. A prototype has been fabricated through MOSIS using 2 μm technology. Supported by the ONR, NIH, and NIMH.

564.9

HIPPOCAMPAL EVOKED POTENTIALS FROM CONTRALATERAL SCHAEFFER'S COLLATERAL STIMULATION SHOW DISCRETE ACTIVITY PATTERN IMAGES.

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We assessed images of reflected light from the surface of the cat dorsal hippocampus together with evoked electrical potentials to determine the spatial organization of neural activation during crossed Schaeffer's collateral stimulation. Cats were anesthetized with sodium pentobarbital, eight stimulating electrodes were inserted in the right hippocampus, and an optic probe coupled to a CCD video camera was lowered to the surface of the contralateral dorsal hippocampus. The camera detected spatial patterns of reflected light intensity through the optic probe. A bipolar electrode, attached to the anterior side of the optic probe, recorded hippocampal commissural potentials evoked by contralateral stimulation.

Evoked electrical potentials and reflected light images were digitized simultaneously, and stored continuously during 20 to 100 single biphasic pulse stimuli at 0.5ms duration, 0.1Hz, 200μA current. Stimuli were directed to each of the eight stimulating electrodes in random order. Optical and electrical responses from stimulation were averaged across trials. Stimuli elicited a complex population synaptic potential lasting 500msec. Image sequences revealed discrete regions of increased and decreased activation, which depended on the position of the stimulating electrode. Activation of the dorsal hippocampal surface shows a distinct topographical organization of contralateral Schaeffer's collateral projections.

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564.10

COMPARISON OF GRANULE CELL IPSPs PRODUCED BY ORTHODROMIC AND ANTIDROMIC STIMULATION OF THE DENTATE GYRUS.

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The local circuitry of the dentate gyrus contains multiple inhibitory pathways, including complex feedforward and feedback systems which terminate on granule cells, the output cells of the dentate gyrus. The two inhibitory systems may exert different effects on the activity of granule cells. We have begun a comparison of the effects of feedforward and feedback inhibition on granule cells and in our initial studies have characterized the IPSPs produced by orthodromic and antidromic stimulation of granule cells.

Simultaneous intracellular and extracellular recordings were made from granule cells in transverse hippocampal slices from rabbits. Both feedforward and feedback systems were activated by suprathreshold s. moleculare stimulation. The feedback system, including pathways activated by area CA3 neurons, was activated by suprathreshold stimulation of s. lucidum of area CA3c. Stimulation of either pathway evoked biphasic IPSPs that were blocked by the $GABA_A$ antagonist bicuculline (early IPSP) and $GABA_B$ antagonist saclofen (late IPSP). Orthodromically-elicited IPSPs were always larger than antidromically-elicited IPSPs, indicating that simultaneous activation of both feedforward and feedback systems from perforant path stimulation results in greater inhibition of granule cells than activation of feedback systems alone from s. lucidum stimulation. In future studies we will dissect the components of the feedforward and feedback pathways and use nonlinear systems analysis techniques to evaluate the behavior of the pathways at different frequencies of stimulation. Supported by an NDSEG Fellowship to JSF; grants from ONR, NIMH, NIH, and Biomedical Simulations Resource to TWB; and NINDS 30831 to HES.

564.11

ELECTROPHYSIOLOGICAL CHARACTERISTICS OF MORPHOLOGICALLY-IDENTIFIED PYRAMIDAL "BASKET" CELLS OF RAT DENTATE GYRUS RECORDED IN HIPPOCAMPAL SLICES.

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Pyramidal-shaped cells located at the base of the granule cell layer are one of the most common types of inhibitory interneurons in the dentate gyrus. They are often assumed to be basket cells and play a critical role in inhibition of granule cells. However, little is known about their physiology. Therefore, membrane properties and synaptic responses were recorded from these cells, which were identified as pyramidal by intracellular injection of Neurobiotin (n=15). The morphology of these cells revealed that they are not necessarily basket cells, because a basket-like plexus was rare and axon collaterals in other layers were common. Antidromic action potentials could often be evoked by weak stimulation of the hilus or molecular layers. Membrane properties were similar to fast-spiking cells (putative interneurons) that have been described elsewhere. For example, input resistance was high (mean 95.4 megohms), time constant was short (mean 7.4 msec) and there was a large afterhyperpolarization following single action potentials. However, action potential duration was usually longer (mean 1.1 msec total duration) and spike frequency adaptation was usually stronger than classic interneurons. Responses to molecular layer stimulation evoked EPSPs primarily; IPSPs were absent or not robust. EPSPs could be surprisingly small (less than 2 mV amplitude, 30 msec duration at threshold) in light of the large dendritic tree in the molecular layer. Even when EPSPs were large, suprathreshold stimuli rarely evoked more than one action potential at resting potential. This may be one of the reasons why they are relatively resistant to prolonged perforant path stimulation. Supported by NINDS grant 30831.

564.12

OLFACTORY BULB, PIRIFORM AND LATERAL ENTORHINAL PROJECTIONS TO THE HIPPOCAMPUS: A CURRENT SOURCE DENSITY STUDY.

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Current source density was used to evaluate inputs to the hippocampus provided by the olfactory bulb, piriform cortex, lateral entorhinal cortex and lateral perforant path (LPP) in the urethane anesthetized rat. Field potentials were recorded at 50 μm depth intervals in tracks through CA1 and dentate gyrus (DG) of the dorsal hippocampus. Cathodal stimulation of the olfactory bulb, piriform cortex, lateral entorhinal cortex and LPP resulted in a current sink at the outer molecular layer (OML) of the dorsal blade of the DG with an onset latency of 20, 13, 7 and 4 ms respectively. Stimulation of the LPP but not olfactory bulb, piriform cortex or lateral entorhinal cortex resulted in a larger and earlier sink at the dorsal than the ventral blade of the DG. Paired pulse facilitation (PPF) (50 ms interpulse interval) at the DG was facilitated by more than 100% following olfactory bulb and piriform stimulation but only approximately 5% following LPP stimulation. LPP also resulted in a short latency (<6 ms) CA1 stratum lacunosum moleculare (SLM) sink, likely provided by excitatory currents at the distal apical dendrites of CA1. The SLM sink is independent of the OML sink because, 1) the SLM sink is later than the DG sink and 2) it has large PPF (~50%) while the OML sink has small PPF (~5%). In conclusion, it is postulated that the olfactory bulb and piriform projections to the DG OML were mediated through the lateral entorhinal cortex generating an excitatory synaptic current sink at the distal dendrites of DG granule cells (OML), and most of the PPF of inputs to the DG occurs at or prior to the entorhinal cortex. Physiological inputs from the olfactory bulb and piriform cortex probably result in equal and simultaneous involvement of the dorsal and ventral blades of the DG, even though LPP stimulation resulted in larger and earlier dorsal than ventral activation. Supported by Human Frontier Science Program.

564.13

CHARACTERIZATION OF HIPPOCAMPAL INTERNEURONS INHIBITED BY OPIOID PEPTIDES

J. Brunton, L. Bernheim* and S. Charpak. Dept of Physiology, University Medical center, 1211 Geneva 4, Switzerland.

In the hippocampus, opioid peptides induce a disinhibition of pyramidal cells that results from a reduction in firing of unidentified inhibitory interneurons as well as from a reduction of GABA release directly at the level of the terminal. Since interneurons form a heterogeneous population, we combined the whole cell recording technique with biocytin injections to characterize the CA3 interneurons sensitive to opioids in brain slices of young rats. Of 50 cells located in the strata lucidum and radiatum, 18 were inhibited by DAMGO (2.5 μM), 2 by DPDPE (2.5 μM) and none by U50469 (2.5 μM), respective agonists for μ, δ and κ-opioid receptors. The response to DAMGO was antagonized by CTOP and persisted in the presence of tetrodotoxin. It proved difficult to classify DAMGO sensitive cells : 8 were localized in the stratum lucidum and 7 in the stratum radiatum; most had a fusiform shape with an axis either parallel or perpendicular to the pyramidal layer; all cells presented an I_H current with variable kinetics, 2 cells presented a prominent I_A current; finally 2 cells exhibited subthreshold oscillations underlying clusters of 30-40 Hz activity. Our preliminary results suggest that opioid peptides depress the activity of a very diverse population of CA3 interneurones.

564.14

CALCIUM CURRENTS IN sAO INTERNEURONS OF THE RAT HIPPOCAMPUS. G.C.Faas* and W.J.Wadman. Inst. for Neurobiology, Univ. of Amsterdam, 1098 SM Amsterdam, The Netherlands.

Interneurons were located in stratum Alveus / Oriens (sAO) of 225 μm thick rat hippocampal slices using infrared microscopy. The surface of the slice was cleaned and the in-situ patch-clamp technique was used to gain voltage control over the cell. Sodium and potassium currents were blocked with the appropriate intra- and extracellular solutions. In this way isolated calcium currents were recorded as has been described before. A transient low voltage activated (LVA) calcium current was evoked by depolarization from -130 mV to -50 mV (threshold > -70 mV). Depolarization from -65 mV to -10 mV evoked a sustained high voltage activated (HVA) component, which was larger and more transient when evoked from -130 mV.

The pipette solution contained Lucifer Yellow and Biocitin which allowed a morphological identification of the patched neurons after the electrophysiological measurements. More than 18 sAO interneurons were investigated. Of this group 6 had a clear stellate morphology and 11 a polar morphology. All neurons contained the HVA calcium currents with a mean amplitude of 134 pA, but a large variation from neuron to neuron. The non-stellate cells contained a LVA current (135 pA) that was significantly larger than the LVA current in the stellate cells (38 pA).

The sAO interneurons are strategicaly placed in the local hippocampal network to participate in recurrent inhibition. Their excitability will in part be determined by on the types and location of calcium currents presence

564.15

OPEN LOOP CIRCUIT ANALYSIS OF RAT ENTORHINAL CORTEX USING CURRENT SOURCE DENSITY MEASUREMENTS OF EVOKED POTENTIALS. K. E. Ahrens* and W. J. Freeman, Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

The limbic system receives olfactory input via the lateral olfactory tract (LOT) at synapses on the apical dendrites of layer II and III pyramidal cells in the entorhinal cortex (EC). The primary input to the hippocampus, the perforant path (PP), originates in the superficial layers of the EC, as well. In order to map out the functional circuitry by which olfactory information is processed in the EC, we recorded simultaneous field potentials in anesthetized Sprague Dawley rats using a 16 channel silicon microelectrode. Using one dimensional current source density (CSD) analysis on the evoked potentials resulting from electrical stimulation of the LOT and the PP, we have observed the distribution of current sinks and sources corresponding to synaptic currents and passive return currents as a function of depth in the EC. By blocking polysynaptic activation within the entorhinal cortex with deep pentobarbital sodium anesthesia, we see only the current sources and sinks due to monosynaptic inputs. Furthermore, the elimination of recurrent excitation and inhibition yields the "open loop" state, which is useful for modeling the dynamics of olfactory-entorhinal interactions.

564.16

ACTION OF CENTRAL OXYTOCIN IN THE BED NUCLEI OF THE STRIA TERMINALIS (BST) IN THE PRE-PARTUM PERIOD AND AFTER STEROID TREATMENT. S.J. Housham, M.G. Terenzi and C.D. Ingram*. Neuroendocrine Research Group, Department of Anatomy, University of Bristol, Bristol BS8 1TD, UK.

During lactation i.c.v. administration of oxytocin (OT) has a facilitatory effect on the bursting of hypothalamic OT neurones which is mediated via OT-sensitive neurones in the BST (Ingram *et al*, J. Neuroendocr. 1995: 7, 1-13). There is an increase in both the facilitatory efficacy of OT (Hughes *et al*, Neuroendocr. Lett. 1993: 15, 363-367) and the density of OT receptors in the BST (Insel, J. Neuroendocr. 1990: 2, 539-545) during the peri-partum period. This study examined the changing responses of BST neurones to central OT in the pre-partum period and their possible steroid regulation.

Studies were performed on Wistar rats on days 19 or 22 of gestation. Animals were anaesthetised (urethane 1.25 g/kg i.p.), cannulated for measurement of intramammary pressure responses to increasing doses OT i.v. (0.2, 0.5, 1.1, 2.2 ng) and prepared for electrophysiological recording. Mammary glands of day 22 animals were more sensitive to OT i.v. than those of day 19 (response to 0.5 ng: 6/6 vs. 1/6) consistent with receptor up-regulation. Responses of BST neurones in day 22 animals were also larger both in the proportion of responsive neurones (42 vs. 33%) and magnitude (mean peak 8.4±1.1 vs. 6.5±1.6 sp/s) but were characterised by a significantly delayed onset (mean onset 14.0±0.5 min). To investigate steroidal regulation, a second group of animals underwent hysterectomy and bilateral ovariectomy on day 19 of pregnancy followed by 3 daily injections of either 10 μg oestradiol (E_2) or 5 mg progesterone (P). Mammary glands of all E_2-treated animals responded 2.2 ng OT but only 60% P-treated did so, although those which did respond showed no difference in sensitivity. BST cells from E_2-treated animals showed a higher baseline firing (1.9 Hz) than P-treated animals (0.8 Hz) and a higher proportion of neurones responding to 1.1 ng OT i.c.v. (55% for E_2 and 25% for P), consistent with increased OT receptor expression. These data demonstrate dynamic pre-partum changes in responses of limbic neurones to i.c.v. OT and suggest the importance of the changing steroid environment for the regulation of OT receptors.

564.17

SIMULTANEOUS RECORDING OF MULTIPLE CELLS IN THE MEDIAL SEPTUM OF FREELY MOVING RATS. C. King, M.Recce* Dept. of Anatomy, University College London, Gower St., London WC1E 6BT, U.K.

Cells of the medial septum and diagonal band of Broca act as a pacemaker for the generation of the 6-8Hz theta rhythm in the hippocampus (Bland and Bland, Br. Res. 375:102-116, 1986). These cells have been studied extensively in urethane-anaesthetised animals (Alonso et al Br. Res 413:135-146, 1987), in which cell firing activity was found to be phase-locked to the hippocampal theta rhythm. Furthermore, reversible inactivation of activity in the medial septum leads to loss of the hippocampal theta rhythm as well as causing an increase in the number of errors made in a working memory task on a radial maze (Mizumori et al J. Neurosci 9:3915-3928, 1989).

We recorded from medial septal cells using chronic implantation of tetrodes, allowing the examination of multiple cells simultaneously. The rats were allowed to move freely and were trained to run from end to end of a linear track. The autocorrelation function of approximately 30% of the cells showed an 8Hz rhythm of varying strength, and, in certain cases, these cells also demonstrated phase-locking to the hippocampal theta rhythm. The firing behaviours of these cells were similar to those found in anaesthetised rats (Alonso et al ibid).

Those cells that were most clearly phase-locked with the theta rhythm also showed an increase in frequency of burst firing corresponding to an increase in the rat's running speed. Occasionally rhythmic interactions could be observed between cells recorded simultaneously, in which cross-correlation plots showed clearer oscillations over a narrow time period than the autocorrelation plots. Cells were also found whose rhythmic firing depended on the spatial orientation of the linear track and the direction of motion of the rat.

This work was supported by the Wellcome Trust and the MRC (UK).

564.18

TEMPORARY SUPPRESSION OF MEDIAL SEPTAL ACTIVITY GREATLY REDUCES THE ACTIVITY OF CA1 PLACE CELLS. E. Brazhnik*, R.U. Muller and S. E. Fox. Dept. of Physiol., SUNY Health Sci. Ctr., Brooklyn, NY 11203

Septohippocampal neurons have well known influences on hippocampal activity. We sought to clarify such influences on the spatial firing properties of pyramids (place cells) and theta-related firing of interneurons (theta cells).

Preliminary results of tetracaine inactivations of MS/DB done as rats chased food pellets in a 76 cm dia. cylinder indicate that the location-specific firing of CA1 place cells is greatly reduced. In 16 min sessions shortly after tetracaine injections (1 μl, 2%), the average in-field and overall firing rate of 19 cells decreased by about 80%; some cells ceased firing for large parts of the session. The effect on firing rate is considerably greater than reported by Mizumori *et al.* (J Neurosci 9: 3915-3928, 1989) for CA1 place cells but comparable to what they reported for CA3c/hilar place cells. Mizumori *et al.* also reported that positional firing specificity was preserved for CA1 units. We observed residual firing within the region of the field, but the rate was usually too low to conclude that spatial specificity was preserved.

To help test if tetracaine acts by suppressing discharge of MS/NB neurons or fibers of passage, injections of the GABA-A agonist muscimol (0.1-0.3 mg) were made into MS/DB. Muscimol reduced the in-field firing rate of 15 CA1 place cells by about 90%; overall rate was also reduced. Muscimol action persisted for more than 3 hr whereas firing rates mostly recovered 30 min after tetracaine. The result suggests that profound reduction of place cell discharge can occur when only the input from MS/NB neurons is decreased; additional effects of tetracaine on fibers of passage are not necessary to explain the effect.

Both muscimol and tetracaine abolished theta rhythm recorded at the fissure and reduced the firing rates of theta cells (interneurons). The recovery of location-specific firing followed the same time course as the recovery of theta rhythm, corroborating the notion that activity of MS/DB neurons plays a critical role for both hippocampal EEG and place cell activity. (Supported by NIH NS 17095 and NS 20686.)

564.19

THE ANTIDEPRESSANT EFFECT ON DEGENERATIVE PROCESS IN THE SUPRAOPTICAL AND PARAVENTRICULAR HYPOTHALAMIC NUCLEI INDUCED BY BILATERAL OLFACTORY BULB ABLATION IN C57BL/6J MICE. N.V.Bobkova, I.V. Nesterova, E.V.Gurevich*, I. Aleksandrova, N.A. Otmakhova. Int. of Cell Biophysics,142292 Pushchino, Moscow Region, Russia

Bilateral bulbectomy in rats and mice induces behavioral, hormonal, a neurochemical changes, counteracted by chronic antidepressant treatment. In this study we examined morphological changes in the neurosecretory neurons in the supraoptical (SO) and paraventricular (PV) nuclei of the hypothalamus resulted from bulbectomy in C57Bl/6j mice. SO and PV are know to receive projections from the raphe nuclei and locus coeruleus as well as from the olfactory bulbs. The brains (28 days after surgery) were fixed and embedded in paraffin. Serial 5μm sections were stained according to Gomory-Gabu method and counterstained by Geidengainu method. 1000 cells were counted in each mouse in each nucleus. Bulbectomy caused increase in the number of picnomorphic cells (4.5 times in SO and 3 times in PV, p=0.001), while the proportion of normal neurons decreased by 45.6 and 60%, respectively. The proportion of neurons with low secretory activity insreased 2.5 time in SO and 5.5 times in PV. Antidepressant trazodone (20 mg/kg, for 14 days starting 14 days after surgery) counteracted the degenerative process in both nuclei. Bulbectomized mice treated with trazodone did not differ from sham-operated mice in the proportion of picnomorphic and normal neurons in either nucleus. However, the proportion of the neurons with decreased secretory activity was higher in both nuclei in the trazodone-treated mice comparing to sham-operated control. The same treatment with amitryptiline counteracted the effect of bulbectomy in SO only. Trazodone is a 5-HT2 receptor antagonist and has been shown to down-regulate 5-HT2 serotonin receptors increased by bulbectomy as well as alleviate degenerative process in the dorsal raphe nucleus induced by bulbectomy. It seems possible that trazodone counteracts bulbectomy-induced degeneration by preserving serotonergic input into both nuclei.

564.20

SLEEP, MONOAMINES, AND EFFECT OF IMIPRAMINE (Imip) ON MOTOR ACTIVITY IN OLFACTORY BULBECTOMIZED RATS. H.H. Webster[1], K.M. Dewar[1]*, R. Godbout [1], D. Perry[1], and M. Vézina[2]. Depts. Psychiat.[1] & Pharmacol.[2] Univ. of Montréal, (Qué) Canada H3C 3J7.

Altered behavioral and physiological characteristics in rats following bilateral olfactory bulb ablation (OBX) are known collectively as the OBX syndrome, which is analogous to depression in humans. In the present study we investigated the effect of OBX on paradoxical sleep (PS) latency and on levels of serotonin (5-HT), noradrenaline (NA,) dopamine (DA), and of their metabolites in the raphé, hippocampus and amygdala. Motor activity before and after imipramine was also investigated. Sleep was recorded for periods of 24 h using EEG, EMG and eye movements before and weekly up to 56 days postOBX (lights on from 6am. to 6pm). Analysis of baseline and postOBX day 21 data for 3 rats showed that PS latency was reduced by 36% from 4am to 4pm. HPLC analysis showed that 5-HT was decreased in the hippocampus on postOBX day 21. NA declined in the raphé on day 21 and in the amygdala on days 14 and 21. 5-HIAA was increased in the raphé on days 14 and 21 and in the hippocampus on days 7, 14, and 21; and decreased in the amygdala on day 7. No change was found in DA, DOPAC, or HVA. Imip administration in 10 rats (5mg/kg/day s.c.) with minipumps began 7 days post-OBX and lasted 21 days. Compared to untreated sham-operated rats, motor activity was increased both in untreated OBX rats and in OBX rats treated subchronically (<14 days) with Imip. Motor activity in OBX rats treated chronically (> 14 days) with Imip was normalized to the level of untreated sham-operated rats. We conclude that a reduced PS latency is part of the OBX syndrome, that MAs are affected in specific ways in some structures, and that hyperactivity responds to chronic but not acute Imip. Supported by MRC Canada and CAFIR, Univ. Montréal.

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS VII

565.1

COMPARISON OF MOTOR SKILL LEARNING IN ELDERLY AND YOUNG HUMAN SUBJECTS. D. B. Willingham*, and E. Winter. Dept. Psychology, Univ. of Virginia, Charlottesville, VA 22903. Elsewhere we have suggested that different types of motor skill learning are subserved by different neural circuits: specifically, motor sequencing by the basal ganglia and supplementary motor area, and learning new perceptual-motor mappings by the cerebellum, parietal cortex and premotor cortex. Because these areas are not implicated in normal aging, we predicted healthy elderly subjects should not be impaired in these types of motor skill learning tasks. Healthy elderly subjects (mean = 76 years) and young subjects (mean = 20 years) were compared on two sequence-learning tasks and two perceptual-motor mapping tasks. The sequencing tasks included the serial response time (RT) task, in which a repeating sequence is embedded in a four-choice RT task and a stepping-stone maze in which subjects responded using a button board. Previous work has shown that part of the learning is sequence-specific. The perceptual-motor mapping tasks were a four-choice RT task in which subjects must learn a new mapping between stimuli and responses (press one key to the right) and; the stepping-stone maze task, but subjects responded with a mouse. Previous work has shown that in this task subjects primarily learn the mapping between cursor movement and mouse movement. As predicted, elderly subjects showed learning equivalent to that of young subjects on all tasks.

565.2

NETWORK ANALYSIS OF RECOGNITION OF FAMILIAR AND NOVEL PICTURES. R. Cabeza*, A. R. McIntosh, E. Tulving. Rotman Research Institute, North York, ONT M6A 2E1.

In Tulving et al. (Neuroreport, 1994, 5, 2525-2528), subjects saw colored pictures of scenes and landscapes, and 24 hr later they distinguished between seen and unseen pictures, while undergoing rCBF PET scans. During the scan window, pictures were all familiar (OLD scans) or all novel (NEW scans). Familiarity activations (OLD-NEW) were found in the prefrontal cortex and anterior cingulate, whereas novelty activations (NEW-OLD) were observed on the right hippocampal gyrus, medial prefrontal and orbitofrontal, and left temporoccipital cortex.

Network analysis (McIntosh AR, Gonzalez-Lima F. Human Brain Mapp., 1994, 2: 2-22) of these data yielded an elaborate pattern of interactions involving a number of brain regions. In both OLD and NEW scans, activity in prefrontal areas was positively correlated with activity in temporal and cingulate areas, and negatively correlated with activity in occipital and hippocampal areas. We interpret this pattern to reflect a functional network involved in recognition attempt, irrespective of the familiarity of the stimuli. The main difference between OLD and NEW conditions was a negative correlation, stronger in the NEW than in the OLD condition, between hippocampus and contralateral prefrontal cortex, especially between the left hippocampus and right prefrontal. We interpret this correlation to reflect an opposing relation between the left hippocampus and right prefrontal cortex for novel items, an idea consistent with the assumed role of this latter area in retrieval of familiar items (Tulving et al., Proc. Natl. Acad. Sci. USA, 1994, 91, 2016-2020).

565.3

PET, MULTIVARIATE STATISTICS, AND THE NEURAL SYSTEMS INVOLVED IN IMPLICIT AND EXPLICIT MEMORY FOR VISUAL OBJECTS. K. Chen, A.Uecker, P. Jones, E. Reiman, L. Yun, D. Schacter, L. Cooper, and R. Lansing*. ARL Division of Neural Systems, Memory and Aging and the Dept. of Psychiatry, Univ. of Arizona; Dept. of Psychology, Harvard Univ.; Dept. of Psychology, Columbia Univ., and the Samaritan PET Center, Good Samaritan Regional Medical Center, Phoenix, AZ, USA.

Positron emission tomography (PET), ^{15}O-water, and the Statistical Parametric Mapping (SPM) were used to characterize regions of the brain that participate in implicit memory, explicit memory, and the structural representation of visual objects.

In contrast to object decisions about previously studied structurally impossible objects, object decisions about previously studied structurally possible objects were associated with priming, postulated blood flow increases in the vicinity of inferior temporal and fusiform gyri, and blood flow increases in several other regions. In contrast to recognition decisions about previously studied structurally impossible objects, recognition decisions about previously studied structurally possible object were associated with better recognition memory, a postulated blood flow increase in the vicinity of the right hippocampal formation, and blood flow increases in several other regions.

Four multivariate statistical techniques (principal components analysis, multidimensional scaling, cluster analysis, and structural equations modeling) were used post hoc to investigate interactions among the implicated brain regions, consider the potentially complementary role of multivariate statistics in the analysis of brain mapping studies, and provide a better understanding of the neural systems involved in implicit and explicit memory for visual objects. Supported by the Flinn Foundation.

565.4

NEUROANATOMICAL CORRELATES OF VISUAL WORD RECOGNITION. R. Habib*, L. Nyberg, E. Tulving, R. Cabeza, L.-G. Nilsson, S. Kapur, S. Houle, and A. R. McIntosh. Rotman Research Institute of Baycrest Centre, 3560 Bathurst Street, North York Ont, Canada. M6A 2E1.

Brain regions involved in episodic memory retrieval (visual word recognition) were studied using positron emission tomography. Prior to scanning, subjects were auditorily presented with two lists of words. In one list, the subjects' attention was focused on verbal, semantic aspects of the stimuli; in the other on nonverbal, nonsemantic aspects. Each subject underwent 8 scans; 2 word reading scans and 6 recognition scans (in 2 recognition scans, nonstudied words were shown; in another 2, words from the nonsemantic encoding condition, and in still another 2, words from the semantic encoding condition).

A region in the right prefrontal cortex was significantly activated during word recognition compared to word reading. Activation patterns across the recognition conditions showed that a high memory performance (following semantic processing) was associated with increased activation in the left middle/superior temporal gyrus. Retrieval following semantic processing involved increased activation in the left prefrontal cortex. Retrieval following nonsemantic processing was associated with increased activation in regions in the right hemisphere, including the parahippocampal gyrus.

It is concluded that activation in the right prefrontal cortex during episodic retrieval is independent of retrieval success and that it may be related to effortful search processes implicated in episodic retrieval attempt. Moreover, it is suggested that retrieval of information partly activates brain regions engaged during the acquisition of the same information.

565.5

DIFFERENT ASPECTS OF WORKING MEMORY SHOW SELECTIVE CHANGES WITH AGE. A. Wingfield*, K.C. Lindfield, P.A. Tun, K.S. Richards, & M.J. Rosen. Department of Psychology and Volen National Center for Complex Systems, Brandeis University, Waltham, MA 02254.

Working memory is usually defined as involving components of memory storage and processing of information, operating in a limited work space (Baddeley, 1986). However, the ways in which working memory have been defined operationally in the neuroscience literature have been varied. In this experiment we tested a battery of measures believed to involve important aspects of working memory (Carpenter, Miyake, & Just, 1994). These included: (1) holding and storage capacity (as measured by simple memory span tests that require only passive storage of words or digits), (2) computational work space (as measured by span tests that require both storage and manipulation of information), (3) processing speed (as measured by a speeded test requiring transposition of digits and symbols), and (4) inhibitory processes (as measured by a modified version of the Stroop color-word test).

In this study we probed the contribution of these various aspects of working memory by comparing young adults with elderly adults, who are often selectively compromised in certain cognitive capacities. Twenty young (mean age = 18.8 years) and 20 elderly (mean age = 72.4 years) normal functioning adults participated in the battery of tests. Results showed larger age differences for tests tapping computational space and speeded processes than for those measuring simple memory storage. This dissociation stresses the importance of a careful specification of the features of working memory being examined from one experiment to another.

565.6

WHERE IS WORKING MEMORY WORKING? C.M. Cowey, C. Polkey[1], B. Meldrum*, R. Morris, Institute of Psychiatry, University of London, U.K.[1]Maudsley Hospital, London, U.K.

The aim of this study is to demonstrate the neuro-anatomical substrate of the Central Executive: a component of a theoretical cognitive model of short-term memory: Working Memory. The Central Executive allocates attentional capacity to "slave-systems" which process visual and verbal material in the execution of short-term memory tasks. A dissociation between attentional capacity for single, and that for dual-tasks has been demonstrated in work involving patients with Alzheimer's disease, with dual-task performance selectively declining as a function of the progression of the disease. From this exploration a suggested frontal lobe site for the division of attention has emerged. The present study tested this hypothesis with an investigation of patients with highly localised neural atrophy. Individuals with epilepsy and either lesions of the frontal lobe or hippocampus were required to perform tests of attention and memory, in single and concurrent trials. Subjects tracked a moving target across a touch-sensitive computer screen. Against this baseline a second task was introduced. Preliminary results suggest that individuals with frontal lobe lesions display the greatest decrement over single and concurrent trials. It is argued that this Janusian ability is mediated by the frontal lobes.

565.7

CEREBRAL ACTIVATION BY A WORKING MEMORY TASK: A HUMAN FMRI STUDY. A.S. Bloom*, S.M. Rao, J.A. Bobholz, T.A. Hammeke, S.A. Fuller, J.R. Binder, R.C. Cox, J. Pankiewicz, H.H. Harsch, J-K. Cho M. Rossing and E.A. Stein. Departments of Pharmacology, Psychiatry, Neurology and Biophysics Research Institute, Medical College of Wisconsin, Milwaukee, WI 53226.

Brain systems involved in human working memory are not well described. A recent PET study by Jonides et al, (1993) reported that a visualspatial working memory task activated right frontal, parietal, occipital and premotor cortex. We have used a very similar task in a whole-brain functional MRI study. While undergoing FMRI, young right-handed subjects were presented with spatial working memory and perceptual control tasks. Imaging was conducted using a 1.5T GE Signa scanner equipped with a 3-axis local gradient coil and an endcapped quadrature birdcage RF coil, using a blipped gradient-echo echo-planar pulse sequence. Sixteen contiguous 7 mm saggital slices covering the entire brain were collected with a TR=4 s producing 64 images per series. Functional images were generated for each slice using a cross-correlational method then warped into stereotaxic space. Activation by the perceptual control task was subtracted from that produced by the working memory task. The results of this study are in good agreement with the PET study of Jonides. Both studies observed activation in the right prefrontal and occipital cortex and bilateral activation in superior parietal and premotor regions. In addition, we observed activation in right thalamus, left anterior cingulate and bilateral insular regions. The results of this study demonstrate that multiple brain systems subserve working memory in humans and that whole-brain FMRI is an effective method for the investigation of these systems. (Supported by DA09465.)

565.8

PET STUDIES OF THE RECALL OF PICTURES AND WORDS FROM MEMORY R.L. Buckner*, M.E. Raichle, F.M. Miezin, and S.E. Petersen. Washington Univ. Sch. of Med., Box 8111, St. Louis, MO 63110.

Recent neuroimaging studies have demonstrated activation in areas of prefrontal cortex during memory retrieval (Buckner and Tulving, 1995 *Handbook of Neuropsychology*, Elsevier). Two new PET studies were conducted to determine if, during the retrieval of pictorial information, prefrontal brain areas would again be activated in addition to brain areas related to picture processing. In study one, 10 subjects were scanned during the naming and viewing of pictures. Compared to analogous conditions using word stimuli, visual areas were activated including a right fusiform area that has been previously observed during face processing and attention to stimulus shape (Haxby 1994, Corbetta 1991 both *J. Neurosci.*). In study two, 14 subjects learned picture-word and word-word pairs *before* PET scans. During PET scans, subjects either recalled the names (labels) of the pictures or the words. For both conditions, recall was auditorily cued with word members of the pairs. There was no differential activation between picture and auditory recall for any of the visual areas activated during picture processing (study one). Moreover, comparison of picture recall to another task (auditory word repetition) also did not reveal differential activity in visual cortex. However, robust activations in frontal cortex were reliably observed in both the picture and auditory recall conditions as compared to repetition. These frontal activations were in bilateral anterior insular cortex and in anterior right prefrontal cortex. Medial and lateral parietal, anterior cingulate, SMA, and cerebellum were also activated. PET-MRI coregistration was used to localize the responses within several of the individual subjects. The right prefrontal activations were closely localized (mean x,y,z = 27,49,16) to similar areas activated during the stem-cued recall of words (32,50,6; Buckner 1995 *J. Neurosci.*).

565.9

RIGHT PREFRONTAL PET ACTIVATION DURING SEMANTIC MONITORING A.K. MacLeod*, R.L. Buckner, S.E. Petersen, and M.E. Raichle. Washington Univ. Sch. of Med., Box 8225, St. Louis, MO 63110.

Areas of the normal human brain used for semantic monitoring were identified using positron emission tomography (PET). For a series of tasks, subjects (N=12) viewed a list of animal names and decided whether the names represented "dangerous" animals. Surface characteristics of the task such as stimulus rate, number of targets, and mode of monitoring were varied across multiple conditions within- and between-subjects. Common to all of these conditions was the semantic monitoring requirement. A passive word viewing condition was used as the control. A subset of these data has been previously analyzed in a limited fashion and revealed activation in left prefrontal cortex, anterior cingulate, and visual extrastriate cortex (Petersen 1989, *J. Cog Neuro.*; 1993 in *Brain Mechanisms of Perception and Memory*, Oxford). In the current analysis, more subjects were included and the entire brain was surveyed for activation. Using a replication approach, reliable activations were identified in anterior and dorsal right prefrontal cortex (mean x,y,z = 25,57,10 and 11,35,46 both $p<.01$). The locations of these areas are similar to areas that have been activated during several memory recall tasks (Buckner 1995 *J. Neurosci.*; companion abstract). Importantly, the right prefrontal activations in the current experiment demonstrate that participation of these areas is not limited to recall of recently learned information or for information associated with a unique temporal event (episodic memory retrieval). Perhaps these areas of right prefrontal cortex facilitate the maintenance of a task dependent context, or template, for matching. During episodic memory retrieval tasks, the context is supplied by the specific source in which the information was learned. In the present experiment, the context is semantic information about characteristics and qualities of dangerous animals.

565.10

GENERALIZATION OF PRACTICE-RELATED EFFECTS IN MOTOR LEARNING USING THE DOMINANT AND NON-DOMINANT HAND MEASURED BY PET. H. van Mier*, L.W. Tempel, J.S. Perlmutter, M.E. Raichle and S.E. Petersen. Washington Univ. Sch. of Med., Box 8111, St. Louis, MO 63110.

Using PET, functional effects of practice on motor performance have been examined in 32 normal right handed subjects. In a study presented last year 16 subjects practiced moving a pen with their dominant right hand continuously in a clockwise direction through a cut-out maze and square pattern as fast and accurately as possible with their eyes closed. Several control conditions were used to distinguish practice related from motor performance changes (e.g. changes in movement velocity). Some areas, including left primary motor cortex, showed changes in activation which could be attributed to motor performance. Decreases in activity attributable to practice were found in **right** premotor and parietal areas, cingulate cortex and **left** cerebellum, while practice-related increases were found in supplementary motor area (SMA).

Because of the surprising lateralization of the practice-related effects, a second group of 16 normal right handed subjects was scanned while performing the same tasks, but using the non-dominant left hand. As would be expected areas relating directly to motor performance shifted lateralization (e.g., primary motor cortex activity was now seen in the right hemisphere). The practice-related changes, however, were seen in the same hemisphere and areas as those found with right hand performance (e.g, right premotor and parietal regions and left cerebellum). These results clearly suggest that processes related to this motor learning task activate right cortical and left cerebellar areas independent from the hand used, and thus must be coding information abstract from the motor performance of the task itself.

565.11

FUNCTIONAL MRI OF THE PREFRONTAL CORTEX DURING SEMANTIC ENCODING. A.M. Golub[1]*, P. Erhard[2], J.P. Strupp[2], P. Andersen[2], G. Adriany[2], X.Hu[2], K. Ugurbil[2]. Department of Psychology, California State University, Sacramento, CA 95864 [1] and Center for Magnetic Resonance Research, University of Minnesota Medical School, Minneapolis, MN 55455 [2].

The left prefrontal cortex has been implicated in memory and attention. We used functional MRI to investigate neuroanatomical correlates of the depth of processing effect. Our goal was to replicate and extend, using fMRI at 4 Tesla, recent PET reports of left inferior prefrontal cortex activation during a semantic information processing task. Seven normal volunteer subjects viewed two types of word lists in an ABABA design. Subjects made non-semantic judgments (Task A) or semantic judgments (Task B). Each word was presented only once and subjects indicated a yes/no decision by pressing one of two buttons on a joystick. Experiments were conducted on a 4 Tesla Sisco/ Siemens system using a quadrature birdcage coil and a head gradient set (30 mT/m). Following acquisition of anatomic images, a total of 160 consecutive multislice (12 slices) T2* weighted EPI images were acquired with a 64x64 matrix, a FOV of 20x20cm and a slice thickness of 10 mm. Both hemispheres were covered (A-a-1 to A-d-10 to F/G-a-1 to F/G-d-10 in Talairach coordinates). Data were zero padded to 128x128 before Fourier transformation. The delay due to hemodynamic response was estimated by searching for the maximal cross correlation with a sliding reference function and was used to define two t-tests, which compared each activation period with both neighboring periods, i.e., with each ABA. Only pixels significant on both t-tests ($p = 0.001$) and part of an activated cluster of at least 16 pixels were used for the activation time course. Significant activation was observed in the left prefrontal cortex in Brodmann's areas 10, 9, 45 and possibly 11. These activations are consistent with other recent fMRI reports and with PET findings and may reflect a hemispheric encoding asymmetry, activation of novelty detectors, or an asymmetry in prefrontal cortex activation associated with other aspects of the task. Supported by NIH Grant RR08079 and a CSUS SFS grant.

565.12

AN FMRI ANALYSIS OF MEDIAL TEMPORAL LOBE ACTIVATION IN MEMORY ENCODING. M.P. McAndrews*, S. Taylor, A.P. Crawley, M. Wood and D.J. Mikulis. The Toronto Hospital, Western Division, Toronto, Ontario, M5T 2S8, Canada.

Several recent studies using PET and fMRI have shown activation of medial temporal structures in memory tasks during the presentation of novel stimuli in comparison with previously studied or more familiar stimuli. The present research examined the difference in activation patterns to visual presentation of orthographically regular non-words (Study 1) and word pairs (Study 2). In the novel conditions, a unique stimulus was presented for study at the rate of 1 per second (non-words) or 1 per 2 seconds (word pairs). In the familiar conditions, a small set of items that had been studied prior to scanning were presented repeatedly throughout image acquisition at the same rates as novel stimuli. Six young volunteers were scanned using a conventional 1.5 Tesla magnet (gradient echo, 256x128 matrix, TE=40, TR=68, FOV=48). Functional images comprised a single 4 mm oblique axial slice, oriented through the longitudinal axis of the body of the hippocampus. For each subject, a statistical t-map was generated by comparing activation in the novel versus familiar conditions.

Results for the non-word task indicated small (1-3%) but significant activation in the posterior medial temporal lobe regions in all subjects. This effect was considerably weaker, appearing in only 3 of 6 subjects, for the word pair task. We are currently collecting three-dimensional isotropic voxel scans to permit more precise anatomical localization of the activated regions in a new group of subjects. These findings are consistent with other data indicating the activation of the hippocampal formation in response to novelty, presumably reflecting the operations involved in encoding new information in memory.

565.13

RETRIEVAL OF REMOTE KNOWLEDGE VERSUS LEARNING OF NEW KNOWLEDGE: PET EVIDENCE FOR DISTINCT NEURAL SYSTEMS. R.D. Jones*, T. Grabowski, D. Tranel. Dept. of Neurology, Division of Cog. Neuroscience, Univ. of Iowa College of Medicine, Iowa City, IA 52242 USA

Lesion studies have suggested that retrieval of old, factual, concrete knowledge and learning of new, factual, concrete knowledge depend on distinct systems in temporal lobe. Here we report a positron emission tomography (PET) study which supports this notion.

A right-handed 70 year old woman with limbic encephalitis caused by thymoma was evaluated four months after diagnosis. Severe multimodal anterograde and retrograde memory defects were found. An (18F) flourodeoxyglucose (FDG) PET scan showed thalamic and diffuse cortical, but especially bilateral mesial and right anterolateral temporal hypometabolism. Two years later there was marked resolution of the remote memory defect, with persistent anterograde memory impairment. Retrieval of nonunique knowledge and other neuropsychological functions were normal. A second FDG PET then showed increased metabolism in the thalamus and most cortical regions, including the lateral right temporal lobe. Metabolism in the right temporal pole and orbital cortex were unimproved, whereas metabolism in the left temporal pole and in both mesial temporal regions declined markedly. Recovery of retrograde memory occurred despite reduction in mesial temporal metabolism. These findings provide further support for the implication of non-mesial temporal lobe structures in the retrieval of remote factual knowledge.

565.14

NEURAL REPRESENTATIONS FOR CONCRETE, ABSTRACT, AND EMOTIONAL WORD LEXICA: A POSITRON EMISSION TOMOGRAPHY STUDY.

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Evidence from cognitive neuropsychology suggests that there is a permanent representation for the visual form of known words in the brain. Neuropsychological and functional imaging studies studies have suggested that this representation, termed the orthographic lexicon, is localized either in the left occipital lobe or, alternatively, in the left temporal lobe. In order to localize the brain regions involved in the representations for concrete, abstract, and emotional word lexica, we have carried out a $H_2{}^{15}O$ PET study on 10 normal right-handed male subjects whose age ranged between 18 and 28 years. A series of scans were performed during different cognitive tasks. In each of the conditions, subjects were told to simply relax and passively look at the material presented on the screen, either flashing Plus Signs (baseline), random letters, concrete words, abstract words, or emotionally-laden words. Results were analyzed for the following subtraction pairs: Random letters minus Plus Sign baseline; Concrete words minus Plus Sign baseline; Abstract words minus Plus Sign baseline; and Emotional words minus Plus Sign baseline. Each of these last three subtractions produced peaks of significantly increased cerebral blood flow (CBF) in the left inferior and middle temporal gyri, and in the left (and right occipital lobe, for Abstract and Emotional words) occipital lobe. In addition, the Emotional minus Plus Sign baseline subtraction produced peaks of increased CBF in the left orbital frontal region and in the midline superior frontal gyrus area. These results suggest that the left temporal lobe region is involved in the neuroanatomic substrate for the orthographic lexicon and that additional regions of the frontal cortex are implicated in the processing of affective information.

565.15

BRAIN MECHANISMS IN HUMAN CLASSICAL CONDITIONING: A PET BLOOD-FLOW STUDY.

Kenneth Hugdahl[1], Anna Berardi[2], William L. Thompson[2], Stephen M. Kosslyn[2], Robert Macy[2], David P. Baker[2], Nathaniel M. Alpert[3], and Joseph E. LeDoux[4]. [1]Department of Biological and Medical Psychology, University of Bergen, Norway [2]Department of Psychology, Harvard University, Cambridge, MA. [3]Department of Radiology, Massachusetts General Hospital, Boston, MA. [4]Center for Neural Science, New York University, New York, N.Y. (SPON: European Brain and Behaviour Society).

Five healthy male subjects participated in a classical conditioning experiment, and positron emission tomography (PET) was used to compare regional cerebral blood flow before and after conditioning. The subjects participated in three different experimental phases. In the first (habituation) phase they listened to 24 repetitions of a tone with random intervals. In the second (acquisition) phase, the tone was paired with a brief shock to the hand whenever it occurred. In the third (extinction) phase, the tone was presented alone again. 15-O PET scans were taken during the habituation and extinction phases. Because the habituation and extinction phases were identical, any difference in blood flow to the tones presented during extinction should be due to conditioning that occurred during the acquisition phase. Statistical parametric mapping (SPM) analysis of the PET data showed increased activation in frontal and temporal regions of the brain during extinction, particularly in the right hemisphere. The results are interpreted as evidence for the involvement of cortical areas in human classical conditioning.

565.16

LONG-TERM RETENTION OF EYELID CONDITIONING AND SKILL ACQUISITION IN AMNESIA. I. Daum, M. M. Schugens, F. M. Eblen* and N. Birbaumer. Institute of Medical Psychology and Department of Neurology, University of Tübingen, 72074 Tübingen, Germany.

Nondeclarative learning, as indicated by classical conditioning or skill acquisition, has been shown to be intact in amnesia, but so far little is known about the long-term retention of these abilities across weeks or months. In the present study, classical eyelid conditioning (tone-airpuff pairings) and tracking performance were assessed in a baseline session, 10 days and 2 months after initial learning in patients with an amnesic syndrome and matched control subjects.

Amnesic patients acquired classically conditioned eyelid responses as readily as control subjects and showed significant savings at both intervals testing retention. The amount of savings was similar to that of controls. None of the patients remembered the previous testing sessions or recognized equipment or stimuli. Despite a lack of awareness of stimulus contingencies, amnesic patients showed significant cortical negativities in the interval between conditioned and unconditioned stimulus. In the motor skill acquisition task the amnesic patients also showed significant learning and savings of the acquired skill over the 10 day and 2 month intervals, without remembering any previous experience with the task.

In summary, the results indicate that amnesic patients are able to retain newly learned motor behavior over an extended period of time to a degree similar to control subjects. It is interesting to note that although EEG recordings indicate cortical processing of stimulus contingencies in the conditioning task, amnesic patients fail to become aware of them.

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565.17

TEMPORAL FACTORS IN MEMORY FOR NONOBJECTIVE IMAGES VERSUS WORDS. Andreas E. Savakis, Robert W. Doty* and Ruiming Fei, Department of Physiology, University of Rochester School of Medicine, Rochester, New York 14642.

From among several hundred multicolored, abstract, nonverbalizable images and over 1100 four-letter, nonoffensive words, test items were chosen at random. In 5 sessions, 3 for images and 2 for words, at weekly intervals, 8 subjects were shown 240 targets on a computer monitor and were asked to identify rapidly and accurately whether a target was being presented for the first time, "new", or was a repetition, "old". Repetitions occurred after 1, 2, 4, 8, 16, or 32 presentations, randomly placed throughout a session. In session 1, images were viewed for 2 sec each, with a 3-sec intertrial interval; while for session 2, with different images, viewing time was 200 msec followed by a 2-sec colored mask. For the 3rd week, 60 randomly selected images each from weeks 1 and 2 were "old" to be discriminated from 120 entirely "new" images, with 2-sec viewing time. In week 4, words were displayed for 200 msec, followed by the 2-sec mask; and in week 5, "old" words from week 4 were to be distinguished from "new" words. In week 1 recognition was 81.8% (d'=1.82) vs 73.5% (d'=1.26) for week 2; while week 4 (words) was at 93.6% (d'=2.97). Long-term memory was strikingly different: 65.2% (d'=0.65) for week-1 images, twice viewed for 2 sec 2 weeks earlier, vs 55% (d'=0.39) for 200-msec views a week previously; and 67% (d'=0.89) for words after 1 week. Reaction times (RTs) were shorter for shorter exposures, averaging 850 and 800 msec for weeks 2 and 4, vs 1 sec in week 1; and were relatively flat throughout a session. However, RT consistently incremented, and accuracy decremented, as a function of number of intervening images yet, surprisingly, showed no significant indication of interference from the inevitably increasing mnemonic load as the session progressed. Thus, a linkage of specific image or word pairs can be deduced, such that it takes longer to identify a previously perceived item the longer it has been in memory. Whether this is purely an effect of time (unlikely given weeks 3 and 5), or a mixture of time and interference, remains a puzzle.

565.18

INTENSITY DISCRIMINATION OF VIBRATORY STIMULI CAN BE IMPROVED BY TRAINING. S.J. Bolanowski, K.L. Hall, J.C. Makous* and M.M. Merzenich Inst. for Sensory Res., Syracuse Univ. Syracuse, NY 13244 and Keck Center for Integrative Neurosci. UCSF, San Francisco, CA 94143.

While it is generally accepted that the magnitude of sensation is related to the physical intensity of the stimulus, the exact form of this relationship and its neural basis have been debated since the time of Weber. Fechner, using the ability of observers to discriminate stimulus intensities (Differenz Limen, DL) and integrating along the intensity domain, proposed a logarithmic relationship between perceived magnitude and stimulus intensity, but this notion has been supplanted by the still controversial power law of Stevens. Because the perception of sensation magnitudes and their differences must be reflected in the DLs, their possible modification should permit studies to access the largely unknown mechanisms underlying intensity perception. We report that the DLs for vibratory stimuli can be transiently modified with training on a DL task (2AFC, gated pedestal). Specifically, DLs were found to decrease by approximately 50% during training (23 daily sessions), and after 3 months post-training, approached pre-training levels. Although no change in threshold sensitivity occurred, DLs for the homologous, contralateral, untrained site were also affected, but in a complicated way that may be channel- (Pacinian, P, versus non-Pacinian, NP) and intensity-specific. Training in the vibratory-DL task also appeared to produce changes in spatial discrimination as assessed by two-point thresholds. This vibratory-based spatial effect, and the bilateral and stimulus-specific effects, may implicate cortical neural-plasticity and suggest that the perception of sensation magnitude comes about through complex (learned?), spatio-temporal mechanisms. Supported by NIH DC00380 and DC00098.

565.19

LEARNING OF VISUOMOTOR COORDINATION WITH ROTATED VISUAL FEEDBACK F.A. Kagerer*, J. Bloedel♦, G. Stelmach, Motor Control Lab, Arizona State University, Tempe AZ, 85287-0404 ♦Barrow Neurological Institute, Phoenix, AZ 85013

The purpose of this experiment was to investigate learning and transfer in a point-to-point aiming task. The task required subjects to make horizontal movements to three target locations on a digitizer board that were also displayed on a monitor. The visual feedback displayed on the monitor was either normal or rotated by 90°. Without vision of their arm, subjects connected the targets by moving the pen between the target locations. After practicing the task under normal and 90° rotated conditions, subjects transferred to a different set of three target locations which required a novel movement pattern. Group 1 (n=8) learned and transferred in the same workspace, which was located in front of the ipsilateral shoulder. Group 2 (n=8) learned in one workspace and transferred to a workspace located just to the opposite side of the subjects's midline. 20 trials were given during baseline (with normal visual feedback), and 60 during learning/transfer. Transfer was assessed based on a statistical comparison of the mean movement times during the first five trials for the learning and transfer conditions in each group. The findings indicate that subjects in Group 1 showed significant positive transfer. In contrast, when learning and transfer were carried out in a different workspace, no significant transfer occurred. These data suggest that when a new sequential visuo-motor task is learned, transfer related to the acquisition of a new pattern can occur, providing the workspace remains the same. Supported by Flinn Foundation.

565.20

SELF-GENERATED ERRORS IN PROSE RECALL IN AN AFRICAN-AMERICAN POPULATION WITH UNILATERAL BRAIN LESIONS N. Winrow*, L. H. Hicks, A. Campbell, Jr. Howard University. Washington, DC 20059; J. Grafman, NIH NINDS/MNB: COGNITIVE NEUROSCIENCE SECTION, BETHESDA,MD.

Webster et al (1992) reported a greater incidence of self-generated errors (SGE) on a memory task in patients with right hemisphere damage than in patients with left hemisphere damage or normal controls. The present study analyzed whether these errors occur more often in specific quadrants of the brain. SGEs were defined as distortions or additions by the subjects to the themes or plots of the story. Results revealed that there were no differences between brain injured patients and non-neurological controls in SGE production. Subjects in each group produced more dependent SGEs (misinterpretations caused by words or phrases with ambiguous meaning) than the other two types of SGE.

The results indicate that this present study did not replicate the findings of Webster (1992). The reason behind comparable performance among groups and disparate results between this present investigation and that of Webster et al is that in our study, non-neurological subjects' errors frequently represented a misinterpretation in what was portrayed by the characters in the story, rather than a failure to remember what the tester said. This finding indicates that the stories of the Logical Memory Subtest may contain words and phrases that are ambiguous to subjects from minority cultures.

565.21

USING COMPUTER PROGRAMS AND BRAIN PUZZLES FOR DETECTION OF READING IMPAIRMENT IN CHILDREN. I. Zarco de Coronado,*J.G. Coronado Zarco and E. Montes de Oca . Dpto. de Fisiologia, Fac de Medicina, Universidad Nacional Autónoma de México A. P. 70250; CP 04510 and Instituto Nacional de la Comunicación Humana (National Institute of Human Communication). Mexico.

Some of the learning problems are related to neurophysiological alterations of the students. Previous studies in our laboratory indicate that computer programs are effective to test visuo spatial abilities in children. It is well known that initial reading requires auditory, visual and associative components. The advanced skills in reading need also visual coordination, extraction of the conceptual content in the phonological structure of the language in order to build the semantic networks. *Objective.* This study was conducted in order to examine mexican children abilities for reading learning as revealed by using computer games with traditional prehispanic illustrations and schematic brain representations. *Methods.* Computers were carried to suburban communities and hearing children were instructed to use the programs. Data recording about the number of essays to complete the game was automatically recorded. The velocity of the reading was also determined. *Results.* About 40% of the examined children were slow readers and half of them showed visuo motor deficiency. This work suggests that normal children are most of the time good readers. Half of the slow readers showed visuo-motor disabilities and the rest of the children probably need better individualized or tutorial educational programs.

566.1

PAIN WITH A STRONG AFFECTIVE DIMENSION REPRODUCED BY STIMULATION OF THE HUMAN SOMATOSENSORY THALAMUS.
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The thalamic substrate of both the affective-motivational aspect of pain and memory for pain has been poorly understood. We report results of studies carried out in the region of the thalamic principal somatosensory nucleus (ventralis caudalis - Vc) prior to thalamotomy for tremor in a patient with a diagnosis of panic disorder. Cells located in the 14mm lateral plane had cutaneous receptive fields on the hand. Microstimulation in the region posterior to Vc evoked sharp chest pain, including the affective dimension, almost identical to atypical chest pain occurring during his panic attacks. His pains were not associated with any change in cardiac function. As measured using a questionnaire, the description of stimulation-associated pain was more similar ($P<10^{-7}$) to that of atypical chest pain than would be expected by random selection so that the two were rated 'almost identical' by the patient. In a retrospective review, stimulation-associated pain with a strong affective dimension only occurred in patients who had experienced such pain spontaneously. Sharp chest pains without an affective dimension were evoked by stimulation at 3 sites in patients (n=100) without prior experience of sharp chest pain with a strong affective dimension.

The region posterior to Vc is connected to SII, parasylvian and insular structures which may be involved in corticolimbic circuits subserving somatosensory memory. Therefore thalamic stimulation in this patient may have activated corticolimbic circuits involved in somatosensory memory that are conditioned by prior experience of pain. (Supported by Lilly and the NIH - NS28598, K08 NS01384, P01 NS32386).

566.2

DISSOCIATION BETWEEN INTERHEMISPHERIC INTEGRATION OF PROCEDURAL AND DECLARATIVE LEARNING OF A VISUOMOTOR SKILL IN CALLOSAL AGENESIS. E. de Guise[1], M. Lassonde[1] and L. Richer[2]*. 1- Groupe de Recherche en Neuropsychologie Expérimentale, Univ. de Montréal Qué., Canada. and 2- Univ. du Québec à Chicoutimi, Canada.

Acallosal subjects show impairments on simple tasks requiring bilateral interdependent motor control. However, few studies have assessed the ability of these subjects to learn a skill that would require the simultaneous contribution of each hemisphere in its acquisition. The present study thus examined whether acallosal subjects could learn a visuomotor skill that involved a motor control from either both or only one hemisphere. The performance of five acallosal subjects aged 20 to 35 years was compared to that of five control subjects, matched for age and I.Q. on a modified version of the serial reaction time task developed by Nissen and Bullemer (1987). On each trial a light appeared at one of four locations. Subjects were instructed to press the one key, out of a set of four keys, that was directly below the light. For the bimanual (interhemispheric) condition, subjects rested the middle and index fingers of both hands on the four keys. In the unimanual (intrahemispheric) condition, subjects rested the index, middle, third and little fingers of the dominant hand on the four keys. In each condition, the subjects received 16 blocks of 100 trials. Each block comprised 10 repetitions of a 10-trial sequence. Following the training, subjects received one block of trials in a self-generated sequence task in order to evaluate their declarative knowledge of the repeated sequence. Results indicated that acallosals learned the visuomotor skill on the unimanual condition as well as their controls. However, they did not learn the sequence in the bimanual condition despite the fact that they knew the sequence explicitely. This finding indicates first, that the corpus callosum is important for procedural learning of a visuomotor skill that requires interhemispheric integration and second, that the development of kwowledge in the declarative memory system does not affect knowledge, or performance, in the procedural system.

566.3

ALZHEIMER'S DISEASE PATIENTS SHOW IMPLICIT LEARNING OF NOVEL PATTERNS. S. Corkin*[1], B. R. Postle[1], and J.H. Growdon[1,2]. [1]Department of Brain and Cognitive Sciences and the Clinical Research Center, Massachusetts Institute of Technology, Cambridge, MA 02139; [2]Department of Neurology, Massachusetts General Hospital, Boston, MA 02114.

Priming is a kind of implicit learning (learning without awareness) that does not depend upon the medial temporal-lobe system. For example, the amnesic patient H.M., who underwent bilateral medial temporal-lobe resection, shows intact priming with novel patterns (Gabrieli et al., 1990), suggesting that perceptual priming with nonverbal material does not depend upon areas critical for explicit memory. A logical candidate for the neural substrate that supports this kind of priming is peristriate cortex, an area that is relatively spared in AD (Arnold et al., 1990). We therefore predicted that AD subjects would be unimpaired on pattern priming. Subjects copied each of six target figures onto dot patterns. After performing a 3-minute distracter task, they were given the same dot patterns (without lines) and asked to draw the first figure that came to mind by connecting the dots with straight lines. In a test of recognition (explicit) memory, subjects viewed each of the six patterns of dots that they had copied previously and were asked to indicate which of four possible completions corresponded to the figure that they had copied 3 minutes earlier. The AD and NCS groups both achieved a mean priming score of 13.1%. In contrast, the recognition score for NCS was higher (mean = 58%) than that for AD subjects (mean = 43.2%). Our finding of intact pattern priming in AD suggests that nonverbal perceptual priming depends upon peristriate cortex. This finding is consistent with previous studies from our laboratory in which AD subjects showed intact perceptual identification priming with words and pseudowords (Keane et al., 1991; 1994).
Supported by NIH grant AG-06605 and an NSF Graduate Research Fellowship to BRP.

566.4

EMOTIONAL MODULATION OF COVERT SPATIAL CUING OF ATTENTION: A CLASSICAL CONDITIONING STUDY.
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Spatial cuing of attentional shifts were investigated when the cue had acquired emotional significance through a classical conditioning procedure. In the conditioning phase, half of the subjects (Conditioning group) received an aversive white noise tone (UCS) contingent upon presentation of a frame-lit rectangle, which was thus turned into a conditioned stimulus (CS+). The Control group received equal number of noise presentations, but they were never paired with the rectangle. During the attentional cuing phase, both groups participated in the covert attentional shift task where the location of the frame-lit rectangle validly or invalidly cued attention to the location a response-target would appear in. The Conditioning group tended to show overall faster reaction times, with no difference between invalid and valid trials, while the Control group showed the predicted delayed RTs on invalid trials. The N1-component of the Event-related Potential to the cue was also larger in the Conditioning group. The emotional salience the cue attracted through the conditioning procedure thus faciliated rapid attentional shifts from the cued to the uncued location on invalid trials.

566.5

Involvement of the Human Amygdaloid Complex in Emotional Memory. L. Cahill*[1], R. Babinsky[2], H. Markowitsch[2] and J.L. McGaugh[1]. [1]Center for the Neurobiology of Learning and Memory, and Department of Psychobiology, University of California, Irvine, CA 92717-3800; [2]Department of Physiological Psychology, University of Bielefeld, D-33501, Bielefeld, Germany.

Considerable evidence from studies of infra-human, and to a lesser extent human, subjects suggests that the Amygdaloid Complex (AC) is particularly involved with memory during emotionally arousing learning situations. We have begun to test this hypothesis in a patient (B.P.) with bilateral, circumscribed amygdala damage (resulting from Urbach-Wiethe disease) using a paradigm previously shown to be sensitive to beta-adrenergic blockade (Cahill et al., 1994). Subjects viewed a brief slide, narrated slide show in which emotional events are introduced in the middle phase of the story. Immediately after, subjects rated their emotional reaction to the story. Retention of the story was assessed one week later. Control subjects demonstrated superior memory for the emotional story phase compared to the non-emotional phase. In contrast, B.P. exibited normal retention of the non-emotional story phase, but showed no evidence of enhanced retention for the emotional story phase. Further, B.P.'s self-described emotional reaction to the story was normal. These results support the view that AC function in humans underlies, at least part, enhanced memory associated with emotional arousal.

Cahill. L., Prins, B., Weber, M., and McGaugh, J.L. β-adrenergic activation and memory for emotional events, Nature, 371:702-704.

566.6

CEREBELLAR SIZE CORRELATES WITH MEMORY FUNCTIONS IN YOUNG NORMAL HUMANS. Paradiso S., Robinson R.G., O'Leary D., Arndt S., Woodworth C.H.*, Andreasen N.C. Univ. of Iowa Dept. of Psychiatry, Iowa City, IA 52242.

Cerebellar and total brain size were estimated through automatic, atlas-based volume measurements using MR images obtained with a T1 weighted 3-D SPGR sequence on a 1.5-T GE Signa scanner (Andreasen et al., JAMA 272, 1994) and locally developed software (Andreasen et al., JNCN 5, 1993). Sample consisted of 83 healthy subjects (46 males; 24.8 ± 4.5 years of age; 14.7 ±1.7 years of education). Full Scale (FS) IQ was 113 ±12.2 (mean and SD); Verbal IQ, 109 ±12.8; Performance IQ, 113 ± 2.3). The study evaluated the relationship between *in vivo* volume of the cerebellum (CV) and intelligence (WAIS-R V&P FSIQ), motor dexterity (WAIS-R Finger Tapping), attention (WAIS-R Digit Span), verbal production (MAE Controlled Oral Word Association), verbal (% ret., WMS Logical Memory), and visual (% ret., Rey-Osterrieth Figure) memory. One-tailed Pearson partial correlation coefficients were calculated using volume of cerebrum as a covariate. For each significant correlation between neuropsychological function and total CV, correlations with left and right cerebellar hemisphere were determined as replications. CV correlated significantly with motor dexterity (left hand r=.218 p=.045, right hand r=.211 p=.050) and logical memory (r=.27 p=.017). Motor dexterity correlation with left (right hand r=.22, p=.04; left hand r=.24, p=.03) and right hemisphere (r hand r=.18, p=.07; l hand r=.15 p=.11) did not show a clear lateralization effect. Logical memory correlated with right and left hemispheres (rhem r=.28, p=.012, C.I=.472-.064; lhem r=.22, p=.034, C.I.=.416-.0). These results show that structural/functional relationships exist between cerebellum and effortful memory, and timed motor dexterity.

566.7

SELECTIVE DISRUPTION OF EYEBLINK CLASSICAL CONDITIONING BY CONCURRENT TAPPING: EVIDENCE FOR CEREBELLAR PROCESSING. M. Papka[1], R. B. Ivry[2], & D. S. Woodruff-Pak[1]. Lab. of Cognitive Neuroscience, Phila. Geriatric Cntr. &Temple Univ., Phila., PA 19122[1], Univ. of California, Berkeley, CA 94720[2].

The purpose of this study was to test the hypothesis that memory systems may be distinguished on the basis of their neurobiological substrates. A total of 140 young, normal adults participated in one of seven conditions. Eighty subjects were simultaneously engaged in EBCC and either a tapping task, a recognition task, a choice reaction time task, or the viewing of a video. Sixty control subjects were simultaneously engaged in the explicitly unpaired control paradigm and either the tapping, recognition, or choice reaction time task. The presumed critical neurobiological substrate for both EBCC and the tapping task was the cerebellum, and the presumed critical substrate for the recognition task was the hippocampus. Choice reaction time and video viewing were chosen as control tasks. The results revealed a selective disruption of eyeblink classical conditioning (EBCC) when it was performed concurrently with tapping. Subjects who simultaneously performed EBCC and tapping were impaired in EBCC compared to both control subjects and subjects who simultaneously performed the recognition task. The groups did not differ in unconditioned response control measures or in their awareness of testing procedures and learning. Tapping, recognition, and choice reaction time performance was comparable for subjects in the paired and unpaired control groups, although subjects in the paired group sometimes showed slower reaction times on the choice reaction task. The results provide evidence for the existence of neurobiologically distinct memory systems, and suggest that the selective disruption of EBCC, when concurrently performed with tapping, may be attributed to cerebellar involvement in both tasks. *Supported by NIA 1 RO1 AG09752 to DSW-P and NINDS 1R29 NS30256 to RBI.*

566.8

A RELATIONSHIP BETWEEN COGNITIVE PROCESSING AND PERCEPTION OF TEMPORAL DURATION IN PATIENTS WITH FRONTAL LOBE OR CEREBELLAR LESIONS. J. A. Mangels,* R. B. Ivry, & N. Shimizu. Dept. of Psychology, University of California, Berkeley, CA 94720.

Both the frontal lobes and cerebellum have been associated with deficits in temporal information processing. Patients with frontal lobe lesions often exhibit deficits on tests of temporal order. Lesions to the cerebellum can impair timing functions. Recent studies indicate a neuroanatomical connection between the cerebellum and frontal lobes. Thus, temporal processing deficits of patients with frontal lobe lesions may extend to timing abilities, such as perception of duration.

In a previous study, we found that patients with frontal lobe lesions were impaired on perceptual judgments of long durations (4000 msec). These patients were not impaired relative to control subjects on perception of short durations (400 msec) or a non-timing control task (frequency perception). Given that temporal processing of long durations (>1000 msec) may be cognitively mediated, the present study explores whether timing deficits exhibited by patients with frontal lobe lesions are related to cognitive deficits in attention and strategic processing.

Patients with dorsolateral prefrontal lesions and control subjects were tested on a series of five long duration (4000 msec) perception tasks in which sustained attention (open vs. subdivided intervals) and strategy (no strategy vs. experimenter-provided strategy) were manipulated. Across all tasks, subjects judged whether a standard interval was longer or shorter than a comparison interval of varying duration. Frontal patients and control subjects did not differ on perception of open durations. Frontal patients were impaired relative to control subjects when the standard interval was subdivided into 400 msec intervals and no strategy was provided. Yet, these groups performed comparably on the subdivided condition when the experimenter provided a counting strategy. These findings suggest that impairments of frontal patients on tasks of temporal perception result from deficits in cognitive processes, rather than deficits in perception of duration. Performance of patients with frontal lobe lesions will be compared to patients with cerebellar lesions, who may have primary deficits in time perception.

566.9

MOTOR SKILL ACQUISITION IN PATIENTS WITH PARKINSON'S DISEASE OR CEREBELLAR DAMAGE. M. M. Schugens, I. Daum, H. Ackermann and H.Topka*. Institute of Medical Psychology and Department of Neurology, University of Tübingen, 72074 Tübingen, Germany.

Models of multiple memory systems discuss the role of different brain structures in the acquisition of declarative and nondeclarative memory. The cerebellum as well as the basal ganglia have been implicated in the acquisition of motor skills. In the present study a group of patients with selective cerebellar damage and a group af patients suffering from Parkinson's disease were investigated on two versions of a computerized tracking task. The first task involved the tracking of a small target on a computer screen with a pointer controlled by a joystick. The horizontal movement of the target was regular whereas the vertical movement of the target was irregular. In the second task both directions of of target movement were irregular; the movement of the joystick and the target in the horizontal direction were reversed.

Patients with damage of the cerebellum displayed less learning than the respective control group in task 1 where a regular movement of the target allowed automation of the tracking movement whereas no such deficit was observed in the mirror reversed task (task 2). The patients suffering from Parkinson's disease acquired the task with the regular component as well as the control group, but learning deficits were observed in task 2 when the joystick movement was reversed and the target moved irregularly.

The results indicate that damage to the cerebellum impairs the acquisition of motor skills that involve the preprogramming of movement whereas damage of the basal ganglia circuitry is detrimental to the learning of motor skills that put higher demands on hand-eye coordination.

Supported by the German Research Society (DFG Da 259/1-2, SFB 307).

566.10

CONTRIBUTION OF THE STRIATUM AND CEREBELLUM TO THE AUTOMATIZATION OF A VISUOMOTOR SKILL ASSESSED WITH A DUAL-TASK PARADIGM. J.Doyon*[1,2,3], R.Laforce Jr.[1,2], D.Gaudreau[1,2], J.-P. Bouchard[2,3], and P.J.Bédard[2,3]. [1]Ecole de Psychologie, [2]Centre de Recherche en Neurobiologie, Québec, Canada, G1K 7P4, Université Laval, et [3]Département des Sciences Neurologiques, Hôpital de l'Enfant-Jésus, Qc, Canada, G1J 1Z4.

Research from our laboratory has recently demonstrated that the disruption of either the striatal or the cerebellar circuitry in patient populations may adversely affect the implicit learning of a repeated visuomotor sequence late in the acquisition process. The present study examined whether this impairment was due to a lack of automatization of the skill using a dual-task paradigm. To this end, the performance of nine Parkinson's disease patients (PD) (Stage 1, n=6, Stages 2-3, n=3) and of 11 cerebellar patients (CER) was compared to that of groups of normal subjects (n=9 and n=12, respectively) on the Repeated Sequence Test, while subjects were performing the Brooks Matrices presented auditorily as a secondary task in both early and late phases of learning. Subjects were required to complete 8 matrices concomitantly with 8 separate blocks of three embedded sequences after having received either 40 (Session 1), or 240 (Session 6) presentations of the sequence. As expected, a dual-task interference was observed in both Sessions 1 and 6, although the groups never differed significantly in their pattern of results on the primary task. By contrast, an impairment on the Brooks Matrices was observed only in the patient groups that had already shown a learning deficit in our previous studies (ie. PD[2-3] and CER), hence suggesting that the latter subjects did not have access to the same level of residual cognitive resources to complete the matrices compared to controls. Such findings are consistent with the notion that both the striatum and the cerebellum play an important role in the automatization of a visuomotor skill.

566.11

TEMPORAL OCCIPITAL LESIONS IMPAIR PRIMING DURING WORD STEM COMPLETION L. Nielsen-Bohlman,* D. Swick, M. Ciranni, R. T. Knight & A. P.Shimamura, Dept. Neurology & Center for Neuroscience, UC Davis & Dept. Psychology, UC Berkeley

Posterior cortical involvement in priming processes is supported by deficits in word priming in Alzheimer's dementia and PET studies of word priming. We examined word stem completion priming in five patients with unilateral temporal-occipital vascular lesions (3 left with hippocampal involvement, 2 right with hippocampal sparing) identified from CT or MRI scans (average lesion volume 33cc) and eight age-matched control subjects. The patients scored within the normal range on the WAIS-R and were variably impaired on the WMS-R. The Warrington Recognition Memory Test showed that while left hemisphere lesion patients showed impaired word recognition, possibly related to their hippocampal lesion or syndrome of alexia without agraphia, all lesion patients had normal recognition for faces. In the study phase, subjects were sequentially shown 10 words (e.g. MOTEL) and asked to rate the pleasantness of each word (i.e., incidental learning). Immediately following word presentation, twenty word stems (eg. MOT) were presented, and subjects were asked to complete the stems with the first words that came to mind. Study words could be used to complete 10 stems, and the other stems were used to assess baseline guessing rates. Lesion patients and control subjects showed comparable baseline word generation rates (control=8%, patient=12%). However, patients completed fewer word stems with previously presented words (control=43% patient=22%, p=0.064), showing impairment of word priming. No difference was seen between left and right lesion groups. These data suggest that word priming is mediated by inferior temporal and occipital cortex.

566.12

SELECTIVE LOSS OF MEMORY FOR MUSIC AFTER BILATERAL DAMAGE TO THE AUDITORY CORTEX. **I. Peretz*. G.R.E.N.E., Univ. of Montreal, Montreal (Qué), H3C 3J7, Canada.**

C.N. is a 40-year old right-handed nurse who suffered from a severe auditory agnosia limited to music after bilateral damage to the auditory cortex. C.N., whose cognitive and speech functions are otherwise normal, is totally unable to identify or to experience a sense of familiarity with musical excerpts that were once highly familiar to her. However, she can recognize the lyrics that usually accompany the songs. She can also identify familiar sounds, such as animal cries. Thus, her agnosia appears highly specific to music (Peretz, Kolinsky, Tramo et al., *Brain*, 1994). A follow-up study shows that C.N. has now recovered most perceptual skills for music. This recovery is not accompanied by any signs of improvement in music recognition, which remains extremely poor. She is still markedly impaired at naming a tune and at judging its familiarity, as well as at memorizing familiar as well as novel music. This deficit is found to be not only modality-specific but music-specific as well. The findings suggest the existence of a perceptual memory that is specialized for music and that can be selectively damaged so as to prevent most forms of recognition ability.

566.13

ARENAMAZE - A VIRTUAL 'WATERMAZE' FOR HUMANS I.C. Reid, N.F. Wright and L.J Whalley* Aberdeen Univ., Dept. of Mental Health, Aberdeen, UK.

The Morris Open Field Watermaze has become a widely used test of spatial learning in the rat. Few spatial tests used with human subjects capture the navigational aspects of the watermaze in a convenient way. Previous attempts have generally used real world solutions, such as the large radial maze painted out on a field by Glassman et al (Brain Res. Bull. 34:151-159, 1994). Such tests are expensive and unwieldy.Virtual space, modelled on a computer, may be more convenient, reliable and cheaper.

We used a modification of the popular computer game "DOOM" (ID software) running on a conventional 486PC to create a "virtual" open field maze for humans. "DOOM" can be edited using a shareware package (DEU2) to create the 2-D illusion of a 3-D arena surrounded by "virtual" extra-maze cues and containing a fixed "hidden" goal escape platform viewed as if from a first person perspective. Using features available in the regular game, subjects can be "teleported" to random start locations within the arena from which to search for a hidden goal area using a trackball mouse. Escape latency measures (and probe trials to measure spatial bias in the absence of the goal platform) can easily be recorded in a manner analogous to those used in the rat Watermaze. The apparent dimensions of the environment were scaled up from the rodent version of the task.

The performance of 7 amnesic (Korsakoff) patients was compared on the task with 6 control subjects. Following a non-spatial practice session, each group was given 9 trials in the spatial task in a single session, followed by a probe trial. The Korsakoff group showed significantly elevated latencies across trials and reduced spatial bias. Further modifications of the program permitted us to develop control non-spatial tasks and a"virtual" radial maze. While this work is clearly at a preliminary stage (and the use of dedicated 3-D modelling software may produce a more professional-looking interface) our findings demonstrate the potential value of simple and inexpensive "VR-like" approaches to testing cognitive function.

566.14

SPATIAL-ARRAY LEARNING BY PATIENTS WITH FOCAL TEMPORAL-LOBE EXCISIONS. J. Crane, B. Milner*, & G. Leonard, McGill University, Montreal, Quebec, Canada, H3A 2B4.

Smith and Milner (1989) demonstrated a role for the right hippocampal region in delayed but not in immediate recall of object locations. Because all these patients' removals included lateral temporal neocortex, a combined-lesion effect could not be ruled out. We examined this question directly by testing patients who had undergone selective amygdalo-hippocampectomy (AH), or anterior temporal-lobectomy (TL). The paradigm involved learning supra-span spatial arrays over successive trials. Included were 18 AH patients [7 left (LAH) and 11 right (RAH)], and 31 TL patients (15 L and 16 R) who were categorized further based on extensive (11 LTH and 8 RTH) or minimal (5 LTh and 7 RTh) hippocampal encroachment. Fourteen age- and education-matched normal control subjects (NC) were also tested. No group differences were found in recall immediately after the first presentation (Trial 1), but ANOVA revealed a strong group effect for number of trials to criterion. Post hoc tests confirmed that both the LTh and NC groups required fewer trials than the RTH and RAH groups. In addition, the LTH group was superior to the RTH group. Our findings suggest that right medial temporal-lobe structures are critically involved in the learning but not in the encoding of object-location information.

566.15

INTACT IMPLICIT MEMORY FOR NEWLY-FORMED VERBAL ASSOCIATIONS IN AMNESIC PATIENTS. Y. Goshen-Gottstein, Dept. of Psychology,Tel-Aviv University, and Morris Moscovitch*, Rotman Institute of Baycrest Centre and Erindale College, University of Toronto, Mississauga, Ontario, L5L 1C6. Canada.

Implicit and explicit memory for newly-formed associations between two random words was tested in ten amnesic patients with various etiologies. At study, subjects saw two simultaneously-presented words on a screen (120 pairs in all) and formed a sentence containing both words. At test, subjects saw 40 of the same old pairs, 40 recombined pairs in which a word from one pair was recombined with a word from another and 80 pairs containing new words which had not been studied. In the implicit test, subjects saw two letter strings and had to determine whether both formed words. In the explicit test, subject had to determine whether both words had appeared at study. Despite being severely impaired on the explicit test, amnesic patients performed like control subjects on the implicit test: responses were fastest to old pairs, intermediate to recombined pairs, and slowest to new pairs. Contrary to many theories, the results indicate that amnesic patients, even those with bilateral medial temporal-lobe damage, can form and retain new associations. Evidence indicates that these associations are perceptually based.

566.16

PERIRHINAL CORTEX IN HUMAN AND MONKEY: TOPOGRAPHY, NEUROCHEMISTRY AND PATHOLOGY IN ALZHEIMER'S DISEASE. A. Solodkin*, C. Dennison , J. Towsend and G.W. Van Hoesen. Anatomy and Neurology. University of Iowa College of Medicine. Iowa City, IA 52242.

The perirhinal cortex (area 35) is a classic proisocortex located "around" the rhinal sulcus in mammals. It is bordered medially by the entorhinal cortex (area 28) and laterally by the inferior temporal cortex (area 36), however, it has not been easy to establish its limits with these areas. Our findings in monkey and human temporal cortex, based on multiple methods, were aimed to precisely delimit area 35.

Fixed tissue from monkey and human temporal cortex was stained for calcium-binding proteins, non-phosphorylated neurofilament protein, AChE, NADPH-d and in AD with Thioflavine-S and Alz-50.

Results in the monkey using calbindin-D28 and SMI-32 revealed that the perirhinal cortex, lies along the lateral bank of the rhinal sulcus, and is formed at least by two sub-areas. These can be segregated clearly from areas 28 and 36.

In the human, area 35 has a columnar organization which can be highlighted by parvalbumin, AChE staining and in AD pathology. This columnarity, conveniently marks the anterior-posterior extent of this cortex, and reveals that area 35 in anterior areas, can occupy both banks of the rhinal sulcus, whereas in posterior parts, it is located along the medial bank of the collateral sulcus. Additionally, at least two sub-areas, were noted.

In summary: (1) good markers exist to locate area 35; (2) there are differences in area 35 among primates, with humans having evidence for a columnar or vertical organization; (3) the perirhinal cortex is greatly expanded in the human brain and segregated into modular units. These are affected early in AD and may be linked to its early memory impairments. Supported by NS 14944 and PO NS 19632.

566.17

EFFECTS OF LESIONS OF EITHER THE AMYGDALA OR ANTERIOR RHINAL CORTEX ON CROSSMODAL DNMS IN RHESUS MACAQUES. S. Goulet* and E.A. Murray. Lab of Neuropsychology, NIMH, NIH, Bethesda, MD 20892, U.S.A.

Naive rhesus monkeys were trained preoperatively on both visual and tactual versions of delayed nonmatching-to-sample (DNMS) using a set of 40 objects. Each day, the 40 objects were randomly assigned anew to 20 pairs, to be used for the 20 trials comprising a test session. Ten seconds intervened between sample presentation and choice. Monkeys were trained until they scored 90 correct responses in 100 trials, first in vision then in touch. Next, they were confronted with the crossmodal version of the task, which used a sample presentation in the dark but a choice test in the light. After reaching the same performance criterion as in the intramodal phases or completing 1000 trials of training, monkeys received either bilateral aspiration lesions of the anterior rhinal (i.e. entorhinal plus perirhinal) cortex (AntRh, N=2), bilateral excitotoxic lesions of the amygdala (A, N=2) based on magnetic resonance images from those animals, or were retained as unoperated controls (CON, N=2). Following surgery or rest, we assessed each monkey's retention of the crossmodal task by calculating its deficit score (mean percent correct over last 100 trials pre-op or pre-rest minus mean percent correct over first 300 trials post-op or post-rest). Group A was not impaired relative to Group CON. These preliminary results argue against the idea that the amygdala is critical for crossmodal associations. By contrast, Group AntRh was impaired relative to Group CON (deficit score of 22 versus 9). Moreover, neither of the AntRh subjects reattained the criterion within the 1000-trial training limit, whereas all subjects in Groups A and CON did. These data suggest that the anterior rhinal cortex mediates retention of tactual-to-visual crossmodal associations.

566.18

EARLY LESIONS OF THE HIPPOCAMPAL FORMATION IN PRIMATES IMPAIR CONTEXT-DEPENDENT RECOGNITION MEMORY. O. Pascalis and J. Bachevalier.* Dept. Neurobiology & Anatomy, Univ. Texas Sch. Med. Houston Tx, 77225.

Both early and late lesions of the hippocampal formation in primates are known to yield few if any effects on visual recognition memory (Mishkin & Murray, 1994 Curr. Op. Neurobiol., 200, 4). However, this lack of impairment may not reflect intact recognition memory, but rather it might indicate that the experimental parameters were not appropriate to detect the impairment. To test this possibility, 3 normal adult monkeys (N) and 3 with neonatal lesions of the hippocampal formation (H), including the hippocampus proper and parahippocampal gyrus, were trained in a modified version of a paired-comparison task. In this task, the animals were familiarized with a picture of an object presented over a background for a cumulative 20-sec period. After a 10-sec delay, the same object was paired with a novel one for two retention trials of 5-sec each. In these retention trials, the familiar and novel objects were presented in six different conditions. In three conditions, the familiar object was over the same background used during the familiarization period and in the other three, it was over a new background. Animals in Group N showed a preference for the novel objects in all conditions (average 63%), whereas those in Group H showed a preference for the novel objects only when the familiar objects were presented over their original backgrounds (average 63% vs 49%). These results suggest that neonatal lesions of the hippocampal formation impair recognition memory when the context in which an object is learned has been changed, and strengthen the proposal that the hippocampal formation may be critical for contextual and/or relational memory (Eichenbaum, et al., 1994, Beh. & Brain Sci., 17,3). *Supported by NIMH grant MH49728, the John D. and Catherine T. MacArthur Foundation and a Fellowship from the Fondation Fyssen to OP.*

566.19

DIFFERENTIAL CHOLINERGIC DYNAMICS ASSOCIATED WITH PERFORMANCE ON DELAYED NON-MATCHING TO SAMPLE AND DELAYED RESPONSE TASKS: AN *IN VIVO* MICRODIALYSIS STUDY IN NON-HUMAN PRIMATES. T.D. Smith*, R.C. Saunders and D.R. Weinberger. Clinical Brain Disorders Branch, NIMH, Bethesda, MD 20892.

In vivo microdialysis was used to monitor extracellular levels of acetylcholine (ACh) in hippocampus (HC) and prefrontal cortex (PFC) in alert rhesus monkeys. Previously we demonstrated that substantial increases in extracellular PFC and HC ACh levels are associated with performance on control motor tasks, as well as on memory tasks. Because no difference was seen between tasks, the present study was undertaken in order to determine whether or not differential ACh overflow is associated with task difficulty. ACh was monitored in HC and PFC while monkeys performed delayed response (DR) (retention intervals of 1 and 10 s), a spatial working memory task, or delayed non-matching to sample (DNMS) (list length of 1, 10, or 20 items), a visual recognition memory task. Microdialysis probes placed into HC and PFC were perfused with artificial CSF at a rate of 1.1 ul/min. Samples were collected every ten minutes and assayed for ACh using HPLC with electrochemical detection. After a stable ACh baseline was established, monkeys performed a task for 20 min and then sat quietly during recovery of neurochemicals back to baseline. This same protocol was then repeated with another task. As previously demonstrated, an increase in extracellular PFC and HC ACh levels was associated with performance on both tasks, with peak overflow occurring after completion of the tasks. Enhanced ACh levels could be abolished by tetrodotoxin infusion. Preliminary results from one monkey, but with several replications, suggests a double dissociation in ACh overflow related to task difficulty and the region sampled. Thus, in PFC a greater ACh overflow was associated with a 10 s delay during performance on DR as compared to a 1 s delay (peak 35% vs 15%, respectively). In contrast, HC ACh overflow was similar at both delays. Conversely, differential levels of HC ACh overflow (35% and 15%) were related to increased list length (1 and 20, respectively) during performance on DNMS, while PFC ACh overflow was not differentiated. These preliminary results suggest that ACh may be differentially involved in PFC and HC during cognitive activation.

566.20

INCREASE IN CEREBRAL ACETYLCHOLINE ASSOCIATED WITH BEHAVIORAL PERFORMANCE IN MONKEYS: AN *IN VIVO* MICRODIALYSIS STUDY. Y. Tang* and T. G. Aigner. Laboratory of Neuropsychology, National Institute of Mental Health, Bethesda, MD 20892-4415

Previous lesion studies in monkeys suggest that rhinal cortex, temporal cortex, and hippocampus are involved in recognition memory. In addition, pharmacological studies have identified the neuromodulator acetylcholine (ACh) as playing a significant role in mnemonic processes. In the present study, extracellular levels of ACh and choline in perirhinal cortex (PR), inferior temporal cortex (IT), and dentate gyrus (DG) of the hippocampus in 2 awake, behaving rhesus monkeys (*macaca mulatta*) were monitored using *in vivo* microdialysis. Animals were trained to perform an automated version of a recognition memory task, delayed nonmatching-to-sample (DNMS) with lists of 10 symbols, and a non-memory control task, plus-symbol-touching (PST). Magnetic resonance imaging scans were used as a guide to place microdialysis probes into PR, IT, and DG. Probes were perfused with lactated Ringer's solution containing 10 µM neostigmine at the rate of 1.0 µl/min; samples were collected every 20 min beginning 1 hr after probe placement and analyzed for ACh and choline concentrations by HPLC coupled with electrochemical detection. Performance on DNMS was associated with 41%, 26%, and 24% increases in ACh overflow in PR, IT, and DG, respectively. During performance of the PST task, ACh overflow increased 34%, 24%, and 7% above baseline in PR, IT, and DG, respectively, then returned to baseline levels when the monkey again sat quietly. The increases of ACh overflow are statistically significant ($p<0.05$, $n=9$, *vs* the levels of ACh while the monkeys sat quietly). By contrast, choline levels decreased without any relation to performance on the behavioral tasks. The results indicate that cholinergic activity in PR, IT, and DG increases during processes of memory, attention, and arousal in nonhuman primates.

566.21

INCREASED METABOLIC ACTIVITY IN THE ENTORHINAL AND PERIRHINAL CORTICES AND SUBICULUM IN MONKEYS PERFORMING WORKING MEMORY TASKS AS REVEALED BY THE 2-DEOXYGLUCOSE METHOD.

Davachi, L.*, Friedman, H.R. and Goldman-Rakic, P.S. Yale School of Medicine, Section of Neurobiology, New Haven, Connecticut, 06510, U. S. A.

As a continuation of our investigation on the role of the hippocampal formation and adjacent cortices in memory function, we have most recently analyzed metabolic activity in the entorhinal and perirhinal cortices as well as the subicular complex of the hippocampus in monkeys performing working memory or control tasks. Rhesus monkeys were trained in a Wisconsin General Test Apparatus to perform a manual delayed spatial alternation (DA), delayed object alternation (DOA) or a control task. Local cerebral glucose utilization (LCGU) during the performance of these tasks was then measured and quantified using a single label (^{14}C) 2-deoxyglucose paradigm. LCGU was significantly elevated ($p < .05$) in the medial and lateral entorhinal regions (17.9% and 21.2% higher, respectively), rostral perirhinal area 35 (19.3%), caudal perirhinal area 36 (19.6%) and the subiculum (25.6% increase) of monkeys performing working memory tasks (DA and DOA) compared to those performing control tasks. As a measure of individual differences in blood flow , the LCGU of the medial geniculate body was analyzed in all animals because each was exposed to the same white noise during the experiment. This analysis revealed no significant difference between groups. The finding that the entorhinal and perirhinal cortices are more active during working memory tasks than control tasks is consistent with lesion data in monkeys, demonstrating a memory impairment in delayed match-to-sample tasks after rhinal cortex lesions with sparing of concurrent object discrimination and other tests of associative (long term) memory (Gaffan, D. and Murray, E. A.., 1992, Behavioral Neuroscience, 106, 30-38). Not only do these data provide further evidence of medial temporal structure involvement in working memory, they also provide a functional analysis of the anatomical network connecting the prefrontal cortex to the hippocampal formation (Goldman-Rakic, P. S. et al., 1984, Neuroscience, 12, 719-743). Supported by ONR grant N00014-91-J-1251.

566.22

AFFECTIVE AND ATTENTIONAL SHIFTING: A FUNCTIONAL DISSOCIATION BETWEEN THE DORSOLATERAL AND ORBITAL PREFRONTAL CORTICES OF THE MONKEY.

R. Dias, T.W. Robbins and A.C. Roberts*. Department of Experimental Psychology, University of Cambridge, Cambridge, CB2 3EB, UK.

Perseverative responding on visual discrimination reversal tasks has been reported widely following lesions of orbital prefrontal cortex (pfc) in monkeys. This has been likened to the perseverative responding described in patients with frontal lobe damage on the Wisconsin Card Sort Test, a test of set-shifting ability. However, in man, dorsolateral pfc, and not orbital pfc, has been identified as the critical locus for the deficit. This discrepancy between the monkey and human literature may be misleading as there are fundamental differences in the cognitive processes underlying performance of these two types of task. To reconcile this discrepancy, the present study investigated the effects of excitotoxic lesions of dorsolateral and orbital regions of the prefrontal cortex in the marmoset on both attentional set-shifting and visual discrimination reversal learning within the same paradigm.

The results demonstrate that dorsolateral pfc, and not orbital pfc, is the critical locus for attentional set-shifting. In contrast the orbital pfc, and not the dorsolateral pfc, is the critical locus for visual discrimination reversal learning. This double dissociation would suggest that the dorsolateral pfc provides flexibility at the level of attentional selection whilst the orbital pfc provides flexibility at the level of affective processing. These results have implications for how we analyse the cognitive and affective changes associated with neuropsychiatric disorders including schizophrenia and depression.

566.23

A NOVEL TEST OF SPATIAL WORKING MEMORY IN PRIMATES: CONTRASTING EFFECTS OF EXCITOTOXIC LESIONS AND DOPAMINE DEPLETION OF THE PREFRONTAL CORTEX IN THE MARMOSET. P. Collins, B. J. Everitt, T.W Robbins and A.C. Roberts. Department of Experimental Psychology, University of Cambridge, Cambridge, CB2 3EB U.K. (SPON:European Brain and Behaviour Society)

Patients with frontal lobe damage or Parkinson's disease (PD) are impaired on a self-ordered searching task designed to measure spatial working memory. We have previously described the development of a primate analogue of this task, in which on any given trial, a computer presents a pre-defined number of identical blue squares at random sites across a touch-sensitive screen. The monkeys task is to touch each square once, and once only, in a self determined sequence to obtain reward. In control animals performance declined from 90% correct on sequences of 2 squares to 30% correct on sequences of 5 squares and was correlated with the development of a spatial searching strategy. The present experiments assessed the contribution of the prefrontal cortex and it's dopamine input to the performance of this task. Excitotoxic lesions of prefrontal cortex produced a marked impairment in task performance which was associated with profound perseveration. In stark contrast, selective lesions of the mesocortical dopamine input to the prefrontal cortex, which degenerates in PD, did not effect task performance. However, both lesion groups were impaired on a manual spatial delayed-response task, a classical measure of spatial working memory in which strategies are not necessary for successful performance. Since the utilisation of a spatial strategy is a key constituent of the self-ordered searching task, it may be that this cognitive function protects spatial working memory from the effects of prefrontal dopamine depletion in tasks in which strategies aid performance. These data suggest that the dopamine dependent deficit seen in PD may be due to striatal dopamine depletion and will be discussed in the light of the effect of these lesions on alternative tests of frontal lobe function in monkey and man.

567.1

HEMIDECORTICATION AND REVERSAL LEARNING IN THE RAT: HEMISPHERIC ASYMMETRY FOR TENDENCY TO PERSEVERATE.

M. Noonan* & S. Axelrod. Canisius College and SUNY at Buffalo.

Noonan and Axelrod (1992) reported a consistent hemispheric asymmetry in rats for the ability to master the second task of a two-task sequence. In the present experiment we tested the hypothesis that this effect was due to an asymmetrical representation of the tendency to perseverate.

Rats were taught a left-right response differentiation in a water-maze, and, based on their turning biases during acquisition, were categorized as either left-turners or right-turners. Rats from both groups were then subjected to either left- or right-hemidecortication, and tested six weeks later on a reversal of the initial task. Scores on the reversal task revealed significant hemispheric differences which were in opposite directions for the turning groups.

The results are taken as compatible with the hypothesis that the tendency to perseverate is asymmetrically represented in the rat. The data suggest that, for each group, the more perseverative hemisphere is contralateral to the turning bias shown during acquisition of the initial learning.

567.2

CLASSICALLY CONDITIONED JAW MOVEMENT AND HEART RATE RESPONSES IN RABBITS WITH MEDIAL PREFRONTAL LESIONS. Joselyn McLaughlin and D.A. Powell.* VA Medical Center and University of South Carolina, Columbia, SC 29208.

Damage to the medial prefrontal cortex (mPFC) prevents acquisition of classically conditioned heart rate (HR) decreases during aversive conditioning. This experiment focused on whether similar effects would be observed during appetitive conditioning. Rabbits with bilateral (mPFC) lesions were compared to sham lesioned rabbits in an appetitive conditioning experiment in which tones were CSs and an intraoral injection of sweetened water was the US. A third group of sham rabbits received unpaired tone and water presentations. Significant jaw movement conditioning occurred in the sham and lesioned animals compared to the unpaired sham control group. However it was accompanied by tone-evoked HR decelerations early during training but only in the sham-paired group. All animals showed accelerative HR CRs during later sessions; moreover, animals with mPFC lesions showed greater conditioned accelerations than the unpaired or sham-paired group. These findings suggest that the lesion prevented normal parasympathetic inhibitory control of the heart, resulting in greater sympathetic activation.

Supported by VA Institutional Research Funds

567.3

THE EFFECTS OF MEDIAL FRONTAL LESIONS ON OBJECT DISCRIMINATION AND REVERSAL TASKS USING A NEW COMPUTER-AUTOMATED TOUCHSCREEN TESTING PROCEDURE FOR THE RAT.

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Recently it has been reported that lesions of the anterior cingulate cortex in the rat produce a variety of behavioural impairments. For example, while such lesions fail to affect simple visual discrimination learning, they do impair the acquisition of an 8-pair concurrent visual discrimination task (Muir et al., Soc. Neurosci. Abstr. 20:1211, 1994). Furthermore, rats with lesions of the anterior cingulate cortex are able to learn a conditional visual discrimination task at a faster rate then control animals (Bussey et al., Soc. Neurosci. Abstr. 19: 1233, 1993). In attempting to provide an explanation for these results, we have suggested that the anterior cingulate cortex may be involved in the acquisition of stimulus-reinforcement associations.

However, the connections between the rostral medial frontal area (MF) and structures such as the nucleus accumbens suggest that this region may also be important in the acquisition of stimulus-reinforcement learning. In addition, Petrides (Behav. Brain Res. 16: 95-101, 1985) has suggested that anterior frontal cortex in the primate is important for conditional learning. It remains to be demonstrated whether the anterior cingulate and medial frontal cortices in the rat are functionally dissociable. Thus, in the present study, following excitotoxic lesions of the pre-genu MF cortex, rats were tested on tasks previously shown to be sensitive to cingulate cortex damage including 8-pair concurrent and conditional discrimination learning. Somewhat surprisingly, lesions of the MF cortex had no effect on any of these tasks but did produce a significant impairment on reversal of a simple discrimination, even though acquisition of this simple visual discrimination was unimpaired. Together with our earlier findings, these results suggest that the anterior cingulate cortex may mediate stimulus-reinforcement learning while the medial frontal cortex in the rat may be important for the inhibition of prepotent responding.

567.4

THE EFFECTS OF CINGULATE CORTEX LESIONS IN THE RAT ON THE ACQUISITION OF SIMPLE AND CONDITIONAL VISUAL DISCRIMINATION OF COMPUTER GRAPHIC STIMULI IN A TOUCHSCREEN TESTING APPARATUS. T.J. Bussey*, J.L. Muir+, B.J. Everitt and T.W. Robbins. Dept. of Experimental Psychology, University of Cambridge, Cambridge CB2 3EB and +School of Psychology, Univ. of Wales College of Cardiff, Cardiff CF1 3YG, U.K.

It is becoming increasingly clear that the cingulate cortex in the rat can be subdivided into anterior and posterior components which are functionally dissociable. For example, we have recently reported that anterior cingulate lesions produce enhanced learning of a conditional visual discrimination task carried out in a Skinner box, whereas posterior cingulate lesions produce an impairment (Bussey et al., Soc. Neurosci. Abstr. 19:1233, 1993). Furthermore, using a new automated touchscreen testing procedure involving the presentation of computer-graphic stimuli, we have shown that on an 8-pair concurrent discrimination task, the opposite pattern of results is obtained with these lesions (Muir et al., Soc. Neurosci. Abstr. 20:1211, 1994).

In the present study, we employed the touchscreen testing method to determine whether the pattern of effects obtained on the conditional task would be obtained using a touchscreen version of this task, in which the opportunity for stimulus sampling and the position of the animal prior to responding were tightly controlled. In the touchscreen task, a discriminative stimulus was presented in the central of three response windows. The rat was required to nose-poke this stimulus, the identity of which provided a cue as to which of two responses (nose-poke right or left) would result in reward. Posterior cingulate lesioned animals were impaired in acquisition during the late stages of learning, thus replicating our findings using the Skinner box version of this task. Anterior cingulate lesions had no effect. There was also no effect of either anterior or posterior cingulate cortex lesions on acquisition of a simple visual discrimination or reversal, suggesting that the effects obtained on the conditional tasks were not due to perceptual factors. This pattern of results will be discussed in terms of our recent proposal that anterior and posterior cingulate cortices may be involved in the acquisition of stimulus-reinforcer and stimulus-response associations respectively.

567.5

THE RELATIONSHIP BETWEEN TACTILE DISCRIMINATION LEARNING AND ACETYLCHOLINE RELEASE IN FRONTAL AND SOMATOSENSORY CORTICES: AN *IN VIVO* MICRODIALYSIS STUDY IN FREELY-MOVING RATS. **A.E. Butt* & R.W. Dykes. Département de Physiologie, Université de Montréal, Québec, Canada H3C 3J7.**

We examined the relationship between cortical acetylcholine (ACh) release and tactile discrimination learning in rats. After microdialysis probe guides were implanted in the frontal cortex and in the vibrissae field of the somatosensory (SI) cortex, animals were pre-trained to enter either of two chambers from a common starting location for food reinforcement. Microdialysis probes (3 mm) were then inserted and animals underwent five daily sessions (30-trials) of either tactile discrimination training or control procedures. Microdialysis samples were collected from frontal and SI cortices for 1 hr in the homecage prior to testing and for the duration of testing each day by perfusing the probes with Ringer solution and neostigmine (2 nM). Based on reports of enhanced ACh release in response to appetitive reinforcement-based conditioning procedures (Inglis, Day, & Fibiger, 1994; Sarter & Bruno, 1994), it was hypothesized that ACh release in the frontal cortex in both groups would be greater during testing, compared to baseline release in the homecage. And as demonstrated by Inglis et al. (1994), it was expected that ACh release in frontal cortex would increase with training in both groups. It was further hypothesized that ACh release in the SI cortex would be greater in the discrimination-trained animals than in controls, and that SI ACh release would increase over the course of training in the tactile discrimination group in parallel with increased discrimination performance accuracy. Results confirmed the hypothesis that appetitively reinforced conditioning procedures would cause a progressive enhancement in the release of ACh in frontal cortex in both groups. Moreover, the pattern of ACh release in frontal and SI cortices differed between discrimination-trained and control animals; during the task, discrimination-trained animals showed greater ACh release in SI cortex than controls, and the extent of this release was related to degree of discrimination training. (Supported by the MRC of Canada).

567.6

PRETEST INFUSION OF LIDOCAINE INTO THE INSULAR OR MEDIAL PREFRONTAL CORTEX IMPAIRS RETRIEVAL OF REMOTE MEMORY IN AN INHIBITORY AVOIDANCE TASK. K. C. Liang* & W.-L. Liao. Psychology, Natl.' Taiwan Univ. & Neuroscience, Natl.' Yang-Ming Univ., Taipei, Taiwan, R.O.C.

Extensive evidence implicates the amygdala in formation of affective memory. Our previous findings further indicate that in an inhibitory avoidance task, inactivation of the amygdala impaired retrieval of a recent (1-day) but not a remote (21-day) memory. The cerebral cortex is implicated in long-term storage of memory. In search of the neural circuitry critically involved in expression of a remote memory, this study examined the roles of three cortical regions receiving amygdala projections in retrieving memory formed 1 or 21 days ago in an inhibitory avoidance task.

Male Wistar rats received surgery to implant cannulae bilaterally into the insular, medial prefrontal or perirhinal cortex. Rats were trained on a one-trial step-through inhibitory avoidance task with a 1.14 mA/1 s footshock. Five minutes prior to the retention test given 1 or 21 days after training, rats received intra-cortical infusion of vehicle or 2% lidocaine. Results indicated that in the 1-day retention test, independent or conjoint inactivation of the insular and medial prefrontal cortices had no effect on retention. In contrast, such treatments given in the 21-day test severely impaired memory expression. Pretest infusion of lidocaine into the perirhinal cortex, while did not impaired retention in the 1-day test, would impair memory expression in the 21-day test only if the rats had previously been tested on a 1-day test.

These data, taken along with previous ones, suggested that the amygdala and its associated cortices, at least the insular or medial prefrontal cortex, play important roles in retrieving, respectively, recent and remote memory of affective experience. They are consistent with a notion that the long-term storage of affective memory might involve cortical circuits.

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567.7

MEMORY FOR OBJECT-OBJECT PAIRED ASSOCIATES: ROLE OF THE PREFRONTAL CORTEX. N. Kurzina*, M. Granholm and R.P. Kesner. Dept. of Psychology, University of Utah, Salt Lake City, UT 84112

It has been shown previously that infralimbic-prelimbic (IPL) cortex lesions impair working memory for visual object information. In order to test whether this working memory deficit would also contribute to paired associate learning for visual objects, the role of the IPL of the rat frontal cortex was investigated in an object-object paired associate task.

Based on a successive discrimination go/no-go paradigm, rats were trained to remember that two pairs of objects (AB and CD as well as BA and DC) resulted in reinforcement (positive trial). All other combinations of the same objects (AD, BD, DB, etc.) were non-reinforced (negative trials) and were considered to be mispairs.

After animals learned this task to a criterion of at least a two second difference between positive and negative trials during two consecutive weeks (200 trials), the animals received under Nembutal anesthesia either electrolytic lesions of the IPL or served as sham-operated controls. After a one week recovery period they were tested for retention of the task for 3 sets of 200 trials each.

The results indicate that rats with damage of the IPL areas did not have a severe deficit in performance of this task compared to presurgery level and sham-operated rats. It is likely that the IPL of the rat frontal neocortex is not critical for object-object relationship performance, even though IPL cortex is involved in working memory for visual object information.

567.8

A NEURAL SYSTEM ANALYSIS OF SPATIAL RECOGNITION MEMORY: ROLE OF THE PREFRONTAL CORTEX. T. Janis, R.P. Kesner*. Dept. of Psychology, University of Utah, Salt Lake City, UT 84112

Based on prior research implicating the ventral lateral orbital cortex (VLO) and prelimbic and infralimbic cortex (IPL) as part of the prefrontal cortex in processing spatial information, the role of VLO and IPL in mediating working memory for spatial information was investigated using a continuous recognition spatial memory task. Rats were each allowed access to 12 arms of a 12 arm maze from a central platform. Access required that the rat orient to a cue on a clear Plexiglas door of the designated arm; upon orientation, the door was opened and latency to reach the end of each arm was measured. Of the 12 presentations per trial, three or four of the arms were repeated, but did not contain reinforcement. Repeated arms were presented with lags ranging from 0 to 6 (from 0 to 6 different arm presentations between the first and the repeated presentation). The dependent variable consisted of the difference in latencies between the first and the second (repeated) presentation of an arm. Rats were trained to a criterion of a grand mean (all lags) 3 second difference between the first presentation and the repetition based on 16 trials (one per day) for a total of 8 presentations per lag. After they reached criterion, the rats received under Nembutal anesthesia electrolytic lesions of the VLO, IPL or served as sham operated controls. One week after recovery they were retested for 32 trials. Results indicated that, relative to controls, rats with IPL lesions, but not VLO lesions, were impaired in continuous recognition memory performance. These results indicate that IPL cortex, but not VLO, may be part of a working memory for spatial information ciruit.

567.9

IDENTIFICATION OF VISUAL TARGETS BY SPATIAL CONTEXT IN THE GERBIL: THE EFFECTS OF CORTICAL LESIONS. M. G. Bigel*, L. Sharma, and C. G. Ellard, Department of Psychology, University of Waterloo, Waterloo, ON, N2L 3G1

Mongolian gerbils (*Meriones unguiculatus*) use retinal image size (RIS) to calibrate distance in a jumping task. The RIS algorithm depends on correlation of image size with target distance. When gerbils are trained to jump to targets of varying size, the use of RIS relies upon target identification. Our goals were to determine whether gerbils use local features or spatial context to identify targets and to assess the effects of cortical lesions on target identification in a jumping task. Sham controls and gerbils with either parietal or temporal lesions were trained to jump across a gap of randomly varying size to either a wide or narrow target. Targets had distinctive local features and were associated with unique spatial contexts. Test sessions were identical with training sessions but included probe trials using an intermediate sized target. The probe was presented with local features and spatial context that either matched or mismatched those seen during training. If gerbils were using RIS, they would over-jump when the probe target was narrower than the training target and underjump when it was wider. Results showed that normal and temporal gerbils could use spatial context but not local features to identify targets and parietal gerbils could use neither cue. We conclude that, for purposes of object identification, spatial context takes precedence over local features and that parietal lesions impair the ability to identify spatial contexts.

567.10

INVOLVEMENT OF THE MEDIAL PREFRONTAL CORTEX CHOLINERGIC SYSTEM IN PRECLINICAL MODELS OF WORKING MEMORY IN THE RAT. A.H.J. Herremans, J.A.D.M. Tonnaer*. Dept. of psychopharmacology, Faculty of Pharmacy, Utrecht University, Sorbonnelaan 16, 3584 CA, Utrecht, The Netherlands.

The involvement of the medial Prefrontal Cortex (mPFC) cholinergic system in working memory (WM) was investigated by assessing the effects of bilateral infusion into the mPFC of scopolamine on performance in a Delayed Matching to Position (DMTP) task and a Delayed Conditional Discrimination task (DCD) in rats. In the DMTP-task scopolamine infusions dose dependently disrupted performance independent of delay, indicating no specific role of the mPFC cholinergic system in WM. Moreover, rats trained on a DMTP-task displayed mediating behavior during delays in 50% of the trials to solve the task which seriously affects the validity of the DMTP-task as a model for WM. Furthermore, a time sampling method indicated that scopolamine infusions into the mPFC disrupted mediating behavior during the task. In a post-hoc analysis scopolamine was found to impair discriminability in a delay-dependent manner only in animals that used mediating behavior in the majority of the trials instead of using WM. Rats that displayed little mediating behavior, and as a consequence used WM showed no delay dependent effect. We developed a DCD task that better prevents mediating behavior. Bilateral infusion of scopolamine into the mPFC dose-dependently impaired DCD-performance. No delay dependent effect was found indicating that scopolamine did not specifically affect WM. This result agrees with the result of the rats using little mediating behavior in the DMTP task suggesting no specific involvement of the mPFC cholinergic system in WM.

567.11

DISCRIMINATORY CHANGES IN NUCLEUS ACCUMBENS DOPAMINERGIC ACTIVITY IN RATS AS ASSOCIATED WITH SOCIAL BEHAVIOR HABITUATION-DISHABITUATION RESPONSES. Y. Shang, B. Liu, and D.E. Dluzen* Department of Anatomy, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

The *in vivo* activity of the nucleus accumbens (N. Acc.) dopaminergic system was evaluated in adult male rats, who were subjected to either a two-time, 5-min exposure to the same (A-A) or different (A-B) ovariectomized (OVX) rats with an inter-trial interval of 25 min. Extracellular DOPAC release was measured at 5 min intervals throughout these social behavior habituation-dishabitution trials. In the A-A condition (n = 5), no changes in DOPAC release between the first and second social stimulus exposure (t = 0.57, N.S.) were obtained. Associated with these neurochemical responses were reductions in behavioral investigation (t = 2.27, $p<0.05$). In contrast, for the A-B condition (n = 6), DOPAC release significantly increased to the second stimulus exposure (t = 2.89, $p<0.05$) which was associated with increased social investigation (t = 2.89, $p<0.05$). These results demonstrate discriminatory responsiveness of the N. Acc. dopaminergic system as a function of exposure to the same or different social stimulus and suggest a role for this system in the mediation of memory/recognition processes to social stimuli.

567.12

REVERSIBLE LESIONS OF STRIATUM, AMYGDALA, AND SUBSTANTIA NIGRA AFTER INHIBITORY AVOIDANCE: DIFFERENTIAL EFFECTS OF HIGH AND LOW FOOTSHOCK INTENSITY. R. Salado Castillo, M. Sánchez-Alavez, G. L. Quirarte, and R. A. Prado-Alcalá*. Fac. Med. Natl. Univ. of México, México, D.F. 04510.

Interference with synaptic activity of the striatum (STR), amygdala (AMY), and substantia nigra (SN) produces marked memory deficits of inhibitory avoidance trained with relatively low intensities of footshock. It has been reported that when high intensities are used, amnesic treatments administered intrastriatally become totally ineffective; given to the amygdala a partial protective effect against amnesia is obtained. To further caracterize this protective effect, and its possible generalization to another structure, rats were trained in inhibitory avoidance with 1.0, 2.0 *or* 3.0 mA and then injected into the STR, AMY *or* SN with lidocaine; retention of the task was measured 24 hr later. A significant amnesic state was found after training with 1.0 mA, about 50% reponding after training with 2.0 mA, and no deficits after 3.0 mA. These results support the hypothesis that after an enhanced learning experience there is a functional rearrangement in those structures involved in memory functions.

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567.13

INVESTIGATION OF THE EFFECTS OF COOLING THE PARABRACHIAL NUCLEUS, MEDIAL PREOPTIC AREA, AND CAUDATE PUTAMEN IN RATS. Y. Wang, D. G. Lavond, K. C. Chambers*, Department of Psychology, University of Southern California, Los Angeles, CA 90089-1061.

Permanent lesions of the area postrema (AP) produce a constellation of symptoms which includes learned aversions to novel dicts. When temporary lesioning of the AP by cooling is paired with consumption of a sucrose solution, a learned aversion to the sucrose solution occurs. Three experiments were conducted to determine whether this effect of cooling is limited to the AP or whether cooling other brain areas also can induce a CTA. Three brain areas were selected to be cooled, the parabrachial nucleus, which is involved in the mediation of CTAs, the medial preoptic area, which is thought to be involved in the modulation of CTAs, and the caudate putamen, which is not known to be involved in CTAs. For each brain area, two groups were tested: Cooling and No-Cooling. On acquisition day, after all of the rats had access to a sucrose solution for 1 hour, the selected brain area of the rats assigned to the Cooling group was cooled for 60 minutes. For each brain area, the Cooling group displayed a significant decrease in sucrose solution consumption on the first test day after acquisition while the No-Cooling group did not show any change in consumption. This suggests that the CTA induced by cooling of the AP is not specific to this brain region but is due to a more general effect of cooling on brain regions. Supported by USC FRIF

567.14

COMPETITIVE "PLACE-CUE" RETENTION IN THE WATER MAZE: MEDIAL VERSUS LATERAL CAUDATE-PUTAMEN. B.D. Devan*, R.J. McDonald & N.M. White, Deptartment of Psychology, McGill University, Montreal, Quebec, Canada.

This study investigated functional differences between the medial and lateral parts of the caudate-putamen (dorsal striatum) in the acquisition and performance of water maze behaviors guided by either complex spatial or simple cues. Rats with dorsomedial (DM) or dorsolateral (DL) caudate-putamen lesions and sham-operated controls were initially trained on the standard hidden platform task. Rats with DM lesions, but not DL lesions, took longer to escape to the hidden platform and spent significantly more time swimming in the peripheral portion of the maze (i.e., thigmotaxic navigation). However, the DM group eventually achieved control levels of performance, and exhibited a significant bias for swimming in the training quadrant and crossing the location of the platform even when it was removed from the pool. In the second phase of the experiment, the three groups were given visible platform training in a different room/maze with the platform moved to a new location each day. Rats with DM lesions returned to thigmotaxis on the first visible trial block, but thereafter did not differ from controls. In the third phase of the experiment, the rats were brought back to the original room/maze. Following 4 invisible platform trials they were given a competitive "place-cue" retention test consisting of 4 trials from two start points that were equidistant from the invisible platform, located in its original position, and the visible platform, which was now positioned in the center of the diagonally opposite quadrant. The following results are expressed as the percentage of escapes to the visible versus invisible platform: Controls (61/39); DM (89/11) and DL (44/56). These results suggest that the DM caudate-putamen, by virtue of its connections with limbic and prefrontal cortical regions, may mediate responses based on spatial (S-S) information processing. In contrast, the DL region, by virtue of its direct sensory input from cortex and thalamus and its output to high level motor control systems, may mediate simple stimulus-response learning.

567.15

RADIAL ARM MAZE PERFORMANCE AND OPEN FIELD ACTIVITY IN STRIATAL KAINIC ACID-LESIONED RATS. Y. Ichitani* and T. Iwasaki. Inst. of Psychology, Univ. of Tsukuba, Tsukuba, Ibaraki 305, Japan.

Striatal kainic acid (KA) lesions, which induce neuronal cell loss in the striatum without affecting fibers and terminals passing through it, are used to make an animal model of Huntington's disease. In this study we investigated whether these animals show any impairment of radial arm maze (RAM) performance and any changes of activity level in the open field test. Female adult Wistar rats were trained to run down all 8 arms rapidly to obtain reward pellets in an elevated RAM. One or two days after reaching the criterion, they were injected with KA (0.5 or 1.0 μg/1.0 μl / each side) or 0.02M phosphate buffer (PB) into the striatum bilaterally. After recovery from operation, they were retrained in the RAM task until they reached the criterion (maximum 15 trials). Then the open field test (90 x 90 x 30cm) was conducted 5 min a day for 3 consecutive days (26-28 days after KA or PB injection). In postoperative RAM test, although control animals could reach the criterion immediately, the number of trials to reach the criterion was increased dose-dependently in KA-lesioned rats and most rats injected with KA 1.0 μg could not relearn the task after 15 trials. In the openfield test, KA-lesioned rats showed more ambulation than control rats. Results indicate that animals with lesions of striatal neurons had difficulty in performing RAM task and this impairment could not be attributable to motor impairment. Thus our results suggest that striatal neurons play an important role in performance of this kind of spatial or working memory task.

567.16

EFFECTS OF UNILATERAL DORSO-STRIATAL LESION ON THE PERFORMANCE ON A LATERALISED REACTION TIME TASK.

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The effects of various doses of systematically administered amphetamine and unilateral dorso-striatal lesions on the performance on a Skinner box adaptation of a visual reaction/movement time task were separately investigated. Food deprived rats were trained to hold the central perplex panel for a variable delay after which a visual stimulus was presented on either side of the animal. The rats were rewarded following a lateralised movement toward, and a press on, the previously extended lever above which the stimulus appeared. In the amphetamine study the pattern of responses reflected a dose dependent state in nearly all of the measured variables. The reaction and movement time also decreased with the low and the middle amphetamine doses. The delay dependent decrease in reaction time observed with the saline treated animals essentially disappeared as a consequence of the amphetamine. This data suggests an enhanced motor readiness exacerbating the increased anticipation of cue presentation as the delay progresses, potentially mediated by dopamine surplus in the striatum. Unilateral dorso-striatal lesions resulted in the increase of error trials, while not differing from the controls in the total number of trials attempted. The lesioned animals showed a bias to respond towards their ipsilateral side, a tendency which is mirrored in the decreased accuracy to respond to contralateral visual stimuli. The overall mean reaction time to contralateral stimuli, was retarded compared to their pre-lesion performance. The results of the lesion study suggest that the lateralised effects are rooted in executive and not in sensory or attentional processes. Contralateral visual cues did instigate responses. The anticipation of the stimulus, directly related to response execution, which shortened the reaction time the longer the required panel hold, was less apparent on the contralateral side following the lesion.

567.17

LEARNING-INDUCED CYTOGENESIS IN THE BRAIN OF FOOD-STORING MARSH-TITS (*Parus palustris*). S.N. Patel, N.S. Clayton, & J.R. Krebs. (**SPON: Brain Research Association**). The University of Oxford, Depts of Zoology and Pharmacology, South Parks Road, Oxford, OX1 3PS, U.K.

Food-storing species of passerine birds have a larger hippocampal region relative to the rest of the telencephalon, than do non-storing species. Experience of storing and retrieving results in an increase in hippocampal volume and in neurone number (Clayton and Krebs, 1994, *PNAS*, 91: 7410-7414). In juvenile hand-raised marsh tits, there is an increase in the intensity of food-storing on day 44. Here we sought to determine whether hippocampal neurogenesis occurs as a result of food storing behaviour (thereby accounting for the increase in hippocampal neuronal number), and whether cytogenesis occurs prior to the increase in food storing intensity seen at day 44.

Hand-raised marsh tits were divided into experienced and control groups. Experienced birds were allowed to store and retrieve seeds in an experimental arena every third day from nutritional independence (day 35) while age-matched controls were prevented from storing and retrieving by providing them with powdered seeds while they were in the arena. Three experienced birds and three controls were sacrificed at day 41, 47 and 56. Three 35 day old birds served as pre-experimental controls. All birds received a single injection of 0.05µCi of ^{3}H-thymidine i.p. one hour before intracardiac perfusion with saline flush and 4% paraformaldehyde fixative. The rate of cytogenesis was measured by examining the percentage of cells labelled (by autoradiography) in the ventricular zone in 6µm thick paraffin embedded sections.

The results of 5 experienced and 7 control birds show that the rate of cytogenesis is significantly greater in experienced birds compared to controls in the ventricular zones of the hippocampus and the hyperstriatum ventrale (HV), but not the neostriatum, irrespective of the amount of experience. The results show that the HV as well as the hippocampus is involved in some aspect of food storing behaviour which involves the induction of cytogenesis in these regions.

567.18

MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL CHARACTERISATION OF NEURONS IN THE IMHV OF THE CHICK BRAIN P.M.Bradley, B.D.Burns, T.M.King & A.C.Webb. Department of Neurobiology, Medical School, Newcastle upon Tyne, UK. (**SPON: Brain Research Association**).

The IMHV has been shown to be essential for learning in the chick. As a consequence of this learning there are changes in the morphological and electrophysiological characteristics of neurons in the IMHV. In order to be able to understand the neuronal circuitry that underlies these learning related changes we have attempted to correlate the electrophysiological characteristics of IMHV neurons with their morphology.

Biocytin filled intracellular electrodes were used to record the properties of a total of 26 neurons in the IMHV. The cells were then visualised,measured and classified as small or medium non-spiny neurons or as small, medium or large spiny neurons. Neurons were also classified according to their response to external stimulation. Type 1 cells displayed an EPSP sometimes with a spike. Type 2 cells showed prolonged depolarisation accompanied by a train of spikes. Type 3 cells showed an EPSP followed by a late hyperpolarisation.

In both spiny and non-spiny neurons the cells with the most extensive dendrites showed lower (more positive) resting membrane potentials and higher passive membrane resistance. Spiny cells were significantly larger than non-spiny cells and had a significantly lower incidence of spontaneous activity and a lower passive membrane resistance. Type 2 cells were smaller than other neurons, were spontaneously active and displayed significantly larger spontaneous EPSP's. Type 1 cells displayed a shorter time constant and a larger evoked EPSP than type 3 cells.

These results suggest a link between structure and function in the IMHV and provide a basis for modelling neuronal circuitry in the IMHV.

567.19

APV PREVENTS SPINE ELIMINATION DURING AUDITORY IMPRINTING OF DOMESTIC CHICKS. J. Bock and K.Braun*. Fed. Inst. Neurobiol., 39118 Magdeburg, Germany.

Previous studies using Golgi impregnation techniques revealed in the medio-rostral neostriatum/ hyperstriatum ventrale (MNH) of imprinted chicks an up to 45% reduction of spine frequency on type I neurons compared to naive controls (Scheich, 1987, *J. Comp. Physiol. A 161*: 605-619). Type I neurons develop LTP-like potentiation after tetanic stimulation of thalamic inputs, which can be blocked by the selective NMDA-antagonist DL-2-amino-5-phosphono valeric acid (APV) (Wang et al., 1994, *Neurosci. 3*: 689-699.). In behavioral experiments APV prevents imprinting in a dose dependent manner (Bock et al., 1993, *Europ. J. Neurosci., Suppl. 5*: 221). These observations suggest that NMDA-dependent potentiation of synapses is one of the mechanisms which underlie this learning process and we speculate that it may also be causally related to the observed morphological changes. To test this hypothesis we compared the spine frequencies in Golgi-Cox impregnated brains from APV-treated trained but not imprinted chicks, imprinted chicks and naive untreated chicks. Spine frequencies of type I neurons were calculated using the Neurolucida system (MicroBrightField, Inc.), which allows quantitative 3D analysis of microscopic images. The spine frequencies of type I neurons of APV-treated chicks and unimprinted controls were similar and both groups showed significant higher spine frequencies (about 35%) than imprinted subjects ($p < 0,001$, Kruskal-Wallis). Thus, APV-treatment not only blocks the induction of LTP and impairs auditory imprinting but also prevents the elimination of dendritic spines. This suggests an involvement of NMDA-receptor activation in the synaptic selection process during imprinting. The imprinting stimulus may activate a subset of synaptic connections in the MNH, which subsequently are potentiated and thereby stabilized and this potentiation may induce elimination of non-activated synapses.
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567.20

INFLUENCE OF THE EXTRACELLULAR MATRIX PROTEIN TENASCIN ON AUDITORY FILIAL IMPRINTING IN THE DOMESTIC CHICK M. Metzger*, J. Wang, K. Braun and M. Schachner Federal Institute for Neurobiology, POB 1860, 39008 Magdeburg, FRG & Swiss Federal Institute of Technology, Hönggerberg, 8093 Zürich, Switzerland

The extracellular matrix protein tenascin (Tn) has been implicated in a variety of morphogenetic events such as neurite outgrowth and pattern formation. In a forebrain area of the domestic chick, the medio-rostral neostriatum/hyperstriatum ventrale (MNH), we found proliferative as well as regressive changes of synaptic connections during different stages of auditory filial imprinting, hence we speculate that recognition molecules such as Tn may be essential for these events. In order to test this hypothesis we blocked different Tn epitopes by intracranial injections of different monoclonal antibodies against Tn and subsequently we tested the imprinting success in behavioral tests. 15 min prior to each behavioral test newly hatched chicks were injected bilaterally into the MNH by the aid of a specially designed headholder either with 2µl of the monoclonal antibody Tn 68 or monoclonal antibody 578, both of which recognize different epitopes of the Tn molecule. In the Tn 68 injected group there was a significant decrease of imprinting success compared to the control group (PBS) and the 578 injected group. Single- and double labelling immunohistochemistry revealed that (i.) the diffusion of the injected Tn antibodies was restricted to the MNH, (ii.) the general distribution of Tn in the juvenile chick forebrain is widespread and (iii.) Tn is particularly accumulated arround neuronal somata containing the Ca-binding protein parvalbumin. This Tn staining pattern is reminiscent of perineuronal nets. Since, in the MNH, a subpopulation of the PV+ neurons is GABAergic we are currently testing the hypothesis that Tn may influence GABAergic mechanisms.
(Supported by the Deutsche Volkswagenstiftung)

567.21

SICKNESS-CONDITIONED LEARNING IN DAY-OLD CHICKS: MEMORY FORMATION DEPENDS ON TIME OF LITHIUM CHLORIDE INJECTION. T.A. Barber*, M.F. Pearlman, and K.E. Patrick. Psychology Department, Dickinson College, Carlisle, PA 17013-2896.

In the sickness-conditioned learning paradigm, the chick pecks a dry bead and is injected 30 min later with lithium chloride (LiCl). When tested 4 hours later, the chick avoids the "training" bead but pecks novel beads. Memory can be disrupted by injecting glycoprotein synthesis inhibitors around the time of "training", suggesting that memory formation concerning the bead begins well before any consequences have occurred.

The current experiment examined learning in this task following different times between presentation of the training bead and LiCl injection. Chicks injected with LiCl 5 or 10 min after training pecked the bead at test; they did not form an association between the bead and the sickness. In contrast, chicks injected with LiCl 15, 20, 30 or 45 min after training avoided the bead, evidence of memory formation for the association. These results suggest that the chick cannot learn the association unless the "memory" for pecking the bead is at least 15 min old.

Lesions of the intermediate medial hyperstriatum ventrale (IMHV) produce amnesia in passive avoidance learning in the chick, suggesting that the IMHV is involved in associating visual characteristics of the stimuli and consequences of pecking. Experiments in progress are examining the role of the IMHV in the sickness-conditioned learning paradigm.

NEUROETHOLOGY: FISH

568.1

VOCAL-ACOUSTIC PATHWAYS IN SYNODONTID AND ARIID CATFISH. F. Ladich , M. A. Marchaterre*, L. Goldstein and A.H. Bass. Section of Neurobiology & Behavior, Cornell. Univ., Ithaca, NY; Inst. of Zoology, Univ. of Vienna, Althanstrasse 14, A-1090 Vienna, Austria.

Several families of catfish, including Synodontidae and Ariidae, utilize swimbladder "drumming" and pectoral spine stridulation to generate vocal communication signals. Biotin compounds were used as retrograde and transneuronal tracers to identify the positions of motor and premotor neurons associated with the vocal/sonic muscles (see Bass et al., J. Neurosci., 14: 4025, 1994). Dextran-biotin revealed the positions of ipsilateral vocal motoneurons: paired nuclei, located in the caudal medulla and rostral spinal cord, are located on the midline between the central canal and the medial longitudinal fasciculus (MLF) in synodontids, but lateral to the MLF in ariids. Pectoral spine motoneurons were labeled within the ventrolateral motor column in both families. Biocytin transport also identified motoneurons in both families, and premotor neurons only in synodontids where labelled fibers and small somata were found bilaterally in the vocal motor nuclei. Biocytin-filled neurons were also located within the ventrolateral medulla, rostral to motoneurons, and gave rise to commissural and lateral brainstem bundles linking the vocal motor circuitry to rostral regions of the brainstem considered to be acoustic. Vocal-acoustic pathways have been identified in other distantly related teleosts (Bass et al., 1994), suggesting that such links are a widespread trait shared by diverse groups of aquatic and terrestrial vertebrates that vocalize. Research support from US National Science Foundation and Austrian Science Foundation.

568.2

SOUND PRODUCING MECHANISM, HANDEDNESS, AND DEVELOPMENTAL CHANGES IN CHANNEL CATFISH SOUND PRODUCTION. M.L. Fine*, D. McElroy, J. Rafi, C.B. King, K. Loesser and S. Newton, Depts. of Biology, Va. Commonwealth University, Mary Washington College, and Va. State University.

The first pectoral spine of the channel catfish (Ictalurus punctatus) functions in swimming, defensive locking and stridulatory sound production. In this study we examined the mechanism of sound production and developmental changes in the sound producing organ and in acoustic parameters of threat sounds. Stidulation was typically produced by pectoral fin abduction when a convex ridged spinal process was rubbed against a rough but relatively featureless concave surface in the glenoid channel of the pectoral girdle. Typical sounds consisted of a series of 7-15 pulses per fin sweep of 56-177 ms, and we suggest that each pulse is produced by an individual ridge, successively contacting channel bone. Approximately half of the fish had a fin preference, and most of these were right handed. The spine and the girdle grew continuously, and the ridges became worn, with minimal remodeling, and grew further apart. Sound pressure level increased and central frequency decreased with fish size. Although sweep and pulse duration increased with fish size, the number of pulses remained constant, and the pulse repetition rate decreased, correlating with the greater distance between ridges and longer sweep time. Supported by NIH DC01083.

568.3

ULTRAVIOLET AND POLARIZED-LIGHT SENSITIVITY IN REPRODUCTIVE TROUT OF TWO SPECIES. D. C. Parkyn* Dept. of Biology, University of Victoria, Victoria, Canada. V8W 2Y2.

The principal objective of the study was to examine differences in spectral and polarization (E-vector) sensitivity of non-reproductive and reproductive adult brook char (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*). Spectral and (E-vector) sensitivity were characterized using multiunit-recording from axons of retinal-ganglion cells in the optic nerve. In contrast to non-reproductive adults (as based on gonad development), ultraviolet (UV) and vertical e-vector sensitive mechanisms were identified in reproductive individuals. Both UV sensitivity and vertical E-vector sensitivity are mediated by UV cones. However, this UV mechanism is not present in non-reproductive adults in either species. Therefore, spectral and polarized-light sensitivity of the reproductive adults was more similar to that found in juvenile salmonids (i.e. four classes of cone photoreceptors, and two classes of polarization-sensitive mechanisms). These observations concur with studies showing the presence of putative UV-cones in the retinae of fish treated with thyroxine and histological studies of returning migratory salmonids (see Hawrysyhyn, Beaudet, and Novales F.). This supports the hypothesis that UV and polarization sensitivity are used by salmonids during their migration to natal streams for reproduction. To assess the functional role of UV-polarization sensitivity, I measured behavioral orientation using a video-image tracking system. My results revealed that some reproductive *O. mykiss* innately-orient relative to the plane of polarized light. This research was supported by an operating grant from the Natural Sciences and Engineering Research Council of Canada (C.W. Hawryshyn) and the University of Victoria (CWH and DCP).

568.4

WHAT THE FLOUNDER'S EYE TELLS THE FLOUNDER'S SKIN. V. S. Ramachandran*, R. L. Gregory, C. W. Tyler, D. Rogers-Ramachandran, S. Duensing, C. Pillsbury, and C. Ramachandran. UCSD, Brain and Perception Lab, Psychology, 0109, La Jolla, CA 92093-0109

Until recently, it was widely believed that flounders had a remarkable ability to match the pattern on their skin with the background (Sumner, 1911). This claim was recently challenged by Saidel (1977) who suggested that the "blending" was done mainly by the beholder, not the flounder. We placed 5 specimens of the tropical flounder *Bothus ocellatus* on 3 background patterns -- a coarse checkerboard, a fine checkerboard, a uniform grey of the same mean luminance. (The fish were placed for 5 minutes on each pattern and videotaped.) They were able to match the background pattern with surprising fidelity in just 6-8 seconds. A principal components analysis of the radial (Fourier) power spectrum of the fish versus the background showed, objectively, that there were 3 predominant channels of independent information about the background texture being expressed as camouflage patterns on the flounder. When the fish were placed on a sparse polka-dot pattern, each animal became pale and developed two small, but prominent black spots as if to mimic the polka-dots! We conclude that flounders do indeed have a remarkable capacity for rapid adaptive camouflage. We suggest that there are "feature detecting" neurons in the fish tectum that respond to different spatial frequencies and exert direct neural control over corresponding sets of melanophores in the skin.

568.5

FEEDING NEUROECOLOGY OF THE MECHANOSENSORY LATERAL LINE IN THE ATLANTIC STINGRAY, *DASYATIS SABINA*. **K.P. Maruska and T.C. Tricas*. Department of Biological Sciences, Florida Institute of Technology, Melbourne, FL 32901-6988.**

The Atlantic stingray, *D. sabina*, feeds almost exclusively on small benthic invertebrates that it excavates from the sand substrate. This study examined the anatomy, spatial organization and innervation patterns of mechanoreceptors to predict the sequential function of each lateralis sub-system in prey detection, positioning, and capture. Superficial neuromasts extend only on the dorsal surface in bilateral rows along the midline from the spiracle to the tip of the tail. Dorsal hyomandibular canals have long radial neuromast-free tubules that project to the pectoral fin margins. The peripheral ventral canals have a similar arrangement of pores along the rostral margin but are displaced medially on the posterior disk surface. An extensive, confluent, and compliant ventral inner canal system lacks surface pores and extends rostrally from the pelvic region to the head where it expands between the mouth and disk margin. Vesicles of Savi are aligned in bilateral rows on the ventral midline of the snout. The mandibular canal spans the lower jaw and terminates in a single pore at each corner of the mouth. Ventral canal neuromast abundance ($\bar{x}$ = 1028) is approximately twice that of the dorsal ($\bar{x}$ = 532) system. Across size classes (22 - 32 cm disk width, n= 10) the number of neuromasts increases only in the mandibular canal and total number of axons innervating each canal group does not change. We suggest that pore-bearing peripheral canals function in predation as detectors of water movement made by prey near the rostral margin and caudal underside of the disk. The extensive complex of inner canals on the ventrum that lack pores is hypothesized to 1) reduce noise caused by water movement across the skin during prey excavation, and 2) function as modified touch receptors for the guidance of exposed prey to the vesicles of Savi and mouth. Final positioning of prey immediately before capture is facilitated by the mandibular canal on the lower jaw.

568.6

APPROACH STRATEGIES USED BY MOTTLED SCULPIN IN LOCALIZING DIPOLE SOURCES. **S. Coombs and R.A. Conley. Parmly Hearing Institute, Loyola University of Chicago.**

Mottled sculpin respond to the sinusoidal vibration of a small sphere with an initial orientation towards the source followed by a stepwise approach to the source that culminates in a strike. The x,y coordinates of the fish's position at each step in the pathway were digitized from a frame-by-frame videotape analysis and mapped into a computational matrix (Matlab) that used the dipole flow field equations to compute pressure gradients and flow angles at each position. Results from over 300 pathways (6 fish) to a 50 Hz suprathreshold source (varying in start distance (0.5 - 10 cm) and angular (0 - 360°) relationship to the fish) show that paths vary. A common approach is one in which fish maintain a < 45° angle to the source and flowline, but alternate between being to the left and right of the source. The total number of left and rightward positions was nearly equal for all but two fish, one of which had roughly twice as many leftward responses and the other which had twice as many rightward responses. Pressure gradients along the fish's head increased from below 10 Pa for start positions, to 10-100 Pa for pre-strike positions, and to 100-1000 Pa for strike positions. Those across the fish's head remained below 10 Pa for all positions. Taken together, these results suggest that fish find the source by minimizing the pressure gradient across the head and maximizing it along the head.

568.7

CONTRIBUTIONS OF CANAL AND SUPERFICIAL NEUROMAST SENSORY CELLS TO THE ELECTROPHYSIOLOGICAL ACTIVITIES OF THE LATERAL LINE SYSTEM OF A CICHLID FISH (*Astronotus ocellatus*). H. Y. Yan* School of Biological Sciences, University of Kentucky, Lexington, KY 40506-0225

It was demonstrated (Song, Yan and Popper, 1995: Hearing Research, In press) recently that by exposing a cichlid fish, oscar (*Astronotus ocellatus*) to the aminoglycoside antibiotic, gentamicin sulfate, selective damages occurred to one class of sensory hair cells (canal neuromasts) of the lateral line system but no morphological damages could be found in the other class of the sensory hair cells, superficial neuromasts. This study was undertaken to examine electrophysiological changes to the lateral line nerves of oscar after direct exposures to gentamicin sulfate solution (20 ppm) for 1, 2, 3, 4, 8, and 12 days. Extracellular recording techniques were used to measure the changes due to the damage of canal neuromasts. Recovery of the electrophysiological activities was also investigated when damaged neuromasts were allowed to regenerate after four days of exposure to gentamicin sulfate. By using this unique selective damage protocol, contributions of each class of neuromasts in the electrophysiological activities of the lateral line of fish can be assessed.

568.8

PROCESSING OF HYDRODYNAMIC INFORMATION BY NEURONS IN THE MEDULLA OF THE GOLDFISH. J. Mogdans* and H. Bleckmann, Zoologisches Institut, Universität Bonn, Poppelsdorfer Schloß, D-53115 Bonn, Germany.

Fishes use a special sensory system, the lateral line, to detext weak water movements. To investigate central processing of hydrodynamic stimuli, we recorded single unit activity of neurons in the medulla of the goldfish while stimulating the lateral line with a small object moving in the water. Unit responses to various object speeds, object distances, and object sizes were measured. Responses were characterized by unit firing patterns and quantified by the discharge rate during stimulus presentation.

Spontaneous activity of mechanosensory lateral line units in the goldfish medulla was low (mean 2.5 spikes/sec, range 0-16 spikes/sec, n=35). On the basis of temporal discharge patterns, four different response types were distinguished. Twentyfive units responded to moving objects with increases in discharge rate. Five units exhibited oscillating increases and decreases in discharge rate. One unit responded to moving objects with a decrease in neural activity. In general, discharge rates were independend of the direction of object movement. In two units, however, the pattern of the response inversed when movement direction was reversed. For all units tested, discharge rate decreased with decreasing object speed and increasing distance between object and fish. Discharge rates were comparable for object sizes between 1 cm and 2 cm but decreased when object size was reduced to 0.5-0.25 cm. We are currently studying whether medullary lateral line units respond differentially to objects that are accelerated or moved at a constant speed along the side of the fish.

The results indicate that in the goldfish medulla, different lateral line neuron populations may be responsible for the processing of different aspects of hydrodynamic stimuli. Moreover, the data suggest neuronal interaction at the level of the medulla, possibly through lateral inhibition. Lateral inhibition may establish direction and space sensitivity.

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568.9

VISUAL PRIMING OF MAUTHNER INITIATED ESCAPE RESPONSES TRIGGERED BY SOUND IN THE GOLDFISH. R. C. Eaton*, M. M Walli, A. L. Guzik and J. L. Casagrand. Center for Neuroscience, University of Colorado, Boulder CO 80309-0334.

The Mauthner cell initiates escape from aversive auditory stimuli such as those generated by a predatory strike or an object falling into the water. We previously observed that if the escape path was blocked, the animal turned *toward* the aversive stimulus, a ball dropped into the water. This suggested that visual information could override the directionality of the responses triggered by auditory stimuli. In this experiment we dropped two balls into the water, one in front and one behind the fish. The ball drops were separated by 50 ms. The ball that hit the water first always triggered the response, but the ball that was most in the field of view primed the directionality. In cases when the trigger stimulus was behind the fish, the fish turned away from the priming stimulus and toward the trigger stimulus. This apparent tactical error occurred in 70.5% of such trials, compared to a rate of 12.5% with a single ball where there was no conflicting visual priming ($p<0.005$, X^2). Further, in the paradigm with two balls, a blind control fish escaped away from the direction of the trigger stimulus. Thus visual components of the stimulus can control the direction of an escape triggered by sound. [Supported by the ONR].

568.10

THE ROLE OF MAUTHNER CELLS IN FAST STARTLE RESPONSES. S.J. Zottoli*,B.C. Newman, H.I. Rieff and D.C. Winters. Dept. of Biology and Neurosci. Prog., Williams College, Williamstown, MA 12617.

Goldfish respond to an abrupt acoustic stimulus with a rapid, C-type startle response (C-start). A C-start is preceded by Mauthner cell (M-cell) activity in the goldfish,*Carassius auratus*. M-cells appear to initiate C-starts with other reticulospinal neurons contributing to the overall response. To further investigate the role of M-cells in C-start responses, either both M-cells were selectively ablated or their axons were selectively axotomized. M-cells were ablated in 8 goldfish by locating their axon cap, moving the microelectrode 50µm lateral to the cap, penetrating the M-cell soma and mechanically killing the cell. In 6 sham-operated control animals the axon caps were located but the somata were not damaged. Both M-axons were selectively axotomized just caudal to the facial lobe in 7 goldfish by penetrating the axons with a microelectrode and mechanically disrupting them. In 6 control animals the microelectrode was lowered close to the M-axon without damaging it. The probability of eliciting C-start responses and a series of kinematic parameters (i.e.,latency,angle,velocity) were compared between experimental and control animals 3 hrs postoperatively. The success of both operations was confirmed morphologically at the end of the experiment. Fish with double ablations or double axotomies demonstrated a decrease in the probability of C-start responses ($p < .0001$) when compared to their respective control groups. This reduced probability suggests that the M-cells may not only initiate C-starts but also contribute to the excitation of other reticulospinal neurons involved in this type of startle response. (Supported in part by an Essel Foundation grant to Williams College).

568.11

SENSORY GUIDANCE OF MAUTHNER-MEDIATED STARTLE IN GOLDFISH AND CICHLIDS. I.G. Canfield* and G.J. Rose. Department of Biology, University of Utah, Salt Lake City, UT 84112

Acoustically-evoked escape behaviors were compared between goldfish (*Carassius auratus*), a hearing specialist, and the cichlid *Haplochromis burtoni*, a hearing nonspecialist. When unilateral sound stimuli were presented alone, both species avoided the direction of either a compressive or rarefying stimulus. If a light preceded and was contralateral to the sound pulse, goldfish avoided the sound source, whereas cichlids avoided the visual cue and turned toward the sound. Thus, for goldfish, acoustic cues predominate in both initiating and directing the response by activating the Mauthner cell ipsilateral to the sound source. For cichlids sound is the releasing stimulus, but response direction is determined by visual cues; the Mauthner cell contralateral to the sound source presumably is activated in these conditions. Chronic recordings from the goldfish Mauthner cell indicate that only the initial rising phase (1.6 msec) of the stimulus is required to direct the response. After a 24 hour exposure to a 0.1 mmol/l cobalt solution (a treatment thought to inhibit lateral line function), goldfish escape performance improved but cichlids would not startle. Activating both speakers simultaneously with opposite polarity directly accelerates the fish's ears and reduces lateral line and pressure cues. When the speakers are not precisely matched for rate of rise, goldfish integrate sound pressure and particle motion aurally, while cichlids preferentially use lateral line cues to detect the direction of the startling sound source. With matched speakers, both species avoid the direction of the initially compressive sound source. This suggests a primitive mechanism for aurally detecting and avoiding the sounds associated with ram-type predators. [NIMH RO3MH50269]

INVERTEBRATE LEARNING AND BEHAVIOR III

569.1

ASSOCIATIVE LEARNING AND MEMORY IN *C. ELEGANS*. G.E. Morrison*, N. Kumar, J.Y.M. Wen, S. Runciman, H. Negandhi and D. van der Kooy. Neurobiology Research Group, Dept. of Anatomy, University of Toronto, Toronto, ON, Canada, M5S 1A8.

We have developed a discriminative classical conditioning assay for *Caenhorhabditis elegans* based on the chemoattractive properties of the conditioning stimuli (CS), Na+ ($NaCH_3COO$) and Cl- (NH_4Cl). The CS ions are equally preferred under naive conditions. After exposure to one of the CS ions (CS+) in the presence of the US (*E. coli*, a food source), counterbalanced by an equal exposure to the alternate ion (CS-) in the absence of the US, the worms demonstrate a significant preference for the CS+ ion on testing. To ask about the differences between the worms that chose correctly (the CS+ ion) on testing and those that did not (chose the CS- ion), animals were separated into two groups following testing (those that chose the CS+ and those that chose the CS-) and then immediately retested without any further conditioning. On retest, both groups, in significant numbers, chose the CS+ ion over the CS- ion, suggesting that the assay may measure the probability of making a correct response (learning) in individual animals. Preferences for the CS+ ion could be easily reversed by replacing the appetitive *E. coli* food suspension with an aversive garlic extract. Conditioning with garlic as the US caused the worms to significantly avoid the garlic paired ion (CS+ ion) and approach the unpaired ion (CS- ion). US only tests with garlic did not affect the equal ion preference of otherwise naive worms on testing. These learning assays allowed for the isolation and behavioural characterization of two mutant C. elegans lines impaired in associative learning, designated *lrn-1* and *lrn-2*. Both lines show no short- or long-term associative learning, but appear normal in tests of non-associative learning and sensorimotor function. Complementation studies demonstrate that the two mutations are different. Outcrossing the two mutant lines indicates that they are both recessive. Using the sequence tagged site strategy for *C. elegans* and multiplex PCR techniques, we have begun mapping the two mutations through the generation of Bergerac/mutant (N2) hybrids.

569.2

ASSOCIATIVE LEARNING IN *C. ELEGANS* USING OLFACTORY CONDITIONING. Hiten Negandhi*, Joseph Y.M. Wen, Glenn Morrison, Sue Runciman and Derek van der Kooy. Neurobiology Research Group, University of Toronto, Toronto, Canada, M5S 1A8.

C. elegans, a soil dwelling nematode, is an attractive organism for the reductionist study of learning and memory due to its excellent genetics and well defined neuroanatomy. We have previously isolated two learning mutants, *lrn-1* and *lrn-2*, which were impaired in an associative taste learning paradigm based on chemotaxis to Na^+ ($NaCH_3COO$) and Cl^- (NH_4Cl). These mutants did not demonstrate any impairments in non-associative learning or sensorimotor function. We have developed a new associative learning paradigm, this time based on the olfactory ability of *C. elegans* to track certain volatile chemicals. In the unconditioned state the nematode is attracted to diacetyl, the conditioned stimulus (CS), and displays an unmistakable tracking response. Acetic acid, which the animal finds aversive, is used as the unconditioned stimulus (US). Training involves placing a population of worms onto a mesh filter and subsequently exposing them to diacetyl, followed by a brief submersion in acetic acid. After six such rounds of conditioning individual worms are tested for their response to a small drop of the CS. After conditioning, a significant proportion of wild type worms no longer track the CS, suggesting that they have learned the association. Several controls including CS only, US only, explicitly unpaired, and pseudo-random conditioning rule out non-associative effects. The *lrn-1* and *lrn-2* mutants isolated in our previous paradigm are also impaired in this new olfactory associative learning paradigm. This suggests that the associative learning impairment in these mutants extends across conditioning with various sensory modalities. A habituation/dishabituation (non-associative) paradigm has been designed using the same diacetyl smell employed in associative conditioning. We plan to test the *lrn-1* and *lrn-2* associative learning mutants on this paradigm to determine if they show any deficits in non-associative learning.

569.3

RECOVERY FROM HABITUATION AND HABITUATION OF SPONTANEOUS BEHAVIOR IN *C. ELEGANS*: DEPENDENCE ON CIRCUIT CHARACTERISTICS. S. R. Wicks* and C. H. Rankin. Dept. of Psychology, Univ. of British Columbia, Vancouver, BC CANADA V6T 1Z4.

The nematode tap withdrawal reflex demonstrates various forms of non-associative learning. The circuitry which supports this reflex has been identified and consists of seven sensory neurons and nine interneurons. It has been shown that recovery from habituation in *C. elegans* possesses an interstimulus interval (ISI) dependence. However, it is not generally clear whether this dependence is on the ISI *per se* or whether it is on the morphology of the habituation produced at that ISI. It is possible to dissociate these possibilities by producing lesions in the circuitry which supports the plasticity such that the morphology of habituation is manipulated independent of the ISI. Habituation of animals at a 10s ISI—as compared to a 60s ISI—results in a faster rate and deeper asymptotic level of habituation. In addition, animals trained at a 10s ISI show recovery within 5 minutes; animals trained at a 60s ISI do not. PLM- animals which were trained at a 10s ISI habituated like 60s ISI control animals—both in terms of rate and asymptotic levels of habituation. Recovery in these animals resembled recovery of the 10s ISI controls rather than the 60s ISI controls. Thus recovery dynamics are dictated by the ISI rather than by the morphology of the habituation curve.

As a first step in localizing the site of behavioral plasticity in the tap withdrawal circuit we analyzed habituation of spontaneous behavior. Animals reverse both in response to tap and spontaneously. The tap evoked reversals are mediated by the mechanosensory neurons of the tap withdrawal circuit; the spontaneous reversals may not be mediated by these cells. We determined that the spontaneous reversals of animals that had undergone habituation of the tap withdrawal response were not also habituated. This suggests that habituation of the tap withdrawal reflex may be localized to the sensory neurons and their terminals and is not due to intrinsic changes in the interneurons which drive locomotion.

569.4

A DEVELOPMENTAL STUDY OF HABITUATION OF ACCELERATIONS IN C. ELEGANS. T.N. Gannon* and C.H. Rankin. Dept. of Psychol., Univ. of British Columbia, Vancouver, BC, CANADA V6T 1Z4.

We have demonstrated that the tap withdrawal reflex of *C. elegans* displays habituation, dishabituation and sensitization. Through the use of laser ablation techniques, the neural circuit underlying this tap withdrawal response has been elucidated. To study learning in the tap withdrawal reflex throughout the course of development in *C. elegans*, we must first address the problem that, unlike adults, the larval animals do not consistently reverse to tap, but rather accelerate about one-half of the time. We circumvent this problem by selectively laser ablating either the tail-touch neurons (PLML and PLMR) that transduce stimuli that produce the accelerations, or the head-touch neurons (ALML and ALMR) that transduce stimuli that produce the reversals: With PLML and PLMR ablated, worms of all ages consistently reverse to tap; with ALML and ALMR ablated, adult worms consistently accelerate to tap. When the above neurons are ablated in adult worms the reversal and acceleration responses appear to habituate at different rates. In a previous study we examined habituation in the reversal response over the course of development. The results showed that although animals of all developmental stages did demonstrate habituation, there were some developmental differences among the stages.

In this study we are examining the time-course and magnitude of habituation of the acceleration response across developmental stages. We are examining the time-course and magnitude of habituation and recovery from habituation of accelerations at two different inter-stimulus intervals (10 and 60 seconds) for twenty different animals tested at each of the six major developmental stages in *C.elegans*. Preliminary results indicate that even the youngest animals tested accelerate to tap when ALML and ALMR are ablated, and that this response does seem to habituate.

569.5

LONG-LASTING ALTERATIONS IN RESPONSIVENESS AFTER REPEATED BLOCKS OF TRAINING AT DIFFERENT TIMES DURING DEVELOPMENT IN *CAENORHABDITIS ELEGANS*. C. D. O. Beck*, H. Aulakh and C. H. Rankin. Dept. of Psychology, Univ. of British Columbia, Vancouver, BC CANADA V6T 1Z4.

Previously, we demonstrated that during adulthood *C. elegans* is capable of long-term memory (over 24 h) for repeated blocks of stimulation in the locomotory response to a vibrational stimulus, tap. We have now developed a method of examining the long-term memory for repeated blocks of stimulation during early development and at maturity.

C. elegans has a reproductive cycle of 3.5 days, and during development passes through four larval stages, L1-4, each ended by a molt of the cuticle. The development of worms was synchronized by bleaching gravid adults, killing larval and adult worms but leaving eggs intact. Within 3 to 5 h, the hatched L1 larval worms were separated into control and experimental groups. Controls received no stimulation, while experimental worms received 100 trains of taps in 5 blocks of 20 stimuli. Within each block, stimuli were given at a 60-s ISI, and between blocks there was a 1-h rest interval. Testing was done individually with 10 stimuli at a 60-s ISI. Worms were trained together in a large group at one developmental stage (i.e. L1 or young adult) and tested individually 46 and 50 h after the beginning of training. Preliminary results suggest that while the responsiveness of worms trained as adults was not altered, the responsiveness of worms trained early in development at L1 was enhanced. These results are of interest in light of the knowledge that the tap withdrawal circuit undergoes rewiring during development as new neurons arise and are incorporated. It is possible that early stimulation may influence the course of development. Further research into this finding is underway.

569.6

A MUTATIONAL APPROACH TO THE ANALYSIS OF HABITUATION IN THE NEMATODE *C. ELEGANS*. C. H. Rankin*. Dept. of Psychology, Univ. of British Columbia, Vancouver B.C. CANADA V6T 1Z4.

The nematode *Caenorhabditis elegans* is capable of a number of forms of behavioral plasticity. In my laboratory we have focused on the factors influencing both short- and long-term habituation and recovery from habituation. We have investigated the role of a number of behavioral parameters including number of stimuli and interstimulus interval (ISI) on habituation. In addition, we have carried out a circuit analysis and determined the contributions of a number of identified neurons to the rate and degree of habituation in intact animals. With this knowledge about the behavioral and neural characteristics of habituation in *C. elegans* we are now ready to carry out a mutational analysis of habituation in this organism. To do this we have used two approaches. In the first approach we have examined habituation and recovery from habituation in individual worms from a variety of mutant strains identified by other research laboratories. These include mutants missing specific neurons (e.g. *egl-5;* missing a pair of sensory neurons), specific neurotransmitters (e.g. *cat-1* missing catecholemines), and a number of mutants that showed behavioral abnormalities in other tests of plasticity in other laboratories. Mutants missing neurons that play a role in producing the response to tap confirmed our laser ablation studies. Worms that show abnormal thermotactic behaviors (supplied by I. Mori) showed some interesting variations in habituation patterns. Several mutations appear to affect probability of responding to tap without affecting habituation, while one mutant appears to show the initial rapid drop in habituation followed by recovery while stimulation continues. We continue to follow up on this approach. In the second approach we have used computer-assisted tracking to observe large numbers of worms at a time. With this system we have begun a mutational analysis of habituation using the mutagen EMS. This approach will allow us to complete a genetic dissection of the components and processes contributing to habituation and influencing short and long term memory for habituation.

569.7

SIGNAL PROPAGATION IN THE NERVE RING OF THE NEMATODE *C. ELEGANS*. S. R. Lockery* and D. H. Hall. Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403 and Dept. of Neuroscience, Albert Einstein College of Med., New York, NY 10461.

Voltage clamp recordings from neurons in the nerve ring, the major site of synaptic integration in *C. elegans*, indicate that most neurons do not have regenerative potentials. This suggests that signal propagation is largely passive. We tested this idea by constructing compartmental models representing the main types of neurons in the ring: (1) RMD, a short monopolar ring interneuron, (2) AWC, a bipolar sensory neuron, and (3) AVH, a long monopolar interneuron. Membrane resistivity (R_m) was estimated from single channel currents in cell-attached patches from L1 larvae. This was possible because the input resistance of *C. elegans* neurons is so high that the membrane capacitance charges and discharges as the channel opens and shuts. We first estimated the membrane time constant (τ_m) by fitting sums of exponentials to the time course of the discharge current, then calculated R_m by dividing τ_m by the specific membrane capacitance. R_m ranged from 0.5 - 2.6 KΩ cm^2. Axial resistivity (R_i) was assumed to be 400 Ω cm. Cell dimensions were determined from electron micrographs of L1 larvae. Signal propagation was studied by measuring the decrement in voltage from a synapse near the soma to the point furthest away in the nerve ring. Decrements ranged from 56% for RMD to 76% for AVH. Actual decrements are probably smaller, however, because we underestimated R_m and overestimated R_i and process length in the ring. Thus, regenerative potentials are not necessary for signal propagation in the nerve ring in *C. elegans*.

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569.8

CYCLIC AMP, A SIGNAL MOLECULE INVOLVED IN THE BEHAVIORAL SEX-PEPTIDE RESPONSE IN *DROSOPHILA MELANOGASTER* FEMALES? I.Fleischmann[1], B.Dauwalder[2], T.Chapman[3], B.Cotton[1], M.Schlumpf*[1] and E.Kubli[1]. [1]University of Zurich, 8057 Zurich, Switzerland. [2]UT MD Anderson Cancer Center, Houston, USA. [3]University College London, London, UK.

Mating elicits several well defined responses in the female of *Drosophila melanogaster* : Ovulation and oviposition are increased, the remating rate (measured as percentage of females copulating within a given time period = receptivity) is low and the female shows rejection behavior towards courting males by extruding her ovipositor. Central to the control of these responses is the sex-peptide (SP), a 36 amino acid long peptide, synthesized in the male accessory glands. During copulation it is transferred into the female. Injected into virgin females, or ectopically expressed, SP induces the same reactions as observed after normal copulation. From work with gynandromorphs it is known that performance of sex-specific behaviors depends on the sex of certain parts of the brain. This finding and the complex behavioral changes induced by SP imply that the nervous system is involved in the females' reaction to SP.

dunce is known to be a learning and memory mutant. The *dunce* gene product is expressed predominantly in the mushroom bodies, a brain structure thought to be involved in these processes. *dunce* females remate more frequently than the wild type. We tested the mating response of SP-injected *dunce* females. They do not respond to the SP with reduction of receptivity. We used transgenic *dunce* females carrying a heat-inducible *dunce*$^+$ rescue construct to test whether the *dunce* gene product is physiologically required for induction of the SP response at the time of injection. We observed a partial rescue of the SP response in heat-induced *dunce* transgenic females injected with SP. Since the *dunce* gene codes for a cAMP-phosphodiesterase and the cAMP level in *dunce* mutant flies is elevated, we conclude that the cAMP level might be crucial for the behavioral SP-response.

569.9

MOLECULAR CHARACTERIZATION OF THE MUSHROOM BODY DERANGED LOCUS OF DROSOPHILA MELANOGASTER. K. Miyamoto, M. Gasser and K. Furukubo-Tokunaga*. Zoologishes Institut der Universität Basel, CH-4051 Basel, Switzerland.

Mushroom bodies are a pair of neuropil structures that are essential for various higher order behaviors in *Drosophila* such as olfactory learning and courtship. Negatively reinforced olfactory learning is completely abolished in the mutant *mushroom body deranged*, in which the axons of mushroom bodies fail to find the right path leading to abnormally enlarged calyces with degeneration of peduncles and lobes (Heisenberg et al., 1985. J. Neurogenet. 2: 1-30.). By genetic deficiency mapping, we have delimited the *mbd* locus at 17B3-6 on the X chromosome and completed DNA walk that covers the region 17A-C. Molecular mapping of the chromosomal break points of the deficiency lines have defined the *mbd* locus at a region of 30 kb in the distal vicinity of the wint-3 gene. Three of the mushroom body specific enhancer-trap lines (isolated in the R.L. Davis lab) have also been mapped on the DNA walk in the proximal vicinity of the *mbd* locus. A putative *mbd* transcriptional unit, which is transcribed at various developmental stages, has been identified and isolated as a cDNA clone. The structure and the expression pattern of this transcriptional unit will be presented (supported by the Swiss NSF).

569.10

THE EFFECT OF MUSHROOM BODY ABLATION ON LEARNING AND MEMORY IN COURTSHIP BEHAVIOR IN *DROSOPHILA*. S. M. McBride, G. Baker and K. K. Siwicki*, Biology Department, Swarthmore College, Swarthmore, PA 19081.

Evidence that the neural structures called mushroom bodies (MB) are related to learning in *Drosophila* includes recent experiments in which the MBs were specifically ablated by treatments with the DNA synthesis inhibitor Hydroxyurea (HU). MB-ablated flies were unable to learn to associate shock and odor in a classical conditioning paradigm (deBelle & Hiesenberg, 1994, *Science 263*, 692). Using the same ablation technique, we tested the role of the MBs in a behavioral learning paradigm, experience-dependent courtship. Experience-dependent courtship occurs when a virgin male is placed with a fertilized female for 60 minutes and as a result displays less vigorous courtship towards a virgin female (Siegel & Hall, 1979, *PNAS 76*, 3430). The effects of MB ablation on learning in this paradigm were tested in males immediately after training, while the effects on memory were tested 30 and 60 minutes after training. Results indicated that the HU-induced MB ablations had no effect on learning in the experience-dependent courtship paradigm: both MB-ablated and control males learned to reduce their courtship behavior as a result of experience with a fertilized female. In control males the learned behavior persisted for at least 60 minutes after training; MB-ablated males, however, exhibited significantly less memory retention than controls at 30 and 60 minutes after training. Our results implicate the MBs in the process of memory retention, but not in the initial learning of preconditioned courtship behavior.

569.11

INHIBITION OF NO-SYNTHASE SPECIFICALLY IMPAIRS LONG-TERM MEMORY IN THE HONEYBEE, *APIS MELLIFERA*. U. Müller* Institut für Neurobiologie FU-Berlin, 14195 Berlin, Germany.

NO-synthase (NOS) in the bee brain has similar properties as the corresponding vertebrate enzyme. The localization of NOS in the antennal lobes and the lip of the mushroom body calyces, sites of associative learning, suggests a role of the NO system in the processing of chemosensory information and in mechanisms of olfactory learning and memory. Inhibition of the NOS during the associative olfactory conditioning (CS-US pairing) specifically interferes with the formation of long-term memory as measured at 24 h (LTM), while inhibition before or after associative CS-US pairing has no effect on LTM formation. Blocking of NOS does not interfere with acquisition or memory retrieval. Interestingly, inhibition of the NOS during a single CS-US pairing, does not affect conditioned response up to 24 h after conditioning, indicating that this form of LTM requires multiple learning trials. Although the molecular target of NO action, mediating LTM is as yet unknown, a likely effector of NO is the soluble guanylate cyclase. We have shown that the cGMP produced via activation of the NOS modulates different components of the cAMP cascade. Therefore, it is conceivable that the specific loss of the LTM indicates a requirement of NO mediated mechanisms during CS-US pairing, possibly a NO dependent modulation of the cAMP cascade in the Kenyon cells.

569.12

NITRIC OXIDE IS IMPLICATED IN OLFACTORY LEARNING IN *HELIX* BUT NOT IN OLFACTORY ORIENTATION PER SE.

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Nitric oxide (NO) has been implicated in learning in vertebrates and invertebrates. In *Helix*, nitric oxide has been localized in the central ganglia and might also be present in peripheral olfactory sensory neurons. Therefore, the role of NO in olfactory learning (food-attraction conditioning) is contrasted to that in olfactory orientation. Food-attraction conditioning is an olfactory learning phenomenon in which snails, by feeding on a food, acquire the ability to locate this particular food. Prior to feeding, naive snails are unable to detect the food; in control runs only 8% of the experimental animals [n=50] located the food. After these tests, NO synthesis was inhibited by injection of selective NO-synthase inhibitor L-NAME (75 mg/kg) into the haemolymph; a control group received injections of vehicle. Snails were fed 1 hour after injection, and the effect of feeding (food conditioning) was assessed the next day by the animals' ability to locate the conditioned food.

Inhibition of NOS significantly reduced food finding ability. While 72% of the saline injected snails located the food, snails injected with L-NAME showed reduced food finding ability, and only 44% of the animals were able to locate the food. This is a highly significant reduction from the performance of saline injected controls, indicating that some aspects of food-attraction learning require the functional enzyme NOS. Complete abolishment of the learning, however, was not observed, and increasing the dosage to 150 mg/kg had effects that were similar to those caused by the lower dosage.

Olfactory orientation *per se* remained unaffected. When food-conditioned animals (fed 24 hrs before) were injected with L-NAME, their food finding ability was indistinguishable from that of saline injected controls when tested 1 hour or 1 day after injection (83% and 62% of the snails located the food, respectively).

These results suggest that NO is involved in the acquisition of food-attraction learning, and that the inhibitory effect of L-NAME on olfactory learning is not due to impeded olfactory functioning.

569.13

A SINGLE CEREBRAL INTERNEURON INITIATES DIFFERENT ELEMENTS OF FEEDING BEHAVIOR IN THE MOLLUSC, *CLIONE LIMACINA*. T.P.Norekian* and R.A.Satterlie. Dept. Zoology, ASU, Tempe, AZ 85287 and FHL, Univ. of Washington, WA 98250.

Clione limacina is a specialized predator that feeds on actively swimming pteropods of the genus *Limacina*. To seize the prey, *Clione* rapidly everts 6 tentacle-like oral appendages, called buccal cones, which surround and hold the *Limacina* shell. Then, the radula and specialized chitinous hooks are used to pull the *Limacina* body from its shell, to be swallowed whole. Feeding behavior is also characterized by acceleration of swimming and depression of all defensive withdrawal reactions. Several groups of neurons which control different components of feeding, withdrawal and swimming behaviors in *Clione* have been previously identified. However, the question of their coordinated activity, necessary for developing feeding behavior, still remains open. We describe here a pair of cerebral interneurons which have widespread excitatory and inhibitory effects on a number of neurons in the cerebral and pedal ganglia, directed toward the initiation of feeding behavior in *Clione*. This bilaterally symmetrical neuron, designated Cr-PC (Cerebral neuron initiating Prey Capture), produced monosynaptic activation of Cr-A motoneurons which control buccal cone extrusion, and inhibition of Cr-B and Cr-L motoneurons whose spike activity maintains buccal cones in a continuous withdrawn position inside the head in non-feeding animals. Cr-PC neuron also produced monosynaptic activation of a number of swim motoneurons in the pedal ganglia and pedal serotonergic Pd-SW neurons involved in peripheral modulation of swimming. Cr-PC neuron in *Clione* functions much like the C-PR which was suggested to evoke a feeding arousal in *Aplysia*.

569.14

TWO-PHASE MECHANISM OF AVERSIVE ASSOCIATIVE LEARNING IN *IN-VITRO* BREEDING EMBRYOS AND ADULTS OF THE POND SNAIL, *LYMNAEA STAGNALIS*. M. Yamanaka, S. Kojima, Y. Fujito† and E. Ito*. Lab. Animal Behavior & Intelligence, Div. Biol. Sci., Grad. Sch. Sci., Hokkaido Univ., Sapporo 060, Japan and †Dept. Physiol., Sch. Med., Sapporo Med. Univ., Sapporo 060, Japan.

A method for the building-up of an associative learning with an aversive unconditioned stimulus (UCS) in *in-vitro* breeding embryos of the pond snail, *Lymnaea stagnalis*, was established. The embryos were maintained in the individual holes of micro assay plates and covered with extract from the egg cocoons and then with tap water. To train the embryos for the associative learnings, they were settled upside down in the individual small holes of silicon dishes and perfused continuously with Jockusch solution (B. Jockusch, 1968). The conditioned stimulus (CS, sucrose) and the UCS (KCl) were associated 5 times. The feeding responses of the embryos in some later developmental stages, in which the assembly of ganglia had already begun, were successfully terminated by a sucrose application as a test, and their acquired behavior was maintained until adulthood.

Although an aversive associative learning is always built up in both embryos and adults of *Lymnaea*, a favorite learning was also sometimes possible. As previously proposed (S.Kojima *et al.*, 1995) to explain this difference, we have examined the neuromodulation mechanisms in their interneurons. For the aversive learning, it was observed that an excited UCS interneuron inhibited that for the CS for a long time. Namely, the sucrose application (CS) failed to start the feeding response in the behavioral experiments. After many training sessions, the excited CS interneuron eventually activated that for the UCS, and thus the CS caused the shrinkage or the withdrawal of the associative behavior. For the aversive associative learning, however, the first phase was enough and, therefore, it was easily built up. With regards to the favorite learning, the CS interneuron had to directly activate that for the UCS. This meant that the first phase like that in the aversive learning must have been skipped and only the second one was required. Such activation and the CS-induced aversive response were difficult to observe.

569.15

AN OPTICAL AND ELECTROPHYSIOLOGICAL STUDY OF MODULATION OF NEURONAL ACTIVITY IN SLUG CEREBRAL GANGLIA. S. Kawahara*, S. Toda, and Y.Kirino. Faculty of Pharmaceutical Sciences, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, JAPAN

The terrestrial slug *Limax marginatus* has a high ability in odor-taste associative learning. When a slug is exposed to an odor of a food followed by a solution of quinidine sulfate, the slug shows an aversive response to that odor the next time. The locus at which the learning takes place is thought to be the cerebral ganglia, where inputs from the olfactory (tentacle) and gustatory (lip) nerves are integrated.

We attempted to find a cellular correlate of learning and memory in the form of plasticity in the cerebral ganglia, using electrophysiological and optical techniques. The cerebral ganglia with tentacle and lip nerves attached were isolated from the slug. A local field potential was measured from the procerebral (PC) lobe of the cerebral ganglia. An electrical stimulation of the tentacle nerve evoked a negative potential in the neuropil of the PC lobe. The evoked potential was decreased by addition of 0.1 mM $NiCl_2$ to the medium or by replacement of $CaCl_2$ with $MgCl_2$. This implies that the evoked potential reflects the postsynaptic potential of PC neurons. After a tetanus stimulation was applied to the tentacle nerve and the cell mass of the PC lobe, the amplitude of the evoked potential increased. The potentiation lasted for about 15 min after the shock. This potentiation was mimicked by bath-application of serotonin (10 μM). These results suggest that input from the tentacle nerve to the PC neuron can be modulated. Optical measurement of neural activity in the PC lobe using a voltage-sensitive dye and 128 x 128 MOS-FET camera gave results consistent with this idea.

INVERTEBRATE LEARNING AND BEHAVIOR IV

570.1

LONG-TERM POTENTIATION OF *APLYSIA* SENSORY NEURON SYNAPSES AFTER TAIL PINCH: EFFECTS OF POSTSYNAPTIC HYPERPOLARIZATION AND BAPTA INJECTION. M. Cui* and E.T. Walters. Department of Integrative Biology, University of Texas - Houston Medical School, TX 77030.

Recently we found that PTP and LTP produced by intracellularly firing tail sensory neurons (SNs) was reduced by postsynaptic hyperpolarizaton or BAPTA injection into the postsynaptic motor neuron (MN)(Cui & Walters, *Soc. Neurosci. Abstr.* 20:1071, 1994). To test contributions of voltage- and Ca-dependent postsynaptic induction signals during noxious stimulation of the SNs' receptive fields, we activated the SNs peripherally, using 10 intense pinches to the tail at 5 sec intervals. Intracellular test stimuli were applied to an SN every 5 min while recording from 2 MNs. Hyperpolarization of one MN soma to -120 mV or more failed to significantly reduce tail-pinch-induced potentiation at 1 min (mean EPSP amplitude 262 % of baseline vs. 278% in concurrently recorded, non-hyperpolarized control MNs) or 30 min (mean EPSP 170% vs 172% in controls, n=5 animals), perhaps because the injected current could not hyperpolarize the postsynaptic membrane sufficiently during massive MN depolarization. In contrast, injection of BAPTA into one MN reduced 1 min potentiation in 5 of 6 animals (mean EPSP 306% vs 341%) and reduced 30 min potentiation in 4 of 6 animals (mean EPSP 112% vs. 130%). Injection of BAPTA had an even stronger effect on potentiation produced by relatively weak tail shock, reducing both the 1 min and 30 min potentiation in 5 of 5 animals (mean EPSPs 153% and 212%, and 98% and 139%, respectively). These results suggest that postsynaptic induction signals contribute at least partially to LTP underlying site-specific sensitization of the tail-wthdrawal reflex.

570.2

DYNAMICS OF INDUCTION AND EXPRESSION OF LONG-TERM SYNAPTIC FACILITATION IN *APLYSIA*. J. Mauelshagen, G.R. Parker, & T.J. Carew*, Dept. of Psychology, Yale University, New Haven, CT, 06520.

In the tail sensory-motor synapses in *Aplysia*, multiple applications of serotonin (5HT) induce both short-term and long-term synaptic facilitation (STF and LTF) (Emptage & Carew, 1993). In the present study, we examined the temporal dynamics of these two forms of synaptic plasticity.

We first examined the expression of STF and LTF. Five applications (6 min each) of 10 μM 5HT were delivered to isolated pleural-pedal ganglia. EPSP amplitudes were recorded before and immediately after the first 5HT application, and again at either 3, 10, 15, or 20 hrs after the last 5HT application. Data are expressed as % baseline. STF was induced after the first 5HT application ($\bar{x}$=198%; p<.001; n=34), and decayed to baseline by 3 hrs ($\bar{x}$=94%; n=12). At the 10-hr test no significant LTF was observed ($\bar{x}$=135%; n=8);. however, LTF was clearly expressed at both the 15-hr test ($\bar{x}$=199%; p<.02; n=6), and at the 20-hr test ($\bar{x}$=130%; p<.02; n=9). These data show that STF completely decays several hours before the expression of LTF.

We next characterized the induction of LTF by exposing different preparations to a specific number (1 to 5) of 5 min 50 μM 5HT applications. Tests were made: (1) at baseline; (2) immediately after the first application; (3) immediately after the last application; and (4) every 15 min thereafter for up to 3 hrs. The amount of facilitation progressively and significantly declined with additional 5HT applications (1st: $\bar{x}$=207%, n=19; 2nd: $\bar{x}$=182%, n=4; 3rd: $\bar{x}$=139%, n=4; 4th: $\bar{x}$=159%; n=5; 5th: $\bar{x}$=82%; n=4; Comparing 1st and 5th application: $\bar{x}$ reduction=125%; p<.003). Moreover, in 6 preparations a second phase of facilitation appeared within one hour after the last 5HT application and subsequently decayed to baseline. These preliminary results may reflect a separate mechanistic component in the induction of LTF (Ghirardi et al., 1995). They further suggest that the induction and expression of LTF may be composed of multiple phases of facilitation that can be temporally dissociated in this system.

570.3

GLUTAMATE INDUCES A CHANGE FROM SYNAPTIC SHORT-TERM DEPRESSION TO SHORT-TERM FACILITATION IN *HELIX POMATIA*. G.R.J. Christoffersen* and T.S.S. Schilhab. (SPON: European Neuroscience Association). Neuroscience Centre for Cognition and Memory, August Krogh Inst., Univ. of Copenhagen, Copenhagen, Denmark, 2100.

EPSPs in neuron RPa3 in *Helix pomatia* display short-term depression when repeatedly activated by stimulation of an afferent nerve at a low, constant strength near threshold value. In contrast to this, a series of EPSPs activated at a higher, constant strength not only begins at a higher amplitude, but displays short-term facilitation. Presently, we tested the ability of glutamate to induce the shift from depression to facilitation at a constant stimulus strength.

Experiments began by a series of EPSPs, activated by stimulation of the right pallial nerve (1/10 Hz) at a constant strength of less than 1.5 times threshold value. Following that, the ganglia were exposed 4 times for 2 min. at 20 min. intervals to constant concentrations of Na-glutamate (ranging in different experiments from 0.5 μM to 10 μM). 10 min. after the last exposure, a second series of EPSPs were elicited using the same stimulus strength and frequency as before.

The results showed, that glutamate had induced two changes. 1) The amplitude of the first EPSP in the second series had increased by an average factor of 6.4 ± 1.7 S.E.M. compared to the first EPSP of the first series. 2) While the first series displayed short-term depression, the potentiated EPSPs in the second series displayed short-term facilitation. Control experiments in normal saline showed a reduced initial EPSP in the second series and short-term depression of the subsequent EPSPs of that series. The effects of glutamate had not decayed 60 min. after the last exposure.

In conclusion, glutamate induced a long-term EPSP enhancement on top of which, a normal short-term depressing capacity was replaced by short-term facilitation. This change is different from heterosynaptic facilitation in for instance *Aplysia*. Here, the amplitude of depressed EPSPs are also increased, but continued EPSP activation after the facilitation leads to short-term depression - in contrast to the presently observed short-term facilitation.

570.4

MODULATION OF NEURONAL ACTIVITY IN THE HEAD GANGLIA OF *APLYSIA* BY BAG CELL PEPTIDES.

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Egg laying hormone (ELH) and other bag cell peptides are released from the bag cells and other cells of the ELH neural system at the initiation of egg laying. In the abdominal ganglion they modulate activity of target neurons for up to several hours. To identify sites of action and possible neurotransmitter or neurohormonal roles for these peptides in the head ganglia, the effects of application of peptides to the head ganglia were compared to the effects of endogenous release of the peptides during bag cell burst discharges. In an isolated CNS or a reduced CNS preparation, ELH application for 6-15 min inhibited ongoing fictive locomotion, as recorded extracellularly from the parapedal commisure. Subsequent washing out of the peptide produced an excitation lasting 7-45 min. A bag cell burst discharge produced similar inhibition followed by excitation. Using intracellular recordings, we also found cells in the cerebral ganglion that are responsive to ELH and α-BCP. The results support the idea that aspects of reproductive behavior are controlled by neuromodulation of circuits in the head ganglia by these neuropeptides.

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570.5

FMRFAMIDE SUPPRESSES THE DEVELOPMENT OF SENSITIZATION-INDUCED INCREASE IN HEART RATE IN APLYSIA CALIFORNICA

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Previous studies in this laboratory have shown that sensitizing stimulation applied to the tail body wall will produce an increase in heart rate lasting for several hours. Billy and Walters (1989) have demonstrated that tail mechanoreceptors are less responsive when exposed to FMRFamide. This study investigates whether FMRFamide applied to tail mechanoreceptors can alter the development or maintenance of sensitization-induced increases in heart rate.

Heart rate was monitored in an in vitro preparation using a cannula implanted in the anterior aorta. FMRFamide (10^{-5}M) was perfused to the lateral body wall while sensitizing shocks were delivered to the same location via implanted electrodes. When FMRFamide was applied concurrent with sensitizing stimulation, there was no increase in heart rate. At 30 min post-training, heart rate was 80.8% (SEM 5.3) of prestimulation values and at 90 min after stimulation, heart rate was 78.9% (SEM 5.9)(N=8). Thus FMRFamide appears to act peripherally at tail mechanoreceptors to suppress sensitization-induced increases in heart rate.

570.6

PROTEIN PHOSPHATASE INHIBITOR OKADAIC ACID AFFECTS SHORT- AND LONG-TERM DEPRESSION OF *APLYSIA* SENSORIMOTOR SYNAPSES EVOKED BY FMRFAMIDE.

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The sensorimotor synapse of *Aplysia* can be depressed for short and long durations by the neuropeptide FMRFamide. Many of the short- and long-term actions of FMRFamide are mediated by metabolites of arachidonic acid. FMRFamide also activates several protein phosphatases including those blocked by okadaic acid. We therefore examined the actions of FMRFamide on the efficacy of the sensorimotor synapse formed in culture in the presence of 200 nM okadaic acid, a concentration that did not evoke excitability changes in the sensory or motor cells. Short-term depression was measured by testing the efficacy of the connections following bath applying 1 μM FMRFamide after the sensory cells were first given 5 stimuli (30 sec intervals) to stabilize the EPSP amplitude. Okadaic acid had no effect on the kinetics of homosynaptic depression. The expression of long-term depression was measured 24 hr following 4 repeated applications of FRMFamide. Okadaic acid did not affect the initial level of synaptic depression, but affected the time course of short-term depression. The amplitude of the EPSP was reduced by 58% and 66% 30 sec following application of FRMFamide in controls and okadaic acid, respectively. After 2 min, controls recovered by only 7% (N=9) while the okadaic acid-treated cultures recovered by 35% (N=13). Okadaic acid also altered long-term depression. Whereas FMRFamide reduced EPSP amplitudes by -23% (N=8) compared to an increase of 1.5% for controls (N=7), application of FMRFamide in the presence of okadaic acid (N=10) evoked only a -8% change. Interestingly, okadaic acid alone (N=13) affected the cultures by evoking a decline in the EPSP amplitude of -22%. All cultures expressed similar levels of short-term depression 24 hr after treatment (from -57% to -61%). The results suggest that protein phosphatases may contribute to the maintenance phase of short- and long-term depression evoked by FMRFamide.

570.7

HETEROSYNAPTIC MODULATION OF *APLYSIA* SENSORIMOTOR SYNAPSES DEPRESSED BY LOW FREQUENCY HOMOSYNAPTIC ACTIVITY EMERGES LATE DURING SYNAPSE FORMATION IN VITRO.

Z.-Y. Sun* and S. Schacher. Ctr. Neurobiol. & Behav., Columbia Univ. College of P & S, NYSPI, New York, NY 10032.

Previous studies indicate that multiple processes mediated by at least two second messenger pathways, PKA and PKC, contribute to 5-HT facilitation of *Aplysia* sensorimotor synapses. The contributions of each of the second messenger pathways in evoking changes in synaptic efficacy may be dependent on the previous stimulus history of the sensory neuron (SN). To explore the contributions of the second messenger systems in mediating short- and long-term synaptic facilitation evoked by 5-HT, we used cell culture to examine the time course for the expression of 5-HT facilitation of SN-L7 synapses first depressed by low frequency (30 sec intervals) stimulation of the SN, and 5-HT facilitation of nondepressed synapses. Cultures were tested on days 1, 2, and 4. Despite the large increase in synaptic efficacy over time (2-3 fold), the kinetics of homosynaptic depression (HSD) were unchanged between day 1 and day 4. In addition, there was no difference over time in the level of facilitation by 5-HT in nondepressed synapses. By contrast, 5-HT facilitation of depressed synapses was significantly lower in day 1 cultures. These early cultures also responded differently to low frequency stimulation in the presence of 5-HT. On day 4, the kinetics of HSD is altered significantly by 5-HT. However, the kinetics of HSD on day 1 in the presence of 5-HT were identical to controls, despite the initial facilitation produced by 5-HT. Preliminary data suggest that the altered kinetics of HSD evoked by 5-HT on day 4 is mediated primarily via an interaction between HSD and the increase in PKC activity evoked by 5-HT. The results are consistent with previous studies suggesting that activation of the PKC pathway contributes significantly to facilitation of depressed synapses. Morevover, they suggest that the PKA pathway triggered by 5-HT is present early, while components of the PKC pathway activated by 5-HT develop over time in culture.

570.8

NEUROPEPTIDE EXPRESSION IN VARICOSITIES OF *APLYSIA* SENSORY NEURONS IS REGULATED BY THE TARGET AND NEUROMODULATORS EVOKING LONG-TERM SYNAPTIC PLASTICITY.

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The synapses of mechanosensory neurons (SNs) of *Aplysia* undergo long-term functional and structural modulation with behavioral training. Similar changes are evoked in connections established in cell culture by specific neuromodulators. Since the number of SN varicosities can be modulated both up and down for long durations, we examined whether the expression of synapse-specific molecules such as sensorin, the SN-specific neuropeptide (Brunet et al., 1991), are also modulated 24 hr following either 5-HT applications that evoke long-term facilitation, or FMRFamide applications that evoke long-term depression. We measured a) changes in the amplitude of the EPSP, b) changes in the number of sensory varicosities with flurescent dye fills of each SN before and afer treatment, and c) the expression of sensorin in varicosities by immunocytochemical staining with standard methods. Both 5-HT and FMRFamide evoked functional and structural changes in the sensorimotor connections established after 5 days in culture compared to control treatment. Consistent with previous studies indicating that varicosities contactng the major axons of L7 contained transmitter release sites, the expression of sensorin was highest in sensory varicosities contacting the proximal motor axons; 54% of the varicosities were sensorin-positive, compared to 22% of varicosities contacting distal neurites or the substrate. Following treatment with 5-HT, 73% of the pre-existing varcosities and 59% of the new varicosities were sensorin-positive. By contrast, only 31% of pre-existing SN varicosities were sensorin-positive following treatment with FMRFamide compared to 52% in another group of controls. These results suggest that neuromodulators not only regulate overall structure but also regulate both up and down the expression of SN peptide at putative synaptic sites either by modulating synthesis or peptide processing, or by changing turnover.

570.9

A COMPUTATIONAL MODEL OF ACTIVITY-DEPENDENT POTENTIATION AND ITS EXTERNAL MODULATION AT L30 INHIBITORY SYNAPSES IN *APLYSIA*.

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In *Aplysia*, activity-dependent synaptic potentiation from inhibitory interneurons (L30s) is induced by tactile stimulation, providing dynamic gain control in the siphon-withdrawal reflex (SWR) (Fischer & Carew, 1993, 1995). In addition, tail shock (TS) selectively inhibits specific components of L30 potentiation; these TS effects are mimicked by 5-HT and by reduced external Ca^{2+} ($[Ca^{2+}]_o$) (Fischer & Carew, 1994). To examine the role of L30 potentiation and its modulation in the SWR, we constructed a computational model that included both the L30s and the L29s, excitatory interneurons which are reciprocally connected to the L30s.

The model was built using the software package NEURON and incorporated empirically derived biophysical and synaptic features of the L29 - L30 circuit. L30 potentiation was modeled with two processes: frequency facilitation (FF) and post-tetanic potentiation (PTP). In our simulations, the frequency of steady-state L30 activation (1.5 to 6 Hz) was varied to mimic activation by different levels of ambient tactile stimulation. Based on cellular data (Fischer & Carew, 1994) FF and PTP were differentially dependent upon $[Ca^{2+}]_o$, so that only PTP was suppressed by a 50 % reduction in $[Ca^{2+}]_o$.

The model revealed that at low frequencies synaptic enhancement is mainly driven by FF, which saturates quickly, providing minimal temporal integration. At higher frequencies, the build up of PTP is more prevalent and extends the temporal integration underlying synaptic enhancement. With reduced $[Ca^{2+}]_o$ (suppressing PTP), L30 potentiation is primarily regulated by FF across all frequencies, which provides less steady-state synaptic enhancement and less temporal integration, thus changing the fundamental integrative properties of the circuit. These data suggest different functional roles for FF and PTP in the dynamic regulation of this reflex circuit.

570.10

DEVELOPMENTAL EMERGENCE OF IDENTIFIED SIPHON MOTOR NEURONS COINCIDES WITH THE BEHAVIORAL EXPRESSION OF THEIR UNIQUE SIPHON MOVEMENT IN *APLYSIA*.

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The number of neurons in the CNS of *Aplysia* increases markedly during the final stage of juvenile development, Stage 12 (Cash & Carew, 1989). This coincides with the expression of behavioral sensitization of the siphon withdrawal response (SWR) (Rankin & Carew, 1988). To examine whether new neurons may contribute to the emergence of sensitization, we have combined cell birthdating techniques with behavioral observations to track the emergence of a specific class of siphon motor neurons (MNs), the LFS-B cells (4 total), which have been implicated in SWR sensitization (Frost et al., 1988).

Stage 12 was partitioned into two sub-stages: Early 12 (E12: 9-13 mm) and Late 12 (L12: 18-25 mm). The approximate birthdates of LFS-B neurons was determined through the use of BrDU and standard double-label immunocytochemical techniques. Animals were first chronically exposed to 1 μm BrDU for the duration of E12 or L12, then reared to adulthood (>10 cm). LFS-B MNs were then positively identified physiologically by monitoring siphon movements induced by intracellular activation, after which the cells were injected with 2% Neurobiotin. Of the LFS-B MNs identified in L12-exposed animals, 45% (10 of 22) were BrDU positive, whereas only 24% (4 of 17) of MNs identified in E12-exposed animals were BrDU positive.

In adults, LFS-B MNs mediate a unique siphon respoose (flaring) which is elicited by tail stimulation. Studies examining the development of flaring in E12, L12, and adult animals (using suprathrshold tail stimulation) revealed that flaring first becomes apparent only in Stage L12. Collectively, our results suggest that the LFS-B MNs are born and are integrated into a functional neural circuit relatively late in development, raising the possibility that their emergence may contribute to the expression of sensitization in this same developmental stage.

570.11

MODULATION OF CURRENTS IN DROSOPHILA LARVAL AND ADULT CNS NEURONS BY SEROTONIN.

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In order to understand the neural contribution to defective learning and memory in Drosophila mutants we are analyzing how neurotransmitter-induced responses are altered in these mutants. In this study we examine the effects of serotonin. Serotonin has been shown to modulate certain ionic currents by activating secondary messenger cascades. Some of these effects have been implicated in regulating neuronal excitability and synaptic transmission and may be involved in learning and memory. Three distinct serotonin receptors have been cloned from Drosophila. We show by whole-cell patch-clamping that serotonin can affect two distinct currents in both larval and adult Drosophila neurons in acute culture. One of the components is the slowly inactivating, voltage-activated potassium current (K_D) which is reversibly inhibited. The other is a "leakage" current which is activated constantly but can be inhibited by serotonin. This "leakage" current is gated by cation channels which have a reversal potential of around +10mV and have a conductance of between 20-30pS. It's amplitude varies between neurons from 5 to 50pA when clamped at -80mV. Inhibition of this current appears to exert a profound effect on membrane potential: hyperpolarizing neurons from -50mV to as much as -100mV. We have examined over 200 neurons, 70 of which were from adult Drosophila brains, and over 35% respond to serotonin. A study of whether and how these responses are altered in learning and memory mutants is currently in progress.

570.12

IONIC CURRENTS IN DISSOCIATED *APLYSIA BRASILIANA* PARAPODIAL MUSCLE FIBERS. P.J. Laurienti*, G.N. Gamkrelidze and J.E. Blankenship. Marine Biomedical Inst., Univ. Tx. Med. Br., Galveston, TX 77555-1069.

Transmission at the parapodial neuromuscular junction in *Aplysia brasiliana* is enhanced by serotonin (5-HT) and provides a useful model for studying synaptic plasticity. Previous work in our laboratory has demonstrated that 5-HT enhances the calcium component of motoneuron action potentials, indicating that a portion of the serotonergic facilitation occurs at the presynaptic terminal. However, the possibility that 5-HT has a direct effect on parapodial muscle remains. We have used a dissociated muscle preparation to study membrane ion currents under voltage-clamp conditions. Initial surveys of total membrane current utilized voltage ramps to produce I-V curves. All cells had outwardly rectified I-V curves with membrane resistance increasing upon hyperpolarization and decreasing with depolarization. The I-V curves provide evidence that these muscle fibers lack an inwardly rectifying K^+ current. Analysis of membrane currents activated by depolarizing voltage steps revealed two populations of muscle fibers. Type I fibers have two main depolarization-activated currents: a TEA-sensitive, outward K^+ current and a Co^{2+}-sensitive, inward Ca^{2+} current which may have several components. Type II fibers not only express the K^+ and Ca^{2+} currents found in Type I fibers, but also have a transient, outward current and a non-inactivating, outward current. These currents are reduced in 250mM K^+ SW and blocked in 15% Cl^- (isethionate-substituted) SW. Both currents are sensitive to TEA and 4-AP and blocked in $0Ca^{2+}$ seawater. This work is the first description of the membrane currents in *Aplysia* parapodial muscle. Experiments are currently underway to investigate the effect of 5-HT on these muscle membrane currents.

570.13

SEROTONIN ENHANCES CALCIUM CURRENT IN ISOLATED SOMATA OF *APLYSIA* PARAPODIAL MOTOR NEURONS. G.N. Gamkrelidze*, P.J. Laurienti and J.E. Blankenship. Marine Biomedical Inst., Univ. Tx. Med. Br., Galveston, TX 77555-1069.

The pedal ganglia of *Aplysia brasiliana* contain identified motor neurons (MN's) and serotonergic modulatory cells (POP cells) which innervate parapodial muscles and fire rhythmically during swimming. With each parapodial opening during swimming, POP cell activity enhances motoneuron-induced junction potentials and muscle contractions and relaxation rates. We have shown using current clamp that POP cells or exogenously applied 5-HT modulates a Ca^{+2} component of MN action potentials to facilitate neuromuscular transmission while having no effect on motoneuron action potential width. We are now investigating the membrane ion currents in isolated somata of parapodial MN's using single electrode voltage-clamp in order to identify the mechanism whereby 5-HT causes an increase in transmitter release. The head-ganglia were removed from the animal with the parapodia still attached via the parapodial nerves. Pedal ganglia were desheathed and then treated with 0.5% Pronase E (40 min) followed by 1% collagenase (120 min). After the enzymes were washed out, putative MN's were identified by their ability to cause parapodial contractions and then isolated from the pedal ganglion by gently pulling the impaled cell away from the ganglion using the penetrating electrode. The stretched axon was then severed near the soma, and the cell was transferred to a solution of artificial sea water (ASW) containing 50mM TEA, 1 μM TTX and 10mM 4AP. Experiments were performed on isolated somata with resting membrane potentials near -50 mV. Depolarizing voltage steps activated an inward current that was entirely blocked in 0 Ca^{+2} (Co^{+2}) ASW. Part of this current was blocked by 10μM nifedipine. Application of 5 μM 5-HT increased the peak inward current by an average of 40% (n=3), suggesting that 5-HT may mediate the facilitatory effect of POP cells on neuromuscular transmission by directly enhancing Ca^{2+} current.

570.14

DYNAMIC REPRESENTATION OF LEARNED INFORMATION IN A DISTRIBUTED MEMORY IN *APLYSIA*. J.R. Lieb Jr.* and W. N. Frost, Dept. of Neurobiology and Anatomy, Univ. of Texas Medical School, Houston, Texas, 77225.

In the *Aplysia* siphon-withdrawal reflex circuit, the memory for sensitization is represented as a distributed set of circuit modifications (Frost et.al., J.Neurobiol., 1988). Our previous use of a realistic computer simulation of this circuit indicated that these sites of plasticity store separate and distinct aspects of the information encoded in sensitization. Specifically, plasticity at the sensory neuron (LE) to motor neuron (LFS) connection encodes changes in the frequency of firing of the phasic motor neuron firing response, which is related to reflex amplitude. Plasticity within the interneuronal population encodes changes in the long-lasting component of the motor neuron firing response, which is related to reflex duration (Lieb and Frost, Soc. Neurosci. Abstr. 1992).

In our present work we find that the information stored by these sites is expressed differently depending upon the intensity of the test stimulus. First, weak inputs are amplified much more than strong inputs following sensitization. Thus the circuit acts to bias motor neuron firing responses toward a uniform level in the sensitized state, independent of stimulus intensity. Second, the quantitative contribution of individual sites of plasticity to the sensitized circuit output also depends on stimulus intensity. For example, with weak stimuli, enhanced interneuronal recruitment by plasticity at sensory neuron to interneuron synapses contributes more importantly than the interneuronal sites of plasticity. With stronger input, however, this relationship reverses. This phenomenon is also observed when the interneuronal sites of plasticity themselves are dissected; the relative contribution made by the L29 and L30 circuit modifications reverses with increasing stimulus intensity.

These results suggest that while the sites of plasticity encoding memory are fixed, the information represented by these sites can be dynamic, shifting in anatomical location with changes in the intensity of the test stimulus.

570.15

PLASTICITY IN APLYSIA GILL-WITHDRAWAL BEHAVIOR A NEURAL NETWORK MODEL. J.L. Leonard*, Mark O. Hatfield Marine Science Center, Newport, OR 97365.

Studies of behavioral plasticity in the *Aplysia* gill-withdrawal response (GWR) show that even habituation involves changes at multiple sites and the much-studied changes at the identified synapses between LE sensory cells and central gill motor neurons (GMNs) do not account for plasticity in the behavior. There are at least three parallel but interacting pathways which will mediate habituation and facilitation of the GWR: the peripheral nervous system (PNS) (Peretz et al. 1976, the Interneuron II pacemaker system (Int II) (Pinsker 1982-3), and the identified direct CNS system (many authors). Here I propose that plasticity in the GWR is the result of parallel-distributed processing with the three pathways representing subnets in a Nowlan-Jacobs system of mini-nets. In the simplest configuration, both the PNS and Interneuron II systems represent mini-nets, with the direct CNS system acting as a "referee" to choose the "correct" mini-net. The six GMNs, along with some as yet unidentified inhibitory gill motor neurons, act as an output layer of the referee network, serving to determine the response of the periphery. Novel phenomena predicted by the model include a) the as yet unidentified sensory neurons to the GMNs also provide input to the Int II cells (R25/L25); b) the neuromodulators known to change weights in the direct CNS system have no direct effect on Int II; and 3) siphon PNS sensory cells provide input to both CNS and PNS networks.

570.16

PREDICTING AGE IN APLYSIA CALIFORNICA FROM SHELL AREA, GANGLIA AREA, AND WEIGHT. J. Thomas, C. Hong†*, T. Capo‡, V. Chandhoke, and J.M. Flinn. George Mason Univ., Fairfax, VA. 22030. †NIH ‡University of Miami-FL

Shell and ganglia areas and weight were examined across age groups in 115 *A. californica* from the Aplysia Resource Center Miami, Florida. The post-hatch ages of the animals studied were 4 (n=8), 4.5 (n=13), 5.5 (n=28), 7 (n=24), 9 (n=9), 10 (n=25), and 12 (n=8) months. Using a dissecting microscope, we measured both the abdominal ganglia and the shell of each animal. Previous studies (e.g. Peretz & Adkins, 1982) have suggested that age can be determined by shell size. Consequently, we examined age(A) as a function of weight(W), shell area(S), and ganglia area(G), individually. Best-fit equations showed: 66% of the variability in age can be predicted from it's relation with weight, $A = 4.78 + .03(W) + 4.50e\text{-}05(W)^2$; 64% can be predicted from shell area, $A = 4.71 + .17(S)$; and, 63% can be predicted from ganglia area, $A = 4.30 + 1.07(G)$. Both ganglia and shell areas were predominantly linear with age, unlike weight. Age was then analyzed as a function of both weight and ganglia area and weight and shell area. 69% of the variability in age can be predicted from both weight and ganglia area, $A = 3.71 + .02(W) + 3.21e\text{-}05(W)^2 + 1.54(G) + .41(G) + .04(G)^3$. 71% can be predicted from weight and shell area, $A = 3.92 + .09(W) + 2.13e\text{-}05(W)^2 + .26(S) + .01(S)^2 + 8.68e\text{-}05(S)^3$. A combination of shell area and weight appear to be the most reliable predictors of age, with $r = .84$ ($\sqrt{.71}$), for this set of animals.

570.17

EFFECTS OF CROWDING ON THE SEROTONERGIC AND DOPAMINERGIC SYSTEMS IN APLYSIA CALIFORNICA. S. Shirazi, J.M. Flinn* and V.Chandhoke. George Mason University, Fairfax, Virginia 22030.

The effects of crowding were assessed in 105 *A. californica* raised in crowded or uncrowded conditions (C or U), subdivided into tanks with or without a protein skimmer, which removed organics such as pheromones, (S or N). Animals were reared from 82 to 171 days (post-hatch). Mean weights were significantly affected by both skimming and crowding, but neurochemical data suggested that pheromonal levels regulated growth more significantly than tactile stimului; see Table 1. Metabolic pathways were examined for serotonin (5-HT) and dopamine (DA) using HPLC. Both tryptophan and 5-HT were significantly reduced in the unskimmed groups. The availability of tryptophan and constancy in conversion rates for tryptophan to 5-HT in all groups (22%) except the US (29%) suggests that the 5-HT differences were metabolically driven. Previous experiments indicated that crowding reduced learning which could be due to reduced levels of 5-HT. The function of DA within *A. californica* is not as well understood. DA concentrations were higher in the crowded vs. uncrowded groups; however, concentrations were significantly higher in groups with a protein skimmer. Crowding can have an effect via increased tactile or chemical concentrations. These data suggest that the pheromonal effects are more important.

Group Means	US	CS	UN	CN	p(SK)	p(CR)
Weight(g)	46	29	26	17	.004	.001
Trp (ng/mg)	2786	2664	1197	791	.001	ns
5-HT (ng/mg)	803	598	264	176	.008	.08
Tyr (ug/mg)	5257	6255	3580	2202	.001	ns
DA (ng/mg)	476	645	96	152	.001	.08

INGESTIVE BEHAVIOR V

571.1

INVOLVEMENT OF 5-HT2C RECEPTORS IN THE HYPOPHAGIC EFFECTS OF SIBUTRAMINE AND D-FENFLURAMINE IN RATS H.C. Jackson , S.C. Cheetham, L.J. Hutchins, S.E. Mazurkiewicz, D.J. Heal and W.R. Buckett (SPON: Brain Research Association). Knoll Pharmaceuticals Research Department, Nottingham, NG2 3AA, U.K.

The novel 5-HT and NA reuptake inhibitor, sibutramine, reduces food intake in rats. This response is attenuated by non-selective 5-HT antagonists and the β1-adrenoceptor antagonist metoprolol (A. Stricker-Krongrad, personal communication). This study uses the 5-HT2C receptor antagonist, SB200646, to investigate whether these receptors are involved in the hypophagic effects of sibutramine. The 5-HT releaser, d-fenfluramine, was examined for comparison. Male Sprague-Dawley rats (350-500g, n=6-8) were maintained on reversed phase lighting (lights off 09.00-17.00h) with free access to powdered diet and water. Feeding jars and water bottles were weighed at the time of p.o. drug administration (09.00h) and after 2-8h. Sibutramine (3, 10 mg/kg) and d-fenfluramine (1-10 mg/kg) produced dose-related decreases in food and water intake over the dark period. SB200646 (10-40 mg/kg) had no effect on food and water intake. Concurrent administration of SB200646 partially antagonised the decrease in mean food intake (g/kg rat weight±sem) induced by sibutramine (vehicle 34.0±3.4; sib 10 mg/kg 0.9±0.3*; sib+SB 20 mg/kg 10.1±4.1*§; sib+SB 40 mg/kg 10.4±1.6*§; *P<0.05 vs. vehicle, §P<0.05 vs. sib) and d-fenfluramine (vehicle 36.5±1.2; d-fen 10 mg/kg 2.5±1.3*; d-fen+SB 20 mg/kg 8.1±2.7*; d-fen+SB 40 mg/kg 10.4±2.2*§; §P<0.05 vs. d-fen) over the 8h dark period. The inhibitory effects of SB200646 on d-fenfluramine (but not sibutramine) hypophagia were apparent by 2h. SB200646 also partially inhibited the decrease in water intake induced by sibutramine and d-fenfluramine. These results implicate 5-HT2C receptors in the hypophagia induced by sibutramine and d-fenfluramine although clearly other mechanisms contribute to these effects.

571.2

SEROTONERGIC MECHANISMS OF THE LATERAL PARABRACHIAL NUCLEUS INHIBIT SODIUM INTAKE IN RATS. J. V. Menani*, R. L. Thunhorst and A. K. Johnson. Departments of Psychology and Pharmacology and the Cardiovascular Center, University of Iowa, Iowa City, IA 52242, USA, and Department of Physiology, School of Dentistry, UNESP, Araraquara, SP 14800, Brazil.

In this study we investigated the role of the serotonergic pathways of the lateral parabrachial nucleus (LPBN) in the control of NaCl intake in rats. Bilateral injection of the serotonergic $5HT_1/5HT_2$ receptor antagonist, methysergide (4 μg/0.2 μl) into the LPBN produced a marked increase in 0.3 M NaCl intake induced by angiotensin II (ANG II, 50 ng/1 μl) injected intracerebroventricularly (13.5±3.8 vs. 3.5±1.0 ml in 1 h with vehicle into LPBN) or by treatment with furosemide (FURO, 10 mg/kg) + captopril (CAP, 5 mg/kg) injected subcutaneously (18.3±2.9 vs. 4.1±0.9 ml in 2 h with vehicle into LPBN). Injection of the serotonergic $5HT_{2A}/5HT_{2C}$ receptor agonist, DOI (5 μg/0.2 μl) bilaterally into the LPBN reduced the 0.3 M NaCl intake after FURO + CAP (2.1±0.8 ml in 2 h). The water intake observed simultaneously with salt intake was not changed by these treatments into LPBN. The results suggest that a serotonergic mechanism associated with the LPBN has strong inhibitory influence on sodium intake in rats.

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571.3

CONGENITAL SEROTONIN ABNORMALITIES ALTER THE ADVERSE EFFECTS OF EXERCISE STRESS. P.F. Aravich*, T. Hsu, W. Walker, H.A. Funk & T.S. Rieg. Eastern Virginia Med. School, Norfolk, VA 23501; Vets. Aff. Med. Ctr., Hampton, VA 23667; Governor's School for Sci. & Tech., Hampton, VA 23666; Hampton Univ., Hampton, VA 23668; Christopher Newport Univ., Newport News, VA 23606.

There is growing interest in the adverse effects of exercise stress and caloric restriction in the rat. This is relevant to exercise and weight control in normal people and to anorexia nervosa, which is highly correlated with hyperactivity. Our previous data suggest that brain serotonin mitigates the adverse effects of the exercise-stress syndrome. This investigation determined if congenital serotonin abnormalities affect it. Adult female Fawn-Hooded (FH) rats (n=8) and Wistar strain controls (n=9) were the subjects. FH rats have normal 5HT content and turnover but region-specific $5HT_{1A}$ reductions and $5HT_{2A}$ increases. These receptors are linked to, e.g., food intake, locomotor behavior, thermoregulation and/or anorexia nervosa. All animals were subjected to the exercise-stress syndrome (1.5 h/day free access to food; 22.5 h/day free access to activity running wheels). FH rats were reliably sub-sensitive to the wheel running effect of the paradigm and ate less than Wistar controls. Both groups lost weight at the same rate despite reliably higher food-to-running ratios in the FH rats. The "morphological stress triad" (relative adrenal hypertrophy; immune organ atrophy) was reliably worse in FH rats. It is concluded that congenital $5HT_{1A}/5HT_{2A}$ abnormalities exacerbate the adverse effects of the exercise stress syndrome on immune organ atrophy but not weight loss.

571.4

DIURNAL RHYTHMS OF EXTRACELLULAR SEROTONIN WITHIN RAT HYPOTHALAMIC PARAVENTRICULAR NUCLEUS. L. McMahon, R. Thiagarajan, T. Green, and P.J. Wellman*. Dept. of Psychology, Texas A&M University, College Station, TX 77843-4235.

Serotonin-releasing neurons within the rat hypothalamic paraventricular nucleus (PVN) are presumed to suppress carbohydrate intake at the beginning of the dark cycle. A previous microdialysis study revealed a peak in extracellular levels of the 5-HT metabolite 5-HIAA within the PVN at the onset of the dark cycle, but no study to date has assessed potential rhythms of extracellular 5-HT within the PVN across the diurnal cycle. Adult male (n=6) albino rats were prepared with concentric 2 mm microdialysis probes aimed at the PVN. A series of 60 minute dialysate samples (1 uL/min) were collected every hour over a 24 hour period, with half of the rats starting collection at 0700 hrs, and the remainder starting at 1900 hrs. Across the light cycle, there was observed a gradual decline in extracellular 5-HT levels that reached a nadir (1.1 pg) at 1800 hrs. In contrast, extracellular 5-HT gradually increased over the dark cycle reaching a peak (2.1 pg) at 0400 hrs. Extracellular levels of PVN 5-HIAA exhibited a peak at 1800 hrs during the light cycle and then increased in a pattern similar to that of 5-HT with a second peak evident later in the dark cycle. The observed increase in 5-HT evident later in the dark cycle may play a role in the modulation of feeding.

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571.5

SEROTONERGIC MECHANISMS OF THE LATERAL PARABRACHIAL NUCLEUS (LPBN) AND AT_1 RECEPTORS OF THE SUBFORNICAL ORGAN (SFO) ON THE INTAKE OF NaCl AND WATER. D.S.A. Colombari*, J.V. Menani, T.G. Beltz, and A.K. Johnson. Depts. of Psychol. and Pharmacol. and the Cardiov. Center, Univ. of Iowa, Iowa City, IA 52242.

Serotonergic mechanisms of the LPBN play an inhibitory role in the dipsogenic response induced by intracerebroventricular angiotensin II (ANG II). In this study we investigated the effects of a serotonergic receptor antagonist (methysergide) injected into the LPBN, and of an angiotensinergic AT_1 receptor antagonist (losartan) injected into the SFO on the intake of water and 0.3 M NaCl induced by ANG II injected into the SFO. Rats had stainless steel cannulas implanted bilaterally in the LPBN and an other in the SFO. When only water was available, the injection of ANG II (20 ng/200 nL) into the SFO induced water intake (6.5±1.1 mL/1 h) which was not changed after bilateral injection of methysergide (4 µg/200 nL) into the LPBN (10.3±2.3 mL/1 h). When both water and 0.3 M NaCl were available, ANG II into the SFO induced significant intake of water (7.2±1.4 mL/1 h) but not 0.3 M NaCl (1.0±0.5 mL/1 h). Pretreatment with bilateral injection of methysergide into the LPBN increased 0.3 M NaCl intake induced by ANG II into the SFO (10.5±3.6 mL/1 h) and the water intake associated with the NaCl intake (12.7±2.0 mL/1 h). These responses induced by ANG II after methysergide into the LPBN were blocked by pretreatment with losartan (1 µg/200 nL) injected into the SFO (0.7±0.5 and 0.9±0.7 mL/1 h, for NaCl and water, respectively). The results suggest that serotonergic mechanisms associated to the LPBN may be involved with an inhibitory mechanism controlling salt intake induced by ANG II into the SFO. In addition, they also show that the water and NaCl intake induced by ANG II into the SFO is mediated by AT_1 receptors. Support: FAPESP, NIH-HLBI

571.6

REDUCTION OF FOOD INTAKE AND ALTERATION OF LICKING DISTRIBUTION BY THE 5-HT_{1B} AGONIST CP-94,253 IN RATS. M. Lee and K. J. Simansky*, Dept. Pharmacology, Med. Coll. Pennsylvania and Hahnemann University, Philadelphia, PA 19129.

Pharmacological studies have implicated 5-HT_{1B} receptors in central serotonergic inhibitory control of feeding. We analyzed the ability of the pyrrolopyridine CP-94,253, a selective 1B agonist that penetrates the blood-brain barrier (Koe et al., 1992), to reduce food intake in rats. Given i.p. 30 min before the tests, doses ranging from 5-40 µmol/kg decreased 30-min intake of sweetened milk after 18-h deprivation, of pellets in rats maintained on a fixed amount of food per day (ca. 28 g) and of 10% sucrose after 2-h deprivation. Doses of 10, 28 and 20 µmol/kg produced approximately 50% reductions of pellets, milk and sucrose, respectively. Thus, CP-94,253 appeared to be more potent in reducing consumption of a solid, less palatable diet. Time-sampling analysis of the 20 µmol/kg dose in the pellet and sucrose tests revealed that CP-94,253 reduced the frequency of feeding and increased the incidence of standing but not resting. Meal duration was shortened. Analysis of the distribution of licking during the test with sucrose established that 20 µmol/kg reduced the total number of licks, and the size and frequency of bursts of licks (licks occurring with ILI < 250 msec) without altering the efficiency of licking. By comparison with these results, the peripherally acting 5-HT (1A,1B,1D,1-Like) agonist, 5-carboxamidotryptamine (5-CT) is >100-fold more potent than CP-94,253 in the pellet and milk tests. Furthermore, CP-94,253 and 5-CT differed in their behavioral profiles in the sucrose test. Overall, the results suggest that CP-94,253 may be a useful probe for central serotonergic mechanisms in feeding. Further analyses are in progress to determine the sites and establish the receptor mechanism for anorectic actions of CP-94,253. Supported by MH 41987 to KJS.

571.7

ATROPINE DECREASES DRINKING BUT NOT FEEDING IN DIABETIC RATS. E. Murzi*, P. Rada and L. Hernandez. Laboratory of Behavioral Physiology. Medical School. Los Andes University. Merida 5101. Venezuela.

Diabetic rats exhibit polydipsia and polyphagia. Lateral intrahypothalamic injections of acetylcholine (ACh) or carbachol evoke vigorous drinking which is abolished by ip. injections of atropine sulfate (1), a cholinergic blocker, in normal rats. We evaluated the effect of ip. atropine sulfate on food and water intake and monitored by microdialysis the hypothalamic levels of ACh in streptozotocin-diabetic male rats individually housed with food and water ad libitum. Water and food intake were measured by weighing the drinkers and the spillage proof feeders. The animals received four doses of atropine sulfate (0.0, 1.0, 2.5, 5 mg/Kg) and food and water intake was measured 2h, 5h, 8h, and 24h after drug injection. Water intake but not food intake linearly decreased for the first 8h after atropine sulfate injection (the regression coeficient was highly significant: R=0.96). This decrease of drinking is not due to malaise since feeding was not affected. In preliminary experiments with brain microdialysis, variations of lateral hypothalamic ACh levels were observed in diabetic rats after ip. administration of atropine sulfate. These results suggest that an increased activity of the cholinergic neurons in the lateral hypothalamus could induce the polydipsia observed in diabetic rats.

1.- Grossman, S. Am. J. Physiol. 202:1230, 1962.

571.8

OPIOID-INDUCED FEEDING BY MORPHINE MICROINJECTIONS INTO THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS AND THE NUCLEUS ACCUMBENS IS MEDIATED BY AN ENHANCEMENT OF TASTE PALATABILITY. Peciña, S* and Berridge K.C. Psychology Department, University of Michigan, Ann Arbor, MI 48104.

The enhancement in food intake produced by opioid agonists (i.e. morphine) administration, both systemically and intracranially, has now be repeatedly demonstrated. But what are the psychological processes that mediate this effect? Based on observations that opioid agonists selectively increase the intake of highly palatable food much more than the intake of regular chow in rats, it has been proposed that opioid-induced increase in food intake is at least partly mediated by an enhancement of taste palatability. This hypothesis has been supported by recent findings that opioid agonists increase hedonic taste reactivity patterns (e.g., tongue protrusions, etc.) in response to a sucrose solution in rats. But can opioid hedonic enhancement be elicited from the specific brain structures in which microinjections of opioid agonists evoke feeding? We investigated the effects of microinjections of morphine into the paraventricular nucleus of the hypothalamus (PVN; 0, 1, 5, 10, 25, 50 µg), and of bilateral microinjections of morphine into the nucleus accumbe (NAcc; 0, 1. 5, 10, 25 µg) on feeding and on taste reactivity patt Morphine microinjections in these two structures significantly enhance food intake and taste palatability. These results demonstrate the opi induced feeding enhancement into the PVN and the NAcc is mediated by an enhancement of taste palatability.

571.9

THE INVOLVEMENT OF AMYGDALA GABA RECEPTORS IN THE RAPID AROUSAL of SALT INTAKE IN RATS. L.Y. Ma*, D.M. Zhang, S.J. Fluharty and R.R. Sakai. Depts. of Animal Biology, Pharmacology and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

We have previously reported that certain adrenal steroids or their tetrahydro (TH) derivatives implanted in the amygdala arouse sodium intake in rat. The data suggested that the rapid injection of saline immediately after implants in the amygdala may be acting through the GABA/benzodiazepine receptor system. In the present study, we have extended these observations to examine the effect of implants of various adrenal steroids and GABA receptor agonists or antagonists on salt intake.

All rats (n=6-8/group) were fitted with bilateral guide cannula which allowed implantation of agents directly into the medial nucleus of the amygdala. They were given ad libitum food and water but access to 0.25 M NaCl for only 2 hours each day. On the test day, they were injected with aldosterone (ALDO), deoxycorticosterone acetate (DOCA), their tetrahydro (TH) derivatives, flunitrazepam β-carboline or cholesterol (200 ng) and their 2 hour intake of 0.25 M NaCl recorded. The results demonstrated that 1) TH-DOCA and TH-ALDO markedly increased 0.25 M NaCl intake as compared to blank or cholesterol treatment; 2) this salt intake was further facilitated by β-carboline pretreatment and was not inhibited by the adrenal steroid receptor agonists RU-28318 or RU-486; and 3) the salt intake induced by TH-DOCA and flunitrazepam was inhibited by the GABA antagonist RU15-4513, while 10% sucrose intake was not affected by either treatment. Together, these data confirm and extend our previous studies by suggesting a novel mechanism for steroid induction of sodium intake involving a non-genomic action in the amygdala. (Supported by MH43787)

571.10

EFFECTS OF THE BENZODIAZEPINE RECEPTOR INVERSE AGONIST Ro 15-4513 ON CONSUMPTION OF SUCROSE AND SACCHARIN SOLUTIONS ANALYSED USING A LICKING MICROSTRUCTURE APPROACH. S.J. Cooper* and S. Higgs Department of Psychology, University of Durham, Science Laboratories, South Road, Durham, DH1 3LE, England.

The increase in ingestive behaviour observed following administration of benzodiazepine receptor (BZR) agonists is a well known phenomenon, but the anorectic action of BZR inverse agonists is less well characterised. Therefore, in the present studies we aimed to examine in great detail the decrease in ingestive behaviour brought about by the BZR partial inverse agonist Ro 15-4513 (1-10mg/kg). In a 20 min test session rats had access either to a 1% sucrose solution or a 0.1% saccharin solution. We videotaped the animals licking behaviour during this period and then performed a microstructural analysis of the data. Ro 15-4513 was found to dose dependently reduce consumption of both the sucrose and saccharin solutions. In the case of sucrose drinking the reduction in intake was due to a decrease in both the number of bouts and the mean bout duration. The total duration of drinking, total number of licks and duration of the first bout of drinking were also reduced. There was no effect of the drug on intra-bout lick rate, latency to engage in drinking or the post-bout drinking time. The effect of Ro 15-4513 on saccharin drinking was very similar but in this case the reduction in intake was mainly due to a reduction in the number of bouts. The results are discussed with reference to the effects of benzodiazepines on palatability.

571.11

NOREPINEPHRINE INFLUENCES GABA RESPONSE TO GLUCOPRIVATION IN VENTROMEDIAL HYPOTHALAMUS OF RATS. J.L. Beverly[1], M. Beverly[1], M. D'Souza[1], J. Roberts[1], M. Shutt[1], R. van Husen[1], and N.F. Shay[2]*. Dept. Nutrition, [1]Texas Tech Univ., Lubbock,TX 79409 [2]Univ. Illinois,Champaign,IL 61801

In response to glucoprivic episode, extracellular GABA is increased in the ventromedial hypothalamus (VMH) in a bimodal fashion. Norepinephrine (NE) turnover in the VMH may also be increased in response to glucoprivation. To determine if NE influences the GABA response to glucoprivation male Sprague-Dawley rats were fitted with guide cannulae to the VMH (n=8) or lateral hypothalamus (LH; n=8) and chronic jugular vein catheters. After a 7-10 d recovery, a 1 mm microdialysis probe was placed in the VMH or LH and continuously perfused with Krebs Ringers Buffer (KRB) at 2 µl/min. Three hr after placement of probes, baseline GABA was determined in 3 consecutive 10 min samples. Dialysate was changed to KRB+NE (10 µM) and 3 consecutive 10 min samples collected before administering a single dose of 2-deoxyglucose (2-DG; 200 mg/kg i.v.). Samples were collected at 10 min intervals for 60 min after 2-DG. GABA was analyzed by HPLC-EC. GABA concentrations in the VMH increased (44%, $p<0.05$) as a single peak with the addition of NE to dialysate. In response to 2-DG, GABA concentration in the VMH increased as a single broad peak (43% increase, $p<0.05$). GABA concentrations recovered from the LH were not altered by the addition of NE to dialysate or in response to 2-DG. Increased NE activity in the VMH may mediate the increase in GABA concentration after a glucoprivic challenge. Supported by Whitehall Foundation, Inc.

571.12

GENE EXPRESSION OF TYROSINE HYDROXYLASE (TH) IN THE PARAVENTRICULAR (PVN) AND SUPRAOPTIC (SON) NUCLEI OF STREPTOZOTOCIN DIABETIC (STZ-D) RATS: RELATIONSHIP TO CO-EXISTING NEUROPEPTIDES. L.F. Pardo, G. Brennan*, S.E. Bachus, and M. Jhanwar-Uniyal. The Rockefeller Univ. NY, NY 10021 and St. Elizabeths at NIMH, Washington D.C. 20032.

Dopamine (DA) synthesizing neurons in the PVN also synthesize neuropeptides, such as cholestocystokinin (CCK), galanin (GAL) oxytocin (OT) and vasopressin (VP), all known to regulate various endocrine functions. In this study, we induced diabetes in male SD rats by administering the pancreatic β-catalytic agent STZ (60 mg/kg BW, IP). Rats were sacrificed 21 days post-injection when 20% loss in body weight, decrease in insulin and increase in glucose was achieved. *In situ* hybridization histochemistry was used to measure mRNA for TH, the rate-limiting enzyme in DA biosynthesis, and other co-existing neuropeptides. The results show: 1) TH mRNA increased dramatically in PVN ($p=0.008$) and SON ($p=0.009$) of STZ-D rats; 2) PVN TH mRNA was correlated negatively with VP mRNA in controls ($r=-0.91$; $p<0.05$) but positively in STZ-D ($r=0.85$;$p<0.05$) rats; 3) PVN TH mRNA was strongly correlated with CCK ($r=0.87$;$p<0.05$) and GAL ($r=0.82$;$p<0.05$) mRNA in STZ-D rats; 4) SON TH mRNA was positively correlated only with OT ($r=0.83$;$p<0.05$) mRNA in STZ-D rats. These results suggest anatomical specificity in activated DA in VP neurons in PVN and SON. Co-activation of DA with VP, CCK and GAL indicates a complex interaction occurring in the hypothalamo-pituitary axis in diabetes.

571.13

HYPOTHALAMIC HISTAMINE MODULATES INGESTIVE BEHAVIOR AND THERMOGENESIS INDUCED BY INTERLEUKIN-1 β. T. Sakata*, H. Yoshimatsu and M. Kurokawa. Dept. of Internal Medicine, School of Medicine, Oita Medical University, Oita, 879-55 Japan.

Homeostatic involvement of hypothalamic neuronal histamine in adaptive behavior and thermogenesis was investigated when interleukin-1 β (IL-1 β), an endogenous pyrogen, was infused peripherally in rats. IL-1 β decreased food and water intake, and elevated body temperature. Depletion of neuronal histamine in the hypothalamus induced by α-fluoromethylhistidine, a suicide inhibitor of the histamine synthesizing enzyme histidine decarboxylase (HDC), attenuated the suppressive effect of IL-1 β on food intake, facilitated the inhibitory effect on water intake, and enhanced its thermogenic effect. Simultaneously IL-1 β increased activity of HDC, and histamine-N-methyltransferase, a neuronal histamine catabolizing enzyme. Pretreatment with indomethacin completely blocked those increase in turnover of neuronal histamine induced by IL-1 β. Hypothalamic prostaglandin E2 (PGE2) activated by peripheral IL-1 β, but not peripheral PGE2 , increased both activities of HDC and HMT. Ginsenoside Rg1, a major component of panax ginseng, modulated the suppressive effects of IL-1 β on ingestive behavior, resulting in a lowering of body temperature. The findings suggest that the effects of IL-1 β on ingestive behavior and thermogenesis may be modulated by dynamics of hypothalamic neuronal histamine through activation of hypothalamic PGE2 which is elevated by peripheral IL-1 β.

571.14

NITROUS OXIDE-ENHANCED INTAKE OF A PALATABLE FLUID IN WATER-DEPRIVED RATS IS REVERSED BY AN OPIOID RECEPTOR BLOCKER. K.D. Guidinger, D.A. Czech, A.C. Fachting, J.F. Headrick and J.C. Brooks.* Department. of Psychology and School of Dentistry - Basic Sciences, Marquette University, Milwaukee, WI 53233

Our laboratory recently observed that subanesthetic concentrations of nitrous oxide (N_2O) can stimulate food intake in rats in a concentration-related manner, and that this effect is attenuated with an opioid receptor blocker. We now report a N_2O-induced enhancement of consumption of a palatable fluid, which is very sensitive to antagonism by naltrexone, using within-subjects designs.

In expt.1, 16-20 hr (overnight) water-deprived male Long-Evans hooded rats were exposed to three concentrations of a N_2O and O_2 mixture (10, 20 & 30% N_2O) and to compressed room air (RA) control circulated through an enclosed environment. Following an initial acclimation to gases, rats were given access to a 0.15% saccharin-Na solution from a sipper tube in the same environment and intake was monitored electronically at 1-min intervals over a 40 min test period. Food was unavailable during testing, and tests were separated by 3-4 days. Cumulative intakes were analyzed at selected times from access to saccharin solution and from first drinking bout with repeated measures ANOVAs, and Dunnett's or Student's *t*-tests; alpha level was set at $p<0.05$. Relative to RA controls, intake increased significantly at both 20% and 30% N_2O, with 20% N_2O exhibiting a more pronounced shift.

In expt.2, similarly water-deprived rats were pretreated with several doses of naltrexone-HCl (Ntx) (0.01-3.0 mg/kg s.c.) or 0.9% NaCl vehicle prior to being exposed to a challenge concentration of 20% N_2O; subsequent procedures were the same as is expt.1. Significant attenuation of a 20% N_2O-induced increase in intake occurred at the relatively low dose of 0.03 mg/kg Ntx.

These data are consistent with the previously observed N_2O-induced stimulation of solid food intake in the rat, and also implicate an opioid mechanism.

571.15

POSSIBLE INVOLVEMENT OF NITRIC OXIDE IN CHLORDIAZEPOXIDE-INDUCED FEEDING IN THE MOUSE. D.A. Czech.* Department of Psychology, Marquette University, Milwaukee, WI 53233

Chlordiazepoxide (CP) and other benzodiazepine receptor ligands reliably stimulate feeding behavior in a number of animal species. Most recently, it has also been reported that the vasodilating gas, nitric oxide (NO), can have a modulating effect on both feeding and drinking behavior. The current study investigates a possible modulatory effect of NO on CP-induced feeding in mice.

In expt.1, male ICR mice were injected s.c. with several doses of the nitric oxide synthase (NOS) inhibitor L-N^G-nitro arginine (L-NOARG) or 0.9% NaCl vehicle (Veh). Fifteen min later, 10 mg/kg of chlordiazepoxide-HCl (CP) or Veh was administered s.c. Five groups were thus formed: Veh/Veh (baseline feeding control), Veh/CP and three groups given doses of L-NOARG (10, 25 or 50 mg/kg) along with CP. Thirty min later, mice were placed in a polyethylene cage containing a preweighed rodent chow pellet for a 1-hr feeding test. Water was unavailable during testing. Amount of food (g) ingested was evaluated separately at 30 and 60 min with randomized one-way ANOVAs, along with Student's and Dunnett's *t*-tests; alpha level was set at $p<0.05$. A CP-induced hyperphagia was partially reversed in a dose-related manner, reaching significance at all doses of L-NOARG at 30 min and by the two higher doses at 60 min.

In expt.2, mice were injected s.c. with 25 mg/kg of L- NOARG, along with 500 mg/kg i.p. of the natural NOS substrate, L-arginine (L-arg) or the inactive isomer, D-arginine (D-arg). A challenge dose of 10 mg/kg CP, or Veh, was injected s.c. 15 min later. Subsequent food intake tests were conducted as in expt.1. Data were evaluated with Student's *t*-tests, again at 30 and 60 min. L-arg partially, but significantly, reversed the attenuation by L-NOARG of CP-induced hyperphagia; D-arg was ineffective, hence revealing a stereospecific effect.

These data are consistent with previous research reporting effects of L-NOARG in deprivation-induced feeding, thus suggesting NO involvement in CP-induced feeding as well.

571.16

DEFECTIVE SYMPATHETIC ACTIVATION AND INSULIN RELEASE DURING PVN INFUSIONS OF NOREPINEPHRINE IN OBESITY-PRONE RATS. B.E. Levin*. Neurology Svc., VA Medical Ctr., East Orange, NJ 07018.

Male Sprague-Dawley rats gain weight homogeneously when fed chow. But when fed a diet high in energy, fat and sucrose, half develop diet-induced obesity; the rest are diet-resistant. Although both chow-fed, obesity-prone (OP) and resistant (OR) rats weigh the same, OP rats have higher 24h urine norepinephrine (NE) levels and widespread decreases in forebrain α_2-adrenoceptor binding including the hypothalamic paraventricular n. (PVN). Here, a functional test of this receptor deficit was made with bilateral indwelling PVN cannulae in OP (urine NE: 1.86$\pm$0.11ng/24h) and OR (0.88$\pm$0.08ng/24h; $P=0.01$) rats. Correct cannula placement was established by induction of eating in sated rats following bolus injection of NE (20nmol). At baseline, fasted OP rats had 32% higher plasma insulin ($p=0.05$) and 9% higher glucose ($P=0.001$) levels than OR rats. Bilateral 20min PVN NE infusions (150pmol/min) increased plasma NE levels by 96% ($P=0.05$) and insulin by 32% ($P=0.05$) compared to carrier infusions in fasted OR rats but produced no change in OP rats. PVN NE infusions altered neither plasma glucose nor epinephrine levels nor motor activity in either phenotype. Thus, the defective sympathetic activation and insulin release shown by OP rats during NE stimulation of the PVN is in keeping with their reduced PVN α_2-adrenoceptor binding. This finding may be important in the pathogenesis of obesity.

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571.17

AMANTADINE DOES NOT PREVENT NEUROLEPTIC-INDUCED OBESITY IN RATS. L. Teneud*, M.E. López, Q. Contreras, T. Alastre, L. Hernández, E. Musseo, M. Altemus, S. Weiss and T. Baptista. Laboratory of Behavioral Physiology, Medical School, Universidad de Los Andes. Mérida, VENEZUELA, and Biological Psychiatry Branch, NIH, Bethesda, MD.

The efficacy of the antiviral agent Amantadine (AM) to prevent neuroleptic-induced obesity was assessed in a model of sulpiride-induced weight gain in adult female rats. The effects of AM (5,10,20,50 and 100 mg/kg/sc. or ip.) on body weight and food intake were tested in drug-free animals and rats under a chronic regimen of Sulpiride (SUL) administration (20 mg/kg/ip for 21 days). In drug-free rats, acute administration of AM/ip. displayed a dose dependent anorectic effect: $r=0.99$, $F(1,4) = 213$, $p < 0.001$. However, chronic administration of AM significantly decreased body weight gain in drug-free rats only at the dose of 100 mg/kg/sc. Similarly, SUL-induced obesity was prevented by AM only at the dose of 100 mg/kg. AM did not prevent SUL-induced disruption of the vaginal cycle at any dosage. In addition, SUL-induced hyperprolactinemia was not counteracted by AM at the dose of 50 or 100 mg/kg. Finally, as assessed by brain microdialysis, systemic administration of AM (50 and 100 mg/kg) induced a slight and non-significant increase in dopamine overflow in the lateral hypothalamus, a putative brain area involved in the anorectic effect of the drug. The results suggest that AM displays a weak antagonistic effect on SUL-induced obesity in rats. This contrasts with anecdotal reports of prevention of neuroleptic-induced weight gain and hyperprolactinemia in patients with mental disorders.

HORMONAL CONTROL OF REPRODUCTIVE BEHAVIOR: BEHAVIORAL MEASURES

572.1

MICROINJECTION OF OXYTOCIN (OXT) INTO THE MEDIAL PREOPTIC-ANTERIOR HYPOTHALAMUS (MPOA-AH) AND VENTROMEDIAL HYPOTHALAMUS (VMH) OVERRIDES THE INHIBITORY EFFECTS OF PROGESTERONE (P) ON LORDOSIS IN SYRIAN HAMSTERS. D. Carol Whitman* and H. Elliott Albers. Lab. Neuroendocrinol. & Behav., Depts. Biol. and Psychol., Georgia State Univ., Atlanta, Ga.

The levels of lordosis induced by injection of OXT into the MPOA-AH and the VMH of ovariectomized estradiol (EB)-primed hamsters mimics that observed on estrus or following administration of EB and P. Injection of an OXT antagonist into the same brain areas inhibits EB and P induced-lordosis in hamsters. To further investigate the relationship between the effects of OXT and P on lordosis we examined whether the inhibitory effects of P could block the ability of OXT to induce lordosis. OVX hamsters were given EB (10μg, s.c.) followed 44 hrs later by P (500 μg, s.c.) and 68 hrs by a second P injection. Three hrs later each hamster received a microinjection of OXT or saline (counterbalanced) and was tested with a male for lordosis duration (sec). Following the second P (500μg.,s.c.) injection, OXT induced a significantly longer duration of lordosis (472.0±63.8 sec) than did saline (48.3±48.3). These data indicate that OXT can override the mechanisms that inhibit lordosis in response to P.

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572.2

MEASURING INTENSITY OF SEXUAL BEHAVIOR IN FEMALE CATTLE. Frank.J.C.M. van Eerdenburg* and Jan H. van Vliet. Dept. Herd Health & Reproduction, Faculty of Veterinary Sciences, 3584 CL Utrecht, The Netherlands.

Estrus detection on dairy farms is usually done by watching for female sexual behavior. A six weeks long, dayround, behavioral survey revealed that only 37% of the modern dairy cows stood to be mounted, a behavior comparable to lordosis in rodents, which has always been the key in estrus detection. However, other, less specific, components of female sexual behavior were performed by the cows. Although showing these also in diestrus, the frequency is higher during estrus. We calculated a scoring scale (Table 1) for these behaviors in order to be able to compare the intensity of estrous behavior between individual animals and herds in neuro-endocrine and -anatomical studies regarding the regulation of female sexual behavior in cattle.

Table 1: Scoring scale for estrous behavior in cattle:

mucous vaginal discharge	3 points
cajoling	3 points
restlessness	5 points
being mounted but not standing	10 points
sniffing on vagina of other cow	10 points
resting with chin on other cow	15 points
mounting (or attempting) other cows	35 points
mounting headside of other cow	45 points
standing heat	100 points

This scoring system is cumulative during a 24 hour period and can also be used for estrus detection.

572.3

ESTROGEN INCREASES THE STRENGTH OF A MEDULLARY-LUMBOSACRAL MOTONEURONAL PROJECTION, POSSIBLY INVOLVED IN LORDOSIS BEHAVIOR IN THE CAT.
V.G.J.M. VanderHorst*, H. de Weerd and G. Holstege. Department of Anatomy, University of Groningen, Oostersingel 69, 9713 EZ, The Netherlands

The nucleus retroambiguus (NRA) is a compact group of interneurons located in the caudal medullary tegmentum. The NRA forms a relay between the periaqueductal gray (PAG) and motoneuronal cellgroups involved in vocalization and possibly lordosis behavior. NRA interneurons have recently been shown to project directly to distinct motoneuronal cellgroups in the lumbosacral cord innervating hindlimb muscles such as iliopsoas, adductor and hamstring muscles, as well as proximal tail and pelvic floor muscles. This set of muscles is involved in lordosis behavior in the cat, which is typically displayed in the presence of elevated levels of estrogen. Estrogen is known to induce plastic changes in hypothalamic pathways which play a role in lordosis behavior. The question arises whether estrogen induced changes are also present in the NRA-motoneuronal pathway. Therefore, the density of the NRA-lumbosacral projection was studied in six estrous and eleven non-estrous cats at the light and electron microscopical level. The NRA was injected with WGA-HRP and light microscopically, the density and arborization pattern of anterogradely labeled axons in their target motoneuronal cell columns was studied. At the ultrastructural level, the number of labeled terminals was counted in the semimembranosus motoneuronal cell group and their synaptic morphology was studied. The results show marked differences in the NRA-lumbosacral projection in estrous versus non-estrous cats. Light microscopically, the density of labeled NRA axons was much higher in estrous than in non-estrous cats. At the ultrastructural level, the number of labeled NRA terminals increased significantly in estrous cats. The results suggest that estrogen induces axonal outgrowth and synaps formation in the NRA-lumbosacral motoneuronal pathway. Periodical, estrogen related changes in the density of the NRA-lumbosacral motoneuronal pathway might be one of the factors responsible for lordosis to occur.

572.4

BEHAVIORAL EFFECTS OF 3α-ANDROSTANEDIOL: HYPOTHALAMIC AND PREOPTIC AREA ACTIONS VIA A GABAERGIC MECHANISM.
M. S. Erskine* and C. A. Frye, Department of Biology, Boston University, Boston, MA 02215.

We investigated whether 5α-androstane-3α,17ß-diol (3α-androstanediol; 3α-Diol), a neurosteroid whose effects are primarily inhibitory to lordosis behavior, may act through interactions with γ-aminobutyric acid (GABA) receptor complexes (GBRs) in the medial basal hypothalamus (MBH) and the preoptic area (POA). In Exp. 1, ovariectomized (ovx) rats were implanted with bilateral guide cannulae aimed above the MBH and were later treated with estradiol-17β (E_2, 2 injections of 1 µg/0.2 ml in 10% ethanol) and either 3α-Diol (3.0 mg/kg sc) or vehicle. Progesterone (0.5 mg sc) was given 24 hrs after the first E_2 injection and a pre-test for lordosis responsiveness was carried out 4 hrs later. The $GABA_A$ agonist, muscimol (50 ng), then was infused into the MBH and rats were tested 10, 30 and 60 min later. Muscimol infusion facilitated lordosis in vehicle-treated controls, but 3α-Diol-treated animals failed to show this facilitation. To ascertain whether 3α-Diol would also prevent muscimol's action in the POA, a site in which muscimol inhibits, rather than facilitates, sexual receptivity, ovx animals in Exp. 2 were implanted with bilateral guide cannulae aimed above the POA and were treated with E_2, 3α-Diol, and P and infused and tested as in Exp. 1. Muscimol and 3α-Diol each significantly inhibited lordosis; when they were combined, the inhibition was more pronounced. In Exp. 3, POA infusions of the $GABA_A$ antagonist, bicuculline, counteracted the inhibition of lordosis by both muscimol and 3α-Diol. In Exp. 4, *in vitro* treatment of POA and MBH membrane fractions with 3α-Diol (30 µM) enhanced maximal [^{3}H]-muscimol binding without altering the affinity of the binding sites for the agonist. These data suggest that 3α-Diol inhibits E_2 and progestin-induced lordosis behavior via actions at GBRs in both the MBH and POA. *Supported by grants from Eli Lilly Co. and HD21802 to MSE and NRSA MH10744 to CAF.*

572.5

SEXUAL BEHAVIOR REGULATED (PACE) BY THE FEMALE INDUCES A REWARD STATE. Araceli Alonso and Raúl G. Paredes*. Psychology Department, Universidad Anáhuac. México D.F. 11000.

The possibility that female coital behavior induces a reward state of a duration sufficiently long, and an intensity sufficiently high, to allow association between that state and environmental cues was evaluated in the present experiment. Female rats were ovariectomized (OVX) and treated with estradiol benzoate (EB) or EB plus progesterone (P). In these 2 groups the females were not able to control their coital contacts. Another group of OVX females was treated with EB plus P and were allowed to control (pace) her sexual interactions with males. Two additional groups of OVX females were included, one treated with saline and the other with morphine (1 mg/kg). The initial preference for a white or black compartment was determined in a 10 min session. The animals were then paired to a reinforcing event (copulation or morphine injection) followed 24 hrs later by a pairing to the non reinforcing situation (injection of vehicle). After 3 pairings to each event their preference was tested again. Females that were allowed to pace their sexual contacts with males showed a place preference similar to that induced by morphine treatment. The groups of females in the nonpaced situation did not modified their preference after mating. The results indicate that sexual interaction in females induces a reward state when they are able to pace their sexual contacts.

572.6

FACTORS AFFECTING THE FEMALE POSTEJACULATORY REFRACTORY PERIOD IN RATS. L.Y. Yang* and L.G. Clemens. Neuroscience Program and Department of Zoology, Michigan State University, East Lansing, MI 48824

When the female rat is tested in a situation that allows her to escape from the male during copulation, she "paces" her sexual contact with the male. It has been reported that different numbers of intromissions prior to ejaculation affect the female rat's postejaculatory refractory period (PER)(Yang and Clemens, 1994). The objective of the present study was to investigate the effect of other male related stimuli on the temporal aspects of female copulatory behavior. In Experiment I, we examined the effect of the seminal plug, the penile cup and prostate secretions on the female rat's pacing behavior. Results showed that neither the seminal plug, the penile cup nor prostate secretions contributed to female's PER. Experiment II investigated the relation of male's ejaculation duration to the female's PER. Results indicated that the female's PER increased in length as the ejaculation duration increased. In addition, the number of intromissions preceding ejaculation was positively correlated with the duration of the ejaculatory reflex.

572.7

EFFECTS OF TESTOSTERONE (T), PROGESTERONE (P), AND DI-HYDROTESTOSTERONE (DHT) ON THE INHIBITION OF LORDOTIC BEHAVIOR IN THE MALE RAT. P.C. BUTLER, S.E. HUBER, AND G.J. BLOCH*, Dept. of Psychology, BYU, Provo,UT 84602.

In a brief report, Dohanich et al (Horm. Beh. 17, '83) reported that T lowered the incidence of lordosis in EB primed male rats, but we found no inhibitory effect of T on lordosis quotients (LQ), or the incidence of lordosis (Bloch et al, Neurosci 20, '94). P is produced in large quantities by the andrenals and testes, so we tested for its effects on lordosis with and without T. Adult gonad-ectomized EB primed and non-primed male rats were given blank (Bk), T, P, or T&P-filled Silastic capsules. Four behavioral tests for lordosis were spaced one week apart. Repeated measures ANOVA revealed that EB primed males given T&P together showed significantly less lordosis than EB primed males that received Bk, T or P alone (For example, fourth week LQ means±SEM: T&P= 36.2±10.5 vs. 76.7±5.0, 68.9±7.5, 67.5±8.0, for Bk, T, and P treatments, p's from 0.002 to 0.01). Although LQ scores were very low in males not primed with EB, the incidence of lordosis was significantly lower after treatment with T&P than with T alone (2 out of 12, vs. 6 out of 12, $p<0.04$). In an additional study, EB primed gonadectomized and adrenalectomized male rats treated with Bk, T, P, T&P, DHT, and DHT&P showed similar results (LQ: Bk=80.0±3.3, T=71.0±7.8, P=72.7±5.6 vs. T&P=22.8±7.7; DHT=52.7±10.5, DHT&P= 49.4±7.5). DHT was inhibitory as has been reported previously (Baum, Science 182, '73), but DHT&P together did not further reduce LQ's. Thus, P acts synergistically with T, but not with DHT, to inhibit lordosis in the male rat. Supported by HD 27334.

572.8

ALTERED SEXUAL PARTNER PREFERENCE IN MALE FERRETS GIVEN EXCITOTOXIC LESIONS OF THE PREOPTIC AREA/ANTERIOR HYPOTHALAMUS. M.J. Baum* and R.G. Paredes. Dept. of Biology, Boston University Boston, MA 02215

In Experiment 1 prepubertal male and female ferrets were gonadectomized, treated daily with estradiol benzoate (EB), and tested in a T-maze for approach to a stud male versus an estrous female. Males given bilateral infusions of the NMDA excitotoxin, quinolinic acid aimed at the dorsomedial POA/AH, preferred to approach a stimulus male significantly more often than control males which received either a sham- or a unilateral mPOA/AH lesion or bilateral infusions of quinolinic acid which produced no detectible excitotoxic damage to the mPOA/AH. Males with bilateral mPOA/AH lesions also neck gripped the stimulus female on significantly fewer trials. EB-treated female subjects with bilateral mPOA/AH lesions, like control females, preferred to approach the stimulus male. The males used in Experiment 1 had never experienced circulating levels of testosterone (T) characteristic of the breeding season. In Experiment 2 prepubertally gonadectomized males and females were treated with a high dose of T propionate and tested several times with a receptive female prior to brain surgery. Males which received bilateral excitotoxic lesions of the mPOA/AH neck gripped and mounted stimulus females significantly less than control males. Again, when given EB followed by T-maze tests of partner preference, males with bilateral mPOA/AH lesions, like sham-operated female controls, preferred to approach the sexually active stimulus male significantly more often than control males, which preferred to approach the stimulus female. Our results suggest that neurons in the mPOA/AH play an important role in the integration of sensory cues which determine heterosexual partner preference in the male ferret, in addition to facilitating masculine coital performance. (supported by HD21094 & MH00392)

572.9

DAMAGE TO THE TELENCEPHALIC NUCLEUS TAENIAE DISRUPTS SEXUAL APPROACH BEHAVIOR IN MALE JAPANESE QUAIL. R. Thompson*, J. Goodson, M. Ruscio, and E. Adkins-Regan, Cornell University, Psychology Dept., Ithaca, NY 14853.

Nucleus taeniae (nTN) is a prominent cell group of the avian archistriatum proposed to be homologous with the mammalian amygdala. Because nTN appears to be similar to the medial nucleus of the mammalian amygdala with respect to sex-steroid binding sites (Watson & Adkins-Regan, 1989), we designed an experiment to see if it plays some role in sexual behavior toward female conspecifics. Adult, sexually experienced male Japanese quail housed under short-day photoperiodic conditions and given exogenous testosterone proprionate received either bilateral, electroiytic ablations of nTN (n=8) or a sham surgical procedure (n=14). Ablations were confirmed with histological analysis. Birds with nTN damage were slower to approach a female housed behind clear plexiglass at the other end of a runway apparatus (mean of the means for individual birds over 4 test sessions: 1.39 min± .32 SEM, experimental group; .66 min± .27 SEM, sham group, $F = 6.22$, $p < .05$) and spent less time in proximity to the female stimulus during a 5 minute testing session when physical contact was not allowed, although this difference did not reach significance (mean of the means for individual birds over 4 test sessions: 3.78 min ± .23 SEM, experimental group; 4.31 min ± .26 SEM, sham group, $F = 3.02$, $p=.09$). Copulatory behavior itself appeared unaffected. These results have implications for the potential homology of nTN with the medial nucleus.

572.10

ANABOLIC-ANDROGENIC STEROIDS AND SEXUAL BEHAVIOR IN INTACT MALE RATS. A.S. Clark* and A.S. Fast. Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

Anabolic-androgenic steroids (AAS) are synthetic androgen-like substances frequently abused by athletes. Very little is known regarding the impact of chronic AAS use on male reproductive function. The present study compared the effects of long-term treatment with three popular AAS on the display of male sexual behavior in gonadally intact male Long-Evans rats. The three AAS selected for study were methyltestosterone, methandrostenolone and nandrolone decanoate. Doses of each AAS comparable to human abuse dose ranges were administered to sexually active male rats daily for 12 weeks. Sexual behavior was quantified each week by an observer blind to the treatment conditions.

Treatment with methyltestosterone proved the most disruptive to male sexual behavior: 50% of the rats treated with the high dose of this AAS failed to complete an ejaculatory sequence by the end of the testing period. Treatment with the high dose of methandrostenolone resulted in a modest increase in ejaculation latency by the end of the testing period, while treatment with the medium dose of nandrolone decanoate produced subtle but persistent changes in several components of male sexual behavior. These results indicate that individual AAS compounds have unique effects on the components of male sexual behavior. These characteristics may reflect the efficacy of specific AAS compounds at androgen receptors. Supported by NIDA-08574.

572.11

THE INFLUENCE OF ANABOLIC-ANDROGENIC STEROIDS ON THE ESTROUS CYCLE IN RATS.
M.E. Blasberg*, C.J. Langan and A.S. Clark. Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

Anabolic-androgenic steroids (AAS) are synthetic androgen-like substances that have been abused by both male and female athletes with the goal of increasing physical strength and athletic abilities. Because other androgens have been shown to interfere with the estrous cycle of female rats, the ability of two AAS to disrupt specific components of the estrous cycle was examined in two separate groups of adult female Long-Evans rats. Female sexual behavior and vaginal cytology were monitored daily over a baseline period, a two-week drug treatment period, and a recovery period. The AAS selected for this study were methyltestosterone and methandrostenolone, AAS compounds frequently abused by athletes. Animals received either the high, medium or low dose of the AAS or the oil vehicle. The number of days of sexual receptivity and vaginal estrus were compared between groups.

Treatment with methyltestosterone significantly reduced receptive behavior and days of vaginal estrus during drug treatment period in a dose-dependent manner. Treatment with methandrostenolone produced no significant disruption in sexual behavior or vaginal cytology as compared to controls. The unique chemical structures or metabolic products of methyltestosterone and methandrostenolone may underlie their distinct effects on female reproductive function. Supported by NIDA-08574.

572.12

MATING IN MALE MICE LACKING THE GENE FOR NEURAL NITRIC OXIDE (nNOS) SYNTHASE. S.L. Klein*[1], G.E. Demas[1], L.J. Kriegsfeld[1], R.J. Nelson[1], A.L. Burnett[2], P.L. Huang[4], M.C. Fishman[4], V.L. Dawson[3], T.M. Dawson[3], and S.H. Snyder[3]. [1]Dept of Psychology, [2]Dept. of Urology, [3]Dept of Neuroscience, Johns Hopkins Univ., Baltimore, MD 21218, [4]Cardiovascular Research Center, Massachusetts General Hospital, Boston, MA 02129.

To determine the specific role of nNOS in male mating behavior and penile erection, homologous recombination was used to create mutant mice with targeted disruption of the nNOS gene (nNOS-). nNOS- and wild-type (WT) males were paired with estrous WT females for 30 min mating tests. There were no significant differences between nNOS- and WT males in the latency to the first mount, number of mounts, latency to first intromission, inter-intromission interval, number of intromissions, or percentage of animals ejaculating with WT females. WT and nNOS- mice produced equivalent numbers of young when bred to WT females. In addition, bilateral stimulation of the cavernous nerves resulted in penile erection in nNOS- animals. In another mating test, nNOS- and WT males were paired with nonestrous WT females for 8 h. Initially, wild-type and nNOS- males mounted females to the same extent, indicative of a normal male response to a novel female. This behavior diminished rapidly in WT males, but only a modest non-significant decrease in the number of mounts was seen in the nNOS- males after the first hour. nNOS- mice continued to attempt to mount nonestrous females at a significantly higher rate than WT males throughout the 8 h test. Plasma testosterone values did not differ significantly between WT and nNOS- mice either before or after the behavioral observations. nNOS- mice did not display any significant impairments on any other behavioral assessments. These findings provide the first evidence for a role of central nNOS neurons in mating behavior.

572.13

INTEGRATION OF CHEMOSENSORY AND HORMONAL CUES IS ESSENTIAL FOR MALE SEXUAL BEHAVIOR IN THE SYRIAN HAMSTER: ROLE OF THE MEDIAL AMYGDALOID NUCLEUS. R.I. WOOD*, Dep't of Ob/Gyn, Yale University, New Haven, CT 06520

Mating behavior in the male hamster is dependent upon both chemosensory and hormonal cues, and copulation is abolished if either signal is interrupted. Through reciprocal interaction of these signals, chemosensory stimuli increase circulating testosterone, and steroid hormones enhance chemoinvestigation. Furthermore, these cues are transmitted through parallel pathways in separate subdivisions of the medial amygdaloid nucleus (Me), bed nucleus of the stria terminalis (BNST) and medial preoptic area (MPOA). Previously, we demonstrated that a single testosterone implant in Me can increase mounting in castrate males, and that integration of chemosensory stimuli with steroid signals in BNST/MPOA is essential for mating. The present studies determined if Me is likewise capable of chemosensory and hormonal integration. The approach was to place an intracerebral implant of testosterone in Me, combined with removal of a single olfactory bulb (UBx), ipsilateral or contralateral to the steroid implant. Ipsilateral UBx blocks communication between chemosensory and hormonal circuits. Sexually-experienced males were used (n=10 each). 12 weeks after castration, a single olfactory bulb was removed, and each male received a testosterone-filled cannula (23 ga). 2 weeks later, there was no effect on sexual behavior in ipsilateral or contralateral UBx males. To determine if steroid stimulation of mating behavior in Me requires bilateral chemo-sensory input, a second study separated implantation and UBx. Contralateral UBx abolished behavioral stimulation in 7 males with an implant in Me. These results confirm that integration of hormonal and chemosensory cues is required for mating behavior. Moreover, steroid facilitation of sexual behavior in Me requires bilateral chemosensory input. (Supported by HD 32669).

572.14

DIFFERENTIAL ROLE OF THREE BRAIN REGIONS (MPOA, PVH, AND NPGi) IN RAT ERECTILE FUNCTION. Y.-C. Liu*, J.D. Salamone, and B.D. Sachs, Dept. of Psychology, Univ. of Connecticut, Storrs, CT 06269-1020.

Three brain regions, the medial preoptic area (MPOA), paraventricular nucleus of the hypothalamus (PVH) and nucleus paragigantocellularis (NPGi), received radiofrequency or sham lesions to compare their roles in penile erection in three contexts: copulation, reflexive erection, and noncontact erection (NCE; i.e., while in proximity to inaccessible estrous females). In two weekly post-op tests, NCEs were displayed by 3/7 & 5/7 MPOAx males vs. 6/10 & 9/10 Sham males (ns). In copulation tests 1-2 days after each NCE test, 1/7 & 1/7 MPOAx males ejaculated vs. 9/10 & 10/10 Sham males (each $p<0.01$). These results support the view that MPOAx rats are aroused autonomically and motorically by estrous females, but unable to translate their sexual arousal into copulatory behavior. PVHx males had smaller intromission ratios (IR, $p<0.03$) and longer ejaculation latencies ($p<0.02$). In supine tests, 2/8 PVHx males vs. 8/10 Sham males displayed reflexive erection ($p<0.05$). The groups were similar in proportion of males showing NCE in two post-op tests, but PVHx males had longer latencies than Sham rats in the second test ($p<0.01$). Thus, PVH lesions impaired erectile function in all three contexts. NPGix males had fewer mounts ($p<0.03$) and intromissions ($p<0.02$) prior to ejaculation than Sham rats, but the IR was unaffected. Reflexive erections were displayed by 9/10 NPGix males vs. 3/8 Sham males ($p<0.05$) and vs. 3/10 NPGi males before lesion ($p<0.02$). In NCE, tests the groups did not differ reliably. These data support the hypothetical inhibitory role for the NPGi in erectile and/or ejaculatory function during copulation and reflexive erection, but not NCE. Collectively, these data and those from other investigators support the view that brain regions participate differentially in erectile function, depending in part on the context in which erection is evoked. (HD08933)

572.15

DIFFERENTIAL ROLE OF RAT MEDIAL AMYGDALA IN THE REGULATION OF ERECTION IN THREE CONTEXTS. Y. Kondo*, B.D. Sachs[1)], and Y. Sakuma. Dept. Physiology, Nippon Medical School, Tokyo 113, Japan, and [1)]Dept. of Psychology, Univ. of Connecticut, Storrs, CT 06269-1020, U.S.A.

We studied erection in rats during (a) copulation, (b) somesthetic stimulation of the penis (reflexive erection), and (c) proximity to inaccessible estrous females (noncontact erection, NCE). Tests were conducted 7-10 days after the rats received radiofrequency lesions of the medial amygdala (MAL, n=8) or sham operations (Sham, n=10). During copulation tests, 6/8 MAL rats achieved intromission, but because of their slower copulatory rate, 0/6 males ejaculated within the 20-min limit after the first intromission, compared with 9/10 Sham males ($p<.001$). The mean intromission ratio [I/(M+I), in part a measure of erectile function] was similar in the two groups (MAL=0.47; Sham=0.60, p=.25), implying no significant erectile dysfunction in copulating MAL rats. The groups also did not differ reliably on any measure of reflexive erection in response to touch/pressure. However, 0/8 MAL rats displayed NCE, compared with 8/10 Sham rats ($p<.01$). The data suggest an important role for the rat MA in NCE, but not for erection in the other two contexts. The MA has been implicated in chemosensory processing. However, since bedding from estrous rats does not provoke erection (Sachs et al, *Physiol. Behav.* 55:1073; 1994), NCE may be mediated by a non-olfactory function of the MA.

572.16

MOUNTING BEHAVIOR PREDICTS FERTILITY OF MALE TRANSGENIC MICE OVEREXPRESSING HUMAN GROWTH HORMONE (GH) GENE. C. J. Meliska* and A. Bartke. Southern Illinois University School of Medicine, Carbondale, IL 62901-6512.

Male transgenic (T) mice overexpressing the human placental GH variant gene (mMT-hGH.V) exhibit reproductive deficits in spite of normal testosterone levels and normal sperm counts (*J. Reprod. Fert. 95:109, 1992*). To evaluate the relationship of copulatory behavior to fertility, we first measured mount, intromission, and ejaculation indices in 2-5 month-old mice (10 T and 10 non-T litter mate controls) during 1-hr tests with ovariectomized, estrogen and progesterone primed females. After 8 tests, each male was housed with 3 intact females for 27 days. Females were checked daily for vaginal plugs and sacrificed 14 days after insemination to determine numbers of corpora lutea, live, and dead fetuses. Relative to non-T controls, T mice displayed shorter latencies to intromit after the initial mount (311 vs. 28 s, $P = 0.013$); made more pre-ejaculation intromissions (18.2 vs. 61.1, $P = 0.043$); plugged fewer (3.0 vs. 1.8, $P = 0.01$), and took longer to plug intact females (3.6 vs. 11.0 days, $P = 0.008$); and sired fewer live fetuses per insemination (9.0 vs. 2.6, $P = 0.017$) than non-T controls. Among both T and non-T mice, mount rate (mounts/min) between first mount and first intromission in the copulation tests was inversely correlated with number of live fetuses ($r = -.66$, $P = 0.027$), suggesting that reduced fertility is associated with altered male copulatory behavior, including a rapid rate of mounting the female prior to the first intromission. (Supported by NIH Grant HD20001.)

573.1

ENHANCED STIMULANT EFFECTS FOLLOWING CHRONIC ANABOLIC STEROID ADMINISTRATION. J.W. Boja*. Dept. Of Pharmacology, NEOUCOM, Rootstown, OH 44272.

The abuse of anabolic steroids has dramatically increased not only by adult body-builders and athletes but also by adolescents as well. It has been estimated that 250,000 high school students have either tried or are currently using anabolic steroids. A recent study reported that 4.7% of male and 2.9% of female ninth grades were using anabolic steroids. In addition, the subjects were more likely to engage in risky behaviors such as needle sharing and co-administration of other illicit drugs such as cocaine. Chronic stimulant administration has been reported to led to higher feelings of aggression, mania, depression and other behavioral disturbances. These symptoms may be enhanced with the concurrent use of other powerful psychoactive substances like amphetamine or cocaine. In order to study the co-abuse liability of anabolic steroids and stimulant use, juvenile male Sprague-Dawley rats (50-60g) were injected IM once a week with 200 mg/kg nandrolone decanoate or the corn oil vehicle for six weeks. Two days after the last injection, the animals were place into an activity chamber for 30 minutes prior to injection of 10 mg/kg amphetamine sulfate. While there were no differences in baseline activity, amphetamine-stimulated activity was significantly higher in the steroid treated than in the vehicle treated controls. In addition the anabolic steroid treated animals displayed heightened aggression towards resident intruders compared to controls. These results indicate that a significant co-abuse liability between anabolic steroids and stimulants may exist.

573.2

GLUCOCORTICOID RECEPTOR MRNA IS DOWN-REGULATED IN THE HIPPOCAMPUS OF RATS BEHAVIORALLY SENSITIZED TO AMPHETAMINE. PD Shilling*, IR Kelsoe, DS Segal. Dept of Psychiatry and Neurosciences Program, Univ. Calif., San Diego, CA 92093.

Repeated administration of amphetamine-like stimulants to adult rats results in enhanced behavioral responsiveness to subsequent administration of these drugs. The mechanisms underlying these behavioral changes may have important implications for stimulant abuse as well as for various forms of psychopathology. Recent evidence suggests corticosterone may play a role in the development of sensitization(Rivet et al. Brain Res. 198:149;1989), perhaps through the down-regulation of glucocorticoid receptor(GR)(Reul et al. Mol. Endocrinol. 3(10):1674;1989). To test this hypothesis we examined the effects of 5 daily injections of amphetamine(AMPH)(2.5mg/kg) on GR mRNA of adult Sprague Dawley rats. This treatment has been shown to produce pronounced sensitization characterized by a progressively more rapid onset and intensification of stereotypy and enhanced post-stereotypy hyperactivity. Two other groups received saline for 4 days and then either saline or AMPH on the fifth day. All animals were sacrificed 24 hours after the last treatment and *in situ* hybridization was performed with an antisense mRNA GR probe. Message was detected by film autoradiography. Quantification of hippocampal GR mRNA was accomplished by computer analysis of digitized images of CA1 and dentate gyrus using Image 1.55. Acute AMPH did not alter GR mRNA. In contrast repeated exposure to AMPH resulted in significant down-regulation in CA1($P<.04$) and a nonsignificant trend towards down-regulation in dentate gyrus. Further studies will be performed to determine if down-regulation of GR mRNA is critical for the development of behavioral sensitization.

573.3

SEX DIFFERENCES IN OPEN-FIELD BEHAVIOR BEFORE AND AFTER AMPHETAMINE: COMPARISON OF NEONATALLY GONADECTOMIZED (NEOGX) AND ADULT-GX RATS. R.H. MILLS*, J.G. KOHLERT, L.W. JOHNSON, T. SLADE, AND G.J. BLOCH. Dept. of Psychology, BYU, Provo, UT 84602.

Adult-Gx male rats show a lower locomotor response to D-amphetamine sulfate (AMPH) than adult-Gx females (Forgie & Stewart, '93). To further explore this issue, males and females were Gx or sham-Gx at 6-30 hr after birth and at 75-80 days of age. Animals were acclimated on days 6-8 after adult-Gx, then received 2.0mg/kg AMPH i.p. on day 9. Locomotor behavior was analyzed by ranking the behavior by its position on an inverted U-shaped curve based on scores of both ambulatory (beam-breaks) and stereotyped behaviors; this method is unambiguous, giving a clear, positive relation between rankings and AMPH dosage (Kohlert & Bloch, '94). Adult-Gx females showed significantly greater levels of basal locomotor activity than the other groups during the first acclimation session and during the first 6 min of all 3 acclimation sessions (p's .01 to .04, Newman-Keuls); a similar result was reported by Stewart & Cygan ('80) using 3-minute tests. Adult-Gx males responded significantly less to AMPH than animals in the other groups (p's .05 to .005, Mann-Whitney tests). We saw no difference in the acute AMPH response among NeoGx males, NeoGx females, and adult-Gx females. Forgie & Stewart ('94) reported lower total activity scores in NeoGx than adult-Gx females, but results were ambiguous because NeoGx females also had higher levels of stereotypy. No difference in basal and AMPH-stimulated activities in NeoGx males vs. NeoGx females suggests that prenatal testosterone is not responsible for the sex difference in Gx animals. However, estrogen during the post-neonatal period appears to increase basal open-field activities in adult-Gx rats, while testosterone during this period appears to reduce the AMPH-stimulated response in adult-Gx rats. Supported by HD27334.

573.4

MK-801 AND HALOPERIDOL FAIL TO BLOCK AMPHETAMINE SENSITIZATION INDUCED BY CHRONIC IMMOBILIZATION STRESS. L. Ho, B.K. Tolliver, and S.P. Berger*. University of California, San Francisco and SFVAMC, San Francisco CA 94121.

Although the cross-sensitization which develops with repeated exposure to stress or amphetamine is well established, the mechanisms which underlie these processes remain incompletely understood. The present study examined the abilities of MK-801 and haloperidol pretreatment to block the development of amphetamine sensitization induced by repeated immobilization stress in male Sprague-Dawley rats. Fifteen minutes before each of ten 30 minute restraint sessions, rats were admininstered saline, MK-801 (0.01, 0.10 or 0.25 mg/kg i.p.) or haloperidol (0.10 or 0.25 mg/kg i.p.). Control rats received the same injection regimen without restraint. Four days after the final treatment, rats were tested for locomotor response to novelty, saline and d-amphetamine (1.5 mg/kg i.p.). Exposure to stress significantly enhanced the locomotor response to amphetamine ($p<0.003$) but not to saline ($p=0.46$) in each of the drug groups, with no stress X drug interactions present. Secondary analysis of dose effects in each drug group revealed that at 0.25 mg/kg, repeated MK-801 induced amphetamine sensitization in control rats and potentiated stress-induced sensitization in restrained rats ($p<0.05$). In contrast, while pretreatment with 0.10 mg/kg haloperidol enhanced the locomotor response to novelty ($p<0.05$), neither dose of haloperidol affected the locomotor response to saline or amphetamine in control or stressed rats. In the context of previous reports of blockade of amphetamine-induced sensitization by both MK-801 and haloperidol at doses used in the present studies, these results suggest that amphetamine- and stress-induced behavioral sensitization may proceed through divergent mechanisms. (Supported by USPHS grant DA07376-04)

573.5

CHARACTERIZATION OF AMPHETAMINE SENSITIZATION IN MALE AND FEMALE RHESUS MONKEYS: IS IT RELATED TO BEHAVIORAL AND HORMONAL INDICES OF STRESS? S. A. Castner* and P. S. Goldman-Rakic. Yale University School of Medicine, Section of Neurobiology, 333 Cedar Street, New Haven, Connecticut, 06510.

Sensitization to amphetamine (AMPH) has been proposed as one model of AMPH psychosis. In rats, there are sex, individual and strain differences in the response to both acute and chronic AMPH administration. To our knowledge, an in depth characterization of AMPH sensitization in male and female non-human primates has not been described.

For these experiments, young adult male and female rhesus monkeys were assigned to either AMPH or saline groups. Assignment was based on the incidence of ritualistic and stereotypic behaviors exhibited in the home cage over months of baseline behavioral observations. Behaviors, such as stereotypic pacing and self-biting, are considered indicative of a stressful response to single-unit housing and/or early social deprivation. For the AMPH group, both acute and chronic responses to AMPH will be explored via daily behavioral analysis, serum analysis of prolactin, AMPH and cortisol, and in vivo SPECT imaging of dopamine and serotonin receptors. Saline controls will undergo the same procedures to control for possible effects of daily injections and anesthesia. We hypothesize that the baseline behavioral catergorization of an animal (stressed vs. non-stressed) will be predictive of either a high or low response to subsequent AMPH administration, respectively.

Preliminary findings indicate that a high incidence of stereotypic pacing in the home cage is reflected in a basal serum cortisol level that is significantly higher than that of animals which do not exhibit any signs of ritualistic or stereotypic behaviors in the home cage environment. Initial findings of AMPH concentrations in serum post-injection (0.1 mg/kg; i.m.) are higher in stressed compared to non-stressed animals. These results are being replicated at higher doses of AMPH. Sensitization data will be reported at the meeting.
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573.6

ACUTE EFFECTS OF METHYLPHENIDATE ON OPERANT BEHAVIOR IN THE RHESUS MONKEY. P. Morris, M.P. Gillam, C. McCarty, D.L. Frederick* and M.G. Paule. Division Of Neurotoxicology, NCTR, FDA, Jefferson, AR 72079-9502.

In the present study, the stimulant methylphenidate (MPH) was administered to male adult rhesus monkeys (N=7) previously trained on the five food-reinforced tasks of the NCTR non-human primate Operant Test Battery (OTB). Each task is designed to model a different aspect of brain function, including motivation (M), time estimation (TE), learning (L), color & position discrimination (CPD) and short-term memory (STM). When tested 15 minutes after acute MPH challenges (0.03 to 1.75 mg/kg, i.v.), operant performance was differentially affected, as indicated by the minimum dose required for a significant disruption of behavior in each of the tasks (0.3 mg/kg: M, TE, STM; 1.0 mg/kg: L, CPD). Decreased numbers of reinforcements were earned in all affected tasks. In some tasks (M, L) both response accuracy and response rate were decreased, but in other tasks neither were affected (CPD, STM); for the TE task response accuracy was affected, but not response rate. It was concluded that the distinctive profile of behavioral effects observed for MPH, when considered together with those for previously tested stimulants such as amphetamine and cocaine, continues to underscore the varied sensitivities of each OTB task to pharmacological manipulation.

573.7

EFFECTS OF AMPHETAMINE ANALOGUES ON THE PERFORMANCE OF A DISCRIMINATION IN RATS. Z. Zhang, J.M. Moerschbaecher and P.J. Winsauer*. Department of Pharmacology, LSU Medical Center, New Orleans, LA 70112.

A fixed-ratio discrimination procedure was used as a baseline to characterize the effects of four amphetamine analogues on complex behavior. In the presence of a light above the center lever, rats were required to complete one of two fixed-ratios (FR 8 or FR 16). Completion of the ratio turned off the center light and produced a light above each of two side levers. If an FR 16 was completed, a response on the left lever produced a food pellet. If an FR 8 was completed, a response on the right lever produced food. Errors produced a brief timeout. Methamphetamine, fenfluramine, 3,4-methylenedioxy-amphetamine (MDA), and 3,4-methylenedioxymethamphetamine (MDMA) decreased response rate in a dose-related manner. Methamphetamine (1-3.2 mg/kg) disrupted the accuracy of responding. In contrast, fenfluramine, MDMA, and MDA had no effect on accuracy. Fenfluramine (0.56 mg/kg) administered in combination with methamphetamine shifted the methamphetamine dose-response curve for percent errors to the left. Fenfluramine did not alter the dose-response curve for MDMA or MDA. The data suggest that various amphetamine analogues may interact differently with serotonergic systems in terms of their effects on the performance of a discrimination. (Supported by DA03573 and DA04775.)

573.8

THE EFFECTS OF DIFFERENTIAL REARING ON THE PSYCHOSTIMULANT EFFECT OF AMPHETAMINE AND THE DOPAMINE TRANSPORTER. **J.M. Valone*, A.P. Williamson, R.A. Bevins, W.B. Shaw, L.P. Dwoskin and M.T. Bardo. Department of Psychology, University of Kentucky, Lexington, KY 40506.**

The present study assessed the locomotor and rewarding effects of amphetamine (AMPH) in rats raised in either an enriched condition (EC) or impoverished condition (IC). Since AMPH may evoke dopamine (DA) release by reversing the DA transporter, we also assessed the kinetics of [^{3}H] DA uptake (0.1-10 μM) in the striatum of EC and IC rats. To assess the behavioral effects of AMPH, a single-trial conditioned place preference (CPP) procedure was used. Rats were surgically implanted with an intravenous (IV) jugular catheter for drug delivery. Conditioning consisted of injecting rats with AMPH (1 mg/kg) at the beginning of a 30-min placement into one compartment of the CPP apparatus. On a separate day, animals also received a 30-min exposure to the other compartment following saline. Sham controls were exposed to both compartments without drug. On Day 3, rats received a 15-min preference test. Results showed that AMPH increased locomotor activity more on the conditioning day in EC rats than IC rats, but did not differ in AMPH-induced CPP. There were no differences between EC and IC rats in striatal [^{3}H] DA uptake as quantified by K_m or V_{max}, nor were there any differences in cocaine (0.0001-100 μM) inhibition of [^{3}H] DA uptake. Thus, the enhanced sensitivity to the locomotor stimulant effect of IV AMPH observed in EC rats relative to IC rats was not accompanied by any difference in the striatal DA transporter. **(Supported by USPHS Grants DA-05312 and DA-07746).**

573.9

PSYCHOSTIMULANT-INDUCED CONDITIONED ACTIVITY DOES NOT APPEAR TO OBEY TO THE RULES GOVERNING THE FORMATION OF A PAVLOVIAN CONDITIONED BEHAVIOR. S.H. Ahmed, L. Stinus and M. Cador*. Lab. Neuropsychobiologie des désadaptations, Université Bordeaux II, INSERM U.259, Domaine de Carreire, Rue Camille Saint-Saëns, 33077 Bordeaux Cedex, France.

Recently, we have developed a within-subject design in order to study the hypothetical associative basis of psychostimulant-induced conditioned activity. In this design, conditioned activity was evidenced when the activity of a same group of rats following a vehicle challenge was greater in an amphetamine-paired environment (CS+) than in a vehicle-paired environment (CS-). Here, we report that: a) conditioned activity developed readily even when rats were exposed to the CS+ following a delay as long as 50 min between the injection of amphetamine (1.25 mg/kg) and the onset of exposure to the CS+ (temporal contiguity); b) the development of conditioned activity was not diminished by increasing the probability of occurrence of the amphetamine effects in the absence of the CS+ relatively to the probability of their occurrence in the presence of the CS+ (contingency); c) the development of conditioned activity seemed to be unaffected by repeated preexposures (0, 5 or 10 preexposures) to the amphetamine effects in the experimental context before the start of the drug-pairing phase (US preexposure). Taken together, these findings did not fit well with what would be expected if conditioned activity was a true Pavlovian conditioned behavior. At least, these results suggest that the amphetamine event seems not to be processed by rats in a manner similar to conventional US events. Thus, future works should emphasize the differences rather than the similarities with Pavlovian US events in order to describe how drug effects are processed and represented by rats. These studies have important implications to our understanding of how stimuli paired with drug effects may influence the relapse of drug taking behaviors.

573.10

EFFECT OF PRIOR SENSITIZATION OF STEREOTYPY ON THE ACQUISITION AND RETENTION OF TOLERANCE TO AMPHETAMINE HYPOPHAGIA. K.M. Hughes* and D.L. Wolgin. Dept. of Psychology, Florida Atlantic Univ., Boca Raton, FL 33431.

We previously found that prior sensitization of stereotypy did not retard the subsequent development of tolerance to the hypophagic effect of amphetamine (Wolgin and Kinney 1992). Since this result was unexpected, a systematic replication of that study was undertaken. Rats were given intermittent injections of either amphetamine (2.5 mg/kg, ip) to induce sensitization of stereotypy, or saline as a control. Following sensitization, subgroups from each group received daily injections of either amphetamine (2 mg/kg) or saline 20 min before a 30 min milk test. Dose-response (DR) curves conducted before and after the chronic phase showed that both sensitized and nonsensitized groups were tolerant to drug-induced hypophagia. Tolerance was accompanied by a suppression of stereotyped movements. The rats were then given milk tests for 3 wk without injections. A final DR curve revealed the loss of tolerance. These results confirm that prior sensitization of stereotypy does not retard the subsequent development of tolerance and show that tolerance can be extinguished by giving repeated milk tests without injections.

(Supported by USPHS grant DA 04592 from NIDA)

573.11

EFFECT OF CHRONIC PSYCHOSTIMULANT AND OPIATE TREATMENT ON SUBSEQUENT LONG-TERM APPETITIVE BEHAVIORS FOR DRUG, SEXUAL AND FOOD REWARD: INTERACTION WITH ENVIRONMENTAL VARIABLES. C. Nocjar,* J. Panksepp, & R.L. Conner. Dept of Psychology, Bowling Green State University, Bowling Green, OH 43403.

It has been hypothesized that sensitization phenomena may play a role in long-term appetitive behaviors to seek drugs (Robinson & Berridge, 1993). This study therefore assessed whether chronic amphetamine, cocaine or morphine pretreatment would affect amphetamine reward-seeking behaviors following long-term drug abstinence, and, whether these effects would generalize to more natural rewards as well. Since environmental variables do appear to affect the development of sensitization phenomena (Einat & Szechtman, 1993), the drug-paired environment was manipulated as well. Rats received 10 days treatment with amphetamine (1 or 5 mg /kg),cocaine (5 mg/kg), morphine (2 or 10 mg/kg), or saline in a runway (run for injection), shuttle-box, or homecage environment. Following 1 or 14 days drug abstinence, appetitive testing for food reward, sexual reward and low-dose amphetamine place-conditioning was conducted. Rats that received chronic psychostimulant pretreatment in a runway apparatus showed long-term enhanced appetitive behaviors for food and drug reward. Sexual pursuit was also enhanced in these animals when testing began 24 hours following drug cessation. However, if the chronic psychostimulant pretreatment was non-contingently administered in a shuttle-box or home-cage environment, amphetamine place-conditioning was depressed. These results show that repetitive use of addictive drugs produce lasting enhancements in appetitive behaviors for drug reward and more natural rewards as well. Furthermore, expected response contingencies to attain drug reward can enhance the development of sensitized incentive to seek rewards.

573.12

MORPHINE POTENTIATES AMPHETAMINE- AND COCAINE-INDUCED ROTATIONAL BEHAVIOR IN THE RAT. H.L.Kimmel* and S.G. Holtzman.Dept. of Pharmacology, Emory Univ. School of Medicine, Atlanta, GA 30322.

Rats with unilateral 6-hydroxydopamine-induced lesions of the nigrostriatal tract respond to dopaminergic drugs by circling. Amphetamine (AMPH) and cocaine (COC), which act presynaptically to elevate synaptic levels of dopamine, induce turning ipsilateral to the lesion. Morphine (MOR) also elevates synaptic dopamine levels, but induces little turning. The present study determined if MOR would enhance turning induced by AMPH and COC as an approach to assessing possible opioid modulation of brain dopamine systems. Adult male Sprague-Dawley rats given unilateral lesions with 6-hydroxydopamine were injected with MOR (0.03-10 mg/kg, SC) and either AMPH (0.1-1.0 mg/kg, SC) or COC (1.0-10 mg/kg, IP). Beginning immediately after the injections, full and partial ipsilateral turns and direction changes were recorded every 15 min for 4 hr. By itself, MOR had little effect, but it dose-dependently increased the magnitude and duration of full and partial ipsilateral turning induced by COC and AMPH, with a peak effect at 3.0 mg/kg. The opioid antagonist, naltrexone (0.1 mg/kg, SC), which had no effect on rotational behavior alone or in combination with AMPH, blocked potentiation of the effects of 1.0 mg/kg AMPH by 3.0 mg/kg MOR; thus the potentiating effect of MOR involves opioid receptors. These data provide further evidence for opioid modulation of the behavioral effects of stimulant drugs mediated by dopamine in the nigrostriatal tract. [Supported, in part, by Grant DA00541 and by K05 DA00008, both from NIDA.]

573.13

EFFECTS OF DYNORPHIN A-(1-13) ON METHAMPHETAMINE-INDUCED BEHAVIORAL SENSITIZATION IN MICE. M. Ukai*, T. Toyoshi and T. Kameyama. Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Meijo University, Nagoya 468, Japan.

Dynorphin A-(1-13), a κ-opioid receptor agonist, has been reported to modulate the behavioral effects of dopamine receptor agonists. The response to the indirect dopamine receptor agonist methamphetamine (MAP) is sensitized with repeated administrations. The purpose of the present study was to determine the effects of intracerebroventricular administration of dynorphin A-(1-13) on the MAP (2 mg/kg)-induced behavioral sensitization in mice by using multidimensional behavioral analyser which can simultaneously record four different kinds of behavioral responses such as linear locomotion, circling, rearing and grooming. Male ddY mice received MAP (2 mg/kg) or its vehicle combined with dynorphin A-(1-13)(3 and 12.5 μg) 6 times at 3- to 4-day intervals. Behavior was also repeatedly measured after drug administrations. The repeated administrations of MAP (2 mg/kg) markedly sensitized behavioral responses such as linear locomotion and circling but not grooming. The MAP (2 mg/kg)-induced behavioral sensitization remained evident even after withdrawal for 3 weeks. Dynorphin A-(1-13)(3 and 12.5 μg) significantly inhibited the behavioral sensitization induced by MAP (2 mg/kg). These results suggest that the MAP-induced behavioral sensitization is evident in linear locomotion and circling, and κ-opioid receptor agonists inhibit the sensitization.

573.14

IBOGAINE EFFECTS ON SWEET PREFERENCE AND AMPHETAMINE-INDUCED LOCOMTION: IMPLICATIONS FOR ADDICTION. J.R. Blackburn* and K.K. Szumlinski. Dept. of Psychology, McMaster University, Hamilton, Ont, L9S 4K1.

Ibogaine is reputedly efficacious in the treatment of addiction to stimulants, opiates, nicotine, and alcohol. The neural basis of such effects is unclear. One possibility is that ibogaine interferes with the shared capacity of these addictive agents to increase dopamine activity, but reports of ibogaine effects on dopamine activity have been inconsistent. Our study suggests those inconsistencies may result from variations in prior drug exposure.

If ibogaine blocks dopamine activity, then it should, like dopamine blockers, decrease preference for natural rewards such as sweet solutions. However, 20 or 40 mg/kg ibogaine did not decrease preference for a glucose+saccharin solution when it was administered to male Long Evans rats 24 hr prior to test. Nor did these doses of ibogaine attenuate conditioned preference for a neutral flavour previously paired with sweet taste.

Effects of 40 mg/kg ibogaine on amphetamine-induced locomotion were investigated in drug naive and drug experienced (4 prior doses of 1.5 mg/kg amphetamine) rats. Locomotion was significantly lower in those ibogaine-treated rats that had previously been exposed to amphetamine than in those that had not. Thus, ibogaine may serve to decrease induced levels of dopamine activity in drug-experienced animals or humans from elevated, sensitized levels back to baseline levels. This could lead to reduced sensitized drug craving in addiction.

Supported by NSERC Canada.

573.15

MDL 100,907 EFFECTS ON AMPHETAMINE-INDUCED FACILITATION OF SELF-STIMULATION THRESHOLD AND LOCOMOTOR ACTIVITY IN RATS. V.L.Tsibulsky*, S.R.Grocki, S.L.Fledderjohn, D.Wallace, and R.A.Frank. Brain Stimulation Lab, University of Cincinnati, Cincinnati, OH 45221-0376.

There is a common opinion that repeated injections of amphetamine in rats result in sensitization to motor stimulant effects but not to facilitation effects on self-stimulation behavior. In addition, the role for 5-HT2 receptors has been shown in locomotor effects of amphetamine but not in latter. This experiment was designed to test both locomotion measured by stabilimeter platforms and self-stimulation threshold in the course of subchronic 10 day injections of 1 mg/kg amphetamine alone or in combination with 2 mg/kg MDL 100,907, a specific 5-HT2 antagonist, in the same rats. The self-stimulation facilitation effect of amphetamine as well as the locomotor hyperactivity reached the maximal level in the first 30 min after injection and decreased by 50-60% at the end of the 2 h test period. There was no alteration in pharmacodynamics of this effect in the course of subchronic 10 day injections. MDL 100,907 attenuated the effect of amphetamine only on locomotion and only on the first day of injection, but not on the following days. In conclusion, there was no tolerance, sensitization or aftereffects of amphetamine alone or in combination with MDL 100,907 on self-stimulation threshold or locomotion.

This project was supported by NIDA grant DA04483, R.A.Frank, PI.

573.16

EFFECTS OF THE 5-HT_2/D_2 ANTAGONIST, MDL 28,133A, ON APOMORPHINE-INDUCED STEREOTYPED BEHAVIOR IN RATS. D.J. Feldman[1], R.A. Frank[1], R. Flannery[1], G. Byrd[1], G. Metcalf*, And J.H. Kehne. Department of Psychology, University of Cincinnati[1], Cincinnati, Ohio, 45220-0376, and Marion Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, Ohio 45215.

Hyperactive dopamine (DA) systems may contribute to conditions such as schizophrenia and stimulant drug abuse. $5HT_{2A}$ receptors modulate some, but not all, of these effects. DA overactivation induced by amphetamine lowers the threshold for brain stimulation reward (BSR). This effect is reduced by a D_2 (haloperidol), a $D_2/5HT_{2A}$ (MDL 28,133A), but not a selective $5HT_{2A}$ (MDL 100,907) receptor antagonist. The present study evaluated the role of D_2 receptors in the actions of MDL 28,133A by measuring its ability to antagonize stereotyped behaviors produced by direct postsynaptic D_2 receptor stimulation with 0.4 mg/kg apomorphine. Effects on stereotypies produced by indirect stimulation of postsynaptic D_2 receptors with 4.0 mg/kg d-amphetamine were also assessed. The main finding of this study was that haloperidol and MDL 28,133A, but not MDL 100,907, reduced apomorphine-induced stereotypies, and these effects occurred in the dose ranges that were active in the BSR paradigm. These data support the conclusion that a D_2 antagonist action accounts for the ability of MDL 28,133A to reduce d-amphetamine lowering of BSR threshold. Furthermore, given that apomorphine-induced stereotypies are mediated by stimulation of striatal D_2 receptors, these data provide additional support for the conclusion that MDL 100,907, a putative antipsychotic, is devoid of extrapyramidal side effect liability. This research was supported by grants from Marion Merrell Dow and NIDA (DA04483) to R.A. Frank.

573.17

INDIVIDUAL DIFFERENCES IN PSYCHOSTIMULANT-INDUCED FACILITATION OF BRAIN STIMULATION REWARD. S.R. Grocki*, V.L. Tsibulsky, R.A. Frank. University of Cincinnati, OH 45221-0376.

Substantial individual differences in susceptibility to substance abuse have been documented in the human clinical literature. These differences may reflect variation in the central neurochemistry associated with the incentive properties of drugs. We have used stimulant-induced facilitation of brain stimulation reward to evaluate individual differences in the euphoric effects of drugs in rats.

Twenty four Sprague-Dawley rats were implanted with bipolar stainless steel electrodes into the ventral tegmental area (VTA) and were trained to nose poke for stimulation. The number of pulses delivered in each train of stimulation was varied from 0 to 15 to determine self-stimulation threshold using a modified version of the method of constant stimuli. Once the rats achieved stable thresholds, they were injected with amphetamine (0.33, 0.66, 1.0 mg/kg) and cocaine (7.5, 15.0, 30.0 mg/kg). A significant correlation (r=.78) was found between amphetamine and cocaine-induced facilitation of brain reward stimulation. It is hypothesized that the individual differences noted in this study reflect differences in the psychostimulants' effects on brain reward systems.

This research project was supported by NIDA grant DA04483, RA Frank, PI.

573.18

NOVELTY-INDUCED LOCOMOTION AND $GABA_A$ RECEPTOR BINDING. R.J.Gruen*, M. Selim, K. Wenberg, A.J.Friedhoff, and C.W. Bradberry. New York Univ., NY, NY 10016, Yale Univ., New Haven, CT 06510.

The results of recent studies suggest that novelty-induced locomotion (NL) may represent a reliable correlate of vulnerability to drug self-administration. Given that ligands which bind to the $GABA_A$ receptor complex regulate the activity of dopamine (DA) (Gruen et al., 1992), and the DA system has been shown to play an important role in locomotor behavior, we examined the relationship between NL and $GABA_A$ receptor binding in the adult rat using the $GABA_A$ antagonist [^{3}H]SR 95531. NL and amphetamine-induced (1.5 mg/kg; i.p.) locomotion was assessed by placing animals in a circular corridor equipped with photocell beams. [^{3}H]SR 95531 binding was conducted according to the method of McCabe et al. (1988). NL was significantly correlated with amphetamine-induced locomotion ($r = .95$; $p \leq .01$). In addition, we found that there were significant correlations between NL and [^{3}H]SR 95531 binding in the cingulate cortex ($r = .91$; $p \leq .01$), prefrontal cortex ($r = .85$; $p \leq .01$), and ventral pallidum ($r = .85$; $p \leq .01$). These findings suggest that individual differences in $GABA_A$ receptor binding may play an important role in NL and drug self-administration. Supported by DA-08073 (CWB) and MH-08618 (AJF).

573.19

Effects of Concurrent Administration of Ethanol and Methamphetamine on D1 and D2 Binding Sites and Locomotor Activity, Allean O. Fouse and M. A. Blackshear* Department of Biological Sciences, Tennessee State University, Nashville, TN 37209-1561.

The acute and chronic effects of concurrent administration of ethanol and methamphetamine(MAP) on brain dopamine D1 and D2 binding sites and locomotor activity were studied. Male Sprague-Dawley mice (26-30 gm) were divided into four treatment groups: Group I -saline and water, Group-II -MAP and water; Group III - saline and ethanol; Group IV- MAP and ethanol. Ethanol treated mice received 10% ethanol in the drinking water, while mice receiving only MAP or ethanol had free access to water. In acute studies, the mice received a single dose of 0.5 mg/kg of MAP or physiological saline. Locomotor activity was monitored in 15 minute intervals for 75 minutes. For chronic studies, 0.5 mg/kg of MAP was injected daily for 7 days. Locomotor activity was measured on day 9 and again on day 20 after the last injection, after a challenge dose of 0.125 mg/kg of MAP was given. While locomotor activity was higher in MAP treated mice, it was highest in mice receiving both MAP and ethanol in both acute and chronic studies. Maximal stimulation occurred at 45 minutes post MAP treatment. Despite the marked changes that were observed in locomotor activity in animals treated with both MAP and ethanol, no significant changes were observed in D1 binding sites in the striatum. However, a small but significant increase in D2 binding sites was observed in the limbic area. In view of recent studies which demonstrate that MAP and ethanol interactions in humans can cause psychoses and sudden death in humans, these results will provide additional information their combined behavioral and biochemical effects.

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573.20

Neural network aided behavioral analysis of amphetamine and apomorphine sensitization.

Peter Laudrup* & Lane J. Wallace, College of Pharmacy, Ohio State University, 500 W 12th Ave, Columbus OH-43210. E-mail: laudrup@osu.edu.

The purpose of this study was to evaluate a method which can predict the drug and it's dose based on a description of drug-elicited behaviors integrating locomotor activity and stereotypic movements. Naive rats were treated with various doses of either amphetamine, cocaine, or apomorphine. Every fifteen minutes during two hours each animal was monitored for ten seconds for the appearances of well defined behavioral patterns (locomoting, rearing, sniffing in the air, sniffing at the cage floor, or grooming). These data were presented to a neural network designed to match the behavior with the dose and drug that induced it. The network is a three-layer backpropagation network trained with random input noise and momentum.

The network trained successfully to discriminate amphetamine and apomorphine behaviors, but failed to discriminate between amphetamine and cocaine. Interestingly, the network revealed that apomorphine sensitized rats challenged with a low dose of amphetamine elicit a behavior similar to a high dose of apomorphine - a sensitized response. In contrast, rats sensitized with amphetamine and challenged with a low apomorphine dose elicit low apomorphine-like behavior - no cross-sensitization.

These results indicate that sensitization induced by the postsynaptic agonist apomorphine implements changes that are manifested when amphetamine induces dopamine release from the presynaptic site. In contrast, the changes responsible for amphetamine sensitization have to be located somewhere upstream from the apomorphine action site. (Supported by DA07722 and DA06776)

DRUGS OF ABUSE: AMPHETAMINES AND OTHER STIMULANTS IV

574.1

DIFFERENTIAL DISPLAY PCR DETECTS NOVEL CLONES RELATING TO METHAMPHETAMINE-INDUCED REVERSE TOLERANCE. T. Umekage*, S. Sugita and Y. Watanabe. Department of Pharmacology, National Defense Medical College, Tokorozawa 359, Japan

Dramatic behavioral changes occur after exposure to psychomotor stimulator such as methamphetamine (MAP). It is known that repeated administration of MAP induces reverse tolerance to ambulatory activity in rodents. The molecular mechanism in the drug-specific responses is still unknown. In order to understand the molecular nature of reverse tolerance, we have used the Differential Display PCR (D.D.PCR) technique to identify genes that are differentially expressed after single or repeated administration of MAP. Reverse tolerance was induced in seven-week-old ddy mice by repeated administration of MAP (2mg/kg, s.c.) at three or four-day intervals. Four weeks later, total RNA was extracted from the whole brain five or twenty or sixty minutes after another administration of MAP. Using D.D.PCR technique, we obtained twelve clones that have unique expression patterns. These patterns were different from those of the clones from single administration of MAP. Among the sequences of these cDNA, no significant identifications with EMBL sequence were observed. Present results suggest gene expression may be associated with the induction and maintenance of reverse tolerance.

574.2

p53 TUMOR SUPPRESSOR GENE IS A MEDIATOR OF METHAMPHETAMINE-INDUCED NEUROTOXICITY IN MICE. J.L. Cadet*, and H. Hirata. Neuroscience Branch, Molecular Neuropsychiatry Section, NIH, NIDA, IRP, Baltimore, MD 21224

p53-knockout transgenic mice provide a useful toxicity model because p53 has been shown to be involved in the development of apoptosis in a number of cell lines. In order to assess if p53 is involved in the neurotoxicity caused by methamphetamine (METH) on the dopaminergic systems, we have evaluated the effects of this drug in male wild-type as well as heterozygous and homozygous p53-knockout mice. The status of dopaminergic terminals was determined after the administration of 3 different doses of METH (2.5, 5.0, and 10 mg/kg were given 2 hours apart for a total of 4 injections in one day). In wild-type mice, METH caused dose-dependent decreases in DA uptake sites in both the striatum and nucleus accumbens. The percentage of decrease was greater in the striatum. In p53-knockout mice, the depletion caused by METH was attenuated in a strain-dependent fashion, with the homozygous mice showing the greatest protection. These results provide evidence for a role of p53 in the neurotoxic effects of METH on the dopaminergic system. They also suggest a role for p53 in the development of neurodegenerative disorders such as Parkinson's disease.

574.3

ISOLATION OF A NOVEL GENE FROM THE FOREBRAIN OF A RAT TRAINED TO SELF-ADMINISTER AMPHETAMINE INTO THE NUCLEUS ACCUMBENS VIA A MICRODIALYSIS PROBE. S. A. Brooks*[1], R.R. Rule[1], O.R. Javid[2], P.H. Janak[3] and J.L. Martinez, Jr[4]. Dept. of Int. Bio., [1]Dept. of Psych., Univ. of California, Berkeley, 94720; [2]Dept. of Public Health, Johns Hopkins Univ., Baltimore, MD, 21205; [3]Dept. of Phys. and Pharm., Wake Forest Univ.,Winston-Salem, N.C. 27157; [4]Div. Life. Sci., Univ. Texas, San Antonio, 78249.

We have isolated a 2.8 kb clone from a subtracted library generated from the forebrain of rats trained to self-administer amphetamine via microdialysis probe (SNA 1994 vol. 20, part 2, p. 1030) and sequenced 1.5 kb of this clone. Searches of the NCBI database with this sequence revealed no consensus matches in the database. The 1.5 kb of sequence contains 3 putative open reading frames and searches of the NCBI Database with the putative proteins from these open reading frames revealed one that demonstrated a 59% homology to rat succinyl-coA ligase. The other two open reading frames did not demonstrate significant homology to protein sequences in the database. Since the entire clone has not been sequenced, it is possible that we do not yet have the actual coding open reading frame. Screening of a Rat Multi-Tissue Northern blot (Clontech) revealed a 4.4 kb band and a 2 kb band present in all the tissues on the blot. The 4.4 kb band had the strongest binding in heart and the 2 kb band had the strongest binding in lung; brain showed moderate binding of both bands. Additionally, liver showed binding of a small, less than 1 kb, band that was not present in any of the other lanes. Screening of a Genomic DNA Zoo-Blot (Clontech) revealed binding in all lanes. Further analysis will include sequencing the remainder of the clone and screening a rat brain library for full length clones, Northern blot and RPA analysis, and *in situ* hybridization using the brains of both amphetamine self-administration animals and controls. *Supported by NSF 94-11564.*

574.4

CHANGES IN TRANSCRIPTIONAL FACTOR AP-1 BINDING ACTIVITIES IN THE RAT BRAIN AFTER SUBCHRONIC METHAMPHETAMINE ADMINISTRATION.

T. Ishihara, K. Akiyama*, K. Kashihara[1], H. Ujike, T. Hamamura and S. Kuroda. Dept. of Neuropsychiatry and Neurology[1], Okayama University Medical School, 2-5-1 Shikata-cho, Okayama 700, JAPAN.

The present study examined the effect of acute and subchronic administration of methamphetamine (MAP) on transcriptional factor AP-1 binding protein in the rat brain using gel mobility shift assay. A single MAP administration (4 mg/kg) induced robust increase in AP-1 binding in the striatum, nucleus accumbens and cingulate cortex with the maximal level being observed at 3 hours. AP-1 binding increased significantly in the three brain regions at one-week abstinence following cessation of subchronic MAP administration (4 mg/kg for 14 days), whereas it decreased significantly in the cingulate cortex, but did not change in the striatum or nucleus accumbens at four-week abstinence. After an abstinence of 4 weeks, a MAP challenge (4 mg/kg) induced increase in AP-1 binding which was attenuated as compared to that occurred after an acute administration to naive rats. A supershift assay using antibodies raised against Fos/Jun revealed that the increase in AP-1 binding protein that occurred after the acute MAP and at one-week abstinence following cessation of the subchronic MAP administration corresponded to induction of Fos/Jun and lasting elevation of Jun, respectively. A MAP challenge after an abstinence of 4 weeks induced Fos in the three brain regions, and Jun in the nucleus accumbens and cingulate cortex. It is concluded that AP-1 binding undergoes quantitative and qualitative changes after subchronic MAP administration.

574.5

CHANGES IN G PROTEIN MESSENGER RNAS AFTER THE BEHAVIORAL SENSITIZATION TO METHAMPHETAMINE.
H. Ujike*, K. Akiyama, S. Kuroda. Dept. of Neuropsychiatry, Okayama Univ. Med. Sch., Okayama 700, Japan

A possible role of G proteins in the behavioral sensitization to methamphetamine was investigated using in situ hybridization to mRNAs of α subunits encoding Gs, Gi2, and Go proteins. Rats received 4 mg/kg of methamphetamine for 14 consecutive days. Four hours after the last injection, Goα mRNA showed an increase of 16.6-31.6 % in CA1, CA3 and dentate gyrus regions of the sensitized hippocampus. Giα mRNA also increased by 24.2% in CA1, while Gsα mRNA was unaffected. These changes of Goα and Giα mRNA recovered to normal levels after 48-hour abstinence. No other brain regions, such as substantia nigra, ventral tegmentum area, striatum, accumbens and cortices, were affected in the expression of any G protein mRNA. The present study suggests that the increase in the expression of Go and Gi, not Gs mRNA of hippocampus after sensitization to methamphetamine may alter neurotransmission between some receptors and their second messengers and result in adaptation of neural function related to behavioral sensitization.

574.6

EFFECTS OF SELECTIVE ADENOSINE A1 AND A2a AGONISTS ON AMPHETAMINE-INDUCED LOCOMOTION AND *c-fos* INDUCTION IN STRIATUM AND NUCLEUS ACCUMBENS.
S.M.Turgeon*, A.E.Pollack, L.Sushiem and J.S.Fink. Molecular Neurobiology Laboratory, Massachusetts General Hospital, and Dept. of Neurology, Harvard Medical School, Boston, MA 02114

Adenosine receptors and dopamine receptors have been shown to interact at both behavioral and cellular levels. Adenosine A1 and A2a and dopamine D2 receptors are present in the striatum. While the cellular localization of A1 receptors in striatum is unknown, A2a and D2 receptors have been shown to be colocalized to striatopallidal neurons. Low doses of amphetamine produce locomotion which is dependent on release of dopamine in the anteromedial striatum and nucleus accumbens. The effects of selective A1 and A2a adenosine receptor agonists on locomotion and *c-fos* induction following a low dose of amphetamine were assessed in rats. Pretreatment with the A1 adenosine receptor agonist CHA or the A2a adenosine receptor agonist APEC inhibited locomotion following an injection of amphetamine (1.5 mg/kg). This dose of amphetamine induced *c-fos* -like immunoreactivity in an antero-dorsomedial distribution in the caudate-putamen and uniformly in the core and shell of the nucleus accumbens. Pretreatment with the A2a agonist APEC, but not the A1 agonist CHA, attenuated *c-fos* induction in caudate-putamen and nucleus accumbens by amphetamine. These findings indicate that amphetamine-induced behavior is subject to modulation by adenosine receptors through mechanisms which are both related to and independent of *c-fos* induction.

574.7

PRE-EXPOSURE TO STRESS ENHANCES AMPHETAMINE-INDUCED SENSITIZATION: EXPRESSION OF THE IMMEDIATELY EARLY GENE *c-fos*
T.Hamamura*, Y.Ichimaru and H.C.Fibiger. Division of Neurological Sciences, Department of Psychiatry, University of British Columbia, Vancouver, B.C., Canada V6T 1Z3

Amphetamine (AMPH) abuse results in psychosis that resembles paranoid schizophrenia. Moreover, stressful experience is supposed to increase vulnerability for AMPH-induced psychosis. To investigate the neuroanatomical basis of this phenomenon, this study examined the effects of pre-exposure to stress on AMPH sensitization by brain mapping using Fos immunohistochemistry. Rats received footshock or sham-shock for 3 days. Three days after the last cession, rats received repeated injections of AMPH (4 mg/kg i.p. , 8 times in total) or saline. After a 14 day drug abstinent period, rats were challenged with 2 mg/kg AMPH. By itself, the AMPH sensitization procedure enhanced subsequent AMPH-induced Fos expression in only one structure, the medial part of lateral habenula (LHbm). In contrast, when animals were pre-exposed to footshock stress, the AMPH sensitization procedure resulted in significantly enhanced AMPH-induced Fos expression in four additional structures, those being agranular insular cortex, piriform cortex, nucleus accumbens and medial caudate putamen. These results indicate that there are significant interactions between pre-exposure to footshock stress and AMPH sensitization in terms of the subsequent ability of AMPH to enhance regional Fos expression.

574.8

METHYLENEDIOXYMETHAMPHETAMINE (MDMA, ECSTASY) CAUSES INCREASES IN C-FOS EXPRESSION IN CU/ZN SUPEROXIDE DISMUTASE TRANSGENIC MICE.
B.N Ladenheim, E.E., Ladenheim,* C.J. Epstein and J.L. Cadet. Molecular Neuropsychiatry Section, NIH/NIDA, IRP, Baltimore, Maryland 21224.

MDMA is a psychoactive drug of abuse commonly known as Ecstasy. MDMA causes dopamine (DA) depletion in striatum of mice. The toxic effects of this agent seem to involve superoxide radicals since CuZn superoxide dismutase (SOD) transgenic (Tg) mice that express the human gene are protected against the deleterious effects of the drug. In the present study, we tested the possibility that MDMA might cause changes in c-Fos immunoreactivity in mouse brain. In addition, we tested the idea that superoxide radicals might be involved by comparing the effects of MDMA in non-transgenic (Non-Tg) and SOD-Tg mice. MDMA elicited a dose-related activation of c-Fos-like immunoreactivity in both NON-Tg and SOD-Tg mice. MDMA-induced labeling was more intense in brain regions associated with the limbic system. These include the piriform, perirhinal and entorhinal cortices, islands of Calleja, olfactory tubercle, amygdala and hippocampal CA-1 regions. These results suggest that administration of Ecstasy is associated with stimulation of limbic structures and are consistent with the known psychotropic effects of this drug. These results also indicate that c-fos regulation by MDMA might not be dependent on oxidative mechanisms.

574.9

DISTAL FOS INDUCTION BY PCP INJECTIONS INTO A NUCLEUS ACCUMBENS REWARD SITE.
C. Marcangione, W. A. Carlezon, Jr., R. A. Wise*, and J. G. Pfaus. Center For Studies in Behavioral Neurobiology and Department of Psychology, Concordia Univ., Montréal, Que, CANADA H3G 1M8.

Rats learn to lever-press for phencyclidine (PCP) injections into ventro-medial (shell) but not the dorso-lateral (core) region of nucleus accumbens (NAS)[1]. PCP has several actions of possible relevance; it is a dopamine uptake inhibitor, and NMDA channel blocker that also binds to σ receptors. To identify efferent projections from PCP reward sites in NAS, we determined Fos expression after a series of rewarding PCP injections. The rats received 26 unilateral infusions of PCP (12 nmol per 120 nl infusion) or vehicle into the ventro-medial (shell) region of NAS; each animal's injections were "yoked" to the rate and pattern of administration of a rat previously trained to lever-press for similar injections. The rats were sacrificed for histological analysis of Fos immunoreactivity 90 min after the first infusion of PCP. Serial coronal sections were taken from the area of the medial prefrontal cortex (mPFC) through the pedunculo-pontine tegmental nucleus of the midbrain. PCP induced Fos expression at several sites distal to the NAS injection sites; dramatic expression was seen in the rostral pole of the ventral tegmental area and in the ventral premammillary nucleus. The injections also appeared to induce Fos in the mPFC, dorsal striatum, lateral preoptic area, lateral septum, central nucleus of the amygdala, and ventral premammilary nuclei. Our observations will be quantified and will be the basis for hypotheses as to the efferent pathways involved in PCP reward.

[1] Carlezon and Wise, SN Abstr, 1993, 19, 830)

574.10

CHANGES IN *c-fos*, NGFI-A, AND NEUROTENSIN mRNA EXPRESSION IN RATS SENSITIZED TO AMPHETAMINE.
D.L.Evans*, L.M.Figur, M.P. Stone, K.A.Svensson and K.M.Merchant, CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001

Neural mechanisms underlying the phenomenon of stimulant sensitization have relevance for not only abuse potential of drugs but also emotional and therapeutic drug responses in psychotic disorders. In order to identify neurochemical/neuroanatomical correlates of this sensitization phenomenon, we examined the expression of two immediate early genes, c-*fos* and NGFI-A, and a neuropeptide, neurotensin, in rat forebrain regions. Rats were treated with 2 mg/kg s.c. of amphetamine or vehicle once daily for 5 days (priming treatment), withdrawn from the drug for 7 days, and then acutely challenged with amphetamine or vehicle. Immediately after the acute treatment, locomotor behavior was monitored for 1 h, after which rats were sacrificed and brains used for *in situ* hybridization of c-*fos*, NGFI-A and proneurotensin (NT/N) mRNA. As expected, acute amphetamine challenges to amphetamine-primed rats produced a greater locomotor activation than that seen in the saline-primed animals. In contrast, the responses of NGFI-A, c-*fos* and NT/N mRNA to an acute amphetamine challenge were attenuated in rats sensitized to amphetamine in a regionally-specific manner. Greatest attenuation was observed in the expression of NGFI-A mRNA in the medial prefrontal cortex and the neostriatum and NT/N mRNA in the nucleus accumbens. Interestingly, in the neostriatum, NGFI-A mRNA was reduced more in the extrastriosomal matrix as compared to the striosomes. The role of the specific dopamine receptor subtype in the amphetamine sensitization phenomenon is being investigated.

574.11

OVERNIGHT WITHDRAWAL FROM NICOTINE REDUCES LYMPHOCYTE C-FOS EXPRESSION IN HUMAN SMOKERS IN VIVO. Andrew B. Norman*, Michael Tabet, John Ganim. Division of Neuroscience, Department of Psychiatry, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267

Changes in the expression of immediate-early response genes, such as c-fos, in response to stimulants may mediate the long-term neurochemical adaptations leading to dependence. We have previously found that the administration of Nicorette® gum to nicotine-naive human subjects produced a rapid transient increase in lymphocyte fos protein levels in vivo. We investigated whether changes in c-fos expression occurred in cells from nicotine dependent individuals as a result of smoking and during withdrawal. Subjects fasted and refrained from smoking on the morning prior to and during the study. An 8 ml blood sample was obtained at 8 a.m. and additional samples were obtained 30, 90, 150 and 270 minutes later. Lymphocytes were rapidly separated, washed, homogenized and frozen. The fos protein levels were determined by quantitative immunoblotting using [^{3}H]fos protein as a standard. After 4 to 8 weeks, smokers returned and repeated the study with the exception that they smoked ad libitum. There was a marked decrease in lymphocyte fos protein levels in all smokers undergoing overnight withdrawal relative to the same individuals not undergoing withdrawal and relative to nicotine-naive individuals. Lymphocyte fos levels may provide a quantitative biological measure of withdrawal severity. (Supported by the Smokeless Tobacco Research Council, Inc.)

574.12

NICOTINE INJECTIONS IN THE VENTRAL TEGMENTAL AREA INCREASE LOCOMOTION AND *C-FOS* EXPRESSION IN THE LIMBIC FOREBRAIN OF THE RAT. G.G.Nomikos*, A.Malmerfelt, G.Panagis, K.Chergui, M.Nisell and T.H.Svensson. Dept. of Physiology and Pharmacology, Div. of Pharmacology, Karolinska Institutet, S-17177 Stockholm, Sweden.

Results of recent studies have suggested that nicotinic receptors in the ventral tegmental area (VTA) may be of primary importance for the locomotor-activating and reinforcing actions of nicotine. Here we examined the effects of acute injections of nicotine in the VTA on locomotor activity and on Fos immunoreactivity in the limbic forebrain and the basal ganglia. We also studied the effects of repeated nicotine injections in the VTA on locomotor activity to determine whether sensitization of locomotion would develop. Bilateral intrategmental injections of nicotine (0.02, 0.2, 2.0 and 8.0 ug/0.5ul/side) dose-dependently increased all parameters of locomotor activity-with the exception of rearing-in an open field. Nicotine (8.0 ug/0.5ul/side) injection in the VTA increased *c-fos* expression in the nucleus accumbens and the medial prefrontal cortex, but not in the dorsolateral striatum. Similar results on Fos immunoreactivity were observed following acute systemic injections of nicotine (0.5-2.0 mg/kg, s.c). Pretreatment (30 min) with the nicotinic receptor antagonist mecamylamine (1.0 mg/kg, s.c.) prevented nicotine-induced hyperlocomotion and reduced the increased number of Fos-positive nuclei in the limbic forebrain. Repeated VTA injections of nicotine (2.0 ug/0.5ul/side; total of six injections at a rate of one injection every 48 hr) progressively increased locomotion across test days. These findings directly support the notion that VTA represents the critical neural substrate mediating the behavior-activating, sensitizing and consequently the dependence-producing actions of nicotine. (Supported by the Council for Tobacco Research Inc., U.S.A.)

574.13

EFFECT OF ABSTINENCE SYNDROME ON DOPAMINE RELEASE IN THE MESOLIMBIC SYSTEM OF NICOTINE DEPENDENT RATS. A MICRODIALYSIS STUDY. E. Carboni*, C. Giua and G. Di Chiara. Dept. of Toxicology, University of Cagliari, Viale Diaz 182, 09126 Cagliari, Italy.

Drugs of abuse like morphine, nicotine and alcohol share the common feature of increasing dopamine levels in the nucleus accumbens when administered acutely. A decrease of mesolimbic dopamine release follows the withdrawal of morphine in chronically treated rats. In nicotine dependent rats an abstinence syndrome can be precipitated by both the nicotinic antagonist mecamylamine and by naloxone suggesting the existence of an endogenous opioid component in nicotine dependence and abstinence syndrome. The aim of the present study was to investigate the changes of dopamine release induced by mecamylamine, naloxone or withdrawal in rats treated chronically with nicotine and correlate the results with the appearance of the abstinence syndrome. Male Sprague Dawley rats (260 g) were implanted with an Alzet osmotic minipump that delivers 9 mg/Kg/day of nicotine tartrate. Nine days after rats were implanted with a dialysis probe in the nucleus accumbens and 24 hour later dopamine was measured. Basal dopamine levels were increased in nicotine dependent rats by 44 % Mecamylamine at the dose of 1 and 2 mg/Kg s.c. decreased by about 40 % dopamine release in nicotine dependent rats while did not affect dopamine in controls. Naloxone (4.5 mg /Kg s.c. decreased dopamine release controls but not in nicotine dependent rats. Nicotine 0.2 mg/Kg s.c. increased by about 50 % dopamine release in control rats and only by 10 % in nicotine dependent rats. Our data indicate that chronic nicotine treatment induces an increase in dopamine release. Blockade of nicotine receptors by mecamylamine causes the precipitation of the abstinence syndrome and a decrease of dopamine release. Naloxone, although is able to precipitate the abstinence syndrome in nicotine dependent rats, caused a decrease in dopamine release in controls but not in nicotine dependent rats suggesting that, the fall in dopamine release might not be associated with nicotine abstinence syndrome.

574.14

CONDITION-INDEPENDENT SENSITIZATION OF LOCOMOTOR STIMULATION AND MESOCORTICAL DOPAMINE RELEASE FOLLOWING CHRONIC NICOTINE TREATMENT IN THE RAT. M.Nisell*, G.G. Nomikos, P. Hertel, G. Panagis and T.H. Svensson. Dept. of Physiology & Pharmacology, Div. of Pharmacology, Karolinska Institutet, S-171 77 Stockholm, Sweden.

Chronic nicotine (NIC) treatment augments NIC-induced locomotion, an effect that seems critically dependent on activation of brain dopamine (DA) systems. In this study the effects of chronic, intermittent NIC treatment on locomotor activity and brain dopaminergic activity were examined. Male rats received 12 daily injections in their home cage of saline (SAL) or NIC (0.5 mg/kg, s.c.). Twenty-four hr after the last injection, the locomotor activity was measured. Furthermore, microdialysis experiments were performed in awake animals, measuring extracellular concentrations of DA in the prefrontal cortex (PFC) and the nucleus accumbens (NAC). Also, extracellular single cell recordings from DA neurons in the ventral tegmental area (VTA) were performed in anesthetized animals. Nicotine (0.5 mg/kg, s.c.) increased locomotor activity to a greater extent in NIC-pretreated than in SAL-pretreated animals. Nicotine (0.5 mg/kg, s.c.) also increased DA release in the PFC and in the NAC. After NIC-pretreatment, the NIC-induced DA release was enhanced in the PFC, but unaffected in the NAC. Intravenously administered NIC dose-dependently increased burst activity, starting at 12 μg/kg in SAL-pretreated animals and at 6 μg/kg in NIC-pretreated animals, and also dose-dependently increased firing rate in SAL- as well as NIC-pretreated animals, starting at 25 μg/kg. These results demonstrate that behavioral sensitization after chronic NIC treatment is accompanied by an enhanced DA release specifically within the PFC. This phenomenon may be highly significant for the dependence-producing effects of NIC.

574.15

NICOTINE SELF-ADMINISTRATION IN RATS E.C. Donny, A.R. Caggiula*, S. Knopf *Dept. of Psychology, University of Pittsburgh, Pittsburgh, PA. 15260 and Pittsburgh Cancer Institute*

Animal models of drug self-administration have greatly contributed to current knowledge of the factors which influence drug-maintained behavior. To date, however, research into the neuropharmacological and behavioral effects of nicotine in rodents has been based almost entirely on designs in which the experimenter administers the drug. While such designs have considerably expanded our understanding of nicotine's effects, a self-administration model would provide a valuable tool for further characterizing nicotine-maintained behavior. To date, however, other laboratories have had difficulty in obtaining reliable self-administration using iv nicotine in rats.

We have recently replicated Corrigall and Coen's (1989) model of iv nicotine self-administration. Male Sprague-Dawley rats maintained stable responding (18 infusions/1 hr session) on an FR5 to .03mg/kg/infusion. Responding was partially extinguished by substituting saline, and reacquired after nicotine was reintroduced. Responding decreased at .003mg/kg but was unchanged at .06 mg/kg and total intake increased at the higher dose. We are now investigating some of the basic effects of self-administered nicotine and comparing them to those resulting from experimenter-administered drug. Supported by DA07546

574.16

SELECTIVE CENTRAL OR PERIPHERAL NICOTINIC ANTAGONISM BY CHLORISONDAMINE S. Knopf*, A.R. Caggiula, A.F. Sved, A.M. Schreihofer and C.G. McAllister. *University of Pittsburgh and Pittsburgh Cancer Institute, Pittsburgh, PA 15260.*

Chlorisondamine (CHLOR) is a potent nicotinic receptor blocker which does not readily cross the blood-brain barrier due to its *bis*quaternary structure. However, its usefulness as a tool for distinguishing central vs peripheral sites of nicotine's action depends on establishing dose/time parameters which produce exclusively peripheral or central blockade when administered either systemically or centrally. In Sprague-Dawley rats, CHLOR (0.1 mg/kg, s.c.) given 20 min earlier significantly attenuated the increase in mean arterial blood pressure (MAP) induced by either nicotine (NIC) (40 μg i.v.) or the specific peripheral nicotinic agonist dimethylphenylpiperazinium iodide (DMPP) (10 μg i.v.), indicating effective ganglionic blockade, but had no effect on the NIC (1.33 mg/kg s.c.)-induced increases in plasma adrenocorticotrophic hormone (ACTH) and vasopressin, indicating that central receptors were unaffected.

Using the same measures, it was found that 5 μg CHLOR administered into the lateral ventricle 5 hr earlier significantly attenuated the NIC (1.33 mg/kg s.c.)-induced increase in plasma ACTH, but had no effect on DMPP-induced increase in MAP. Therefore, using these doses and times, it is possible to produce selective central or peripheral nicotinic antagonism with chlorisondamine. Supported by DA07546 and DA08505.

574.17

BEHAVIORAL MANIFESTATIONS OF THE NICOTINE ABSTINENCE SYNDROME IN RATS. B.E.Hildebrand, G.G. Nomikos, C. Bondiers, M. Nisell, S.E. Krahl* and T.H. Svensson. Dept. of Physiology & Pharmacology, Div. of Pharmacology, Karolinska Institutet, S-171 77 Stockholm, Sweden.

There are very few behavioral models in use for the nicotine abstinence syndrome. This study has used a somewhat modified model of the opiate abstinence syndrome. Male Wistar rats were infused for 7 days with 9 mg/kg/day nicotine tartrate via subcutaneously implanted Alzet 2ML1 osmotic micropumps. Rats were observed for 30 minutes in a plexiglass box before, during and after infusion (at 16 and 40 hrs after explantation of the pumps). The signs oberved as behavioral measures of spontaneous nicotine withdrawal were: gasps, genital licks, ptosis, teeth chatters, wet shakes, yawns and changes in locomotor activity as compared to baseline session and to sham operated controls. Statistical evaluation revealed a significant increase in overall abstinence signs both at 16 and 40 hrs, ($p<0.05$) as compared to baseline and ($p<0.01$) to controls. Moreover, there was a significant reduction in locomotor activity ($p<0.01$) at both withdrawal sessions. Another group were injected with the centrally acting nicotinic receptor antagonist mecamylamine, 1mg/kg s.c., on the seventh day of infusion. This treatment precipitated the abstinence syndrome, characterized by an even higher increase in overall abstinence signs as compared to all other groups ($p<0.001$), while there was no significant change in locomotor activity. Preliminary results from this laboratory show that a low dose of nicotine (0.15mg/kg s.c.) administered to spontaneously abstinent rats extinguishes the withdrawal syndrome. Moreover, administration of the peripherally acting nicotinic receptor antagonist chlorisondamine, 1mg/kg s.c., seems to elicit at least some components of the withdrawal syndrome, as described in the above model. This model of nicotine abstinence may prove useful in examining further the underlying mechanisms of nicotine dependence and withdrawal. (Supported by the Council for Tobacco Research Inc., U.S.A.)

574.18

A TIME-COURSE ANALYSIS OF BEHAVIOURAL SENSITIZATION TO NICOTINE IN THE RAT. D.H. Johnson*, J.A. Engel and B. Söderpalm. Inst. of Physiol. and Pharmacol., Dept. of Pharmacol., Göteborg University, Sweden.

Nicotine, amphetamine, cocaine, opiates and ethanol share the ability to induce behavioural and neuronal sensitization in laboratory animals. These phenomena have been implicated in the development of psychic dependence to drugs of abuse. Behavioural sensitization to nicotine has been documented as an enhancement of nicotine-induced locomotor activity (LMA; measured over 30' to 60' without any detailed time-resolution) after 5 - 15 daily injections, as compared to nicotine-induced LMA day 1.

The purpose of the present experiments was to obtain more detailed information regarding the time-course (over five-minute intervals) of the LMA response to nicotine throughout chronic intermittent nicotine treatment, the maximal sensitized response and the duration of an established sensitization.

Adult male S-D rats received nicotine daily (0.4 mg/kg, s.c.) and LMA (30' habituation followed by drug injection and recording for another 30') was recorded on days 1, 6, 9 and 14. On day 6, the LMA response during the first 10-minute period after injection (early phase) was sensitized ($p<0.001$), whereas LMA during the last 20-minute period (late phase) was not sensitized until on day 14, and then only weakly. The total LMA response (30') was sensitized from day 9 ($p<0.001$). Some animals received further nicotine treatment and were tested on days 16, 20, 29, 31, 38 and 44. The maximal sensitized response (200 % of the day 1 response, both regarding the early and late LMA phase) was obtained from day 29. Nicotine treatment was then interrupted and 1, 3 and 5 months later the animals were again challenged with nicotine. The early phase LMA response remained significantly sensitized at all time-points (approx. 150 % after 5 months), whereas the late phase response was further enhanced after 1 month (250 %) and sharply declined after 5 months (68 %).

These results indicate that behavioural sensitization to nicotine may involve two processes affecting the early and late phase LMA after nicotine injection differently. Sensitization to the early phase response appears rapidly induced, robust and long-lasting, while that to the late phase develops slowly, appears boosted after interruption of nicotine administration, but is not as long-lasting as the early phase sensitization.

574.19

EFFECT OF NICOTINE (NIC) ON DOPAMINE (DA) RELEASE FROM TERMINAL REGIONS OF THREE DOPAMINERGIC PATHWAYS. D.L. Marshall*, P.H. Redfern and [1]S. Wonnacott. Sch. Pharmacy & Pharmacol. and [1]Sch. Biol. & Biochem., Univ. Bath, Bath BA2 7AY, U.K.

The ability of NIC to enhance DA release in the mesolimbic DA pathway is thought to underlie NIC's reinforcing properties [1]. Systemically administered NIC results in greater DA release from accumbens than from striatum of frontal cortex [2, 3]. Why the mesolimbic system is more sensitive, and the precise site of action of nicotine remain unclear. Here we have compared the release of DA by NIC from the terminal regions of the 3 DA pathways in adult male Sprague-Dawley rats using *in vivo* microdialysis. NIC was delivered to the terminals via the dialysis probe.

NIC produced an immediate, dose-dependent increase in DA release (n=6-8). At a high NIC concentration ($3x10^{-1}$M) peak DA release was increased to 265±119 fold of basal (striatum), 101±51 fold (accumbens) and 21±12 fold (cortex); release was not inhibited by prior addition of 10^{-3}M mecamylamine (MEC) suggesting a non-specific mechanism of action. At a lower NIC concentration ($3x10^{-3}$M) peak DA release was 3.3±1.1 fold of basal (striatum), 3.2±1.1 fold (accumbens) and 1.1±0.1 fold (cortex); release was inhibited by MEC suggesting NIC acts via stimulation of nAChRs. The estimated EC_{50} for specific DA release by NIC was $3.5x10^{-3}$M for the striatum and accumbens, and $5x10^{-3}$M for the cortex. The effective *in vivo* recovery for NIC in the striatum was 0.5%, giving EC_{50} values in the low micromolar range. These data suggest there is no difference in the sensitivity of the terminal regions of the different ascending DA pathways to the specific effects of NIC.

1. Di Chiara, G. *et. al.*, (1994) "Neurochemistry of drug dependence", pp65-82
2. Imperato, A. *et al.*, (1986) *Eur. J. Pharmacol.*, 132, 337-338
3. Nisell, M. *et al.*, (1994) "Effects of nicotine on biological systems II" P74

574.20

ACUTE TOLERANCE TO THE DOPAMINE RESPONSE TO NICOTINE. *I.M. Maisonneuve*, C.R. Deibel and S.D. Glick, Department of Pharmacology and Neuroscience, Albany Medical College, Albany, NY 12208.*

There is increasing evidence that the rewarding effect of nicotine is mediated by the dopamine mesolimbic system. Acute tolerance to the subjective effects of nicotine in novice smokers has been hypothesized to be the basis for the subsequent increase in drug intake. This phenomenon has been observed in *in vitro* paradigms, but not yet reported *in vivo* in naive animals. Using *in vivo* microdialysis in awake and freely moving male Sprague Dawley rats, we investigated the acute dopamine response to two 5 minute i.v. infusions of nicotine, administered one hour apart, in the shell area of the nucleus accumbens. I.v. nicotine infusions (0.16 mg/kg/5min or 0.32 mg/kg/5min) produced increases in extracellular dopamine levels as well as in DOPAC and HVA, the two main dopamine metabolites. The magnitude of the dopamine increases was dose dependent (dose effect, $F_{(1,10)}=5.34$, $p<0.043$). For both doses, the response decreased with the second infusion (infusion effect, $F_{(1,10)}=6.98$, $p<0.024$). Pretreatment, 30 minutes before the first nicotine infusion, with the selective nicotinic receptor antagonist mecamylamine (5mg/kg, i.p.) abolished the dopamine increases. On-going experiments are determining the duration of the observed acute desensitization.

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574.21

ALTERATIONS IN CORTICOSTERONE BINDING SITES FOLLOWING CHRONIC NICOTINE ADMINISTRATION IN RATS. M. Shoaib, V.K. Patchev*, and O.F.X. Almeida. Neuroadaptations Group, Department of Neuroendocrinology, Max-Planck Institute of Psychiatry, Clinical Institute, Kraepelinstrasse 2, 80804 Munich, GERMANY.

The mechanisms underlying the adaptive responses following chronic exposure to nicotine are not known. While increases in nicotinic binding sites are evident after chronic administration of nicotine, there are studies implicating corticosteroids in nicotine tolerance. The present experiment was conducted to examine the influence of repeated nicotine treatment on corticosterone receptor binding. To confirm the nature of the chronic treatments, behavioural tests were conducted on locomotor activity one day prior to sacrifice. Male Sprague Dawley rats were injected chronically twice daily for 7 or 28 days with nicotine (0.6 mg/kg SC) or saline in their home cages. The next day, the rats were challenged with nicotine (0.6 mg/kg SC) or saline and locomotor activity was monitored. The rats were then adrenalectomised and the following day, the hippocampus and hypothalamus were dissected. Cytosol preparations were assayed using ^{3}[H]-corticosterone as the radioligand, and dexamethasone was used to displace binding. In saline-pretreated rats, a nicotine challenge produced robust depression of locomotor activity. Both nicotine pretreated groups of rats displayed tolerance to this depressant response, and over the second half of the 2 hr test, sensitisation was apparent to the activating effects of nicotine. In both nicotine-treated groups, corticosterone binding sites (B_{max}) and binding affinities (K_d) decreased in both the hippocampus and hypothalamus. These results suggest that glucocorticoids may be involved in the adaptive responses to chronic nicotine treatment. Furthermore, changes in binding to corticosterone suggest altered physiological drive of the hypothalamic pituitary adrenal axis, which may account for the tolerance observed to the behavioural effects of nicotine.

575.1

SELECTIVE AREAS ARE DAMAGED IN THE AMYGDALOID COMPLEX AFTER SEIZURES INDUCED BY SYSTEMIC INJECTION OF KAINIC ACID. J.Tuunanen*[1], T.Halonen[1], A.Pitkänen[2]. [1]Dept. of Neurology and [2] A.I.Virtanen Institute, Univ. of Kuopio, P.O.Box 1627, FIN-70211 Kuopio, Finland.

Neuronal damage in the hippocampus is a common finding in patients with temporal lobe epilepsy and in experimental epilepsy. Much less attention has been paid on the damage in the other regions of the temporal lobe such as in the amygdala. We used the systemic injection of kainic acid (9mg/kg) to produce status epilepticus in rats. Two weeks after the induction of seizures the animals were intracardially perfused and the distribution and severity of neuronal damage in different nuclei of the amygdaloid complex was analyzed from sections stained with thionin and Gallyas silver impregnation. The number of GABA- and somatostatin-immunoreactive (SOM-ir) interneurons were analyzed in the lateral and basal nuclei of the amygdaloid complex. The severity of neuronal damage varied between different amygdaloid nuclei and between the nuclear divisions. For example, in the lateral nucleus, the dorsolateral division was well preserved, whereas the ventrolateral and medial divisions were heavily damaged. In the basal nucleus, the magnocellular and intermediate divisions were well preserved compared to the parvicellular division that was severely damaged. In the lateral nucleus, the number of GABA-ir neurons was 49% and the number of SOM-ir neurons 46% of that in controls. In the basal nucleus, the number of GABA-ir neurons was 24% and the number of SOM-ir neurons 34% of that in controls. These data indicate that like in the hippocampus also in the amygdaloid complex there are selective areas which are vulnerable to seizures. In addition we found GABAergic inhibitory interneurons to be severely damaged in the amygdaloid complex in this model of epilepsy.

575.2

SEIZURE FREQUENCY AND SUPRAGRANULAR SPROUTING IN 3 DIFFERENT MODELS OF CHRONIC SPONTANEOUS SEIZURES. R.L.M. Rivero*, C.M. Kohmann, A.P. Simão, P.R. Penna, A. Borin and L.E.A.M. Mello. Depto. de Fisiologia, Escola Paulista de Medicina-UNIFESP, 04023-900 São Paulo, Brazil.

Sprouting of hippocampal dentate gyrus mossy fibers onto the supragranular layer has been described in both human tissue and experimental models of epilepsy. However it is still controversial as to whether it is adaptative (leading to blockade of seizures) or mal-adaptative (contributing to seizure generation or expression). Here we have induced chronic recurrent seizures and mossy fiber sprouting in 3 different models: intrahippocampal injection of kainic acid (KA) (1.25 μg, unilateral); intraperitoneal (i.p.) injection of KA (10 mg/kg); and i.p. injection of pilocarpine (Pilo) (320 mg/kg). Behavioral observations for the assessment of spontaneous recurrent seizures were performed thereafter for a period of 40 weeks, 3-8 h/day, 5 days/week. After 40 weeks the animals were deeply anesthetized and their brains histologically processed according the Neo-Timm method. Frequency of SRS was highest for Pilo-injected animals (2.3 SRS/rat/week), followed by systemically-injected KA (1.5 SRS/rat/week) and intrahippocampal KA (0.4 SRS/rat/week). Similarly, intensity of the supragranular mossy fiber sprouting was highest for the Pilo animals, followed by systemic-KA and intrahippocampal KA. We suggest that the different seizure patterns among these models might be in part related to the amount of the mossy fiber sprouting. The simplest hypothesis for such possible link is that mossy fiber sprouting contributes to seizure generation or expression.

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575.3

MOSSY FIBER SPROUTING FOLLOWING TRANSECTION OF SUBCORTICAL AND CORTICAL AFFERENTS TO THE HIPPOCAMPUS IN RATS TREATED WITH KAINIC ACID. J. Jolkkonen*, E. Jolkkonen and A. Pitkänen. Departments of Neurology and A.I.Virtanen Institute, University of Kuopio, Kuopio, Finland.

Administration of kainic acid induces prolonged seizures that are accompanied with a neuronal loss and mossy fiber sprouting in the dentate gyrus. Mechanisms that regulate initiation of plastic response and guidance of mossy fibers to a new target area (i.e. the supragranular area and the inner molecular layer) are poorly understood. In the present study, we investigated whether the major afferent pathways to the hippocampus could modify a slowly occuring synaptic reorganization induced by an epileptic insult. Thus, the perforant pathway or the fimbria-fornix were bilaterally transected 2 days following generalized seizures induced by systemic administration of kainic acid (9 mg/kg). Two months later, aberrant mossy fiber sprouting was estimated from Timm-stained horizontal sections throughout the hippocampus. The severity of sprouting was scored from 0 (no sprouting) to 7 (most severe sprouting). Somatostatin immunohistochemistry was used to estimate the neuronal loss in the dentate hilus. In sham-operated rats, mossy fiber sprouting to a new target area was increased (212-1420%) following kainic acid treatment compared to that in saline treated rats. The number of somatostatin neurons in the dentate hilus decreased by 50-84% and, in particular, the temporal end of the hippocampus was severely affected. In perforant pathway transected rats treated with kainic acid, Timm staining in the supragranular area and the inner molecular layer was also increased (146-1612%), but the decrease in the number of hilar somatostatin neurons was less significant (34-51%). In fimbria-fornix transected rats treated with kainic acid, aberrant mossy fiber sprouting was significantly lower (227-314%) compared to that in sham-operated rats. In these rats, somatostatin neurons in the dentate hilus were better preserved in the septal end of the hippocampus than in the temporal end. These results suggest that in this particular model of epilepsy, aberrant synaptic reorganization may partly be modulated by mechanism(s) dependend on the subcortical afferents to the hippocampus.

575.4

KAINIC ACID-INDUCED MOSSY FIBER SPROUTING AND SYNAPSE FORMATION IN THE RAT DENTATE GYRUS. H.J. Wenzel*, C.S. Woolley and P.A. Schwartzkroin. Dept. of Neurological Surgery, University of Washington, Seattle, WA 98195-6470.

In an experimental model of temporal lobe epilepsy, kainic acid (KA) administration in rats evokes seizures which are accompanied by hippocampal and hilar neuronal death and synapse degeneration. KA treatment has been shown to result in sprouting of dentate gyrus granule cell axons (mossy fibers) to aberrant sites in the inner molecular layer. It has been hypothesized that these aberrant mossy fibers form synapses with granule cells at sites normally innervated by hilar mossy cells, which have been lost as a result of KA treatment. In order to determine the postsynaptic targets of these aberrant mossy fibers, we have performed light and electron microscopic analysis of electrophysiologically identified granule cells injected with biocytin in hippocampal slices taken from adult rats 2-5 months following injection of KA (12 mg/kg s.c.); Timm staining was performed on sections from the same brains in order to document the overall degree of sprouting for each animal. Examination of Timm stained tissue indicated 1) a progressive growth of mossy fibers in the inner molecular layer and 2) high interanimal variability in the degree of sprouting. Light microscopic examination of biocytin-filled mossy fiber collaterals in KA-treated animals revealed increased collateralization of mossy fibers in the hilus compared to controls as well as mossy fiber growth into the stratum granulosum; sprouted mossy fiber collaterals crossed the s. granulosum and branched in the inner molecular layer. Electron microscopic examination of serially reconstructed biocytin-filled mossy fiber collaterals in the molecular layer revealed mossy fiber boutons that formed asymmetrical synapses with dendritic spines and shafts of granule cells (including autapses on the parent dendrites of a biocytin-filled granule cell). These results support the view that KA-induced synaptic reorganization could provide a basis for recurrent excitation of dentate gyrus granule cells.

575.5

***IN VIVO* INTRACELLULAR RECORDING AND LABELING OF GRANULE CELLS IN THE DENTATE GYRUS OF EPILEPTIC KAINATE-TREATED RATS.** P.S. Buckmaster* and F.E. Dudek. Dept. of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523.

In vivo intracellular recording and labeling techniques were used to examine dentate granule cells in rats that had been treated with kainic acid and, months later, were observed having spontaneous, recurrent seizures. Urethane-anesthetized animals were placed in a stereotaxic apparatus. Granule cells were impaled and synaptic responses to perforant path stimulation were obtained. In some epileptic rats, granule cells displayed a brief EPSP followed by a large-amplitude, high-conductance, biphasic IPSP. In other epileptic animals, a delayed depolarization was superimposed on the IPSP. In an extreme case, a single, low-intensity stimulus evoked repetitive bursts of action potentials with no evidence of synaptic inhibition. Individual granule cells were labeled with biocytin, and their complete axon arbors were reconstructed. Granule cells in epileptic rats consistently showed axon collaterals that projected into the inner molecular layer. Most of the molecular layer axons remained close to the parent cell. However, in some cases, axons crossed the hilus and projected to the molecular layer of the opposite blade of granule cells and spanned over 750 μm, or 15% of the total septotemporal length of the hippocampus. These data suggest that strong synaptic inhibition is present in granule cells of most epileptic kainate-treated rats. Axon projections to the molecular layer and late synaptic depolarizations suggest the existence of a functional recurrent excitatory circuit.

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575.6

CHANGES IN N-ACETYLASPARTATE, N-ACETYLASPARTYLGLUTAMATE AND LACTATE IN KAINATE-INDUCED EPILEPSY. T. Nimura , F.C. Cheng, S.H. Graham, R.A. Swanson and P.R. Weinstein* Dept. of Neurological surgery, Univ. of Calif., San Francisco and VA medical center, SF 94121.

We examine the hypothesis that N-acetylaspartate (NAA), N-acetylaspartylglutamine (NAAG) and lactate have a good correlation with spectroscopic contents in kainate-induced epilepsy. Epilepsy were induced by the injection of kainic acids (10mg/kg) subcutaneously. Animals were sacrificed by microwave at 3, 12, 24, 72 h after injection. Control animal were injected same dose saline. The brains were removed and dissected hippocampus (H), amygdala (A), piriform corte (PC) and frontal cortex (FC). The contents of NAA and NAAG were measured by HPLC and lactate were determined by flurometric assay. NAA decreased at 12 h, 24 h and 72 h significantly ($p<0.05$ or $p<0.01$) in H and A. In PC, NAA at 24 h and 72 h ($p<0.05$ or $p<0.01$) was much lower than control. The concentration of NAA in FC didn't change significantly. NAAG increased at 12 and 24 h ($p<0.05$) in H and at 12 h in A ($p<0.05$). However, NAAG reduced after 12h significantly in PC ($p<0.01$ or $p<0.05$). Lactate were significantly higher than control in every lesion at 12 h ($p<0.00001$) ,but not at 3 h. Lactate decreased at 24 h in everywhere near the control level, but went up at 72 h except FC ($p<0.05$). These data showed that spectroscopic measurements didn't necessarily reflect true concentrations during the acute phase in kainate-induced epilepsy. This observation suggests that this discrepancy may be overestimated changes in NAA and lactate contents by changes in NAA and lactate relaxation time.

575.7

ACTIVATION OF CRF-EXPRESSING NEURONS IN EXTRAHYPOTHALAMIC SITES FOLLOWING SEIZURES. D.T. Piekut* and B. Phipps. Dept. of Neurobiology and Anatomy, University of Rochester, Rochester, NY 14642.

We have previously reported FOS expression in hypothalamic CRF neurons following the elicitation in rats of fully generalized seizures by kainic acid (KA). This finding suggests that seizure induction activates the hypothalamic-pituitary-adrenal axis. Effects of seizures on extrahypothalamic CRF are virtually unknown.

In this study, Sprague-Dawley rats were administered KA (18 mg/kg, ip), and animals typically responded with a fully generalized seizure syndrome. Control animals were injected with saline. Animals were allowed to survive from 5-48 hours following KA. Animals were perfused and brains were processed for CRF immunocytochemistry. Preliminary findings show a significant increase from 5-48 hours following KA injection in CRF immunoreactivity. Immunolabeled cell bodies and fibers are found in extrahypothalamic sites that typically demonstrate little or no CRF in controls. Marked increases in the number of neuronal perikarya are observed in deep layers of piriform cortex along with thick and beaded neuronal processes. Other sites of increased labeling of CRF perikarya and fibers include lateral septum, dorsal endopiriform nucleus, fundus striati, and nucleus of the lateral olfactory tract. Additionally, CRF labeled cell bodies are found atypically in the basolateral and lateral amygdaloid nuclei. In hippocampus, labeling of pyramidal cell bodies is observed, and cells are labeled in the polymorphic layer of dentate gyrus. Many of the aforementioned areas exhibiting increased CRF immunoreactivity are areas of brain exhibiting degenerative changes following kainate elicited seizures. Although it is not entirely clear what function CRF may play at these extrahypothalamic sites, its presence in vulnerable areas suggests a neuroprotective or neurodegenerative role. Supported by NIH grant NS18626

575.8

AGE-RELATED CHANGES IN PARVALBUMIN IMMUNOREACTIVITY FOLLOWING KAINIC ACID SEIZURE. E.F. Sperber*, K. K. Weireter, H. Kubova and M-T. Romero. Depts of Neurology, Albert Einstein College of Medicine, NYC, NY 10461 and Psychology, SUNY Binghamton, NY 13902.

Studies indicate normal cell function is dependent on intracellular calcium homeostasis. An accumulation of calcium in the cell has been linked to neuronal damage and cell death in several pathological states including status epilepticus. It has been shown that proteins as PV (parvalbumin) which sequester calcium may have a protective role.

We have repeatedly demonstrated that immature rat is more resistant to status epilepticus induced hippocampal damage in comparison to adult rat. The purpose of the present study was to determine whether the calcium binding protein, PV, was differentially altered following kainic acid induced status epilepticus with age. Fourteen day old and adult rats were treated with kainic acid. Four days post-seizure, animals were sacrificed and sections prepared for PV immunocytochemistry. Results indicated age-related differences in the distribution and number of PV positive expressed cells. In the adult hippocampus there were only a small number of intact positively PV stained cells and extensive degenerating profiles were observed. In contrast, the kainic acid treated immature hippocampus had extensive positive staining of cells that were similar in distribution, density and morphology to age-matched controls. Results suggest that the presence of parvalbumin may have age-related functional differences.

575.9

ALTERED EXPRESSION OF PLASMA MEMBRANE CALCIUM ATPASE mRNAS WITHIN THE HIPPOCAMPUS FOLLOWING KAINATE-INDUCED SEIZURES. **M.L. Garcia*, K.D. Murray, E.E. Strehler, P.J. Isackson.** ***Department of Molecular Neuroscience, Jacksonville, FL 32224***

Calcium, a ubiquitous second messenger, has been liked to several pathological conditions. The plasma membrane calcium ATPase (PMCA) is one of the primary methods of translocating calcium. As an initial assessment of the possible involvement of PMCAs in kainate (KA)-induced neurodegeneration, we have determined the effect of KA-induced seizures upon PMCA mRNA expression. In situ hybridization was performed on tissue from adult male Sprague-Dawley rats sacrificed at various time points following intraperitoneal injection of KA. KA altered the expression within the hippocampal subfields for mRNAs of all isoforms. PMCA 1, 2, and 3 mRNAs exhibited hybridization below control levels 12 to 48 hours post-injection within CA1 and CA3. Within the dentate gyrus, PMCA 2 mRNA hybridized below control levels 4 hours post-injection and exceeded control levels at 48 and 96 hours post-injection. These observations provide evidence that KA-induced seizures alter the expression of the PMCAs at the mRNA level suggesting a possible role for the PMCAs in the neuronal degeneration within CA1 and CA3 pyramidal cell layers inherent to this paradigm.

575.10

EXPRESSION OF THE INDUCIBLE 70-kDa HEAT SHOCK PROTEIN FOLLOWING KAINIC ACID-INDUCED STATUS EPILEPTICUS IN RAT. J.N. Armstrong*, J-C.L. Plumier, H.A. Robertson, and R.W. Currie. Laboratory of Molecular Neurobiology, Depts. of Pharmacology, Anatomy and Neurobiology, Dalhousie University, Halifax, N.S., Canada, B3H 4H7.

Using both immunohistochemistry and *in situ* hybridization, we examined the rat brain for the expression of the inducible 70-kDa heat shock protein (HSP70) at 1, 3, 6, 12, and 24 hrs after kainic acid-induced status epilepticus (12 mg/kg i.p.). No HSP70 protein was found at 1 or 3 hrs following administration of kainic acid. However, HSP70 immunoreactivity (HSP70-IR) was observed in a small population of neurons in the hilar region of the hippocampus at 6 hrs post-injection. No other immuno-positive cells could be detected in the brain at this time point. Substantial HSP70-IR was observed in neurons of the dentate hilus as well as in hippocampal CA1 and CA3c neurons 12 hrs after kainic acid administration. Other areas including the basolateral, medial and central amygdala, the ventral portion of the perirhinal cortex and layer II of the pyriform cortex demonstrated HSP70-IR at this time. At 24 hrs, HSP70-IR was observed throughout CA3 and the perirhinal cortex. Other small populations of cells were observed to be immuno-positive for HSP70 protein including a small portion of dentate granule cells, the mediodorsal thalamus, the amygdala, layer V pyramidal cells and some deep layer VI cells in the neocortex. HSP70 immunoreactive astrocytes were also observed in the dentate hilus and the molecular layer of CA1 24 hrs after injection of kainic acid. Cresyl violet staining of adjacent sections revealed pyknotic cells in the areas that HSP70 immunoreactive astrocytes were observed. *In situ* hydridization for HSP70 mRNA at 3 and 6 hrs after the injection of kainic acid revealed a similar pattern of expression to that observed at 12 and 24 hrs using immunohistochemistry for HSP70 protein. Work is currently underway to identify the population of dentate hilar neurons that express HSP70 protein at 6 hrs after the administration of kainic acid.
(Supported by MRC and SmithKline Beecham Pharmaceuticals)

575.11

INCREASED FASCIA DENTATA POSTSYNAPTIC AMPA AND GABA RECEPTORS PRECEDE AXON SPROUTING AFTER INTRAHIPPOCAMPAL KAINATE. J.P. Leite*, T.L. Babb, G.W. Mathern, A.C. Chu, C.R. Rosales, K.M. Yeoman, P.A. Kuhlman and J.K. Pretorius. UCLA School of Medicine, Los Angeles, CA, 90024.

Recent studies indicate that receptor changes are associated with axonal sprouting in the epileptic sclerotic fascia dentata (FD), however it is unknown if these receptors increase independently or in response to the ingrowth of the aberrant axons. Intrahippocampal kainic acid (KA) reproduces many behavioral, electroencephalographic and pathological features of hippocampal epilepsy. We correlated the time course of AMPA and GABA postsynaptic receptor changes in KA-treated rats to the axonal reorganizations of mossy fibers (MFs) and GABAergic fibers. KA (0.4μg/0.2μl) was injected unilaterally in the right hippocampus; while the contralateral side received a saline injection. Animals were sacrificed at post-injection days (PIDs) 2, 5, 10, 15, 22 and 30 and their brains processed for Nissl, Neo-Timm (MFs), and immunocytochemistry (ICC) for GAD, GABA-Aß, GluR1 and GluR2/3 receptors. Increased receptor-ICC staining for GluR1 and GluR2/3 occurred as early as PID 2; however more staining occurred at PID 5 and was maintained out to PID 30 ($p<0.05$). Timm positive terminals were detected in the molecular layer only after PID 10. GABA-Aß receptors were also increased at PID 2-5 and maintained above control in all subsequent groups ($P<0.03$), before GAD positive sprouting was observed. Transitory axonal and postsynaptic receptor changes were observed in the contralateral side, but the staining returned to control levels after PID 22. Our results indicate that following KA damage, upregulation of postsynaptic AMPA and GABA receptors precede the ingrowth of MF and GABA neoinnervation of the FD. This supports the hypothesis that early changes in the reinervated target neuron receptors are based on molecular mechanisms that are temporally-independent of those that promote axon sprouting to the FD molecular layer.
(Supported by NS 02802, K08 NS 1603, CNP and.)

575.12

EFFECTS OF MELATONIN ON KAINATE-INDUCED NEUROTOXICITY. M. Lipartiti, D. Franceschini, E. Aromataris, N. Schiavo, M. Gusella and P. Giusti* Deparment of Pharmacology, L.go Meneghetti, 2. 35131 Padova (Italy)

It has been suggested that free radical formation may play a significant role in non-NMDA mediated excitatory aminoacid neurotoxicity (H.S. Chow, J.J. Lynch, K. Rose and D.W. Choi, *Brain Res.* **639**, 102, 1994). To test this hypothesis, the neuroprotective effects of melatonin, a potent endogenous hydroxyl radical scavenger (D.F. Tan, L.D. Chen, B. Poeggeler, L.C. Manchester, and R.J. Reiter, *Endocrine Journal* **1**, 57, 1993), were evaluated against kainate-induced neurotoxicity both *in vitro* and *in vivo*. Seven-day-old primary cultures of rat cerebellar granule neurons were employed in the *in vitro* study. Cells were exposed to different doses of kainate for 30 min in the absence or presence of melatonin (500 μM), and viability was assessed 24 h later by means of MTT technique. Melatonin blocked the neurotoxic effect of kainate. A similar effect was observed after melatonin administration *in vivo* (2.5 mg/kg i.p., 4 times) in kainate (10 mg/kg i.p.) treated rats. Melatonin administration reduced kainate-induced mortality, body weight loss, and also neurodegeneration. Melatonin's protection against the neurotoxic effects of kainate, could be explained, at least in part, by its anti-scavenger profile.

575.13

DIFFERENCES IN ACOUSTICALLY-EVOKED NEURONAL RESPONSE IN EXTERNAL NUCLEUS OF INFERIOR COLLICULUS (IC) OF NORMAL AND GENETICALLY EPILEPSY-PRONE RATS: RESPONSE CHANGES PRODUCED BY MICROINJECTION OF NMDA INTO IC CENTRAL NUCLEUS. D.N.Chakravarty* and C.L.Faingold, Dept. Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62794.

Anatomical studies have demonstrated neuronal connections between IC central nucleus (ICc) and external nuclei (ICx) of IC. In the audiogenic seizure (AGS) network of the genetically epilepsy-prone rat (GEPR), this intracollicular pathway is a potential trajectory for generation of seizure activity, after AGS initiation in ICc. The ICx connection may be a critical pathway, since the ICc unlike the ICx, lacks connections to nuclei with motor outputs, required for behavioral manifestations of AGS. The present study examined acoustically-evoked neuronal responses in ICx of the GEPR and normal animals and examined changes after contralateral infusion of NMDA into ICc (10 nmol/0.5 µl, 2 min). Anesthetized (ketamine-xylazine 85/3mg/kg) GEPR and normal rats were used. Response patterns of ICx neurons to 50 presentations of tone bursts at characteristic frequency (10 dB SPL above threshold) were subject to poststimulus time histogram analysis. NMDA was then infused into the contralateral ICc and changes in ICx response pattern changes were evaluated. Tonic response patterns were observed in 24% of normal ICx cells (5/21), which was significantly lower than the incidence of tonic firing in GEPR ICx cells (71%, 15/21) (Chi square $p<0.01$), pre-NMDA. Mean neuronal firing in the GEPR was also significantly higher than normal ($p<0.001$, ANOVA). This increased incidence of tonic firing and mean firing increase in ICx neurons in the GEPR indicate an increased ICx excitability in the GEPR, further supporting involvement of the ICx in the AGS network. Following NMDA infusion, 60% (9/15) of normal cells displayed excitation (mean increase, 36%), reversibly. NMDA doses, similar to those that result in increased ICx neuronal responses in the present study, also produce AGS susceptibility in normal animals. This suggests a critical mechansism subserving the role of ICx in the AGS network . (Support NIH NINDS NS 21281)

575.14

CHARACTERIZATION OF TRIMETHYLOLPROPANE PHOSPHATE (TMPP), A CHEMICAL MODEL FOR ABSENCE EPILEPSY. G.D. Ritchie*[1], T.K. Narayanan[1], A. Jung[1] and J. Rossi III. Tri-Service Toxicology Consortium: Naval Medical Research Institute Detachment (Toxicology) and Geo-Centers, Inc[1]., Wright-Patterson AFB, OH 45433-7903.

Sub-convulsive doses of TMPP, a convulsant 100 times more potent than PTZ, have been shown both to increase EEG paroxysms in rats for up to 6 hours post-injection, and kindle long-term increases in abnormal EEG activity but not behavioral seizures. TMPP administration in sub-clinical doses reliably disrupted performance of a well-trained operant task requiring attention, although no behavioral seizure activity was observed. The present study characterized the uptake, distribution, disposition, clearance and ligand binding of TMPP. TMPP was shown to cross the blood-brain barrier 6-10 min. post-injection, distribute in highest concentrations in the cerebellum, striatum and hippocampus, and clear from brain tissues 60%+ within 3 hours and 95%+ in 24 hours. A significant increase in blood serum concentration of TMPP was observed 3-6 hours post-administration, perhaps consistent with changes in blood-brain barrier permeability and/or kidney clearance rate. Repeated administration of TMPP for five days did not result in accumulation of the drug in any tissues, including fat, questioning the mechanism of TMPP-induced CNS sensitization. Ligand binding studies indicated possible interference with the GABA/$GABA_A$ inhibitory mechanism, with the interesting additional observation of TMPP-induced enhancement of bicuculline binding.

575.15

NEUROBEHAVIORAL ASSESSMENT OF THE EFFECTS OF SUB-CONVULSIVE DOSES OF A POTENT CONVULSANT (TMPP) IN THREE RAT STRAINS. J. Rossi III*, C. Nocjar[2], M.Y. Vaningan[2], T.J. Moore, C.Y. Ademujohn[1], G.D. Ritchie[1] and J. Panksepp[2]. Tri-Service Toxicology Consortium: Naval Medical Research Institute Detachment (Toxicology) and Geo-Centers, Inc[1]. Wright-Patterson Air Force Base, OH and Dept. of Psychology[2], Bowling Green State University, Bowling Green, OH.

Clinical seizures induced by neuroconvulsants is a topic of extensive study; however, there is little information (with the exception of behavioral kindling) available concerning short- and long-term neurobehavioral consequences of exposure to sub-clinical doses of convulsants. Trimethylolpropane phosphate (TMPP), a neuroconvulsant 100 times more potent than PTZ, was reliably shown in this study to disrupt performance of a well-trained operant task in a time frame consistent with brain uptake of the drug and appearance of EEG paroxysms. In an eight-arm radial maze test, TMPP administration increased latency to find food in the first trial, yet significantly reduced time to find and consume food (compared to controls) during the second trial in which previously unbaited arms contained food. In a plus maze, sub-clinical doses of TMPP were also shown to increase timidity significantly in adult rats, and decrease play solicitation (dorsal pins) in juvenile rats. Repeated administration of low doses of TMPP (0.0125-0.2 mg/kg) sensitized both locomotory response to amphetamine challenge and audiogenic seizure sensitivity in rats tested 30-60 days later. This finding is consistent with the previous research in the laboratory showing long-term kindling of EEG paroxysms but not behavioral seizures. Finally, it was shown that repeated TMPP administration neither increased nor decreased place preference as tested in a shuttlebox.

575.16

SIMULTANEOUS MICRODIALYSIS, EEG AND BEHAVIORAL ASSESSMENT OF EFFECTS OF SUB-CONVULSIVE DOSES OF TRIMETHYLOLPROPANE PHOSPHATE (TMPP) IN SPRAGUE-DAWLEY RATS. J.W. Lindsey, S. Prues[1], C. Alva, G.D. Ritchie[1], J. Rossi III and H.N. Davis*. Tri-Service Toxicology Consortium: Naval Medical Research Institute Detachment (Toxicology), Geo-Centers, Inc[1]. Wright-Patterson Air Force Base, OH and Department of Psychology, Wright State University, Dayton, OH[2]

Administration (i.p.) of TMPP [1.5 ug/kg-0.4 mg/kg] has been shown to reliably induce EEG paroxysms and sub-clinical and clinical seizures in rats in a dose-related manner. The consistent observation of TMPP-induced sub-clinical behavioral seizures *(i.e.,* twitches, *etc.)* that are temporally correlated with EEG paroxysms motivated an effort to temporally correlate behavioral seizure activity, EEG paroxysms and changes in neurotransmitter (and major metabolite) levels in nuclei thought to be associated with seizure induction or recruitment. Rats implanted with both EEG electrodes and microdialysis cannulas were injected with TMPP. A video editing system was used to correlate sub-clinical behavioral seizures with EEG paroxysms and changes in five transmitters/metabolites: dopamine, DOPAC, 5-HIAA, 5-HT, and HVA, as measured by microdialysis. Microdialysis probes in the nucleus accumbens recorded gradual changes in dopamine and serotonin levels, then return to baseline levels, correlating with onset and termination of TMPP-induced behavioral seizures and EEG paroxysms. Integrated behavioral responses to TMPP administration, including activated sniffing, were reliably correlated with occurrence of specific EEG paroxysms.

EPILEPSY: BASIC MECHANISMS IV

576.1

CHANGES IN GENE EXPRESSION IN A HIPPOCAMPAL NEURONAL CULTURE MODEL OF RECURRENT SPONTANEOUS SEIZURES. R. E. Blair*, S. Sombati and R. J. DeLorenzo, Department of Pharmacology and Toxicology and Department of Neurology, Medical College of Virginia, Richmond, VA 23298.

Brief exposure of hippocampal neuronal cultures to a Mg^{++}-free media results in long lasting recurrent spontaneous seizure activity as recorded by whole cell patch-clamp. This "epileptic" phenomenon persists for the life of the hippocampal culture, and is a potential tool for understanding the molecular mechanisms that underlie seizure discharges (Sombati and DeLorenzo, J. Neorophys., in press). Hippocampal neuronal cultures were exposed to a Mg^{++}-free media, and at 1, 3, and 6 days post-exposure cultures were harvested for RNA isolation and analyzed by whole-cell patch-clamp studies. Epileptiform activity and seizure discharges were observed at all time points following Mg^{++}-free exposure. Molecular probes complementary to NMDA receptor subunits (NR2A, NR2B, and NR2C), AMPA/KA receptor subunit (GluR-A) and $GABA_A$ receptor subunits ($GABA_A$-1-α and $GABA_A$-2-α) were used to study mRNA expression. Furthermore, molecular probes complementary to γ -actin, c-fos, and ligatin, a membrane bound protein, were also used to measure mRNA expression. At 24 hours post-treatment, GluR-A, $GABA_A$-2-α, and ligatin mRNA showed significant decreases of 46.3% , 20.4%, and 17.5%, respectively. γ -actin mRNA levels showed a significant decrease of 36.5% and 31.6% at 0.0 and 24 hours, respectively. At 3 and 6 days post-exposure, mRNA levels for GluR-A and $GABA_A$-2-α remained significantly down. mRNA levels for NR2A, NR2B, NR2C, $GABA_A$-1-α, and c-fos showed no significant changes following the Mg^{++}-free exposure. These findings demonstrate that Mg^{++}-free exposure results in selective long lasting changes in mRNA levels, and suggest that these enduring alterations in genetic expression may underlie the recurrent spontaneous seizure activity observed in this model.

576.2

INDUCTION OF HYPEREXCITABILITY BY KNOCK-DOWN OF GABA-A-α2 RECEPTOR SUBUNIT IN CULTURED HIPPOCAMPAL NEURONS. **S. Sombati*, L.Severt, E. Jakoi and R. DeLorenzo. Dept. of Neurology, Medical College of VA, VCU, Richmond, VA 23298.**

Loss of GABA-mediated inhibition has been proposed to underlie seizure initiation and duration [Lothman, et al., Progress in Neurobiology 37(1):1-82, 1991]. Nonetheless, the regulatory role of the GABA receptor and its specific isotype in hyperexcitability remains unclear. We are interested in the possible role of the GABA-A-α2 receptor in neuronal hyperexcitability.

Neonatal hippocampal neurons were cultured at high density and used for experiments after 2 weeks in culture. Antisense strategy was employed by applying antisense oligodeoxynucleotides (1µM, ODN) daily for 3 days to the cultures. Control cultures (n=11 plates) were treated with matching missense oligodeoxynucleotides (n=9 plates). The effects of such treatments on neuronal excitability were examined using whole-cell voltage-clamp intracellular recordings. In normal bath solution (containing 1mM Mg^{2+}) antisense-treated neurons exhibited long-duration epileptiform bursts (1-4sec) and each burst exhibited a large depolarization (30-45 mV) containing 10-20 spikes. "Seizure" activity of 3-10 Hz was often observed in the cultures. Both epileptiform burst and spike frequencies increased dramatically when Mg^{2+} in the bath was lowered to .7 mM. Moreover, paired recordings revealed the activity to be synchronous . These effects were not observed in control neurons unless GABA receptor antagonist bicuculin was included in the bath.

The results indicate that selective down-regulation of GABA-A-α2 receptor subunits by gene knock-down techniques induced neuronal hyperexcitability and seizure discharge. Reduction of the GABA receptor through decreased genetic expression may be a mechanism underlying the generation and maintenance of seizure activity. Supported by NIH grant NS-23350.

576.3

DECREASED EXPRESSION OF THE α5 SUBUNIT OF THE GABA-A RECEPTOR IN A MODEL OF TEMPORAL LOBE EPILEPSY. C.R. Houser*[1], M. Esclapez[1], J.M. Fritschy[2], and H. Mohler[2]. Brain Research Institute[1], UCLA, Los Angeles, CA 90095, and Institute of Pharmacology[2], University of Zurich, Zurich, Switzerland.

The presence of multiple GABA-A receptor subunit-subtypes raises the possibility that specific subtypes could be differentially regulated in disorders such as epilepsy. We have focused our initial studies on the expression of the α5 subunit in the hippocampus of chronic pilocarpine-treated rats with recurrent seizures. In normal animals, immunoreactivity for the α5 subunit is particularly strong in dendritic layers of CA1 and CA2; moderate levels of labeling are present in CA3; and very low levels of labeling are found in the dentate gyrus. However, in rats with recurrent seizures (3-4 months after pilocarpine injections), immunohistochemical labeling for the α5 subunit is substantially decreased in CA1 and is virtually absent in CA2. Expression of α5 mRNA is also significantly decreased in stratum pyramidale of CA1 and CA2. The decreases in receptor and mRNA labeling do not appear to be related to the loss of pyramidal neurons since these neurons are generally preserved in this model. No comparable changes in α1 and α2 subunits were detected. These findings indicate that the α5 subunit of the GABA-A receptor is capable of substantial and prolonged alterations in an in vivo model of chronic epilepsy. Supported by NS29231 and NS21908.

576.4

NMDAR1 mRNA SPLICED VARIANTS AT THE C-TERMINAL BUT NOT AT THE N-TERMINAL DOMAIN ARE ALTERED IN THE KINDLED HIPPOCAMPUS. A. Vezzani[1]*, C. Speciale, F. Della Vedova, M. Tamburin and L. Benatti. [1]Istituto di Ricerche Farmacologiche Mario Negri, Milano, Pharmacia R&D CNS, 20014 Nerviano, Italy.

A lasting enhancement of the NMDA receptor function has been suggested to play a significant role in the chronic hyperexcitability of the hippocampus after kindling epileptogenesis. We have investigated whether hippocampal kindling induces alterations in the expression of NMDAR1(NR1) splice variants, at exon5 which confers different sensitivity to polyamine, and exon21 which encodes a 37-amino acid insert containing the major phosphorilation sites for PKC. One week after stage 5 of kindling in rats, the relative abundance of two alternatively spliced forms at the C-terminal domain, respectively containing (+) or lacking (-) exon21, was reversed compared to controls, in the dorsal hippocampus ipsilateral and contralateral to the electrical stimulation ($exon21^+/exon21^-$ mRNA ratio, mean±SE, controls, 1.3±0.04; kindled ipsi, 0.64±0.05, $p<0.05$; kindled contra, 0.48±0.07, $p<0.01$). Similar bilateral effects were observed in the ventral hippocampus (temporal pole). No changes were found after induction of a single afterdischarge. No significant alterations were induced by kindling in the relative abundance of the spliced variants containing or lacking exon5.

Our findings show selective and lasting changes in the alternative splicing of the NR1 gene after repeated application of an epileptogenic stimulus. This may generate receptors with different functional properties possibly underlying the increased sensitivity to NMDA observed in kindled animals.

576.5

DEVELOPMENT OF RAPID HIPPOCAMPAL KINDLING: RELATION TO GAP-43 AND NEUROTROPHIN GENE EXPRESSION

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Restorative Neurology Unit, Department of Neurology, University Hospital, S-221 85 Lund, Sweden.

In rapid kindling (RK), suprathreshold electrical stimulations every 5 min with low intratrain frequency lead to the development of severe limbic seizures within a few hours. The temporal evolution of the increased seizure susceptibility after RK is not known in detail. We have studied the development of excitability and explored a possible role of neurotrophins and other growth related factors after RK. Rats were given 40 electrical stimulations in the hippocampus according to the RK paradigm (400 μA, 10 Hz, 1 ms square wave pulses for 10 s). To assess excitability, 5 test kindling stimulations were then performed with 5 min interval in separate groups at 6, 12 and 24 h, 5 days and 2 and 4 weeks after RK. We found increased seizure susceptibility already during the first 24 h but that the behavioral seizures in response to test stimulations became progressively more severe up to 4 weeks following RK. Neurotrophin and GAP-43 mRNA expression was analysed using *in situ* hybridization at 2 h up to 4 weeks after RK. BDNF mRNA levels were markedly increased at 2 h in the hippocampus (420% in dentate gyrus, 310% in CA1, 240% in CA3), piriform cortex (400%) and amygdala (200%), compared to non-stimulated controls. TrkB mRNA levels were also elevated at the same timepoint in the dentate gyrus (420%). Both BDNF and trkB mRNAs had returned to basal levels at 12h and no changes were detected at later time-points. Transient increases of GAP-43 mRNA levels were detected at 12 and 24 h after RK (410% in dentate gyrus, 175% in CA1, 160% in CA3, 170% in amygdala and 220% in piriform cortex at 24 h). We hypothesize that the first, acute phase of increased seizure susceptibility after cessation of RK stimulations might be related to modulation of glutamatergic synaptic transmission, possibly caused by alterations in BDNF levels, while the second, long-term phase of kindled state maintenance could be attributed to structural synaptic reorganisation triggered by transient increases in BDNF, TrkB and GAP-43 expression.

576.6

C-FOS MAPPING AFTER HYPERBARIC OXYGEN SEIZURES: COMPARISON WITH PENTYLENETETRAZOLE AND MAXIMAL ELECTROSHOCK SEIZURES. C.R. Auker*, S.T. Ahlers, W. Koller, D. Glerup. Naval Medical Research Institute, Bethesda, MD 20889-5607.

Exposure to hyperbaric oxygen (HBO) causes seizures by an unknown mechanism. To help identify the brain structures involved we mapped c-fos protein distribution in rat brains after HBO seizures and compared it to the distribution seen after seizures induced by either pentylenetetrazole (PTZ) or maximal electroshock (MES). We also characterized the motor activity seen in HBO seizures compared with PTZ and MES seizures. Seizures were elicited in 21 male, Long-Evans, hooded rats by exposure to either HBO (100% O_2, 6 ATA, N=6), PTZ (50 mg/kg ip, N=8) or MES (ear clips, 99 mA, 100 Hz, 1 s, N=7). Rats were anesthetized and perfusion fixed and their brains removed either 1, 2, or 3 h post-seizure. Brain sections were prepared and stained immunohistochemically for c-fos activity using the ABC technique (Oncogene polyclonal primary antibody, Vectastain kits). Behaviorally, HBO motor seizures were characterized primarily by facial/forelimb clonus, "kangaroo" rearing postures, and occasional generalized clonic activity; running/bouncing was never observed. PTZ seizures were similar to HBO seizures, except several rats exhibited running/bouncing activity. MES seizures were tonic extensor followed by limb clonus. HBO seizures induced c-fos staining in more cerebral structures than either PTZ or MES seizures, most prominently in hippocampus, entorhinal cortex, pyriform cortex, amygdala, olfactory bulbs, and other cortex. Staining was prominent at 1 h, peaked at 2 h, and was nearly absent by 3 h post-seizure. PTZ seizures induced c-fos staining most prominently in pyriform and entorhinal cortex; staining peaked at 2 h, but was generally less intense than HBO at 1 h and more intense than HBO at 3 h. Following MES seizures the most prominent staining was at 1 h in hippocampus, entorhinal cortex, and ventromedial hypothalamus; less so in amygdala, olfactory bulbs, and pyriform cortex. Staining was decreased at 2 h and nearly absent by 3 h. Behaviorally, HBO seizures were more similar to PTZ seizures than MES seizures and can be categorized as "limbic". The pattern of c-fos labelling of rat brains following HBO seizures is anatomically and temporally distinct from patterns seen after PTZ or MES seizures. (Support: NMRDC WU 62233N 343P30.004-1519)

576.7

INDUCTION OF NF-κB IN RAT BRAIN FOLLOWING KAINIC ACID INDUCED SEIZURES AND LPS ADMINISTRATION. A.V.Prasad*, W.H.Pilcher and S.A.Joseph. Neurosurgery, Box 670, Univ. of Rochester Med. Ctr., Rochester, NY 14623.

Nuclear factor-κB (NF-κB) is a regulatory transcription factor controlling the expression of numerous genes including cytokines which are associated with cell death. We have previously shown that NF-κB is present in rat brain and its activation by metrazole induced seizures was via a nitric oxide signaling pathway. In these studies, we have extended the knowledge of NF-κB induction mechanisms in rat brain tissue following 1) kainic acid (KA) induced seizures in which correlates were made with the onset of neuronal cell death and 2) lipopolysacharide (LPS), a bacterial endotoxin administered rats in a developmental study. In the first experiment, our results indicate that the induction of NF-κB preceded apparent cell death due to the seizures by several hours. In second experiment involving rat pups (Ages 3 - 38 days post partum), the DNA binding activity of NF-κB was analyzed in brain at 24 h post injections. The data revealed a high level of constitutive NF-κB until day 21 at which time the rats reach their sexual maturity and the density levels are inversely proportional to the increase in age until day 21. After day 21 the DNA binding activity could be induced by LPS. The data on NF-κB subunit proteins, p50 and p65 in both KA and LPS administered rat brains correlate to their corresponding DNA binding activities as determined by the western immunoblotting analysis. The apparent refractory age related mechanisms of transcription factor induction is further investigation.
Supported by NIH grant NS21323.

576.8

PLATELET-ACTIVATING FACTOR IS A MESSENGER IN KAINATE EPILEPTOGENESIS-ENHANCED MITOGEN ACTIVATED PROTEIN KINASE ACTIVITY IN HIPPOCAMPUS. P. Mukherje, M. A. DeCoster, and N. G. Bazan*. LSU Medical Center, Neuroscience Center, New Orleans, LA 70112-2234.

Cellular kinases such as mitogen activated protein (MAP) kinase serve as transducers of both normal and pathological second messenger signalling. In the present study we investigated the activity of MAP kinase in both *in vivo* and *in vitro* models of kainic acid (KA)-induced epileptogenesis and neuronal injury. *In vivo*, treatment of rats by intraperitoneal injection of 10mg/kg body weight of KA caused an induction of MAP kinase activity in hippocampus starting at 4 hours, with maximal activity detected at 3 days, and persisting to 7 days posttreatment. This induction was also found to occur in primary cultures of rat hippocampal neurons, with 100 μM KA treatment for 45 minutes leading to a 3-4 fold increase in activity. Because of the involvement of the potent lipid activator platelet-activating factor (PAF) in both epileptic and ischemic brain injury, we evaluated the role of PAF as a mediator in KA-induced MAP kinase activation. In hippocampal neurons it was found that PAF (100 nM) increased MAP kinase activity 3-fold. This increase in activity was inhibited by 65-70% when the cultures were pretreated 45 minutes with the intracellular PAF antagonist BN 50730 (100 nM). We found that BN 50730 (1 μM) was also effective in inhibiting MAP kinase activity stimulated by 100 μM KA treatement of the hippocampal neurons. This response to KA may include the early signalling events of cell death, since using the release of lactate dehydrogenase as a marker, we found that 100 μM KA treatment resulted in significant cell loss (150% of controls) one day after KA addition. These results indicate that PAF may be a mediator in early signalling events following epileptogenesis and neurotrauma, specifically, at the level of cellular kinase activity (Supported by NS 23002).

576.9

Kainic Acid Induced Seizures Reduces Inositol 1,4,5-Trisphosphate 3-Kinase mRNA Expression in Rat Dentate Gyrus

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It has been reported that convulsion increases phosphatidylinositol 4,5-bisphosphate breakdown and inositol 1,4,5-trisphosphate(IP_3) accumulation. To understand further the role of convulsion in IP_3 metabolism, it seems reasonable to investigate the effect of convulsion on the expression of IP_3-kinase, which mediates turnover of IP_3 to inositol 1,3,4,5-tetrakisphosphate. In the present study we demonstrate the effect of kainic acid(KA) induced seizures on the expression of IP_3 kinase gene using *in situ* hybridization histochemistry. The pattern of expression of IP_3-kinase mRNA was not changed until 2 h after KA injection. However, at 3 h after KA injection, clear reduction in the expression of the mRNA was observed in the dentate gyrus of hippocampus. At 6 h after injection, IP_3-kinase mRNA level in the dentate gyrus was returned to basal level. We could not detect any apparent changes in the expression of IP_3-kinase mRNA in the CA1-CA3 areas of hippocampus, the striatum, and the cerebral cortex at any time point examined. In the temporal pattern, the reduction of the expression in the dentate gyrus was preceded by the induction of c-*fos* after KA induced seizures. These observations suggest that the IP_3-kinase might be one of the genes whose expression can be altered by KA induced seizures.

576.10

EFFECT OF NMDA RECEPTOR ANTAGONIST ON AUDIOGENIC SEIZURE AND SEIZURE INDUCED c-fos mRNA EXPRESSION OF MATURE RATS AFTER NEONATAL EXPOSURE TO PROPYLTHIOURACIL (PTU). N. Ishida, T. Nishimura., H. Kanai, A. Higashiyama, M. Sadamatsu, N. Kato*, Y. Kuroda and B.,S., McEwen.. Dept. of Psychiat. Shiga Univ. Med. Sci, Otsu, 520-21, Japan. , Lab. of Neuroendocrinol., Rockefeller Univ., New York, NY 10021-6399.

Mature PTU rats, which exposed to 0.02% PTU through the mother's milk at 0 to 19 post natal days, show running fits followed by generalized tonic-clonic seizure (GTCS) by acoustic stimulation. Kato et al. reported the specific expression of c-fos mRNA after GTCS in PTU rats (Soc. Neurosci. Abst. 20:406, 1994). It has been reported that NMDA receptors contribute to audiogenic seizure responses in genetically epilepsy prone rats. We used in situ hybridization for c-fos mRNA determination in the brain of PTU rats and the effect of NMDA receptor antagonist on audiogenic seizure and c-fos mRNA expression were examined. NMDA receptor antagonist, dizocilpine at a dose of 0.01 , 0.05 and 0.5 mg/kg were administerd intraperitoneally to 35 PTU rats 30 min prior to acoustic stimulation. Control rats received vehicle before acoustic stimulation. Dizocilpine inhibited audiogenic seizure of PTU rats in a dose-dependent manner. There was a significant difference of c-fos mRNA expression after acoustic stimulation between PTU rats and control rats in septal nucl., bed nucl., dorsomedial hypothalamic nucl., arcuate nucl., central gray, superior colliculus, peripeduncular nucl. and inferior colliculs (IC). In IC , a marked c-fos mRNA expression was obsereved in the external nucl. and not in the central nucli. When high dose of dizocilpine was applied, PTU rats showed neither behavioral GTCS nor expression of c-fos mRNA in external nucli. of IC. These results suggested that NMDA receptors in IC contribute to the audiogenic seizure in PTU rats.

576.11

NEURONAL AND ASTROCYTIC CONNEXIN mRNA EXPRESSION IN TETANUS TOXIN INDUCED EPILEPTOGENICITY. K. Elisevich*, S. Rempel, S. Dodd, K. Ellis, B. Smith, K. Hirst. Epilepsy Research Unit, Henry Ford Hospital, Detroit, MI, 48202.

The mRNA of two connexins, Cx32 in neurons and Cx43 in astrocytes, was studied by Northern blot following injection of tetanus toxin (2-4 pg; 3 MLD_{50}) into the basolateral nucleus of the amygdala. Animals were monitored electrographically at weekly intervals with electrodes implanted in both amygdalas. Cohorts of 3 animals were sacrificed at week 1,2,3,4,6,8 and 10 following injection and the region of the right and left amygdala was collected, frozen and later pooled for Northern blot. Generation of spiking electrographically was manifest in all animals during the first 4 weeks followed by variable attenuation and cessation by 10 weeks. Regression analysis was performed for each connexin with pairing of left (injected)-right values. Over the first 4 weeks, Cx32 mRNA expression showed a significant ($p<0.05$) increasing trend in both left and right amygdalas. Astrocytic Cx43 mRNA showed a significant ($p<0.05$) increasing trend for the left amygdala and a nonsignificant decreasing trend for the right. There was a tendency for both Cx32 and Cx43 mRNA expression to plateau or return to baseline beyond 4 weeks. Injection of tetanus toxin significantly ($p<0.05$) diminished Cx32 and Cx43 mRNA expression when compared to implantation of the electrode alone in the left amygdala. This effect diminished beyond the 4 week point with decline in expression for the noninjected cohort. Regression analysis for Cx32 and Cx43 mRNA expression in the left amygdala in the injected animals however showed no significant trends whatsoever.

These findings provide no convincing evidence of a correlation between Cx32 and Cx43 mRNA expression and the development of epileptogenicity using low titres of tetanus toxin injected into the amygdala. (Supported by the Epilepsy Foundation of America)

576.12

INDUCTION OF AP-1 DNA-BINDING ACTIVITY IN THE RAT HIPPOCUMPUS FOLLOWING AMYGDALA KINDLED SEIZURES. K. Kashihara,*[1] K. Sato,[2] K. Akiyama,[3] T. Ishihara[3] & T. Shomori.[1] Department of [1]Neurology & [3]Neuropsychiatry, Okayama University Medical School, Okayama 700, [2]Clinical Research Institute, National Sanatorium Minamiokayama Hospital, Okayama 701-03, Japan.

In order to ellucidate the role of transcription factor in the plastic changes in the central nervous system associated with the development of kinding, we determined the temporal pattern of AP-1 induction and its component proteins in the rat hippocumpus following kindled seizures. Male Sprague-Dawley rats had once daily electrical stimulations from the left amygdala. After reaching stage 5 (Racine) generalized seizures, rats were decapitated 0, 30min, 1, 2, 4, 8, 24 hr after the final seizure. The hippocampi were dissected, and nuclear protein extracts were prepared. AP-1 DNA-binding activities were determined by using gel-mobility shift assay with ^{32}P-labeled AP-1 probe. In order to determine the component proteins raised against AP-1, antibodies for Fos and Jun family proteins were added to supershift the DNA-protein complex. Autoradiogram of the specific AP-1 bands revealed that AP-1 DNA-binding activity was enhanced, peaked at 2hr after the seizure and returned to the control level within 24 hr. Supershift analysis revealed that Jun B plus JunD and c-Fos were the main component proteins for seizure-induced AP-1. AP-1 may be invoved in the development of long-lasting seizure susceptibility associated with kindling.

576.13

INCREASED EMOTIONALITY AFTER AMYGDALA KINDLING IN RATS MAY BE RELATED TO RECEPTOR REGULATION I. $GABA_A$ AND BENZODIAZEPINES. L.E. Kalynchuk*, J.C. McEachern, K.N. Barr, J.P.J. Pinel, & C.A. Shaw. Depts. of Psychology, Physiology and Ophthalmology, Univ. of British Columbia, Vancouver, B.C., V6T 1Z4.

We have previously reported that amygdala kindling in rats results in increased emotional behavior, which depends on both the site of stimulation (Pinel et al., this meeting) and the number of kindling stimulations (Kalynchuk et al., 1995). However, the physiological correlates of this emotionality have not been identified. In this experiment, we investigated the relation between long-term amygdala kindling, emotional behavior, and $GABA_A$ and BZ receptor plasticity. Bipolar electrodes were implanted in the basolateral amygdala of 15 male, Long-Evans rats. The rats then received either 99 convulsive (kindled, n=10) or sham (control, n=5) stimulations. One day after its last stimulation, each rat was first tested on an elevated-plus maze, then placed in an open field, and finally scored for its resistance to capture from the open field. The next day the rats were killed and their brains immediately frozen. $GABA_A$ and BZ receptors were detected by ^{3}H-SR 95531 and ^{3}H-flunitrazepam binding respectively. These ligands were then visualized by autoradiography and the differences in binding in several brain regions were quantified. The kindled rats engaged in more escape behavior from the elevated-plus maze and were more resistant to capture from the open field than the control rats were. They also had more $GABA_A$ and BZ ligand binding in the dentate gyrus and less ligand binding in the cortex compared to the control rats. These results suggest that long-term amygdala kindling results in a site-specific regulation of inhibitory receptors. This pattern of receptor regulation may contribute to the increased emotionality in these rats and may be important in understanding the behavioral disturbances often experienced by temporal lobe epileptics. (supported by NSERC grants to J.P.J.P. & C.A.S. and by M.R.C. scholarships to L.E.K. & J.C.M.)

576.14

INCREASED EMOTIONALITY AFTER AMYGDALA KINDLING IN RATS MAY BE RELATED TO RECEPTOR REGULATION II. AMPA, KAINATE, AND ACETYLCHOLINE. J.C. McEachern, L.E. Kalynchuk, H.C. Fibiger*, J.P.J. Pinel, & C.A. Shaw . Depts. of Psychology, Physiology, and Neuroscience, U.B.C., Vancouver, B.C., V6T 1Z4.

We have previously reported that long-term amygdala kindling in rats results in increased emotional behavior. However, the potential contribution of excitatory receptor regulation to the development of this emotionality has not been studied. In this experiment, we investigated the relation between long-term amygdala kindling, emotional behavior, and AMPA, KA, and ACH receptor plasticity. Bipolar electrodes were implanted in the basolateral amygdala of 15 male, Long-Evans rats. The rats then received either 99 convulsive (kindled, n=10) or sham (control, n=5) stimulations. One day after its last stimulation, each rat was first tested on an elevated-plus maze, then placed in an open field and finally scored for its resistance to capture from the open field. The next day the rats were killed and their brains immediately frozen. The AMPA, KA, and ACH receptors were detected by ^{3}H-CNQX, ^{3}H-KA, and ^{3}H-QNB binding respectively. They were then visualized by autoradiography and the resulting film quantified using a densitometer. The kindled rats engaged in more escape behavior from the elevated-plus maze and were more resistant to capture from the open field than the control rats were. They also had less CNQX binding in hippocampal CA1, dentate gyrus, and amygdala, and less KA binding in hippocampal CA3 compared to the control rats. QNB binding was lower in hippocampal CA1 and higher in the dentate gyrus and cortex relative to controls. These findings, combined with our report of increased inhibitory ligand binding (Kalynchuk et al., this meeting), suggest that a reciprocal regulation of inhibitory and excitatory receptors occurs after long-term amygdala kindling. This pattern of receptor regulation may be related to the permanent kindled state and to the increased emotionality and defensiveness in these rats.

576.15

ANTICONVULSANT ACTION OF THE STRYCHNINE-INSENSITIVE GLYCINE SITE IN THE NUCLEUS RETICULARIS PONTIS ORALIS. S.L. Peterson*. Department of Medical Pharmacology and Toxicology, Texas A&M University Health Science Center, College Station, Texas 77843.

Previous studies in this laboratory have demonstrated that local microinfusion of the strychnine-insensitive glycine site partial agonist D-cycloserine into the n. reticularis pontis oralis (RPO) inhibits the tonic hindlimb extension (THE) component of maximal electroshock (MES). The purpose of the present study was to characterize further the role of the strychnine-insensitive glycine site in the RPO in inhibiting THE.

Bilateral RPO microinfusion of 100 nmol D-cycloserine inhibited THE in all rats tested. 100 nmol L-cycloserine had no effect on THE. RPO microinfusion of the agonists glycine and D-serine (1-100 nmol) had no effect on THE. RPO microinfusion of the antagonist 5,7-dichlorokynurenic acid (1-20 nmol) significantly reduced the incidence of THE. Neither the partial agonists (+)HA-966 and ACPC (50-200 nmol) nor strychnine (1-20 nmol) altered the MES response.

These results support a hypothesis for subtypes of the strychnine-insensitive glycine site and indicate that antagonist activity at that site regulates anticonvulsant mechanisms in the RPO.
(Supported by NIH grant 32626)

576.16

***IN VIVO* ANTICONVULSANT MODULATION OF SEIZURE-INDUCED CHANGES IN HIPPOCAMPAL GABA AND GLUTAMATE LEVELS.**
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We have previously used the maximal electroshock (MES) model to demonstrate generalised seizure-induced functional changes in GABA and glutamate (GLU) levels *in vivo* (Rowley *et al.*, Br. J. Pharmacol. in press, 1995). Here we report the effects of pre-treatment with phenytoin or sodium valproate (Na-VPA), on these changes.

A concentric microdialysis probe was implanted into the ventral hippocampus of male, Lister hooded rats (250-350g), under Equithesin anaesthesia. 24 hr later, basal samples (5 min collections), were collected prior to MES administration via ear-clip electrodes to conscious animals. Stimulus parameters were 200V for 2 sec which induced tonic hind-limb extensions (HLE). Amino acids were analysed by HPLC with EC detection (Rowley *et al.*, J. Neurosci. Meth., 57, 93-99, 1995).

In saline-treated controls (n=6) following MES, there was a significant ($p<0.001$; ANOVA) immediate increase in basal GABA (0.21 ± 0.01 pmol/20µl) and GLU (0.58 ± 0.02 pmol) levels of 112 ± 28% and 30 ± 8% respectively. Subsequently, GABA levels were reduced to 0.11 ± 0.01 pmol for the remainder of the experiment whereas GLU levels were reduced to 0.52 ± 0.02 pmol over the next 15 min period before a transient increase at 20 min post-ictally. Phenytoin (20mg/kg i.p.), 60 min prior to MES, prevented HLE (n=6) and attenuated the immediate increase in GLU levels by 46 ± 9% ($p<0.01$). No effects were observed on GABA release or on the secondary increase in glutamate. Na-VPA (400mg/kg i.p.), also prevented HLE (n=6) and increased basal GABA levels to 0.31 ± 0.01 pmol ($p<0.01$). Following MES, the initial increase in GABA and GLU was unaffected but the secondary changes in both amino acids were prevented.

These results suggest that phenytoin is an effective anticonvulsant in this model by preventing the stimulated release of GLU, whereas Na-VPA is effective by enhancing GABA-mediated inhibition. This work was supported by BBSRC and Knoll Pharmaceuticals.

576.17

THE RELATION OF THE [^{3}H] γ-HYDROXYBUTYRIC ACID BINDING SITE TO THE GABA$_B$ RECEPTOR. O. Carter Snead III* Div. Neurol. Childrens Hospital Los Angeles, Dept. Neurol. Univ. Southern California, Sch. Med, Los Angeles, CA 90027

γ-Hydroxybutyric acid (GHB) is a naturally occurring compound which has the ability to induce generalized absence seizures when given to animals. GHB has been hypothesized to induce this effect via the postsynaptic GABA$_B$ receptor [Bernasconi et al, J Neur. Trans. 1992;35(Suppl):155]. We sought to test this hypothesis by examining the affinity of GABA$_B$ agonists and antagonists for the [^{3}H]GHB binding site, the affinity of GHB and a GHB antagonist for the [^{3}H]GABA$_B$ binding site, and the effect of guanyl nucleotides and pertussis toxin on both, using autoradiographic binding assays.

GHB and its antagonist, NCS 382, did not compete for [^{3}H]GABA$_B$ binding; nor did (-) baclofen or the [^{3}H]GABA$_B$ antagonists, CGP 35348 or SCH 50911, compete for [^{3}H]GHB binding; however, the [^{3}H]GABA$_B$ antagonists phaclofen and 2-OH saclofen did show an affinity for [^{3}H]GHB binding in frontal cortex with an IC_{50} of 300 mM and 400 mM respectively.

Guanyl nucleotides and pertussis toxin depressed [^{3}H]GABA$_B$ binding throughout the brain, attenuated [^{3}H]GHB binding only in frontal cortex and thalamus, those regions involved in GHB-induced absence seizures, and reversed the affinity of phaclofen and 2-OH saclofen for [^{3}H]GHB binding.

These data raise the possibility that the [^{3}H]GHB binding site may be an important part of the presynaptic GABA$_B$ receptor.

BETA-AMYLOID: NEUROPATHOLOGY I

577.1

EXPRESSION OF AMYLOID PRECURSOR PROTEIN IN SUBSTANTIA NIGRA AFTER A UNILATERAL 6-HYDROXYDOPAMINE LESION IN RATS. M. Eriksdotter Jönhagen*, R. Cowburn, L. Holmberg, B. Wiehager, E. Sundström. Dept. Clinical Neuroscience and Family Medicine, Division of Geriatric Medicine, Huddinge Hospital, 141 86 Huddinge, Sweden.

The β-amyloid peptide forms part of four precursor polypeptides constituting the amyloid precursor protein (APP). APP may play a role in Alzheimer's disease and other neurodegenerative processes. Changes in APP expression have been reported following a number of experimental lesioning paradigms. However, the role of APP in degenerative and regenerative processes remains unclear. To further investigate the role of APP we have used the morphologically and pharmacologically well characterized unilateral 6-hydroxydopamine lesion model to study APP expression in a defined subset of neurons within the substantia nigra (SN). APP-immunoreactivity was determined using the monoclonal antibody 22C11. This antibody recognized the same full length APP isoforms in control and lesioned animals as determined by Western blotting. Two days after the lesion, an increased APP-immunoreactivity was seen in a small number of neurons in the medial, ventral part of the ipsilateral SN while many more neurons in this area were swollen and showed increased tyrosine hydroxylase (TH)-immunoreactivity. After 13 days there was no difference in APP expression between lesioned and non-lesioned sides but with time a decreasing number of TH-immunoreactive neurons was found throughout the SN on the lesioned side. After a 6-hydroxydopamine lesion, it appears that changes in APP-immunoreactivity occur in a small subpopulation of SN neurons. These changes are transient, as compared to the permanent changes in TH-immunoreactivity.

577.2

SOLUBLE Aß OLIGOMERS IN NORMAL AND ALZHEIMER DISEASE BRAINS. Y.-M. Kuo,[1] M. R. Emmerling[2]*, C. Vigo-Pelfrey[3], T. C. Kasunic[1], J. B. Kirkpatrick[4], M. J. Ball[5] & A. E. Roher[1] [1]Department of Anatomy and Cell Biology, Wayne State University School of Medicine, Detroit, MI 48201; [2]Parke Davis, Ann Arbor, MI 48106; [3]Athena Neurosciences, Inc., South San Francisco, CA 94080; [4]Department of Pathology, Baylor College of Medicine, Houston, TX 77030; [5]Departments of Pathology and Neurology, Oregon Health Sciences University, Portland, OR 97201.

Water soluble oligomers of Aß N-40 and N-42 present in normal and Alzheimer disease (AD) cerebral cortex were quantified by a sensitive sandwich ELISA. The soluble peptides were isolated from brain homogenates by ultracentrifugation (220,000 and 435,000 g) and by graded membrane filtration (<10, 10-30, 30-100, >100 kDa). Our results indicated a continuous distribution of soluble monomers and oligomers of Aß. AD brains contained 6 times more soluble Aß oligomers than control brains. Most of the soluble Aß peptides in AD brains were of the N-42 type, which on average amounted to 12 times the values observed in control brains. The amount of insoluble Aß in the cerebral cortex of AD patients was about 100 times more than that found in control brains. Interestingly, the quantity of Aß N-42 measured in AD cortex was about 50 times greater than that present in the CSF of AD patients, using the same ELISA technique. This disparity may result from the integrity of the physical barriers separating the two extracellular spaces. Our data implicates the increased pool of soluble oligomeric Aß in the pathogenesis of AD, since this fraction is toxic to some cells in culture. In addition, soluble Aß has been found capable of generating cation channels in membranes *in vitro*, thus, providing a potential mechanism for cellular injury in AD.

577.3

WITHDRAWN

577.4

IN VIVO MODIFICATIONS OF β-AMYLOID PEPTIDE PROVIDING PROTECTION FROM MAJOR AMINOPEPTIDASES

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Deposition of β-amyloid (Aβ) generating senile plaques in brain is critically involved in the development of Alzheimer's disease, making it essential to clarify the mechanism by which the Aβ peptides form aggregates. One structural factor of Aβ associated with depositability and thus with pathogenicity is a variation in the carboxyl terminal length, i.e. Aβ40 versus Aβ42, whereas information on the amino terminal heterogeneity is yet limited. To assess the possible pathological role of amino terminal modifications in plaque formation, we quantitated Aβ species possessing normal L-aspartate, isoaspartate, D-aspartate, or pyroglutamate at the amino terminus, using anti-Aβ antibodies that distinguish one from another. The majority of Aβ extracted from brains with various degrees of senile plaque deposition possessed nonstandard amino-termini, the ratios of which to the standard L-aspartate form correlated with the ratio of Aβ42 to Aβ40, indicating that Aβ peptides with modified amino termini were initially deposited. Because these modifications render Aβ resistant to major aminopeptidases, our observation suggests that proteolysis of Aβ from its amino terminus is a rate-limiting step in normal Aβ catabolism.

577.5

CLEARANCE OF AMYLOID BETA-PEPTIDE FROM CEREBROSPINAL FLUID AND BRAIN.

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Soluble forms of amyloid beta-peptide (sAβ) are normal metabolites of amyloid precursor protein (APP) and are found at very low concentrations in normal cerebrospinal fluid (CSF) and plasma and in extracts of Alzheimers disease (AD) brain but are not detectable in control, amyloid-free brain. Insoluble forms of Aβ are the major peptide fragments of senile plaques and cerebrovascular amyloid deposits in AD. The distribution of the predominant sAβ in CSF, $sA\beta_{1-40}$, was studied in the CSF-brain-blood system: 1) *in vivo* by stereotaxically infusing 1.0 µl of CSF containing ^{125}I-$sA\beta_{1-40}$ at physiological concentration into one lateral ventricle of the rat for 60 sec and 2) *in vitro* by introducing ^{125}I-$sA\beta_{1-40}$ on either side of a coculture model of the blood-brain barrier (BBB) consisting of a monolayer of bovine brain capillary endothelial cells grown on a collagen-coated filter in the presence of glial cells. ^{14}C-PEG 4000 was used as an inert compound of similar molecular weight as $sA\beta_{1-40}$. The autoradiographic and gel electrophoretic data from the *in vivo* study indicated that $sA\beta_{1-40}$: 1) was more rapidly cleared from CSF and brain than PEG; 2) penetrated less into brain tissue than PEG; and 3) was significantly bound to larger CSF proteins. The flux of $sA\beta_{1-40}$ in both directions across the *in vitro* BBB model was about 40% larger than that of PEG and was not altered when $sA\beta_{1-40}$ concentration was raised from 30 nM to 1.8 µM. *In vitro* the larger flux across the BBB of $sA\beta_{1-40}$ *vis-a-vis* PEG may be related to its greater lipid solubility and transcellular diffusion and not to a specific transporter for $sA\beta_{1-40}$, whereas *in vivo* the rapid clearance of $sA\beta_{1-40}$ from CSF and brain may involve some BBB transporter for CSF protein-$sA\beta_{1-40}$ complexes.

577.6

IMMUNOLOCALIZATION OF RECEPTORS FOR THE GLOBULAR AND COLLAGEN REGION OF THE COMPLEMENT COMPONENT C1q IN ALZHEIMER DISEASE BRAIN.

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Activated complement (C) components are selectively associated with senile plaques in Alzheimer disease (AD) brain. The role of C components in the AD plaques is little understood and might be pertinent to the recruitment and activation of inflammatory cells. C1q, a subcomponent of C1, is found in complex with amyloid ß-peptides (Aß) of the AD plaques and there is evidence that Aß might control the activation state of C1. In this study we explored the presence of C1q receptors (C1qR) in temporal cortex specimens of AD (n=10) and non-demented age matched controls (n=4). We used polyclonal antisera specific for both the 60 kDa low affinity C1qR for the collagen region of C1q (cC1qR), and for the 33 kDa high affinity receptor for the globular heads of C1q (gC1qR) (Ghebrehiwet et al, 1994). We found that in AD brains both the cC1qR and the gC1qR immunolocalized with neuritic Aß plaques, which are associated with activated microglia and dystrophic neurites. No C1qR's immunoreactivity was found in Aß plaques of the diffuse type in brains of non-demented controls. Intense immunostaining of both cC1qR and gC1qR was found in microglia of the white matter in both AD and non-demented control brains. The cellular immunolocalization of C1qR (c and g) in AD neuritic plaques is unresolved. These observations support a possible role for C1q in C mediated inflammatory responses in AD. Ongoing in situ hybridization studies will clarify the cell type expression of C1qR's in amyloid plaques at the mRNA level. Supported by the Nathan W. & Margaret T. Shock Aging Research Foundation to GMP.

577.7

CARBOXYL- AND AMINO-TERMINAL PROPERTIES OF DEPOSITED Aβ IN ALZHEIMER AND DOWN'S SYNDROME BRAINS.

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To investigate the tissue distribution and chronological relationships in the deposition of Aβ molecules with different carboxyl(C)- and amino(N)-termini, we examined by immunocytochemistry different morphological types of Aβ deposits in Alzheimer's disease (AD) brains, and Down's syndrome (DS) brains over a wide range of ages. Consecutive sections from formalin-fixed paraffin-embedded frontal and cerebellar cortices of AD and DS brains were immunostained with the following Aβ antibodies: BC05(Aβ42(43)-specific), BA27 (Aβ40), Ab9204(Aβ L-Asp1), BAN50, BAN52(Aβ 1-16, L-Asp1,7>isoAsp), anti-Aβ3pE (3pyroGlu), anti-Aβ11pE (11pyroGlu), anti-p3N(17Leu). BC05 stained virtually all senile plaques (SP) of both diffuse (DP) and mature type. BA27 stained only a subset of SP, especially mature ones, and cerebral amyloid angiopathy (CAA). Amino-terminal Aβ antibodies, especially Ab9204, preferentially immunolabeled mature SP and CAA in a similar pattern to BA27, suggesting that Aβ1-40 is present in these Aβ deposits. Aβ3pE and 11pE were more widely distributed than Aβ N1- and Aβ40-immunoreactivities in mature SP, especially in core portions, as well as in a proportion of DP. Anti-p3N preferentially stained mature SP and CAA but few DP. Early DP in young DS brains, as well as cerebellar DP, were BC05-positive, but only occasionally positive for Aβ N1, 3pE, 11pE and p3N, and not reactive with BA27. Mature SP in older DS brains acquired Aβ N1, 3pE, 11pE, p3N and BA27 immunoreactivities. These results suggest that Aβ species ending at the 42nd(43rd) residue, with N-terminus other than L-Asp1, initially accumulate in AD and DS brains; deposition of Aβ species with truncated N-termini and Aβ1-40 occurs at later stages of plaque evolution.

577.8

THE AMYLOID BURDEN OF THE CEREBRAL CORTEX IN NON-DEMENTED OLD AGE.

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The Aß amyloid burden in the cerebral cortex of patients with Alzheimer's disease and age-matched controls has usually been assessed by counting the number of neuropil plaques. Since plaques have widely different sizes, this approach may be misleading. We used computerized densitometry to calculate the proportional cortical area covered by immunocytochemically detected Aß in the cerebral cortex of non-demented elderly specimens. Cortical areas and laminae were delineated in matching sections stained with cresyl violet.

Major inter-individual differences were noted. All cortical areas in all specimens contained Aß deposits with microscopic features of neuropil plaques. In general, the amyloid burden (proportional area covered by Aß) was lowest in primary motor cortex and in somatosensory koniocortex (<5%). The results were more variable in visual and auditory koniocortex where the amyloid burden ranged from <1% to 12%. In some specimens, the amyloid burden of paralimbic and association cortices (areas 24,19,21,22) reached levels that were regionally as high as 15-25%. Most cortical areas had distinct laminar patterns of Aß deposits. In general, layers I-IV contained a greater amyloid burden than the deeper cortical layers.

These results indicate that Aß deposits in non-demented elderly individuals may be very substantial. The regional and laminar variations in the distribution of these deposits may shed light on the nature of age-related changes of neural function and the differential regional vulnerabilities to Alzheimer's disease.

577.9

SELECTIVE ALTERATIONS OF AMYLOID PRECURSOR PROTEIN ISOFORM LEVELS IN HUMAN HEAD INJURY. K. Horsburgh*, M. Roberts, J.A.R. Nicoll,^ D.I. Graham,^ Wellcome Surgical Institute and Department of Neuropathology^, University of Glasgow, Glasgow, G61, U.K.

In experimental animal models, expression of amyloid precursor protein (APP) can be altered in response to various forms of injury. There is minimal information regarding APP in human brain following trauma despite the detection of β-amyloid depostion in at least 30% of head-injury cases. The present study sought to determine whether levels of APP are altered in human cortex following head injury.

Brain tissue from nine head-injury cases and eight cases without neurological disease were obtained post-mortem. APP levels were examined in the left and right superior temporal cortex using western iummunoblot analysis with a monoclonal antibody to APP (clone 2C11). APP isoforms were visualised at 135, 130 and 120kDa and the relative levels of each APP was measured using an image analysis system. In the head injury group, levels of the 135kDa APP isoform were significantly reduced from control levels by at least 25% ($P<0.005$) in the cortex of both cerebral hemispheres. Similarly cortical levels of 130kDa APP isoform were reduced compared to control levels but these did not reach statistical significance. In contrast, the levels of the 120kDa APP isoform exhibited a trend towards an increase in head injury cortex compared to control levels. There was no relation between the levels of each APP isoform to post-mortem interval and age of the patients in the groups of post-mortem control or head injury brains. In the head injury group, the length of survival following the initial insult was not related to APP levels.

Thus, APP isoform levels are selectively altered in cortex after head injury. These alterations in APP may be pertinent to the pathology observed in post-mortem human head injury.

577.10

ß-AMYLOID (Aß) PEPTIDES IN DIVERSE NEURODEGENERATIVE DISEASES (ND), ALZHEIMER'S DISEASE (AD) AND AGED INDIVIDUALS. J.A. Schneider, M. Gearing, H. Mori, and S. S. Mirra*. Dept. of Pathology and Laboratory Medicine, VA Medical Center & Emory University School of Medicine, Atlanta, GA 30322; Tokyo Institute of Psychiatry, Tokyo, Japan.

In AD, Aß42 is the predominant species in senile plaques, thought to precede Aß40 deposition (Iwatsubo, et al. Neuron 1994;13:45). We sought to determine whether a similar pattern of peptide deposition occurs in neocortical plaques in ND patients and elderly nondemented individuals. Since apolipoprotein E ϵ4 allele dosage is correlated with Aß deposition, we also investigated the influence of ϵ4 genotype on Aß peptide pattern. We examined 54 brains with neocortical Aß deposition: 32 cases of AD, 18 of diverse ND (progressive supranuclear palsy, Pick's disease, corticobasal degeneration, Parkinson's disease, and multisystem degeneration), and those of 4 elderly nondemented individuals. Nine of the 18 ND cases also met neuropathologic criteria for AD. Immunohistochemistry using C-terminal-specific antibodies revealed that, in virtually all cases, Aß42-positive plaques outnumbered Aß40-positive plaques. The ratio of Aß40:Aß42 plaques was 0.37 in AD and 0.40 in ND cases with coexistent AD pathology. In ND cases without coexistent AD, and in elderly nondemented individuals, however, this ratio dropped to 0.25 and 0.26, respectively. Interestingly, in AD cases and ND cases with coexistent AD pathology, the ratio of Aß40:Aß42 was higher in cases with an ϵ4 allele (0.42 vs. 0.27 in cases without an ϵ4 allele). Although a genotype effect was not observed in the 9 ND cases without AD and the 4 elderly individuals, the number of cases is small. Our results suggest that the pattern of Aß40 and Aß42 deposition in ND and elderly individuals is similar to that of AD; Aß40-positive neocortical plaques are more frequent, however, in cases meeting neuropathologic criteria for AD. Furthermore, ApoE genotype may influence the proportion of plaques that are Aß40-positive in AD.

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577.11

CARBOXYL TERMINAL ß-AMYLOID DEPOSITS IN AGED HUMAN, CANINE, AND POLAR BEAR BRAINS. T. L. TEKIRIAN, G. M. COLE±, M. J. RUSSELL‡, F. YANG±, D.R. WEKSTEIN, E. PATEL, D.A. SNOWDON, W.R. MARKESBERY, J.W. GEDDES* Sanders-Brown Center on Aging, University of Kentucky, Lexington, Kentucky 40536; +Geriatrics, Sepulveda VA Hospital, Sepulveda, CA 91343; ‡Anesthesiology, School of Medicine, University of California, Davis, CA 95616

C-terminal ß-amyloid peptide residues Aß40 and Aß42 were immunocytochemically analyzed to compare plaque and cerebrovascular (CVA) deposits of aged animals with those observed in human brain. Aged beagle dogs (ages 14-17) solely acquire diffuse-type plaques and lack neurofibrillary tangles (NFTs). Aged polar bear brains (ages 35-36) contain diffuse plaques as well as PHF-1 positive NFTs. Diffuse plaques predominate in non-demented, elderly, individuals. Alzheimer's disease (AD) cases had both diffuse & mature (cored) neuritic plaques. In all cases, diffuse plaques were positively immunostained with anti-Aß42 antibody. Anti-Aß40 strongly immunolabeled CVA deposits in all species, however, some CVA was preferentially labeled with Aß42. The shift from Aß42 to Aß40 was more rapid in CVA in contrast to the same transition in plaques. Aß40 selectively stained a subset of mature AD plaques; Aß40-positive immunostaining was not evident in any of the non-AD plaques. The aged dog, polar bear, as well as the human, displayed Aß deposition at the outer molecular layer of the dentate gyrus (DGOML). This region may be one of the earliest sites of Aß deposition. Plaque maturation appeared to correlate with subject age, starting as a diffuse cloud (aged dog), progressing to a more aggregated state (polar bear) and diffuse (non-demented human) vs extremely compacted plaques seen within the AD DGOML. These cross-species data indicate that Aß deposition in aged mammals is similar to the earliest stages observed in human brain.

577.12

DIFFERENTIAL PATTERN OF β-AMYLOID A4, AMYLOID PRECURSOR PROTEIN AND APOLIPOPROTEIN E DEPOSITION IN PLAQUES IN ALZHEIMER´S DISEASE. D. R. Thal[1,2], A. Dietrich[1], W. Härtig*[3], R. Schober[1]. [1]Departments of Neuropathology, University of Leipzig, 04103 Leipzig, [2]Department of Neuropathology, University of Frankfurt a. M., 60528 Frankfurt a. M., [3]Department of Neurochemistry, University of Leipzig, 04109 Leipzig, Germany

In Alzheimer´s disease β-amyloid A4 (β-A4), amyloid precursor protein (APP) and apolipoprotein E (Apo E) have been well demonstrated to be components of both senile plaques and blood vessels affected by amyloid angiopathy. Quantitative regional and stage dependent differences of β-A4, APP and/or Apo E positive plaques have been largely neglected so far, although they may be of pathological relevance. Therefore, we investigated 20 cases of Alzheimer´s disease by semiquantitative immunohistochemistry. The cases were staged according to Braak and Braak (Acta Neuropathol. 82: 239-259;1991). The density of β-A4 (DAKO, 6F/3D), amyloid precursor protein APP 695 (Boehringer, 22C11) and Apo E (Boehringer, 2E1) positive plaques was examined in the Ammon´s horn, the entorhinal cortex, and the occipital and temporal isocortex. The results showed the highest frequency of β-A4 positive plaques in the entorhinal cortex and the temporal isocortex through all stages with increasing density in severe stages. In contrast, the frequency of APP and Apo E positive plaques was pronounced in sector CA 1. The occipital cortex showed a differential staining pattern similar to the temporal isocortex in that the number of β-A4 positive plaques was larger than the number of APP and Apo E positive plaques. This difference was seen throughout all stages investigated but was most pronounced in stages 3 and 4. In conclusion, the immunoreactivity of APP in plaques seems to be pronounced in the region CA 1 of the hippocampus. Possible explanations are either pathological inputs from the entorhinal cortex affect by neurofibrillary changes and / or stage dependent factors in plaque formation.

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577.13

AMYLOID PRECURSOR-LIKE PROTEIN 2 IS ASSOCIATED WITH SENILE PLAQUES IN ALZHEIMERS DISEASE. M-T. Webster[1], C.P.L-H. Chen[2], P.T. Francis[1], F.E.Sherriff[2], R.C.A.Pearson[3]*, D.M. Bowen[1]. Institute of Neurology, London[1], Department of Neuropathology, Oxford University[2], Department of Biomedical Science, University of Sheffield[3], U.K.

Several antibodies, including the commercially available 22C11, raised against amyloid precursor protein (APP) epitopes fail to discriminate APP from amyloid precursor-like protein (APLP2). We have raised and characterised a novel monoclonal antibody (3B11) to a peptide sequence in APLP2 not present in APP (Webster *et al*, 1995, Biochemical Journal, in press). With this antibody, APLP2 was detected by Western blotting in rat and human brain, lumbar and ventricular cerebrospinal fluid, as well as lysate and conditioned media from cell lines including PC12, SH-SY-5Y and cortical and cerebellar granule cells. Using immunocytochemistry, strong labelling by 3B11 was found surrounding plaques in AD brain. The pattern of APLP2 labelling therefore resembles that of APP. More detailed studies in normal and AD brain are underway to verify this. In rat brain, preliminary studies of regional and cellular distribution of APLP2 and APP indicate differences, this was particularly apparent in the brainstem. Localisation of APLP2 is an important step towards understanding the relationship of this protein with APP and its relevance in AD pathogenesis.

577.14

REACTIVE ASTROCYTES AND APP-IMMUNOREACTIVE DYSTROPHIC NEURITES ARE INDEPENDENTLY ASSOCIATED WITH AMYLOID PLAQUES. S.L. Weinstein*, C.J. Pike, B.J. Cummings and C.W. Cotman. Institute for Brain Aging and Dementia, *University of California, Irvine, CA 92717 USA*

The fibrillar ß-amyloid protein (Aß) plaques of Alzheimer's disease (AD) are associated with reactive astrocytes and dystrophic neurites. Previously we reported that in hippocampal sections of AD brains, reactive astrocytes appear to colocalize with plaques during the relatively early stages of AD, and that this association may occur prior to and independent of the presence of PHF-1 immunoreactive dystrophic neurites. We also found plaque types associated with reactive astrocytes but not dystrophic neurites, and plaques associated with dystrophic neurites but not reactive astrocytes. To determine the generalizability of these trends, we extended our examination of cellular colocalization with plaques to include earlier markers of dystrophic neurites, additional brain regions, and Down's syndrome (DS) brains. Fifty µm sections of frontal cortex and hippocampus from 4 DS and 10 AD brains, ranging in neuropathology from mild to severe, were triple immunolabeled with antibodies recognizing Aß, reactive astrocytes (GFAP), and dystrophic neurites (PHF-1, APP). In agreement with our previous observations in AD hippocampus, we report the presence of plaques with the following immunoreactivities in DS and other AD brain regions: GFAP(+)/PHF(+), GFAP(-)/PHF(+), GFAP(+)/PHF(-). In addition, we report similar patterns using APP, a marker of earlier neuritic dystrophy than PHF-1. These data reveal that reactive astrocytes can associate with plaques in the absence of early stage dystrophic neurites, suggesting that plaque-associated reactive astrocytosis may occur in response to specific plaque components (e.g., Aß) rather than solely in response to degenerating neurites.

577.15

SOLUBLE AMYLOID BETA IN DOWN'S SYNDROME BRAINS. J.K.Teller, C.Russo, L.M.DeBusk, D.M.A.Mann[&], M.Tabaton[#], and P.Gambetti[*]. Division of Neuropathology, Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106, U.S.A., [&]Department of Pathological Sciences, University of Manchester, Manchester M13 9PT, U.K., and [#]Institute of Anatomy, University of Genova, Genova, Italy.

We report a quantitative assessment of the presence of the soluble form of amyloid beta (Aβ) peptide in Down's syndrome (DS) and age-matched control brains. Soluble, buffer-extractable Aβ (Tabaton et al., BBRC, 200, 1598-1603, 1994) has been detected in DS brains from individuals of ages ranging from 4 days to 61 years, that included cases lacking amyloid plaques. Aβ peptides were detected in all DS cases regardless of age and plaque presence. The amount of free Aβ increased exponentially concurrently with the appearance of amyloid plaques. In control individuals no soluble Aβ could be detected by using our extraction procedure. The results suggest that soluble Aβ is constitutively present at elevated concentration in DS brain many years before any deposits are detectable. A mechanism is likely to keep the level of soluble Aβ sufficiently low to inhibit the formation of deposits before the critical age of plaque formation. Once plaques are formed the amount of extractable Aβ rises exponentially, most probably representing a mixture of free and loosely bound, aggregated peptide. Supported by NIA grants AG0812, AGNS08155, AG08992, the Britton Fund, and grants from NATO and Telethon (E126).

577.16

SOLUBLE AMYLOID BETA IN FETAL BRAINS WITH TRISOMY 21. M. Tabaton, G. Angelini[#], C. Russo[#], D. Zaccheo, F. Dagna[&], P. Scartezzini[&], J.K. Teller[†], P. Gambetti[†]. (SPON: Society of Neuroscientists of Africa) Institute of Anatomy, University of Genova, [#]Advanced Biotechnology Center, and [&]Hospital Galliera, Genova, Italy, and [†]Division of Neuropathology, Case Western Reserve University, Cleveland, OH 44106.

Soluble amyloid beta peptide (sAβ) has been detected in brain tissue from Alzheimer's disease (AD) and Down's syndrome subjects. The same form of sAβ is normally undetectable in the brain from healthy subjects. Therefore, abnormal accumulation of sAβ might be a primary event in amyloidogenesis. We analyzed sAβ in fetal brains from trisomy 21 cases (20-22 week-old), and in control fetal brains with trisomies 13 and 18, and in cases without chromosomal abnormalities. sAβ was immunoprecipitated from cerebral cortex extracts with an anti-Aβ antiserum, electrophoresed, Western-blotted, and detected with anti-Aβ monoclonal antibodies. Two sAβ peptides were detected with 6E10 antibody (anti-Aβ 1-17) in five out of nine trisomy 21 brains, and none in the control cases. The peptides identical to sAβ peptides extracted from AD brains. There was no difference in Aβ precursor protein mRNA between trisomy 21 brains positive or negative for sAβ. The findings indicate that sAβ accumulates very early in brains of individuals with trisomy 21, most likely due to the presence of an additional copy of the Aβ precursor gene. Supported by NIA grants AG0812, AGNS08155, AG08992, the Britton Fund, and grants from NATO and Telethon (E126).

577.17

INDOMETHACIN REVERSES β-AMYLOID INDUCED GLIOSIS. B.A.Tate*, E.Netland and R.E.Majocha. Dept of Psychiatry and Human Behavior, Miriam Hospital and Brown University, Providence, RI 02906.

Oral indomethacin treatment has been shown to improve cognitive test performance in mild to moderately impaired Alzheimer's patients (Rogers et al., 1993), possibly through effects on glia or its anti-inflammatory actions. Neuropathological evaluations of rats receiving chronic intracranial infusion of β-amyloid peptide indicate gliosis and macrophage infiltration results from β-amyloid exposure. Therefore, the effects of indomethacin in this animal model were evaluated. Indomethacin (2.5 mg/ml in 2% DMSO) or vehicle was concurrently administered via intra-ventricular infusion into rats receiving β-amyloid or 5% glucose vehicle. In amyloid-infused animals, immunopositive amyloid deposits were found in cortical and hippocampal tissue adjacent to the ventricles. Double immunostaining procedures demonstrated co-localization of β-amyloid deposits and GFAP positive cells. Indomethacin treatment dramatically reduced GFAP staining, but not amyloid staining. Therefore, centrally administered indomethacin can reduce β-amyloid induced gliosis.

577.18

ANALYSES OF AMYLOID β PROTEIN SPECIES IN AD BRAINS BY ELISA A.Tamaoka*(1), T.Kondo(2), N.Sawamura(1), A.Odaka(3), N.Suzuki(3), N.Sahara(2), K.Ozawa(2), S.Shoji(1), and H.Mori(2). Department of Neurology, University of Tsukuba, Tsukuba, Ibaraki 305(1); Department of Molecular Biology, Tokyo Institute for Psychiatry, Setagaya, Tokyo 156(2); Discovery Research Division, Takeda Chemical Industries, Ltd., Tsukuba, Ibaraki 300-42, Japan(3).

Alzheimer's disease (AD) is characterized by the accumulation of amyloid β protein (Aβ) in senile plaques and amyloid angiopathy. The production of Aβ from its precursor protein (APP) is known to be a physiological reaction from the finding of soluble Aβ in cerebrospinal fluids as well as cell culture media. Since amyloid deposition is believed to be insoluble and highly specific in AD etiology, a pathogenic reaction could occur leading to conversion of Aβ from a soluble form to an insoluble one. To clarify the mechanism involved in such pathogenic conversion of Aβ, we measured the amounts of total Aβ, Aβ1-40 and Aβ1-42/43 in brain cortices of patients with sporadic AD using the ELISA systems, which could differentiate two types of Aβ(Aβ1-40 and Aβ1-42/43), and found that the amounts of insoluble Aβ1-42/43 and Aβ1-40 were linearly related to the total amount of Aβ deposits at their lower and higher concentrations, respectively. We also performed 5 consecutive washes of the insoluble precipitates of AD brains with buffered saline. Both species of Aβ were found in all 5 supernatant fractions and their amounts were gradually decreased. The ratio of Aβ1-42/43 to Aβ1-40 was increased with the number of washes, indicating that Aβ1-40 existed in an exposed manner as compared to Aβ1-42/43. These findings taken together with the hypothesis that Aβ1-42/43 functions as a "seed" that increases the kinetics of amyloid fibril formation, we conclude that Aβ1-42/43 is the predominant species of initial amyloid formation, Aβ1-40 mainly associated with amyloid deposits in their growth phases.

577.19

BAP LEVELS DECREASE IN THE CSF FOLLOWING CHOLINERGIC LESIONING OF THE FIMBRIA FORNIX. D.M. Tummolo, A.B. Brown, J.S. Jacobsen , K. Rhodes, J. Sonnenberg-Reines* Department of Central Nervous System Biological Research, Wyeth-Ayerst Res, A Division of American Home Products Corporation., Pearl River, N.Y. 10965.

Haroutunian has shown that infusions into the nucleus basalis of Meynert with N-methyl-D-aspartate induced cholinergic deficits (Brain Res., 1989 487:200-203) and amyloid precursor protein (APP) expression in the cortex of the rat (Mol Brain Res., 1991 10: 173) and that this induction results in the constant release of APP into the cerebrospinal fluid (CSF). Increased levels of soluble APP were also present in the CSF collected from lesioned aged rats relative to sham operated controls or lesioned younger rats. (Neurosci.Abstract, 1994, # 435.11).

In the current series of experiments we made bilateral fornix lesions in rabbits (N=8) by transecting the fornix bilaterally using a Scouten wire knife. After 24-96 hours of survival, operated animals and unoperated controls were sacrificed by pentobarbital overdose. The CSF was immediately collected and the brains removed, dissected and rapidly frozen on dry ice. In CSF the levels of BAP were measured by ELISA and Western analysis, and PNII by Western analysis. One hemisphere from each animal was cut into 25 µm horizontal sections and processed histochemically for visualization of AChE and immunocytochemically for visualization of APP metabolites. The other hemisphere was used to measured APP, BAP and C-terminal fragment levels by Western analysis and immunoprecipitation. We found, a transient increase in soluble APP levels in the CSF, while BAP levels decrease. When hippocampus or frontal cortex tissue was analyzed by Western analysis or immunoprecipitation techniques the levels of BAP or APP were unaffected by lesioning. Immunocytochemistry of tissue samples reveals similar results.

The development of a cholinergic animal model may provide a convenient means to assess the utility of novel therapeutic strategies designed to slow the rate of the progression of AD.

578.1

APP on the surface of cultured rat cortical neurons.

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We have previously shown that APP is co-localized with markers of adhesion patches on neurites, but is not present on the surface of growth cones. These results are at first sight surprising in view of the reported short half-life of APP in PC12 cells (20-30 min). Here we provide further evidence that the APP in these segments is actually on the neurite surface, and that the half-life of this surface pool is considerably longer than that of the general cellular pool. Segmentally-distributed APP can be visualised on the surface of unfixed neurons immunostained at 4°C in the presence of azide. Enzymatic treatment of unfixed neurons with calpain 1 substantially removed these segments as judged by immunofluorescence. Confocal laser microscopy shows the segments to be hollow. Metabolic labelling followed by surface biotinylation and two-stage precipitation shows that the labelled, biotinylated pool has a half-life of 3.5-5.5 hours, and is susceptible to surface trypsinization. Other labelled but non-biotinylated APP species are not susceptible to such digestion, and presumably represent the intracellular pool. The reason for the surface pool's stability is unclear: it is not C-terminally truncated (*i.e.* does not lack the clathrin recognition sequence), and does not seem to consist predominantly of Kunitz protease inhibitor-containing isoforms of APP.

578.2

THE DEVELOPMENTAL EXPRESSION OF THE BETA-AMYLOID PRECURSOR PROTEIN IN RAT BRAIN- A PRELIMINARY REPORT. C. Nall, D.K.Lahiri, K. Sambamurti#, M. Zaphiriou and J.I. Numberger*. Lab. of Molecular Neurogenetics, Institute of Psychiatric Research, Indiana Univ. Sch. of Med., 791 Union Drive Indianapolis, IN 46202 and #Dept. of Pathology, Univ. of Texas Health Sci. Cent., Houston, TX-77030

Alzheimer's disease (AD) is characterized by depositions of amyloid ß–protein (Aß) in the brain in the form of extracellular plaques and cerebrovascular amyloid. Aß is derived from a family of large ß-amyloid precursor proteins (ßAPP) which are integral membrane glycoproteins. To understand the biological function we examined the protein expression profile during the early stages of rat development. We extended this study to understand the effects of genetic predisposition to alcoholism on the expression and processing of ßAPP. For this study, we compared the gel patterns of ßAPP immunoreactive proteins between alcohol-preferring (P) and non-alcohol-preferring (NP) rats.

Rat brains from 7 different age groups (4d, 7d, 14d, 21d, 36d, 43d and 78d) were divided into the following four sections: cerebral hemispheres, cerebellum, brain stem and olfactory bulb. Extracts from each region were subjected to western blotting with mAb22C11 which detected proteins in the range of 110-140 kDa normally observed for ßAPP and ßAPP-like proteins. The levels of ßAPP in all regions of the brain, except the olfactory bulb, were significantly higher in earlier days (7d) than in the other days studied. The levels in the olfactory bulb were lower than other areas and remained constant throughout the development. No significant differences were observed between the levels of ßAPP expression in extracts from P- vs. NP-rats. Our preliminary results suggest that ßAPP expression is high during early development of rats and may therefore play a significant role during this period. Supported by a N.I.H. grant R01 AG10297 to D.K. Lahiri.

578.3

IMMUNOCHEMICAL STUDY OF AD BRAIN USING MONOCLONAL ANTIBODY AGAINST APP717 SITE N.Nukina*, K.Keiko, I.Kanazawa
Department of Neurology, University of Tokyo, Tokyo,Japan 113

The amyloid core in senile plaques of Alzheimer's disease is composed of beta protein. Recent discovery of familial Alzheimer's diseases which are linked to APP mutations suggests that the beta protein accumulation is a primary pathology. It is important to clarify how the processing of APP in those cases with the mutation is different from the processing in other cases. To determine whether APP717 site accumulates in amyloid plaques, we raised monoclonal antibodies against the synthetic peptides around APP717.

Monoclonal antibody was raised against APP712-719 with mutation(Val/Ile) or without mutation. Antibodies were screened by ELISA with the synthetic peptide used for antigen and synthetic peptide APP715-723. We obtained several monoclonal antibodies which react with both peptide, suggesting that those antibodies react with APP715-719.One of the antibody called APP63-21 was used in this study.

Tris Saline insoluble fraction were examined by ECL western blot kit(Amarsham) using the antibody. The antibody reacts with APP C terminal fragments. Immunoreaction was relatively stronger in AD samples than in normal samples. The result was compatible with the previous study showing increased APP C terminal immunoreactivity in ADs. This antibody also stained senile plaques, suggesting that APP accumulating in senile plaque includes APP717 site.

578.4

AMYLOID β AND AMYLOID ASSOCIATED PROTEINS ARE INVOLVED IN RIMMED VACUOLES IN CHLOROQUINE-INDUCED MYOPATHY. K. Tsuzuki[1], R. Fukatsu[2]*, Y. Takamaru[3], T. Yoshida[2], K. Kobayashi[4], N. Fujii[1], and N. Takahata[2]. [1]Dept. of Microbiology, [2]Dept. of Neuropsychiatry, Sapporo Med. Univ. Sch. of Med., [3]Sapporo Municipal General Hospital, [4]Dept of Pediatrics, Hokkaido Univ. Sch. of Med., Sapporo 060, Japan.

Deposition of amyloid is the pathological characteristic in Alzheimer's disease (AD). Amyloids are known to be composed of amyloid β (Aβ) and a variety of additional proteins, designated as amyloid associated proteins, which are considered to play an important role in Aβ deposition or amyloid formation. To the present, their significance in the pathogenesis of AD remains unclear. Recently, we demonstrated immunohistochemical evidence that Aβ and cathepsin D accumulate in vacuolated muscle fibers in chloroquine-induced myopathy in rats.

In this study, we examine immunopathologically whether or not Aβ, N-, C-terminals of amyloid precursor protein (APP), a number of amyloid associated proteins, apolipoprotein E (apoE), SP-40,40, α_1-antichymotrypsin (α_1-ACT), and ubiquitin immunoreactivities are present in rimmed vacuoles (RVs) of affected muscle in experimental chloroquine myopathy. Denervated soleus muscle from rats 14 days after chloroquine treatment, were studied. By day 14, RVs were most notable. Transverse serial paraffin sections obtained from affected soleus muscle, were stained using the standard streptavidin-biotin peroxidase technique. Immunopathological studies demonstrated that the Aβ, N-, C- terminal of APP, apoE, SP-40,40, α_1-ACT, ubiquitin, and cathepsin D were present in the RVs.

Further studies are needed to understand the significance of amyloid associated proteins in the formation of RVs in this chloroquine myopathy. This in turn could provide a useful model for insight into the mechanism underlying the expression and regulation of amyloid associated proteins, in addition to APP in the amyloidogenesis and pathogenesis of AD.

578.5

HIGH-RESOLUTION IMAGING OF Aß ATTACHMENT TO NERVE CELL SURFACES AND DOWNSTREAM CYTOARCHITECTURAL CONSEQUENCES. W.L. Klein*, Barber, K., Bodovitz, S., Stevens, G., Krafft, G., MacDonald, R., and Mala, R. Dept. Neurobiol. & Physiol., Northwestern University, Evanston, IL

Amyloid ß proteins in their aggregated state trigger deterioration of SH-SY5Y human neuroblastoma cells (Lambert et al, 1994, J. Neurosci. Res. 39:377). Deterioration is differentiation-dependent, as retinoic acid-induced cells die, but their proliferating precursors are unscathed. Cell death correlates with an evoked upregulation of FAK Tyr(P) (Zhang et al, 1994, J. Biol. Chem. 269:25247). FAK is an unusual protein tyrosine kinase coupled by integrins and other cell surface receptors to cytoskeletal regulation. The current study of SH-SY5Y cells was designed to obtain high resolution images of the cytoskeletal-surface membrane-Aß relationship and its response to differentiation. Cells were exposed to Aß 1-42 aggregates and examined by whole mount transmission electron microscopy (WMTEM; Tsui et al, 1983, PNAS 80:5779); because it omits embedding and sectioning, WMTEM concomitantly provides high contrast images of extracellular, cell surface, and intracellular compartments, making it highly suited to the current work. Association of aggregates with the cell surface was clearly evident in both differentiated and undifferentiated cells, but a local degenerative response occurred only in differentiated cells. Aggregates comprised structurally heterogeneous forms, including flocculant and fibrillar subtypes. The subtype associated with cell surfaces was clearly fibrillar. Surfaces of differentiated neurites stretched out upon the fibrils, which appeared to meld with the cell surface, making it difficult to discern where the aggregate stopped and the cell began. At points of contact, surface integrity was weakened, membranous organelles were less abundant, and microfilaments were withdrawn. Experiments are in progress to monitor cytoskeletal responses in real time using confocal microscopy. To simplify sampling, we have developed cationic liposomes as a new means to deliver specific probes to the interiors of living cells.

578.6

INTRACELLULAR LOCALIZATION OF β AMYLOID PRECURSOR PROTEIN IMMUNOREACTIVITY. A. Dranovsky[1], S. Lyubsky[2], D. Goldgaber[3]*. [1]M.S.T.P. Program, Depts. of [3]Psychiatry and [2]Pathology, School of Medicine, State Univ. of New York at Stony Brook, Stony Brook, NY 11794.

β amyloid precursor protein (APP) has been implicated in cytoskeletal abnormalities associated with neuronal degeneration of Alzheimer's Disease. Although APP is also present in cells of nonneuronal origin, its intracellular function has not been elucidated. Intracellular immuno-localization of APP was investigated by confocal immunofluorescence microscopy on human epithelial (Hep-2) cells. A fibrilar, intermediate filament like, distribution of APP was observed with antibodies (Ab) to synthetic peptides corresponding to the C and N termini of APP but not with preadsorbed controls. Dual label laser confocal microscopy demonstrated colocalization of APP immunoreactivity with vimentin immunoreactivity, partial colocalization with keratin, and a slight overlap with tubulin. Differences between APP C and APP N immunoreactivities were observed.

579.1

ENGRAFTMENT OF P19 CELLS OVER EXPRESSING hAPP751 INTO RAT ENTORHINAL CORTEX. S.M. Grant*, M. Szyf, A.C. Cuello, Dept. of Pharmacology & Therapeutics, McGill University, Montreal, Quebec, Canada, H3G 1Y6.

The aim of this work is to investigate the effect of focal over expression of APP751 on an area of cortex known to be affected early in the evolution of Alzheimer's disease-type pathology in patients with Down's syndrome or Alzheimer's disease. The mouse embryonal carcinoma cell line P19, which is capable of terminal differentiation to a CNS phenotype, was transfected with the full cDNA for the human amyloid precursor protein (APP) 751 isoform. Several stable clones were isolated and verified by Southern blotting. Western analysis of cell lysates and conditioned medium confirmed the over expression of APP and production of the amyloid peptide. For grafting, APP-expressing cells or control (neo^{R}vector-only) cells were placed stereotaxically into the right entorhinal cortex of mature Sprague Dawley rats. The rats were maintained under cyclosporin A 10 mg/kg immunosuppression for various survival times. After perfusion with 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.4, the brains were post-fixed and cryoprotected, then processed for immunocytochemistry and Nissl stained to localize the graft and to reveal epitopes of interest. Work is ongoing to analyse integration of the grafted cells into the host cortex and to examine host brain responses and the potential for amyloid deposition. In addition to their use in grafting studies, it is expected that these clones will yield information on the interaction between aberrant APP expression, cell differentiation and viability of different lineages. This work is supported by the Alzheimer Society of Canada and the MRC of Canada.

579.2

CANINE LEPTOMENINGEAL ORGAN CULTURE: A MODEL FOR STUDYING THE PATHOGENESIS OF ß-AMYLOID FORMATION

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Cerebral amyloid angiopathy (CAA) is characterized by the deposition of amyloid ß protein (Aß) in the cortical and leptomeningeal vessel walls. CAA is the major cause of non-hypertensive cerebral hemorrhage in the elderly and is a neuropathological feature of Alzheimer's disease and Down's syndrome. It is likely that common pathogenetic factors promote Aß deposition in the cortical senile plaques of Alzheimer's disease and in the vascular amyloid. Since dogs are one of the few species which develop age-associated Aß deposits in the cerebral vasculature, we attempted to use old dog leptomeninges as a model for ß-amyloid formation. Leptomeninges were dissected from euthanized old dogs and kept in culture up to 8 weeks. Histological evaluation of paraffin sections showed good tissue preservation with a high number of leptomeningeal arterioles which are the vessel type primarily affected by CAA. Viability of the leptomeningeal vessels was demonstrated by staining with fluorescein diacetate and propidium iodide. Immunofluorescence microscopy of leptomeningeal cryosections stained with antibodies against Aß, amyloid precursor protein (APP), apolipoprotein E, smooth muscle actin and fibronectin, demonstrated the presence of all these antigens in the leptomeningeal vessel walls even after 4 weeks of culture. Immunoprecipitation of metabolically (^{35}S-Methionin) labeled APP showed that the cultured leptomeninges continue to produce different isoforms of full-length APP, which may represent a source of Aß needed for the development of cerebrovascular amyloid. Hence, the canine leptomeningeal organ culture may be a suitable model to study Aß deposition and ß-amyloid formation. (Supported by DFG)

579.3

THIAMINE DEFICIENCY LEADS TO NOVEL PLAQUE-LIKE ACCUMULATIONS OF AMYLOID PRECURSOR PROTEIN-LIKE IMMUNOREACTIVITY IN DAMAGED BRAIN REGIONS. N. Y. Calingasan[1]*, S. E. Gandy[2], H. Baker[1], K.-F. R. Sheu[1], J. D. Smith[3], J. L. Breslow[3], B. T. Lamb[4], J. D. Gearhart[4], D. J. Selkoe[5], D. L. Price[4], S. S. Sisodia[4] and G. E. Gibson[1]. [1]Cornell Univ. Med. Coll. at Burke Med. Res. Inst., White Plains, NY 10605; [2]Cornell Univ. Med. Coll., New York, NY 10021; [3]Rockefeller Univ., New York, NY 10021; [4]Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205; [5]Brigham and Women's Hospital, Boston, MA 02115.

Experimental thiamine deficiency (TD) is a classical model of a nutritional deficit associated with a generalized impairment of oxidative metabolism and selective cell loss in the brain. In rats, TD-induced cell degeneration is associated with accumulation of amyloid precursor protein (APP)-like immunoreactivity in swollen, abnormal neurites and perikarya. Prompted by these data and our previous findings of a genetic variation in the development of the symptoms of TD, we extended our studies to mice. C57BL/6, ApoE knockout and APP YAC transgenic mice received thiamine-deficient diet *ad libitum* and daily injections of the thiamine antagonist pyrithiamine. Immunocytochemistry employed antiserum against human APP$^{645-694}$. Eleven days of TD produced pathological lesions in the thalamus, mammillary body and inferior colliculus. Unlike rats, APP-like immunoreactive neurites in all strains of mice were sparsely scattered and did not delimit the thalamic lesions. In addition, abnormal clusters of intensely immunoreactive materials occurred only in areas of cell damage. These clusters appeared as either "rosettes" or clumps with an unstained central core. While these clusters strikingly resembled Alzheimer amyloid plaques morphologically, immunostaining using an antiserum to synthetic Aβ_{1-40} and thioflavine S histochemistry failed to show positive reactivity. These clusters were not observed in thiamine-deficient rats. Our results demonstrate species differences in the response to central nervous system injury. Although the relevance of these clusters to TD-induced neurodegeneration is still unclear, this is the first report that chronic oxidative deficits can lead to this novel pathology.(Supported by Grant MH48325)

579.4

Establishment of APP-targeted mice with a humanized Aß sequence and the Swedish FAD mutations. A.G. Reaume, S.P. Trusko, D.S. Howland*, D.M. Lang and R.W. Scott, Cephalon, Inc., 145 Brandywine Pk, West Chester, PA 19380.

To create an animal model for examining the role of human Aß in the development of AD-related neuropathology we have utilized a gene-targeting approach to humanize the mouse Aß gene sequence and simultaneously introduce the K670N/M671L mutations linked to familial AD (FAD) in a Swedish kindred. This was accomplished by site-specific mutagenesis of exon 16 of the mouse APP gene using a gene replacement strategy in embryonic stem cells. The positive selection marker, PGKneo , used to enrich for homologous recombinants was excised from intron 15 of the APP-targeted allele in ES cells by means of a site-specific recombination system. Mouse lines carrying the APP-targeted locus in which the neo selectable marker was (neo-) and was not (neo+) removed have been established. In mice created by this strategy, normal processing of mouse APP should result in production of human Aß in the absence of mouse Aß. Spatial and temporal patterns of Aß production are expected to be normal. Furthermore, incorporation of the Swedish FAD mutations should enhance Aß production while APP levels remain normal. This will eliminate potential complication of APP overexpression associated with conventional APP transgenic approaches on the development of any AD-related neuropathologies. Expression of humanized APP has been verified in the mutant ES cells and mouse lines using antisera specific for the humanized APP domain. An accompanying abstract (Howland et al.) describes the characterization of APP processing and Aß production in the APP-targeted mice.

579.5

EXPRESSION OF HUMAN APP770 IN RATS DISRUPTS PLACE, BUT NOT CUE OR MOTOR, MEMORY. D.P. Binsack*[1], M. Montoya-Zavala[2], and C.A. Marotta[3] , Depts. of Psychiatry and Human Behavior[1,3] and Neuroscience[3], Brown University, Providence, R.I. 02912; and Alteon, Inc.[2], Ramsey, NJ 07446.

The most common symptom of Alzheimer's Disease (AD) is memory loss, however the relationship between the clinical disorder and the accumulation of extracellular senile plaques containing beta amyloid is unclear. To examine this issue transgenic rats were made and examined behaviorally. A metallothionein promoter linked to the human APP 770 cDNA followed by an SV40 late poly(A) region was used. A pUC19 vector was used as a backbone for this construct. The construct was microinjected into fertilized embryos from a Sprague-Dawley rat. The embryos were placed into a foster mother and allowed to come to term. After birth and weaning, DNA was isolated from a tail biopsy and the presence of the transgene was confirmed via PCR in the F2 generation. Male rats (6-8 m/o), [APP+: n = 10; controls: n = 15], were trained in a multi-component water maze task in which each rat served as its own control. The task simultaneously assessed long-term (LT) and short-term (ST) place memory, cue memory and motor memory. Amyloid rats exhibited severe deficits in LT and ST place memory but not in cue or motor memory. Further, the deficits were not attributable to visual, attentional, or motivational factors. Preliminary neuropathological examinations suggest that widespread accumulation of plaques were not a prerequisite for the observed specific behavioral disruption.

579.6

DOPAMINE RECEPTOR AUTORADIOGRAPHY IN TRANSGENIC MOUSE MODELS OF ALZHEIMER'S DISEASE. M.D. Roberson, C.A. Singer, A. Horita*, R.C. LeBoeuf, K. Fukuchi, D.M. Dorsa and A.S. Unis. Dept. of Psychiatry & Beh. Sci., Box 356560, Univ. of Washington, Seattle, WA 98195-6560.

Disruption of dopaminergic neuronal pathways in Alzheimer's disease (AD) has been proposed and is supported by clinical observations of psychotic symptoms early in the course of the illness. Since the degenerative processes seen in AD are hypothesized to result from the toxic effects of accumulated β-amyloid (Aβ), mitigated or worsened by apolipoprotein E (ApoE) isoforms, we examined dopamine receptors using tissue slice autoradiography in two C57BL/6 transgenic mouse strains in which either Aβ or ApoE expression was modified. In the first strain, Aβ was over-expressed using a construct containing amino- and carboxy- regions of the amyloid precursor protein (APP) linked to the chicken β actin promoter. In the second strain, ApoE expression was knocked out using gene targeting to replace the endogenous gene with a dysfunctional construct. Receptor autoradiography was conducted using ^{125}I-labeled antagonists for D_1-like (SCH 23982) and D_2-like receptors (epidepride) in frozen brain sections from the striatum and hippocampus.

Preliminary analysis of relative optical density of autoradiograms derived from brain sections revealed strain-specific alterations in striatal dopamine receptor antagonist binding. Specifically, ^{125}I-SCH 23982 specific binding was elevated (137% of the control C57BL/6 mouse strain) in the caudate-putamen (CPu) of the Aβ overexpresser strain while no change was observed in the ApoE knockout strain. In contrast, ^{125}I-epidepride specific binding was significantly reduced (to 25% of controls) in the CPu of the ApoE knockout strain with no differences observed in the Aβ overexpresser strain. Experiments are underway to better characterize these strain-related differences in binding. Nevertheless, these data suggest that altered expression of Aβ or ApoE, two major constituents of the neuritic plaques seen in AD, may contribute to the disruption of dopaminergic neuronal systems.

579.7

Herpes virus-mediated transfer of genetic components implicated in Alzheimers' Disease into the mouse brain.
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Our goal is to study the phenotypic response of neuronal cells of the normal or transgenic murine brain after introduction of genes involved in Alzheimer's disease such as mutant forms of the amyloid precursor protein gene (APP) and the ε4 allele of the apolipoprotein E gene (ApoE4). For these gene transfer experiments we used an amplicon type expression vector, pHermes-L, which carries the β-galactosidase reporter gene under the control of SV40 promoter and can be packaged into Herpes viral particles in the presence of replication deficient helpter virus.

Two mutated forms of the APP have been cloned into pHermes-L amplicon under the CMV promoter, creating: 1) pHLAD37 with the APP695 gene carrying a single mutation (Val_{642} to Ile) and 2) pHLAD40 with the same gene with a double mutation (Lys_{595} to Asn and Met_{596} to Leu). Expression from these constructs has been confirmed by Western blot of transiently transformed Vero derived cells. The ApoE cDNA has been cloned from a human liver cDNA library and the mutation generating the ε4 allele has been introduced.

Injections of the mouse brain with 5μl of the HLAD40 viral stock in the hippocampus region showed no gross tissue damage in the injection site although immunostaining detected increased number of astrocytes and microglia as a result of the injection. Under the conditions used only limited number of cells were infected with the amplicon vector and currently we are working on improving the viral stock to obtain higher efficiency of gene transfer.

579.8

Comparison of Neuritic Dystrophy and Amyloid Plaques in APP Transgenic Mice and Alzheimer's Disease.
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Overexpression of mutated human amyloid precursor protein (hAPP) under the control of platelet derived growth factor promoter (PDAPP minigene) in transgenic (tg) mice results in neurodegenerative changes similar to Alzheimer's disease (AD) (Nature 373:523,1995). We examined the neuritic component of amyloid plaques in this model and compared these abnormalities with other hAPP tg mice (Brain Res 666:151,1994) and AD. Serial reconstructions were performed on images derived using laser confocal and electron microscopy. Amyloid fibrils 9-11 nm in diameter were abundant in the PDAPP tg and were strikingly similar to those observed in AD. In both cases, the amyloid was surrounded by interdigitating membranes and associated with dense core vesicles. Neurons were associated with plaques in both PDAPP tg mice and AD. In PDAPP tg mice, distal neuronal processes contained amyloid fibrils and electron dense material, less evident in AD. Dystrophic neurites were also observed in high expressors NSE-hAPP695 (717 V-->I) tg mice, but were much less abundant than in PDAPP tg mice. Dystrophic neurites formed occasional synapses and contained many dense multilaminar bodies and neurofilaments (10nm); no paired helical filaments have yet been observed. Low expressor hAPP tg mice did not develop dystrophic neurites. In the PDAPP tg mice, synaptic alterations were most prominent in the periplaque region. The above results indicate that β-amyloid can occur within neuritic processes and that such neuritic alterations may contribute to plaque formation in AD. This work was funded in part by Athena Neurosciences, Inc., NIH AG5131, AG10689, AG11385 and the State of California Department of Health Services.

579.9

SEX-DEPENDENT COGNITIVE IMPAIRMENTS IN TRANSGENIC MICE THAT OVEREXPRESS THE CARBOXY-TERMINAL FRAGMENT OF THE AMYLOID PRECURSOR PEPTIDE (APP-C100)
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The cause of the degeneration of neurons in Alzheimer's disease (AD) is not fully understood. We hypothesize that abnormal processing of amyloid precursor peptide (APP) results in the generation of a carboxy-terminal peptide fragment (C-100) which is responsible for the characteristic neurodegeneration and subsequent cognitive impairments in AD patients. The current study examined the effects of overexpression of this C-100 fragment on cognitive function in mice. Three lines of C-100 transgenic mice (C57Bl/6J x SJL/J hybrids) were generated using the dystrophin brain promoter. At one year of age, 44 transgenics and non-transgenic littermates were tested on cued, spatial and reversal tasks in the Morris swim maze. The most consistent finding in the three lines was that transgenic females exhibited a severe impairment on the reversal task as compared to transgenic males and controls; while transgenic males performed either equal to, or better than, controls. In one transgenic line, the transgenic females also exhibited a slight cued, and a more pronounced spatial deficit with respect to transgenic males and controls. Furthermore, there were no significant differences among the groups in locomotor activity or swim speed which could account for the impaired performance of the transgenic females. These results suggest that overexpression of C-100 differentially affects cognitive performance in the two sexes. Interestingly, gender differences in cognitive performance have also been noted in patients with AD.

ALZHEIMER'S DISEASE: ApoE

580.1

APOJ BUT NOT APOE RETARDS APOPTOSIS IN PC12 CELLS.
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This study examines the survival of PC12 cells after serum and growth factor deprivation in the presence of ApoE or ApoJ. PC12 cells were grown for 10 days in the presence of 15% serum (naive) or in a NGF-containing-serum free medium (primed). After extensive rinsing, cells were replated in serum and NGF-free medium, and treated with ApoJ or one of the 3 isoforms of ApoE. Purified human ApoJ (Quidel) was used at concentrations of 0.16, 16 and 160 ug/ml, while recombinant ApoE (Panvera Corp.) was used at concentrations of 16 and 32 ug/ml. At 24 hours of treatment with ApoJ, the number of cells alive was significantly increased (70% vs. 40% in untreated cells). By day 4 all control cells were dead, while 10% of the ApoJ treated cells remained viable. Treatment with ApoE had no effect on cell survival. No significant difference was detected between naive and primed cells.
These results suggest that ApoJ may trigger a protective mechanism in response to deprivation of trophic factors, leading to a retardation of apoptotic cell death.

580.2

APOLIPOPROTEIN E MODULATES THE ACTIVITIES OF SECRETED βAPP IN AN ISOFORM-SPECIFIC MANNER.
S. W. Barger* and M. P. Mattson. Sanders-Brown Center on Aging and Department of Anatomy & Neurobiology, University of Kentucky, Lexington, KY.

Specific alleles of both apolipoprotein E (ApoE) and β-amyloid precursor protein (βAPP) are genetically linked to Alzheimer's disease. Rare mutations in βAPP appear to be sufficient to cause AD, implicating an abnormality in the function of βAPP or one of its proteolytically derived fragments. With respect to ApoE, polymorphisms common in humans are linked in a rank order to the age of onset of AD: ApoE4 < ApoE3 < ApoE2. We have investigated possible molecular interactions between these proteins that would explain their relationship to the disease. Secreted forms of βAPP (sAPP) protect primary neurons from metabolic and excitotoxic insults and modulate their electrophysiological excitability (Furukawa et al., this meeting). Therefore, we reasoned that ApoE might interfere with these activities in a manner consistent with their rank in predisposing for AD. Surprisingly, we found that ApoE actually enhanced the ability of sAPP to acutely depress rest calcium levels and ameliorate excitotoxicity in primary neurons, with the E3 isoform being more potent than E4. A second activity of sAPP which appears to result from a different region of the molecule, the elevation of inositol phosphate generation, was inhibited by ApoE. These effects depended on a direct molecular interaction between ApoE and sAPP as they were dependent on time and concentration of coincubation of the two proteins; furthermore, the proteins were coprecipitated from mixtures. These data suggest that ApoEs bind with isoform-specific affinity to a specific region of sAPP and modulate the bioactivity of individual sAPP domains. Supported by the Alzheimer's Association, NINDS, and French Foundation.

580.3

AMYLOID P COMPONENT AND APOLIPOPROTEIN E IN ALZHEIMER'S DISEASE. T.Duong*, P. J. Acton and E. J. Chaney. Terre Haute Center, Indiana Univ. Sch. of Med., Terre Haute, IN 47809.

Amyloid P component (APC) and apolipoprotein E (Apo E) have been localized previously to the lesions of Alzheimer's disease (AD): the senile plaques (SP) and the neurofibrillary tangles (NFT). In this study, the distribution of APC and Apo E in the lesions of AD have been compared by immunohistochemistry in a set of 10 AD patients staged histopathologically for the degree of disease severity (H. Braak and E. Braak, Acta Neuropathol. 82, 239-259, 1991). The patients were grouped into transentorhinal (I-II), limbic (III-IV) and isocortical (V-VI) stages. Tissue blocks from the hippocampus CA1 region and entorhinal cortex were fixed in phosphate -buffered 4% paraformaldehyde and cut on a cryostat (40 µm section thickness). Adjacent sections were processed free-floating using a monoclonal antibody to APC (Sigma; 1:1000) or a polyclonal antibody to Apo E (Midland Bioproducts; 1:3000). The number of SP and NFT labeled by APC or Apo E were determined and expressed as a density (number/mm^2). The results show that in stages I-IV, the density of APC- and Apo E-immunoreactive SP in both brain regions studied were similar but in later stages (V-VI), the density of Apo E-immunoreactive SP was higher compared to APC. The density of NFT immunoreactive to APC or Apo E were similar at all stages in both brain regions although the density of APC-immunoreactive NFT was slightly higher in the hippocampus CA1 region in stages V-VI. **These results suggest that, in our patients, both APC and Apo E accumulate at the same rate in AD lesions in early stages of the disease; but, in late stages, there may be a greater deposition of Apo E in SP or APC in NFT.** This work was supported by NIH NINDS grant NS31524

580.4

APOLIPOPROTEIN E (APOE) ISOFORMS BIND DIFFERENTIALLY TO TAU AND OTHER CYTOSKELETAL PROTEINS. G.V.W. Johnson*[1], L.M. Fleming[1], W.J. Strittmatter[2] and K.H. Weisgraber[3]. [1]Dept. of Psychiatry, Univ. of Alabama at Birmingham, Birmingham, AL 35294-0017. [2]Dept. of Neurology, Duke Univ. Med. Ctr., Durham, NC 27710 and [3]Gladstone Institute, Univ. of California, San Francisco, CA 94140-9100.

The frequency of the ε4 allele of apoE is significantly increased in late-onset familial and sporadic Alzheimer's disease. In vitro apoE3, but not apoE4, binds to the microtubule-associated proteins (MAPs) tau and MAP-2C, as evidenced by the presence of apoE/MAP complexes on SDS-polyacrylamide gels run under non-reducing conditions. In this study the associations of apoE2, E3 and E4 with several cytoskeletal proteins were examined using both gel shift and overlay assays. The association of the longest isoform of human recombinant tau (T4L) and the shortest isoform (T3), with the different isoforms of apoE were examined under non-reducing conditions on SDS-polyacrylamide gels. As expected, apoE3 formed SDS-resistant complexes with T4L and T3. ApoE2, however, did not form detectable complexes with T4L, although apoE2 did bind to T3. ApoE4 did not bind to either T3 or T4L as determined by the gel shift assay. ApoE3 also formed SDS-stable complexes with bovine tau and neurofilament proteins, but not with actin. The association of apoE3 and apoE4 with T4L, actin or tubulin was further examined in an overlay assay with known amounts of the proteins slot-blotted onto nitrocellulose. The resulting blots were incubated with 5 µg/ml of apoE3 or apoE4, rinsed thoroughly and then probed with an apoE antibody. The results indicated apoE3 and apoE4 bound T4L and tubulin equally well. In contrast, apoE3 bound actin significantly better than apoE4. In addition, apoE3 bound tubulin to a significantly greater extent than T4L. These results indicate that apoE isoforms interact with cytoskeletal proteins with at least two different binding affinities. The stronger interaction which results in the formation of complexes which are SDS-stable only occurs with apoE3, while the other interactions between apoE and cytoskeletal proteins are apparently not isoform specific.

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580.5

THE DISTRIBUTION OF APOLIPOPROTEIN AND APOLIPOPROTEIN RECEPTOR mRNAS IN THE RAT FOREBRAIN. Keith Page* and Bradley Hyman. Neurology Research, Harvard Medical School, Massachusetts General Hospital, Boston 02114, USA.

In order to map the distributions of Alzheimer pathology related apolipoproteins and their receptors in the rat CNS, *in situ* hybridizations were performed. Serial 16µm sections were examined using 45mer oligonucleotide probes to apolipoproteins E,J and A1 mRNAs, low density lipoprotein receptor (LDLr), very low density lipoprotein receptor (VLDLr), low density lipoprotein receptor related protein (LRP) and the LDL receptor-associated protein (RAP) mRNAs. Non-specific hybridizations were assessed using the appropriate sense probes and a 45mer probe of random sequence which produced no hybridization signal. Apolipoprotein E, J and A1 mRNAs were abundant in rat forebrain; ApoE and ApoA1 mRNAs were expressed diffusely across all brain regions studied with particularly high expression in the CA-subfields and dentate gyrus, while ApoJ mRNA was also strongly expressed in neocortex and choroid plexus. Emulsion dipped sections revealed that ApoE and ApoJ mRNA expression was largely restricted to non-neuronal elements while ApoA1 mRNA appeared to be localised to neurons. The distributions of LRP and RAP mRNA were nearly identical, both were located in neurons and densely expressed in the hippocampus, both LRP and RAP mRNA were present in choroid plexus. VLDLr and LDLr mRNA were diffusely expressed, indicating their likely glial localisation. These data show a differential distribution of apolipoproteins and different ApoE receptors in various cell populations in the rat CNS.

580.6

NEUROTOXIC EFFECTS OF APOLIPOPROTEIN E SYNTHETIC PEPTIDES AND PROTECTION BY HEPARIN AND HEPARAN SULFATE. M.Tolar*, M.A.Marques, J.A.K.Harmony# and K.A.Crutcher. Dept. of Neurosurgery and Dept. of Pharmacology and Cell Biophysics#, University of Cincinnati, Cincinnati, OH 45267-0515

Apolipoprotein E (ApoE) is localized to amyloid plaques and neurofibrillary tangles in Alzheimer's disease (AD) brains and its ε_4 isoform is implicated in familial and sporadic AD. However, the specific role that apoE may play in the etiology of AD is unknown. ApoE-related peptides have been found to cause extensive degeneration in vitro when applied to explant cultures of chick sympathetic ganglia (Exp. Neurol. 130: 120-126; 1994). These findings have been extended in the present study of dissociated chick sympathetic neurons, and undifferentiated and nerve growth factor-differentiated PC12 cells, as well as testing agents that might be expected to block toxicity. ApoE can be internalized by the low density lipoprotein (LDL) receptor, or the LDL receptor-related protein, and all toxic apoE peptides include the receptor-binding domain of apoE, as well as the overlapping heparin-binding site.

Dose-response curves of apoE peptides were found to be similar for both sympathetic neurons and PC12 cells. Half-maximal effective concentrations of toxic peptides in the various cell culture systems ranged from 2-4 µM. To determine the site of action and neurotoxic mechanism of the apoE peptides, we undertook blockage studies designed to interfere with their anticipated interaction with cell-surface receptors. The peptides were applied at half-maximal effective concentrations and protective effects of heparin and heparan sulfate (HS) were tested. A 10 min preincubation of any of the apoE peptides with either heparin or HS abolished their neurotoxic effects in a dose-dependent manner in all cell-culture systems studied. Complete abolition was achieved at 2-4 µM. These results suggest that the toxicity of apoE peptides is mediated by the cell interaction with either receptor- or heparin-binding site of the peptides and provide evidence that the heparin-binding site located on apoE peptides is functional. (Supported by a Res. Challenge Grant and NIH grants NS31410 and HL 27333.)

580.7

EFFECTS OF APOE3 AND APOE4 ON THE PHOSPHORYLATION OF TAU PROTEINS D. Kapkov*, Christine Guenzi, John C. Lehmann, Dept. Neurosurgery MS 407, Medical College of Pennsylvania and Hahnemann University, Philadelphia PA 19102-1192.

Both ApoE3 and ApoE4 significantly reduced [32P] incorporation into tau by protein kinases CK1 and CK2, but the effect was more robust for ApoE3. When ApoE3 was added to tau together with protein kinases, it almost completely prevented phosphorylation of tau by CK1 or CK2 added separately. Preincubation of ApoE3 with tau reduced this effect, in contrast to ApoE4, whose effect was generally independent of preincubation with tau. Such an insensitivity of ApoE4 in comparison with ApoE3 to starting conditions suggests the importance of a dimer structure, which is much more stable for ApoE3.

Antibody recognition of AD-specific sites of tau protein failed to correlate as predicted by the level of phosphorylation in the presence and absence of ApoE. Remarkably, when ribosome-free brain extract was used as a source of total protein kinase activity, ApoE3 and ApoE4 did not reduce the level of phosphorylation of tau. But if ApoE4 was preincubated with tau at pH 7.7, the phosphorylation by brain extract protein kinases was significantly increased. These results suggest that ApoE4 and ApoE3 have differential effects on the phosphorylation of tau protein, which may be pertinent to the relatively early development of AD pathology in patients homozygous for ApoE4.

580.8

APO E EXPRESSION IN PLAQUE TYPES IN ALZHEIMER'S DISEASE. W.S.T. Griffin*, J.G. Sheng, and R.E. Mrak. Ark. Child. Hosp. Res. Inst., Univ. Ark. Med. Sci., Dept. Vet. Aff. Med. Ctr., Little Rock, AR 72202.

β-Amyloid-containing plaques are a salient histopathologic feature of Alzheimer's disease. These plaques have been shown to contain apolipoprotein E (Apo E), and a potential pathogenic role for Apo E in neuritic plaque formation has been suggested. This predicts that Apo E should be present in early (diffuse, non-neuritic) amyloid deposits. To examine this possibility, we used immunohistochemical double labelling of temporal lobe tissue sections from 12 Alzheimer patients, age 66-88. Amyloid-immunopositive plaques were classified into four types according to the pattern of amyloid distribution (diffuse *vs* dense core) and the presence or absence of β-amyloid precursor protein-immunoreactive dystrophic neurites. Only 6% of diffuse non-neuritic plaques contained Apo E, and these had very low immunoreactivity. In contrast, 83% of diffuse neuritic plaques and 86% of dense-core neuritic plaques were highly immunoreactive for Apo E. Only 6% of dense core non-neuritic ("burnt out") plaques showed Apo E immunoreactivity. In each case, the area of Apo E immunoreactivity completely overlapped with that of β-amyloid. These results suggest that Apo E deposition occurs concomitant with, or following, but does not precede dystrophic neurite formation in amyloid plaques. These findings, together with the previous demonstration of Apo E immunoreactivity in the non-progressing diffuse amyloid deposits in cerebellum in Alzheimer's disease, suggest that plaque-associated Apo E is not the critical initiating factor in dystrophic neurite formation in amyloid deposits in Alzheimer's disease. This work was supported in part by NIH AG10208, NS 27414, and AG12411.

580.9

DOES THE APOLIPOPROTEIN E ε4 ALLELE ACCELERATE THE FORMATION OF HIPPOCAMPAL NEURITIC PLAQUES? J.M. Olichney, L. Hansen, D. Galasko, T. Saitoh, C.R. Hofstetter, D. Connor*, R. Katzman, L.J. Thal. Dept. of Neurosciences, University of California, San Diego, CA 92093.

Objective: To test if the apolipoprotein E (APOE) ε4 genotype increases the proportion of neuritic plaques (NP) versus diffuse plaques (DP) present in the hippocampus of autopsied Alzheimer's disease (AD) and Lewy Body Variant (LBV) cases. Methods: Thioflavin-S stained sections of the posterior hippocampus (CA1 region of the pyramidal cell layer, including what previously has been called the prosubiculum) were examined for NP and DP counts in 92 cases of AD (n=56) and LBV (n=36). Effects of APOE genotype, age and duration of clinical symptoms upon NP and DP were analyzed. Results: For AD and LBV combined, cases without any ε4 alleles had a mean number of 6 NP per 1.6 mm² (comprising 66% of the total plaques). For cases with one ε4 allele, the mean NP count was 8 (70% of total plaques). For cases with two ε4 alleles, the mean NP count was 10 (84% of total plaques). Differential effects of APOE genotype upon the NP/DP proportion was seen between the AD and LBV groups. Conclusions: The APOE ε4 allele may favor the transformation of diffuse plaques into neuritic plaques.

580.10

EXPRESSION OF HUMAN APOLIPOPROTEIN E4 BLOCKS NEURITE OUTGROWTH FROM MURINE NEUROBLASTOMA CELLS. S. Bellosta, B.P. Nathan, R.W. Mahley,†‡ and R.E. Pitas.*‡ Gladstone Institute of Cardiovascular Disease, Cardiovascular Research Institute, Departments of †Medicine and ‡Pathology, University of California, San Francisco, CA 94141-9100.

We have previously shown that, in the presence of a source of lipid, apolipoprotein (apo) E4, a protein associated with late-onset familial and sporadic Alzheimer's disease (AD), inhibits neurite outgrowth from cultured dorsal root ganglion neurons, whereas apoE3 stimulates outgrowth. In the current studies, murine neuroblastoma cells (Neuro-2a) were stably transfected with human apoE3 or apoE4 cDNA, and the effect on neurite outgrowth was examined. The transfected cells secreted 44 to 89 ng of apoE per mg of cell protein in 48 h. In the absence of a source of lipid, neurite outgrowth was the same in the apoE3- and apoE4-secreting cells. Incubation of the cells with β-migrating very low density lipoproteins (β-VLDL) or with very low density lipoproteins (VLDL) resulted in inhibition of neurite extension from the apoE4-secreting cells and an increase in neurite extension from the apoE3-secreting cells. Similar effects were observed when cells were incubated with lipid emulsions containing only phosphatidylcholine and triolein (1:4 ratio). Incubation of the cells with apoE HDL_C, a specific type of high density lipoprotein that is similar to the HDL in cerebrospinal fluid, resulted in a moderate isoform-specific effect, which was not as dramatic as that observed with β-VLDL and VLDL. Low density lipoproteins did not produce a differential effect on neurite outgrowth in the apoE3- and apoE4-secreting cells. Whether the lipid particle serves only as a vehicle to deliver apoE to the cells or if specific lipids are required for the differential effects remains to be determined. These data suggest that apoE4 in the brain together with lipoproteins, which are known to occur in the central nervous system, may inhibit neurite extension *in vivo*, thus contributing to the progression of AD.

580.11

INCREASED EXPRESSION OF APOLIPOPROTEIN D IN OLIGODENDROGLIA IN ALZHEIMER BRAIN

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Apolipoprotein D (apo D), a 27-32 kDa glycoprotein component of human high density lipoproteins (HDL) and a member of the lipocalin-calycin superfamily of small hydrophobic ligand carrier proteins, has been implicated in the transport of cholesterol, progesterone, bilirubin and a variety of similar ligands. Apo D is widely expressed in many tissues including the nervous system. In the crushed rat sciatic nerve, levels of apo D increase by 500-fold as the nerve regenerates, suggesting a role for the protein in the repair of neural tissues after injury. In the current studies, we investigated apo D expression in the normal human brain and in brains from select neurodegenerative disorders. Using an affinity purified antibody to human apo D and biotin-strepavidin enhanced immunocytochemistry, we observed that in the normal human brain there was little apo D expression which was restricted to scattered astrocytes and to perivascular fibroblasts. In contrast, apo D was readily detected in the brains of three individuals with histopathologically confirmed Alzheimer's disease. Most of the apo D immunoreactivity in AD brain was found in oligodendroglia; lower levels of apo D expression were found in reactive astrocytes and in microglia. No immunoreactivity was observed in neurons. A similar pattern of immunoreactivity, but of much lesser intensity was found in a patient with ALS-dementia. In general, the intensity of apo D immunoreactivity in the three AD brains paralleled the occurrence of established neuropathological features of AD, including amyloid plaques and neurofibrillary tangles. Our results suggest that induction of apo D in oligodendroglia may play a role in Alzheimer and other human neurodegenerative disorders.

580.12

CHARACTERIZATION OF APOLIPOPROTEIN E FRAGMENTS FROM ALZHEIMER'S DISEASE AND CONTROL TISSUE. M.A. Marques, M. Tolar, J. A.K. Harmony[#] and K.A. Crutcher*. Dept. of Neurosurgery and Dept. of Pharmacology and Cell Biophysics[#], Univ. of Cincinnati, Cincinnati, OH-45267-0515.

The role of apolipoprotein E (apoE) in the nervous system is unknown. The lipid-binding function of apoE has led to the hypothesis that this protein plays a role in peripheral nerve regeneration by recycling lipids, especially cholesterol, thereby providing membrane components to regenerating axons. Furthermore, the striking association between inheritance of the allele for the E4 isoform of the protein and the risk of developing Alzheimer's disease (AD) suggests a role for this protein in this disease, perhaps through its association with beta amyloid or tau proteins. However, the exact role played by apoE has not been determined yet.

We have found that apoE synthetic peptides are toxic to chick sympathetic and cortical neurons, as well as to PC-12 cells in vitro (Exp. Neurol. 130:120-126; 1994, and Tolar et al., this meeting). These results suggest the possibility that peptides generated from apoE play a role in neurodegeneration in AD. As an initial approach to test this hypothesis, we have studied post-mortem tissue from AD and age-matched control patients to determine whether apoE peptide fragments are present. Two different biochemical approaches were used: immunoprecipitation and heparin-sepharose chromatography column followed by ion-exchange chromatography, reverse phase HPLC, SDS-PAGE and electroimmunoblot analysis. CSF samples from both AD and control patients were found to contain apoE fragments in size from 22 kDa to 5 kDa. Brain tissue homogenates contained three distinct apoE fragments: 22kDa, 16kDa and 10 kDa. (Supported by Research Challenge Grant and NS 31410.)

580.13

INFLUENCE OF APOLIPOPROTEIN E GENOTYPE ON DIFFUSE PLAQUE FORMATION IN CORTEX OF NONDEMENTED ELDERLY. J.S. Bains, S.S. Bou, T. Fung, D. Hoffinger, C.L. White III, A.W. Clark*. U. of Calgary, Alberta T2N 4N1 Canada; and U. of Texas Southwestern Medical School, Dallas, TX 75235 USA.

Apolipoprotein E (ApoE), a facilitator of lipid metabolism, is encoded by a gene on chromosome 19. The most common alleles are epsilon 2, 3, and 4. Previous evidence suggests that the epsilon 4 allele predisposes to sporadic and familial Alzheimer's disease (AD). The pathogenetic point of entry for ApoE in AD-type degeneration is not known. In neocortex, the earliest stage of AD-type degeneration is formation of diffuse plaques (DP), seen to varying extent in nondemented elderly subjects. We have initiated a study to determine whether ApoE genotype influences this stage. Postmortem samples of neocortex from nondemented subjects (ages 60-99 years) are studied with a Steiner silver stain highly sensitive and specific for DP. Each subject is thus assigned a DP score from 0 (normal) to 5 (extensive DP, similar to AD but without other AD pathology). ApoE restriction isotyping is then done on DNA from each case, PCR-amplified with selected oligonucleotide primers. For cases studied to date, analysis of covariance was used to compare the 3/3 and 3/4 genotype groups, with the DP score as the dependent variable and age as the covariate. Those of genotype 3/3 (n = 25) and 3/4 (n = 16) yielded age-adjusted DP scores of 1.07 and 2.00, respectively. The difference was significant (p = 0.019). Sample sizes from other genotype groups (n = 7) were too small for analysis. Our findings support an influence of the ApoE epsilon 4 allele in formation of DP in neocortex of nondemented elderly, a very early stage of AD-type degeneration.

580.14

APOLIPOPROTEIN E GENOTYPE AND NEUROPATHOLOGICAL PHENOTYPE IN SPORADIC ALZHEIMER'S DISEASE.

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Several studies have demonstrated an association between apolipoprotein E (APOE) allele ε4 and Alzheimer's disease (AD). As interactions between APOE gene products and the ßA4 peptide are considered to play an important role in the pathogenesis of AD, we have studied the relationship between APOE genotypes and quantitative aspects of AD neuropathology. For genotyping of APOE, a new polymerase chain reaction (PCR) assay was developed which allows analysis of formalin-fixed and paraffin-embedded brain tissue (Egensperger et al., in press). Quantitative evaluation of histological phenotypes was performed by counting the number of amyloid plaques with τ-positive neurites, measuring the tissue area covered by cortical ßA4-immunoreactive deposits and counting the number of τ-positive neurons using a computer-assisted image analysis system. The frequency of the APOE allele ε4 in 19 clinically characterized and neuropathologically verified cases of AD was 39% (9 ε3/ε4-heterozygotes, 3 ε4/ε4-homozygotes). Detailed correlations between histological phenotypes and APOE genotypes are currently being carried out. Preliminary results suggest that not only the severity of AD lesions but also the extent of perivascular amyloid deposition is positively correlated to the number of APOE ε4 alleles. In addition, the extent of microglial activation as indicated by increased expression of major histocompatibility complex class II molecules appeared to be highest in APOE ε4 homozygotes.

580.15

PSYCHOMETRIC PERFORMANCE AND APOE4 IN SUBJECTS WITH DEMENTIA OF THE ALZHEIMER TYPE (DAT). K. Mann Koepke*, A. Goate, M. Storandt, C. Talbot, E. Grant. Departments of Neurology, Psychiatry, Psychology & Biostatistics, Washington University, St. Louis, MO 63110.

The presence of at least one apolipoprotein E4 allelle (APOE4) has been demonstrated to significantly increase the risk for the development of dementia of the Alzheimer type (DAT) and to lower the age of onset in these individuals. That APOE4 may directly and substantially contribute to the etiology of DAT in these patients has been hypothesized but not yet confirmed. This hypothesis would suggest that at any stage of dementia, subjects with at least one APOE4 allelle should perform less well or have a different distribution of decline on tests of cognitive function than age- and stage-matched non-APOE4 subjects. Reed et al (Arch Neurol 1994;51:1189-1192.) tested this hypothesis in "normal" male faternal twin pairs discordant for APOE4 and found poorer mean performance on cognitive tests in the E4-positive twins.

Subjects with a Clinical Dementia Rating (CDR) of 0 (no dementia), 0.5 (very mild), or 1.0 (mild dementia) and recruited for study by the Washington University Memory and Aging Project were evaluated for cognitive function using the Washington University ADRC Psychometric Battery and genotyped for APOE using PCR methods. Grouped by APOE4 genotype (i.e., no, one, or two APOE4 alleles), controlling for age (inverse relationship found between E4 dosage and age at time of testing) and CDR (i.e, CDR=0, 0.5, or 1), no group differences were found in performance on any of the 15 psychometric variables assessed ($F=1.44$, $df=19/187$, $p=0.12$). These results fail to support the conclusions of Reed and colleagues of poorer psychometric performance in APOE4 positive individuals.

580.16

EXPRESSION OF APOLIPOPROTEIN E ALLELES IN ALZHEIMER'S DISEASE BRAINS. J. PÉREZ-TUR[1,*], JC. LAMBERT[1], JP. DAVID[2], C. DIMENZA[2], P. VERMERSCH[1], A. DELACOURTE[1], MC. CHARTIER-HARLIN[1]. [1]INSERM-Unité 422. Place de Verdun. F59045 LILLE cedex (France). [2]Centre Hôpitalier Emille Roux. 1 av Verdun, F94456 Limeil-Brévannes (France).

Several studies had demonstrated that the apolipoprotein E (apoE) is one of the most important risk factors for the development of either familial or sporadic late-onset (LO) Alzheimer's disease (AD). This genetic association, found by a number of groups, has been reinforced by the finding that apoE may bind both Aß and tau proteins in a isoform-specific fashion. This has lead to the proposal of a hypothesis by which the binding of the E4 isoform to the Aß-peptide should enhance its rate of deposition and then increase the risk for developing AD. But an important number of people producing the E4 isoform are not affected by the disease, even in their 9th or 10th decades, so other factors than the APOE genotype should be involved.

In 1991, Diedrich and colleagues, showed an increased expression of the APOE gene in the brain of Alzheimer's disease patients. Then, the isoform-specific effects of this protein in AD should be due not only to the differences in sequence, but also to differences in the expression of each allele.

In the present work, we study the amount of mRNA for each allele present in 5 AD brains and in control brains. We performed a RT-PCR on RNA obtained from brains with a post-mortem delay ranging from 2-8 h. After restriction enzyme digestion, the samples were loaded on a polyacrylamide gel, subjected to electrophoresis and then stained in a ethidium bromide bath. The gel was photographed and the image was scanned and analysed with a computer program.

The relative data obtained for each of the three heterozygote genotypes are presented.

Diedrich et al. (1991) *J. Virol.* 65:4759-4768.

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580.17

APOE IS EXPRESSED IN SKNSH-SY 5Y NEUROBLASTOMA CELLS. C. Soulié[1], L. Wallois-Dupont[1], J. Perez-Tur[1], M.C. Chartier-Harlin[1], J. Lépagnol[2], A. Delacourte[1] and M.L. Caillet-Boudin[1]*. 1: Inserm U422, 59045 Lille, France. 2: Institut de Recherches SERVIER, 78290 Croissy sur Seine, France.

In human brain, apoE is synthesized and secreted by astrocytes, and is generally reported to be absent in neurons. In Alzheimer brain, apolipoprotein E is present within senile plaques and neurofibrillary tangles, suggesting that apoE may be involved in this disorder. In the present study, apoE was found in the neuroblastoma cell line, SKNSH-SY 5Y.

Presence of apoE was demonstrated by both immunochemical analysis and reverse transcription polymerase chain reaction. Using a monoclonal antibody against apoE, apoE immunoreactivity was found in the cytoplasm and neurites of neuroblastoma cells SY 5Y. ApoE distribution varied during NGF-differentiation time. SY 5Y cells were genotyped E3/E3.

ApoE synthesis in SY 5Y cells is an important finding because these cells were used as a model of Alzheimer-type neurodegeneration. After okadaic acid treatment, SY 5Y express hyperphosphorylated Tau proteins, Tau 55 and Tau 64, with Alzheimer-type epitopes. These results suggest that neurons could express apoE, and SY 5Y may be used to study apoE-Tau interactions.

580.18

AD2, A MAB THAT DIFFERENTIATES BY WESTERN BLOTTING BETWEEN NORMAL AGING, ALZHEIMER'S DISEASE, PROGRESSIVE SUPRANUCLEAR PALSY, PICK DISEASE AND A SUBTYPE OF FRONTAL LOBE DEGENERATION. A. Delacourte[1]*., C. Gilles-Mourton[2]., V. Buée-Scherrer[1]., N. Sergeant[1]., T. Bussière[1]., P.Vermersch[1]., Y.Robitaille[3]., D.Gauvreau[3]. 1:U. 422 INSERM, 59045 Lille France. Fax (33) 52.37.94. 2: CNRS-UMR 9921, Faculté de Pharmacie, 34060 Montpellier I, France. 3: Image Project, Hospital Côtes des Neiges, H3W 1W5, Montreal, Canada.

AD2 is a phosphorylation-dependant IgG mAb raised against Alzheimer PHF. Its epitope is located on the C terminal part of Tau. AD2 is able to specifically detect pathological Tau proteins in brain homogenates, in less than 10 µl of homogenate (1W/10V), on western blots and at a mAb concentration of 0.2 µg/ml. AD2 detects a Tau triplet (Tau 55, 64, 69) in Alzheimer's disease, which is different from the biopsy-derived normal "hyperphosphorylated" Tau triplet. For PSP as well as Corticobasal Degeneration, AD2 immunodetects Tau 64 and 69, which provides a biochemical signature for PSP-type lesions. In the same way, we were able to characterize Pick 's Disease and a subtype of Frontal Lobe Degeneration.

580.19

SPECIFIC TAU PROFILE IN THE BRAIN FROM PATIENTS WITH MYOTONIC DYSTROPHY. P. Vermersch[1,3*], N. Sergeant[1], M.M. Ruchoux[2], Ch. Daems[3], H. Petit[3], Ph. Dewailly[4], A. Delacourte[1]. [1]INSERM U422, Place de Verdun, Departments of [2]Pathology, [3]Neurology and [4]Geriatrics and Internal Medicine, University of Lille, 59045 France.

Pathological Tau proteins (PTP) are immunodetected in SDS brain extracts from patients with Alzheimer's disease (AD) or progressive supranuclear palsy. These PTP, which are the basic components of neurofibrillary lesions (NFL), are different from normal Tau proteins which are soluble and rapidly dephosphorylated after death. It has been reported that NFL are present in the brain from patients with myotonic dystrophy (MyD). Using AD2, a specific monoclonal antibody against PTP, we performed a mono and two dimensional immunoblotting and immunohistological studies of the brain of two MyD cases, aged 53 and 61 at death and compared the Tau profile with those obtained in AD. In both cases, we found NFL in the superficial layers of the limbic and temporal lobes. No amyloid deposits were found. PTP were immunodetected in the same areas and in the amygdala. The densitometric data showed different Tau profiles in MyD and AD. We found a low amount of the Tau 69 isoform in MyD and in most of the cortical areas, Tau 55 were in higher amounts compared with AD homogenates. This study shows evidence for a specific Tau pathology in MyD, probably due to the overexpression of the myotonine protein kinase in the brain of these patients.

581.1

INCREASED RISK OF DEMENTIA IN MOTHERS OF ALZHEIMER'S DISEASE CASES: EVIDENCE FOR MATERNAL INHERITANCE. S. D. Edland, J. Silverman, E. R. Peskind*, D. Tsuang, E. Wijsman, J. C. Morris. Department of Environmental Health, Univ. of Washington, Seattle, WA 98195-4709.

Duara et al. [Neurol, 43:1377-1384] found a 3.6:1 mother to father ratio among affected parents of familial Alzheimer's disease (AD) subjects. This observation is consistent with several speculative hypotheses regarding the etiology of AD. The present study tested the hypothesis of maternal inheritance in 118 AD subjects from the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) cohort. Age adjusted relative risk was calculated using the Cox proportional hazards model. 24 dementia cases were ascertained in the parental generation. Among these, the age adjusted mother:father relative risk was 2.9 (95% CIs 1.1 to 7.7). Among a subset of 10 families with one parent and at least two siblings affected, the ratio of affected mothers to fathers was 9:1. These findings are consistent with the hypothesis of maternal inheritance in AD. Confirmation with a larger sample or by genetic investigations of families with maternal inheritance patterns is required.

581.2

ANALYSIS OF THE 19Q13.2 CHROMOSOMAL REGION IN ALZHEIMER'S DISEASE. S.E. Poduslo*, M.R. Neal, J.D. Schwankhaus. Department of Neurology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Alzheimer's Disease is characterized by progressive dementia with cortical neuronal loss associated with β-amyloid plaques and neurofibrillary tangles. The disease is heterogeneous. There is strong evidence for a genetic component due to multiple affecteds in large families. The gene for apolipoprotein E, located on chromosome 19, has an $\epsilon 4$ allele which has been implicated in late onset Alzheimer's Disease. In our studies of 163 probable Alzheimer's patients from Texas, we found that the late onset patients had a frequency 0.412 for the $\epsilon 4$ allele while the early onset patients had a frequency of 0.537. Spouses, used as the control group, had a frequency of 0.087 for the $\epsilon 4$ allele. We examined markers and genes surrounding ApoE for associations with the disease. These included BCL3, LIPE, D19S47, D19S178, ApoCII, and ApoCI. We found no association of the disease with BCL3, LIPE, or D19S47. There may be a weak association with ApoCI and D19S178 as described by Chartier-Harlin et al. *Human Mol Genet* 3:569, 1994, and Schellenberg et al. *Ann Neurol* 31:223, 1992. We found a strong association of the $\epsilon 4$ allele with a restriction site in ApoCI. Of the Alzheimer's patients homozygous for the $\epsilon 4$ allele, over 90% had the CI restriction site. However, CEPH family members homozygous for the $\epsilon 4$ allele also had the CI restriction site. Taylor's group found a tight linkage of ApoE and ApoCI as well as a common regulatory element between the 2 genes (Lauer et al. *J Biol Chem* 263:7277, 1988; Simonet et al. *J Biol Chem* 266:8651, 1991). We are sequencing this area of the chromosome to further delineate this association.

Supported by the Texas DNA Bank of Alzheimer's Families for Genetic Studies.

581.3

CYSTATIN C: HCHWA-ICELANDIC-LIKE MUTATION IN SQUIRREL MONKEYS WITH CEREBRAL AMYLOID ANGIOPATHY. L. Wei, L.C. Walker and E. Levy*. Depts. Pharmacology and Pathology, New York University Med. Center, New York, NY 10016; Neuropathology Lab. The Johns Hopkins University Sch. Med. Baltimore, MD and Dept. Neurology, University of Greifswald, Germany.

The primary histological locus of cerebral amyloid deposition varies in aged humans and in different species of non-human primates. In aged rhesus monkeys (*Macaca mulatta*), amyloid deposition occurs most frequently in senile plaques, whereas in aged squirrel monkeys (*Saimiri sciureus*) the cerebral blood vessels are most affected. Cerebral amyloid angiopathy (CAA) can occur as a sporadic disorder in aged humans, as a frequent component of Alzheimer's disease, or in hereditary cerebral hemorrhage with amyloidosis (HCHWA). Immunohistochemical studies have demonstrated dual staining of the vascular amyloid of CAA patients with antibodies to amyloid ß-protein (Aß) and to cystatin C, a cysteine protease inhibitor.

We found that in aged squirrel monkeys the amyloid, previously shown to be immunoreactive with anti-Aß antibodies, reacts also with antibodies to cystatin C. The cystatin C cDNA was sequenced to determine if CAA in these monkeys is related to a species-specific amino acid change. While the predicted amino acid sequence in rhesus monkeys differs from the human sequence by four residues, that of the squirrel monkeys has seven additional amino acid substitutions, one of which is Leu68Met. The amyloid protein isolated from the leptomeninges of Icelandic patients with HCHWA-I is composed of cystatin C containing a single amino acid substitution, Leu68Gln. We propose that specific amino acid substitutions in cystatin C may modulate amyloid deposition in the walls of cerebral blood vessels.

Supported by NIH grants AG11481, AG05146 and NS20471.

581.4

PREPARATION AND TESTING OF YAC-DNA FOR THE GENERATION OF TRANSGENIC RODENTS Ulrich Kosciessa and Claus Braestrup*, Schering AG Research Laboratories, Müllerstr. 170-178, 13353 Berlin, Germany

The principal advantage of Yeast artificial chromosomes (YACs) as transgenes compared to cDNAs is essentially the copy number dependent and integration site independent expression. YACs contain gene loci which are not influenced by flanking expression regulators (enhancers/ silencers). They guarantee protein expression and because they represent intact genomic loci they also produce splice variants. The YAC transgenes are controlled by their natural promoters and 3'UTRs.

A major disadvantage of YACs is their size. They are very sensitive to shearing forces which occur during preparation and microinjection. We overcame these problems by avoiding concentrations by centrifugation during DNA preparation as this leeds to significant degradation of the YAC. In our proccdure the YAC is purified and concentrated in two pulsed field gel electrophoresis (PFGE) runs. In a conventional first PFGE the YAC is separated from other Yeast DNA. In a second PFGE (high percentage of agarose) with the slice (first PFGE) containing the purified YAC the DNA is concentated while entering the second gel with higher agarose concentration. The excised concentrated YAC band can now be stored for several month until usage or eluted by conventional agarasing.

Because of their sensitivity against shearing forces the YACs need to be tested for integrity after passage through the microinjection needle. For this we agarase the con-centrated YAC slice in a polyamines containing buffer. After removal of residual agarose we microinject the YAC into the same buffer. Yeast strains deficient for the selection marker located on the YAC are then transformed with the injected YAC. Resulting transformants obtained by plating on selection agar are further analysed by PFGE.

581.5

KYNURENIC ACID AND KYNURENINE AMINOTRANSFERASES KAT I AND KAT II IN THE BRAINS OF PATIENTS WITH DOWN SYNDROME AND ALZHEIMER'S DISEASE. H.Baran,*N.Cairns, **N.Singewald and G.Lubec. Dept. of Pediatrics, Univ. of Vienna, Austria, * Brain Bank, Dept. of Neuropathology, Institute of Psychiatry, London, England, **Dept. of Pharmacology and Toxicology, Univ. of Innsbruck, Austria.

The present study was designed to examine the biosynthetic machinery of the excitatory amino acid receptor antagonist kynurenic acid (KYNA) in frontal cortex of patients with DOWN syndrome (DS) and Alzheimer's disease (AD). We measured the content of KYNA, determined by HPLC and activities of enzymes synthesizing KYNA, kynurenine aminotransferases I and II (KAT I and KAT II) in the presence of the 1 mM co-substrates 2-oxoglutarate, 2-oxoisocaproate and pyruvate at their pH optima of 10.0 for KAT I and 7.4 for KAT II. KYNA content was normal in frontal cortex from AD patients (0.28 ± 0.05 pmol/mg tissue) and increased in DS (205 %) as compared with control (0.22 ± 0.05 pmol/mg tissue). In DS and AD the activities of KAT's were remarkably altered. KAT I was significantly reduced (75-86 %) in the presence of all three 2-oxo acids in both diseases. Significantly lowered KAT II activity was observed only in the presence of 2-oxoglutarate (34 %) in frontal cortex from AD. Reduction of KAT I and KAT II activities in frontal cotex did not correlate with the decline of choline acetyltransferase in frontal cortex from both diseases. Measurement of amino acids revealed no changes in the levels of γ-aminobutyric acid, taurine, glycine, aspartate, glutamate and arginine in frontal cortex from DS and AD patients. Our data demonstrated changes in the parameters of kynurenine metabolism and suggest the involvement of KAT's deficit in the brain in DS and AD patients. As KYNA antagonizes competitively the N-methyl-D-aspartate (NMDA) receptors, a system responsible for memory, learning and cognitive functions, our present data may suggest a link to the neuronal degeneration mediated by overstimulation of NMDA receptors. Supported by grands from Red Bull and Austria Science Research Fund (No H0043-MED to H.B.)

581.6

PROSPECTIVE CLINICAL-PATHOLOGIC INVESTIGATION OF PARKINSONISM IN ALZHEIMER'S DISEASE (AD). D.A. Bennett*, D.W. Gilley, R.S. Joglekar, E.J. Cochran. Rush Alzheimer's Disease Center, Chicago, IL 60612

We evaluated 25 consecutive patients with clinical and pathologic diagnoses of AD and a brain autopsy within 12 months of the last examination. Parkinsoism was assessed with the Unified Parkinson's Disease Rating Scale. Cortical neuritic plaques and neurofibrillary tangles, and substantia nigra neuronal loss (SNloss) and Lewy bodies were quantified on 5-micron thick paraffin-embedded sections stained with modified Bielschowsky and hematoxylin-eosin as recommended by CERAD. Average age at death was 77.5 years and average mini-mental state examination (MMSE) was 9.2. Four persons had one parkinsonian domain, 6 had two, 4 had three, and 2 had all four. Nine persons had at least mild neuronal loss, 4 with Lewy bodies. Age at death, SNloss and MMSE were all related to parkinsonism; after adjusting, only SNloss and age at death remained independent correlates. These data support the view that extranigral, age-related changes contribute to parkinsonism among persons with AD.

(Supported by AG10161 and AG09466).

581.7

MICROTUBULE-ASSOCIATED PROTEIN TAU IN CEREBROSPINAL FLUID:IMPLICATIONS FOR A POTENTIAL DIAGNOSTIC MARKER IN ALZHEIMER'S DISEASE. H. Arai*[1], M. Terajima[1], M. Miura[2], S. Higuchi[3], S. Takase[4], C. Clark[5], V. M.-Y. Lee[6], J. Q. Trojanowski[6] and H. Sasaki[1].
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Cerebrospinal fluids (CSFs) from 67 patients with Alzheimer's disease (AD) and 94 patients with non-AD neurological diseases as well as 19 normal subjects were surveyed by sandwich enzyme-linked immunodorbent assay (ELSIA) to quantitate levels of the microtubule-associated protein tau in CSF (CSF-tau). The CSF-tau level was significantly increased ($P < 0.001$) in AD patients (80.7 ± 46.6 pg/ml) as compared with that in patients with non-AD neurological diseases (21.4 ± 32.3pg/ml)and controls (9.0 ± 4.5 pg/ml). In AD, the significant increase was found irrespective of age at onset, apolipoprotein E genotype and clinical stage. Western blots of AD CSF proteins revealed two to three tau immunoreactive bands with apparant molecular weight between 50 and 65 KDa consistent with phosphorylated CSF-tau. Taken together, our results suggest that CSF-tau might reflect the accumulation of altered tau due to the progressive death of neurons in the AD brain, and that the assay of CSF-tau by ELISA may prove to be a reliable and early diagnostic test in AD.

581.8

ANTEMORTEM CSF TAU IS RELATED TO NEURONAL PATHOLOGY AT AUTOPSY IN ALZHEIMER'S DISEASE. D. Galasko*, L. Hansen, C. Vigo-Pelfrey, D. Schenk, P Seubert. Dept. of Neurosciences, University of California, San Diego, CA 92093 and Athena Neurosciences, South San Francisco, CA 94080.

Antemortem biological markers of Alzheimer's disease (AD) such as cerebrospinal fluid (CSF) levels of tau, APP and amyloid beta protein have been proposed. For example, CSF tau is increased in many AD patients, even very early in the course of their dementia. Comparison between CSF measures and autopsy data allows the relationship between CSF markers and brain lesions to be clarified.

We have measured CSF tau in patients with clinical probable AD, using a sensitive 2-site sandwich ELISA. From this cohort, 9 patients have come to autopsy, from 3 - 8 years after the CSF was obtained. Brains were examined histologically in a standard protocol, that included counts of neurofibrillary tangles and senile and neuritic plaques.

CSF tau ranged from 148 - 538 pg/ml. Of the 4 patients with the lowest tau levels, 3 had pathology of Lewy body variant of AD, with cortical and subcortical Lewy bodies, and one had "plaque only" AD; NFT were exceedingly rare in these 4 patients, while plaque counts met criteria for AD. The remaining 5 patients had abundant NFT in the hippocampus and neocortex. The 2 patients with tau levels over 500 pg/ml had the highest NFT counts, especially striking in the hipocampus. In patients with AD, CSF tau levels therefore appear to be related to the extent of neurofibrillary pathology; this damage presumably releases soluble tau that can diffuse into CSF.

581.9

EVIDENCE OF BLOOD-CERBROSPINAL-FLUID-BARRIER IMPAIRMENT FOR HIGH MOLECULAR WEIGHT PROTEINS IN A SUBGROUP OF ELDERLY PATIENTS WITH MAJOR DEPRESSION AND ALZHEIMER'S DISEASE H. Hampel*, C. Hock, A. Haberl, C. Berger, M. Ackenheil, F. Müller-Spahn and H.-J. Möller. Department of Psychiatry, University of Munich, 80336 Munich, Germany and Department of Psychiatry, University of Basle, 4025 Basle, Switzerland

Serum and cerebrospinal fluid (CSF) from 44 patients with clinical probable Alzheimer's disease (AD) (subdivided into two groups with 18 early onset [EO] and 26 late onset [LO] cases) and 24 patients with Major Depression (MD) were assayed for concentrations of albumin and IgG. The severity of dementia was assessed with the Mini Mental State Examination (MMSE). The CSF/serum ratio for albumin and IgG as well as the IgG-index were used to evaluate blood-CSF barrier (BCB) function. Various patients showed signs of BCB dysfunction for high molecular weight proteins like albumin more pregnant in the MD than in the AD group. This was significantly correlated to the total CSF protein content. The permeability of the BCB and the total CSF protein content were not correlated to measures of dementia severity. Our data indicate a BCB leakage in a subgroup of 16 % demented and more pregnant in 21% of the depressed patients. Wether BCB impairment, in a possible yet undefined neurobiological subgroup of patients with MD and AD, playes a role in the pathophysiology of the diseases or is only an epiphenomenon, remains to be investigated. In total BCB impairment is unspecific either for AD or MD.

581.10

CEREBRAL BLOOD FLOW CHANGES IN RESPONSE TO VISUAL STIMULATION IN DEMENTED PATIENTS H.A. Buchtel*, R.A. Koeppe, J. Mountz, N.L. Foster, S. Berent, B. Giordani and D.E. Kuhl. VA Medical Center, Ann Arbor and University of Michigan Departments of Psychiatry, Internal Medicine, Division of Nuclear Medicine, Neurology, and Psychology.

The visual-spatial disorders that are commonly seen in dementia are usually attributed to a disturbance of higher-order mechanisms rather than to reduced responsiveness of the visual system. However, EEG evoked potentials are slowed or reduced in amplitude in patients with Alzheimer's Disease (AD) and Parkinson's Disease (PD) with dementia. To test the possibility that visual-spatial deficits seen in dementia may be secondary to reduced input to higher cortical centers, we carried out [O-15]-water activation PET scans with two groups of demented patients (PD with dementia, N=5; probable AD; N=5), and two control groups (normal control subjects, N=7; and cognitively intact patients with PD, N=4). Overall metabolic values were lower for the patient groups compared to normals, and stimulation effects were significantly lower in association cortices for the demented patients compared to the non-demented individuals. We are uncertain if the effect observed would entirely account for the behavioral findings in visual-spatial tasks, and plan to study these phenomena further. We do feel that responsiveness in the visual system should not be excluded as one of the factors contributing to cognitive deficits in demented patients.

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581.11

A STUDY OF SENSORYMOTOR COORDINATION IN ALZHEIMER'S DISEASE. P. Demers [1,2], M.Lassonde *[1] and A.Robillard [2]. [1] Hôpital Maisonneuve-Rosemont [2] Département de psychologie,Université de Montréal, Montréal, CANADA.

This study examined movement accuracy and movement time in 11 subjects with probable Alzheimer's disease and in 8 normal elderly controls. Subjects had to point to targets (red light emitting diodes), randomly presented on a board. This board was covered with an isotropic resistent surface that measures terminal movement accuracy in two dimensions. The task was executed under two conditions: with visual control and without vision of the arm. Results indicate that subjects with dementia of the Alzheimer type were as accurate as normal controls only in the experimental condition where both proprioception and vision were available. The measurement of variable error and constant error revealed significant differences between groups but only in the no-visual feedback condition. Indeed, demented subjects had more variations in their performance and a larger constant error in that condition. Undershooting was present in all subjects as revealed by the direction of the constant error. However, the magnitude of the undershooting movement was more important in AD subjects than in controls in the absence of visual feedback. Finally, movement time was significantly longer in demented subjects than in normal controls. This effect was especially evident with the non-dominant hand in the absence of visual feedback. Longer movement time did not result in an increased movement accuracy in the group with dementia. These findings suggest that DAT subjects need to rely on more than one source of afference to accurately execute a fine motor movement. They also support the idea that movement disturbance, other than limb apraxia, may be present in Alzheimer's disease.

581.12

SPARING OF PRIMARY SENSORY AND MOTOR ABILITIES IN INTERMEDIATE STAGES OF ALZHEIMER'S DISEASE. Y. Lakmache[1]; S. Gauthier[2*]; F. Leporé[1]; and M. Lassonde[1].
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Higher-order cognitive functions have been extensively investigated in patients with probable Alzheimer's disease (AD). Additionally, modality-specific elementary sensory-motor integration remains under-reported. The aim of this study was to evaluate visual, somesthetic and motor performance in early AD's patients using six screening tasks. Ten probable ADs patients (stages III & IV on the Reisberg Scale) were compared to 10 age and sex matched controls. Visual acuity was mesured with the Rosenbaum test and visual field integrity (confrontation) was also assessed. The Face-hand test, digital agnosia and single and double finger localisation were used to assess somesthetic function. Finally, the Purdue Pegboard was administered in order to verify unimanual and bimanual dexterity. Results showed that there were no differences in visual, somesthetic and motor modalities in all subjects tested irrespective of the stage of illness. However, a difference was noted in the face-hand test. Thus, stage IV patients showed deficits when crossed and uncrossed distal-proximal stimulation was applied. The findings of this study suggest that, in spite of higher order cognitive deficits in probable AD, less elaborated motor and sensory (visual and somesthetic) functions are preserved even in intermediate stages of the illness. Furthermore, these observations confirm that the primary sensory and motor areas are dysfunctional only late in AD and concord well with neuroanatomical findings demonstrating that these areas are not affected in the early stages of the illness.

581.13

PREHENSILE ADAPTABILITY IN DEMENTIA OF THE ALZHEIMER TYPE. V. Buckles*, J.C. Morris, J. Duchek, L. Hunt. Alzheimer's Disease Research Center, Washington University School of Medicine, St. Louis, MO 63110.

Global cognitive loss in dementia of the Alzheimer type (DAT) is associated with reduced functional abilities, e.g. driving. Previous studies have rarely controlled for dementia severity, particularly the mildest stages of DAT. Our purpose was to examine a simple but overlearned movement in subjects in the earliest stages of DAT. In this experiment, three task characteristics of prehension were manipulated to study movement adaptations made by DAT subjects compared to elderly controls: object size (cylinder size: 5cm vs 2.5cm), movement direction (straight vs left), and speed/accuracy directions (fast but accurate vs fast). DAT subjects were staged in 2 groups according to dementia severity: very mild (n=8) and mild (n=8). Controls (n=8) and DAT subjects were asked to grasp a cylinder 20cm from the starting hand position, lift and place it on a target 20cm from the cylinder's original location when they heard a 'go' tone. Except for the 'fast' condition, subjects were instructed to "move as quickly and accurately as possible." **Results:** Mildly demented subjects had longer reaction times and movement times, used lower peak velocities, and spent a greater proportion of time in deceleration than controls. An interesting question is how well did DAT subjects adapt to changes in the task characteristics compared to controls. Only time to peak aperture showed a group X condition interaction, where the mildly demented subjects reached peak aperture (opening of the fingers to grasp) later in fast and left movements compared to other groups. DAT subjects adapted similarly to controls. The ability to adapt overlearned skills to meet task demands appears preserved in very mild and mildly demented subjects, and may have implications for more complex motor skills such as driving. Support: NIH AG10145.

581.14

MATHEMATICAL ANALYSIS OF THE MINIATURE EYE MOVEMENTS OF BOTH EYES IN DEMENTIA PATIENTS

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It is known that object recognition is impossible under conditions where miniature eye movements do not occur due to anesthesia. Also , as it has been suggested that miniature eye movements are chaotic (Yoshimatsu et al,1993), we carried out fractal analysis of the miniature eye movements. The results showed that abnormal findings are characteristic of dementia patients with a frontal lobe deficit,suggesting a close relationship between the attention function and miniature eye movements (Fujii et al,1994).Using an eye movement analyzer(Takei Co.LTD.),we applied bandpass filter to the data of miniature eye movements of both eyes from the results of tests carried out on Alzheimer's disease patients,vascular dementia patients and healthy subjects,and obtained the drift component. We then carried out mathematical analysis using a newly developed analytical software program. Analysis was carried out to obtain the following:correlation coefficient,pseudo topographical space,topographical space in time series,and waveform in time series.

The results of these analyses were different for Alzheimer's disease patients compared to healthy subjects and vascular dementia patients. Although the clinical significance of these analytical results is not clear,the results of this mathematical analysis did show that vascular dementia patients and elderly healthy subjects were similar,and we were also able to obtain characteristic findings for Alzheimer's disease patients. Thus,the results of this analysis are thought to be useful in the differential diagnosis of vascular dementia and Alzheimer's disease. It is thought that the difference in the analytical results for these two diseases may also reflect the difference in the nature of the recognition deficit between vascular dementia and Alzheimer's disease.

581.15

DISCRIMINATION OF ALZHEIMER'S AND PARKINSON'S DISEASE BY ANALYSIS OF REGIONAL CSF PROFILES USING QUANTITATIVE CT BRAIN IMAGING. D.H. Mathalon, E.V. Sullivan, P.K. Shear, K.O. Lim*, H.J. Sagar, J.A. Yesavage, J.R. Tinklenberg, and A.Pfefferbaum. Dept. of Psychiatry and Behavioral Sciences, Stanford University School of Medicine and DVAMC, Palo Alto, CA 94304; Dept. of Clinical Neurology, University of Sheffield, England.

The primary pathology of Alzheimer's disease (AD) is cortical whereas that of Parkinson's disease (PD) is nigrostriatal; yet, cortical atrophy has been observed in PD. We used CT measures of CSF volumes to compare the regional pattern of brain atrophy in AD and PD across 6 regions of interest (ROI; frontal, parieto-occipital, temporal sulci, Sylvian fissures, lateral and 3rd ventricles). Subjects were 81 normal controls (NC; 62±11y), 117 AD (69±8y), and 54 PD (62±8y). Data were expressed as age- and head size-corrected Z-scores (standardized to controls; NC ROI mean=0±1sd). A MANOVA revealed group differences across the ROIs, with AD and PD showing greater CSF volumes than NC in every ROI except the lateral ventricles, which were not abnormally enlarged in PD. Moreover, AD showed more severe atrophy than PD in every ROI except the parieto-occipital sulci, where the groups did not differ. A discriminant analysis of AD vs. PD using the 6 ROIs as discriminators correctly classified 81.2% of AD and 83.3% of PD. However, this discriminative power may have simply reflected the overall CSF volume profile elevation of AD relative to PD. Accordingly, we performed a multivariate profile analysis to compare the relative *distributions* of regional atrophy in the two diseases, independent of the overall difference in profile elevations. AD and PD exhibited different patterns of regional brain atrophy; a discriminant function using only within-subject *differences* between ROIs as discriminators correctly classified 82.1% of AD and 83.3% of PD. In AD, the lateral ventricles, Sylvian fissures, and temporal sulci were equally abnormal and more enlarged than the frontal, parieto-occipital, and 3rd ventricle. By contrast, in PD, the cortical and 3rd ventricle ROIs were atrophic relative to the lateral ventricles, with Sylvian and temporal atrophy comparable to that in the other cortical ROIs. Thus, AD and PD are distinguishable by their regional profiles of brain atrophy as measured with CT. *[Funded by MH 40041, MH 30854, AA 05965, DVA]*

NEUROMUSCULAR DISEASES

582.1

RUBRAL, VESTIBULAR, AND RETICULAR PROJECTIONS TO SPINAL CORD IN THE MUTANT *MDX* MOUSE. D. Minciacchi*, D. Carretta, M. Santarelli, R. Carrai, F. Pinto, A. Sbriccoli§, and A. Granato‡. Dept. of Neurol. and Psychiat. Sci., Univ. of Florence, Italy, and §Lab. of Exp. Neurol. and ‡Inst. of Anat., Catholic Univ., Rome, Italy.

The mutant *mdx* mouse is acknowledged model of the human disease Duchenne Muscular Dystrophy. Previous investigations from our laboratory demonstrated that the cortico-spinal system of *mdx* is altered impressively (Minciacchi et al., Soc. Neurosci. Abstr., Vol. 20, p3, 1994).

In the present study we analyzed, in *mdx* mice, the spinal projections of the red nucleus (RN), and the medial and lateral vestibular (MVe and LVe), gigantocellular reticular (Gi), and raphe magnus (RMg) nuclei. Injections of aqueous solutions of horseradish peroxidase were performed into the cervical spinal cord of *mdx* and normal mice, and retrogradely labeled neurons were studied in the brainstem.

Populations of labeled neurons are present in RN, MVe, LVe, Gi, and RMg of both normal and *mdx* mice. In contrast to normal animals, spinal projecting neurons in the RN of *mdx* mice are reduced of about 40% and the difference is significant ($p<0.05$). Conversely, in MVe, LVe, Gi, and RMg, the cell populations projecting to spinal cord are quantitatively matching; in no case the difference between *mdx* and normal animals is significant. No major comparative variation were observed in the morphology and size of spinal projecting cell populations in RN, MVe, LVe, Gi, and RMg.

The present data show that the structural alterations of the cortico-spinal system we observed previously in *mdx* mice are not unique since the rubro-spinal system is altered as well. The damages of the central nervous system in *mdx* selectively involve structures implicated in the control of motor behaviour. The fact that only a part of supra-spinal motor centers is affected represents an information of pivotal interest and opens the possibility to reconsider the pathogenetic hypotheses of Duchenne Muscular Dystrophy.

582.2

MEMBRANE ELECTRICAL PROPERTIES OF SKELETAL MUSCLE FIBERS OF DYSTROPHIC (MDX) MICE DURING AGING. A. De Luca*, S. Pierno, A. Roselli, F. Natuzzi and D. Conte Camerino. Unit of Pharmacology, Dept. of Pharmacobiology, Faculty of Pharmacy, University of Bari, Italy.

Mdx mouse is genetically homolog with human Duchenne muscular dystrophy *(Engel et al., in Myology, 1133, 1994)*. We previously recorded a transient increase of macroscopic chloride conductance (GCl) in extensor digitorum longus (EDL) muscle from mdx mice during the 2nd month of age, a period characterized by a succesfull regeneration of the hind limb muscles. On the contrary a decrease of this parameter has been observed in the non-regenerating diaphragm (DIA) muscle of the same strain *(De Luca et al., Biophys. J. 68: A274, 1995; Engel et al., 1994)*. To better evaluate the relationship between the changes of GCl and the regeneration and degeneration events, the component conductances to chloride and potassium (GK) ions of EDL and DIA muscles were recorded *in vitro* by means of computerized intracellular microelectrode technique from mdx mice undergoing aging (14 month-old), a spontaneous degenerative event characterized by a decrease of GCl and an increase of GK *(De Luca et al., J. Pharmacol. Exp. Ther. 269: 948, 1994)*. The EDL muscles from aged control mice (C57BL/10) were characterized by a significant decrease of GCl from the adult value of 2248±96μS/cm^2 (n=60) to 1860±100μS/cm^2 (n=20) and by a 20% increase of GK. Conversely, the GCl value of EDL muscle from aged mdx mice was not significantly different with respect to adult value (2021±96μS/cm^2; n=86), being 2108±125μS/cm^2 (n=28). Also GK was unaffected being 429±44 μS/cm^2 (n=27) vs. 404±50μS/cm^2 (n=24) of adult mdx EDL muscles. In the control mice, the GCl of DIA was 2186±143μS/cm^2 (n=16) in adult and 1095±115μS/cm^2 (n=11) in aged and GK was 30% higher in the aged animals. In the DIA muscle from aged mdx mice GCl was dramatically low being 660±94μS/cm^2 (n=13) vs. 1755±136μS/cm^2 (n=12) of adult mdx DIA. Thus, the EDL muscle from mdx mice has a high regenerative potential, which has in GCl a sensitive target, that allows the muscle to counteract degenerative conditions such as aging. On the contrary a non-regenerating mdx muscle, such as DIA, is more sensitive to the progression of aging-induced degeneration (Telethon-Italy project n° 322).

582.3

NITRIC OXIDE SYNTHASE IS COMPLEXED WITH DYSTROPHIN IN SKELETAL MUSCLE AND ABSENT FROM SARCOLEMMA IN DUCHENNE MUSCULAR DYSTROPHY. J.E. Brenman, D.S. Chao, H. Xia, D. Copenhagen*, and D.S. Bredt. Dept. of Physiology, UCSF, CA 94143.

Nitric oxide (NO) is synthesized in skeletal muscle by neuronal NO synthase (nNOS), which is localized beneath the sacrolemma of fast twitch muscle fibers. We now show that nNOS associates with both microsomal and cytoskeletal fractions of skeletal muscle. This subcellular localization is mediated by anchoring of nNOS to dystrophin, the protein product of the gene mutated in Duchenne and Becker muscular dystrophy. Specificity of binding is demonstrated by copurification of nNOS and dystrophin by both succinylated wheat germ agglutinin and 2' 5'-ADP affinity chromatography procedures. Dystrophin interacts with the amino-terminal domain of nNOS which contains a 66 amino acid motif that is shared by a family of cytoskeletal associated proteins including syntrophin, a known binding partner of dystrophin. *mdx* mice and humans with Duchenne muscular dystrophy (DMD) evince a selective loss of nNOS protein and catalytic activity from membrane and cytoskeletal fractions and an accumulation of nNOS in muscle cytosol. These data demonstrate a novel role for dystrophin in localizing a signaling enzyme to the myotube plasma membrane. Aberrant regulation of nNOS in dystrophic fast twitch muscle fibers may contribute to selective degeneration of this fiber type in DMD.

582.4

A POSSIBLE FUNCTIONAL CORE REGION OF DYSTROPHIN. Cheng Zhang*, Youmei Xie[1], Genbin Shi, Zhoulin Liu, Jianhua Chai. Dept. of Neurology, First Hospital, Sun Yat-sen Univ. of Med. Sci., Guangzhou, 510080 and Dept. of Obstetric and Gynecology[1], West China Univ. of Med. Sci.,Chengdu, 610041, P. R. China.

Dystrophin is the protein product of DMD gene. The observation that abnomalities of dystrophin result in different phenotypes (DMD and BMD) suggests the possibility that mutations in different critical functional regions of the DMD gene might result in different phenotypes. Hydrophobicity and hydrophilicity plots of the 3685 amino acid sequence of dystrophin were analyzed using Genepro software (window 56). Our results demonstrated that dystrophin contains four hydrophobic regions located in dystrophin amino acid domains 95-120, 1990-2010, 2438-2493, and 3150-3300, respectively. Among the four regions, the hydrophobicity in the third one (amino acids 2438-2493) is the strongest. We have named the third hydrophobic region the Deletion Hot Spot Hyrophobic Peptide (DHSHP) of dystrophin because the amino acids belonging to this region are coded by the deletion hot spot exon 51 of the dystrophin gene. In order to assess the reletionship between deletions in DHSHP and phenotype, we collected 189 cases of DMD and BMD with DNA in-frame deletions of the dystrophin gene from nine published papers. Among the 189 cases, 70 were DMD and 119 were BMD. Of the DMD patients, 55.7% (39) had deletions in the DHSHP. Of the BMD patients Only 3.4%(4) had deletion in the DHSHP (Chi-squire test, $P<0.01$). This result suggests that the presence or abscence of the DHSHP region is one significant determinant of DMD/BMD phenotype.

582.5

Ca^{2+} CURRENT RECORDINGS FROM MYOCYTES TREATED WITH LES SERUM K.D.García*, M.Mynlieff● and K.G.Beam#. Depts. of Anatomy and Neurobiology* and Physiology#, Colorado State Univ., Fort Collins, CO 80523, and Dept. of Biology●, Marquette Univ., Milwaukee, WI 53233.

Lambert-Eaton Myasthenic Syndrome (LES) is a human autoimmune disorder characterized by decreased quantal content at the neuromuscular junction. LES antibodies do not appear to specifically react with one type of Ca^{2+} channel since they decrease both the LVA and the HVA components of Ca^{2+} current in dorsal root ganglion cells (DRGs) and neuroblastoma cells. Past research has concluded that LES antibodies do not react with myocytes since they do not alter the strength of muscle contraction elicited by direct stimulation. However, no one has recorded Ca^{2+} currents in myocytes treated with LES serum. In the present study we examine the possible effects of LES serum on murine cardiac and skeletal myocytes. Serum from three patients previously tested on DRGs was used. Serum from patients I and II, who were definitively diagnosed as having LES, decreased both HVA and LVA components of Ca^{2+} current in DRGs. Serum from patient III, who was diagnosed as having myasthenia gravis and some characteristics of LES, did not decrease DRG Ca^{2+} currents. Cells were maintained in culture for 3-10 days. Serum from normal individuals or one of the three patients was added to the culture medium 24 h before currents were recorded. Ca^{2+} currents were measured with the whole-cell variant of the patch clamp method (10mM Ca^{2+} as charge carrier). Currents were evoked by 300-ms depolarizations applied from a holding potential of -80 mV. In myocytes, sera from patients I and II did not decrease Ca^{2+} currents while serum from patient III decreased the HVA component of Ca^{2+} current. These results suggest that LES antibodies do not react with Ca^{2+} channels in myocytes and that muscle inflammation in myasthenia gravis may result in production of antibodies to muscle type Ca^{2+} channels. Supported by NIH grant NS 26416 to Kurt Beam.

582.6

A β SUBUNIT MUTATION IN THE PUTATIVE GATE OF THE ACETYLCHOLINE RECEPTOR (ACHR) CHANNEL CAUSES A CONGENITAL MYASTHENIC SYNDROME (CMS). Jason Gammack, Ricardo Maselli, Cesar Labarca, Christopher Gomez*. Univ. of Minn., Minneapolis, MN 55455.

A number of CMS are due to kinetic disorders of the muscle AChR. In some, mutations have been found in the gene encoding the ε subunit of the AChR. We used intracellular and extracellular microelectrode studies to detect a kinetic disorder of AChRs in a patient with a severe CMS. One population of channels had open times of approximately 6-8 ms. We screened for single strand conformation polymorphisms in DNA amplified from the ion channel-(M2) coding regions of the patient's four AChR genes. We found an abnormal conformer in the β subunit M2 domain that was present in the patient, but not in his asymptomatic parents or siblings. By allele-specific sequence analysis we identified a C-to-A transversion in the β subunit gene. This mutation codes for the substitution of a methionine residue for leucine263, a highly conserved residue that is believed to participate with homologous leucine residues to form the ion channel gate. Only the wild type sequence was present in the β subunit M2 regions amplified from the patient's parents and siblings. To confirm the role of this mutation in the pathogenesis of his disease we generated the homologous mouse mutation in cloned mouse β subunit cDNA and compared ACh-induced currents induced in oocytes expressing AChRs with the βL263M mutation to those with wild type AChRs. We found that AChRs with this mutation had ten-fold greater sensitivity to ACh, as judged by EC50, than do wild type AChRs. We conclude that a spontaneous mutation in codon 263 of the β subunit gene is responsible for this patient's CMS. Supported by NIH# R01 NS33202-01A1.

582.7

CALCIUM OVERLOAD AND POSTSYNAPTIC VACUOLATION IN TRANSGENIC MICE AS A FUNCTION OF CHOLINERGIC RECEPTOR ION CHANNEL KINETICS. C. M. Gomez, R. Maselli, J. W. Day* and R.W. Wollmann, Univ. of Minn., Minneapolis, MN 55455.

Changes in acetylcholine receptor (AChR) channel kinetics occur in neuromuscular junction (NMJ) development and underlie some congenital myasthenic syndromes (CMS). To assess the role of such changes on NMJ calcium (Ca^{+2}) overload and NMJ integrity we created transgenic (TG) mice expressing mutant AChRs with a range of ion channel abnormalities. Muscle was studied in vitro using voltage-clamp and patch-clamp single-channel analysis, using a histochemical stain for ionized Ca^{+2}, glyoxal bis-hydroxyanil, and by electron microscopy (EM). Mice expressing AChRs with the mutation, δS262T (open times prolonged 3-fold) had no evidence of Ca^{+2} overload and normal motor endplates by EM. Mice expressing AChRs with the mutations, αC418W (open times prolonged 5-fold) or αL251T (open-times prolonged 10-fold) had histochemical evidence of Ca^{+2} overload at a mean of 1 % of NMJ when studied at rest. This proportion increased ten-fold (10% of NMJ) after exercise or nerve stimulation. Ca^{+2} overload was totally prevented in the gastrocnemius of exercised mice by prior sciatic nerve ligation. NMJ of αC418W-TG mice appeared normal by EM. In contrast, in forelimb flexor muscle of exercised αL251T-TG mice the junctional sarcoplasm was filled with membrane-bound vacuoles between and beneath the secondary synaptic clefts in multiple NMJ examined. Mitochondria in the region were morphologically normal. These findings suggest that increased AChR channel open time interferes with Ca^{+2} homeostasis at the NMJ and is responsible for the pathologic changes seen in many CMS. Supported by NIH-CIDA# K08NS01540 and the Muscular Dystrophy Association.

582.8

ELECTRON MICROSCOPIC INVESTIGATION OF MUSCLE MITOCHONDRIA IN CHRONIC FATIGUE SYNDROME. A.V. Plioplys*, S. Plioplys, Chronic Fatigue Syndrome Center, Mercy Hospital, Stevenson Expwy at King Drive, Chicago, IL 60616.

Chronic Fatigue Syndrome (CFS) patients suffer from disabling physical and mental fatigue. Abnormalities in mitochondrial function can lead to fatigue and weakness. Other researchers have reported ultrastructural mitochondrial abnormalities in CFS patients (W.M.H. Behan, J Pathol 1992; 166: 213-214).

We obtained percutaneous needle muscle biopsies from 15 CFS patients and 15 age and sex-matched controls. We investigated previously reported ultrastructural abnormalities in CFS: subsarcolemmal mitochondrial aggregates, intermyofibrillar mitochondrial aggregates, mitochondrial circumference, area, pleomorphism and the presence of compartmentalization of the inner mitochondrial membrane. All of the steps of tissue processing, electron microscopy and data abstracting and analysis were performed in a totally blinded fashion. All of our data was rigorously quantified and statistically analyzed. We studied over 2,500 mitochondria.

We observed all of the previously reported abnormalities of mitochondrial ultrastructure in our CFS patients. However, in all of the parameters studied the results obtained from the controls were identical to those of the CFS patients.

Although there is no ultrastructural mitochondrial abnormality in CFS patients, other lines of evidence suggest the presence of a possible functional mitochondrial abnormality. This study was supported by the Chronic Fatigue Syndrome Association of Minnesota and by Sigma-Tau Pharmaceutical Company.

582.9

CHARACTERISTICS AND FUNCTIONAL CORRELATION OF THE EMG AND TORQUE OF THE STRETCH REFLEX IN SPASTICITY. F. Lin and M. Sabbahi*. School of Physical Therapy, Texas Woman's University, Houston, TX 77030.

The purpose of this study was to evaluate the contribution of stretch reflexes in the volitional and functional wrist performance in spasticity. The stretch reflexes of wrist flexors, evoked by ramp-and hold stretches, were recorded at four different stretch velocities and four levels of background muscle contraction in ten spastic stroke patients. The reflexive EMG (flexor carpi radialis) and wrist flexion torque responses were recorded. When compared with ten age-matched healthy subjects, the reflex gain and amplitude of the EMG short-latency response in spasticity, but not the torque response, were significantly higher than normal. The EMG to torque ratio of the stretch reflex response was also significantly higher in spastic patients. Unlike normal, the EMG short-latency reflex in spasticity was independent of the background muscle contraction. Three patterns of EMG long-latency response were recorded among spastic patients. Patients of pattern A (n=3, prolonged and enlarged long-latency reflex) tended to have lower spasticity and higher functional level. Patients of pattern B (n=2, reduced or absent long-latency reflex) tended to have higher spasticity and lower functional level. Patients of pattern C (n=5, delayed and enlarged long-latency reflex) were a mixed group. In average, the EMG long-latency reflex in spasticity was not higher than normal, which suggested that the long-latency reflex response has little contribution to spasticity. The reduced long-latency reflex response in pattern B was probably a result of inhibition from the preceding short-latency reflex. The degree of clinical spasticity (measured by the Ashworth scale) showed strong reverse correlation with the volitional (active range of motion, grip strength) and functional (Fugl-Meyer tests, Box & Blocks Test) movement performance and moderate correlation with the EMG response of the stretch reflex. No correlation was found between the degree of spasticity and the torque response of the stretch reflex. (This study was funded by training grant #40341 from NIDRR.)

582.10

THE EXPRESSION OF C-*FOS* AND C-*JUN* FOLLOWING DENERVATION AND REINNERVATION OF MUSCLE. H.D. Soares*, S.-C. Chen, T. Curran, and J.I. Morgan. Roche Inst of Molec. Biol, Nutley, NJ 07110

Previous studies in our laboratory have demonstrated prolonged increases of c-*fos* and c-*jun* mRNA in chronically denervated muscle suggesting a regulatory role for these two cellular immediate early genes during muscle fiber atrophy. We compared muscle fiber c-*fos* and c-*jun* expression following either sciatic nerve transection (chronic denervation atrophy) or sciatic nerve crush (reinnervation) in transgenic mice harboring either c-*fos*-lacZ or c-*jun*-lacZ transgenes. Sciatic nerve transection resulted in prolonged elevations of both c-*fos* and c-*jun* mRNA in denervated muscle for up to four weeks after trauma. However, sciatic nerve crush elicited only transient increases in c-*fos* and c-*jun* mRNA with levels returning to baseline values by two weeks post injury. Although increased c-*fos* levels were associated with nuclear Fos protein expression, elevated c-*jun* message was not accompanied by concomitant increases of nuclear Jun protein expression in denervated muscle. Interestingly, Fos-lacZ induction appeared first in muscle fiber nuclei adjacent to the motor endplates 24 hours following either sciatic nerve transection or crush. However, Fos-lacZ expression spread to adjoining muscle fibers at 2 and 4 weeks after sciatic transection while Fos-lacZ expression following crush injury was absent in muscle at later timepoints. In summary, these results suggest that c-*fos* expression is dependent upon the innervation status of muscle and may participate in the responses that contribute to degenerative muscle diseases elicited by muscle denervation.

582.11

HYDROCORTISONE (HC) INCREASES MEROSIN AND CAUSES ITS AGGREGATION IN ANEURALLY CULTURED HUMAN MUSCLE. J. McFerrin, V. Askanas* and W.K. Engel. USC Neuromuscular Center, Los Angeles, CA 90017-1969.

Merosin, a laminin isoform, is a newly-discovered component of the basal lamina of striated muscle and peripheral nerve. Merosin deficiency has been described as a cause of certain congenital myopathies (CM). Previously we demonstrated in aneurally cultured human muscle that glucocorticoids increase amounts of dystrophin and acetylcholine receptors (AChRs), and causes aggregation of dystrophin, AChRs, and utrophin. Aggregates of all three co-localize with each other.

Now we have studied the influence of HC on the distribution and amount of merosin in aneurally cultured human muscle. In 7 experiments, each from a different normal muscle biopsy, human muscle cultures were established from the satellite cells. Treatment with 20 uM HC was initiated after myoblast fusion was completed (approximately 8 days after cultures were established) and it was continued for 1,2, and 3 weeks. In both HC-treated and control cultures, increase of merosin was developmentally regulated. As compared to controls, in the HC-treated cultures the merosin was: a) increased approximately 2-fold; and b) localized along the surface of the fiber in very pronounced irregular aggregates, in distinct contrast to a uniform distribution in the controls. Unlike dystrophin and utrophin, merosin aggregation did not co-localize with AChRs. Our study demonstrates for the first time that treatment with glucocorticoid increases merosin and influences its localization. These results suggest that treatment with glucocorticoid may benefit some CM patients.

582.12

EXPRESSION OF INTERLEUKIN 10-MRNA IN WALLERIAN DEGENERATION AND DEMYELINATION OF THE RAT PNS G. Stoll*, S. Jander, J. Pohl and C. Gillen
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Interleukin 10 (IL10) is a potent immunosuppressant which blocks macrophages and TH1 cell mediated immune responses. In this study we localized mRNA for IL10 in the rat PNS by in-situ hybridization using a digoxygenin-labeled riboprobe specific for rat IL10. In normal sciatic nerves some Schwann cells (SC) expressed IL10-mRNA. After nerve transection the number of IL10-mRNA positive cells dramatically increased at days 2 and 4. Most IL10-mRNA positive cells could be identified as S100 positive SC by immunocytochemistry while ED1 positive macrophages were IL10-mRNA negative. By day 14 only occassional cells were IL10-mRNA positive. In immune-mediated demyelination induced by active immunization with peripheral nerve myelin macrophages transiently expressed IL10-mRNA. In conclusion our data suggest that SC provide a immunosuppressant system in the PNS that is activated after nerve transection. Moreover, IL10-mRNA is differentially regulated in macrophages in response to axotomy and during immune-mediated demyelination.

582.13

T Cell Receptor Vß15 Gene Usage in Guillain-Barré Syndrome A Khalili-Shirazi[1], NA Gregson[2], MA Hall[3], RAC Hughes[1], P Doherty[4], JS Lanchbury[3] Departments of Neurology[1], Anatomy & Cell Biology[2], Molecular Immunogenetics[3], Experimental Pathology[4], UMDS, Guy's Campus, London SE1 9RT, UK.

We set out to determine whether the T cell receptor (Tcr) Vß gene usage in acute inflammatory demyelinating polyradiculoneuropathy (AIDP) is restricted. We separated activated from non-activated peripheral blood T cells with anti-IL2 receptor (anti-CD25) antibody-labelled magnetic beads from four AIDP patients and 4 normal control (NC) subjects. The Tcr Vß gene usage of circulating activated and non-activated T cells was heterogeneous in all the patients and controls, but the activated T cells of all 4 of the AIDP patients showed a more limited usage of Vß genes and enhanced Vß15 usage, as compared to the unactivated T cells. This was not seen in the healthy controls. The activated and non-activated T cells from a patient with acute motor and sensory axonal neuropathy (AMSAN) showed a similar Vß gene usage to that of the controls. From a further patient with AIDP, we studied the Vß gene usage of short-term T cell lines reactive to the peripheral nerve myelin proteins P_2, P_0 and the P_0peptide amino acid sequence 184-208. The Vß gene usage of the lines was heterogeneous, but the cell line responsive to the P_0 peptide showed prominent usage of Vß15. We conclude that T cells activated during the immune response associated with AIDP preferentially use Vß 15. The limited Tcr usage of activated T cells may indicate a restricted response to a common antigen, or a role for an as yet undefined superantigen in the pathogenesis of AIDP.

582.14

EXPERIMENTAL MONONEUROPATHY: MICROENVIRONMENTAL MECHANISMS OF SENSITIZATION. H. Sasaki, M. Kihara, K. K. Nickander, P. J. Zollman, I. L. Smithson, J. D. Schmelzer*, E. E. Benarroch and P. A. Low. Department of Neurology, Mayo Clinic, Rochester, MN 55905.

The application of 4 loose ligatures to sciatic nerve results in spontaneous nerve activity and a reduction in thermal and mechanoreceptor thresholds. Fiber degeneration occurs, but the mechanism of fiber degeneration and sensitization is unknown. We evaluated nerve blood flow (NBF), morphometric alterations, norepinephrine, and neuropeptides at the lesion and the ipsilateral L5 dorsal root ganglia (DRG). Tissues were evaluated from days 2 to 45 in Sprague-Dawley male rats. NBF was measured using microelectrode H_2-polarography and was significantly increased within the lesion but reduced by >70% at the lesional edge. Norepinephrine (NE), measured by HPLC/electrochemical detection showed a large increase (x6) within the ligatures at day 5, x2 above the lesion and normal distal to the ligature. By day 14, there was a mild (40%) reduction in NE distal to the ligatures. Glyoxylic acid fluorescence confirmed increased noradrenergic fluorescence within the ligatures. Morphometric analysis showed that >75% fibers had undergone axonal degeneration at the lesion. We postulate that the ligatures create a dam where vasoactive agents (NE, CGRP, NPY) accumulate, increasing NBF and nociceptor sensitization. Ischemia occurs at the lesional edge, causing degeneration of myelinated fibers with relative sparing of unmyelinated fibers. A second site of sensitization occurs at the DRG (see Zollman et al. abstract), involving α-adrenoreceptors and changes in neuropeptide expression.

582.15

PERIPHERAL NEUROPEPTIDE PLASTICITY IN A QUADRUPLE LIGATURE MODEL OF PAINFUL NEUROPATHY. P. J. Zollman, E. E. Benarroch*, D. A. Wittrock, I. L. Smithson, L. M. Ivanjack, J. D. Schmelzer and P. A. Low. Department of Neurology, Mayo Clinic, Rochester, MN 55905.

Peripheral axotomy produces plastic changes in neuropeptide expression in the dorsal root ganglion (DRG) (Hokfelt et al., TINS 17:22-30, 1994). We sought to determine whether changes in concentration and distribution of neuropeptide Y (NPY), and calcitonin gene-related peptide (CGRP) in the DRG, sciatic nerve (ScN), and lumbar sympathetic ganglion (LSG) occur in the neuropathy model produced by application of 4 loose ligatures in the ScN. Male Sprague-Dawley rats underwent quadruple ligature on the left ScN; controls underwent ScN sham manipulation. Rats were sacrificed (pentobarbital) at 2 to 45 days thereafter. Bilateral ScN, DRG and LSG (L4-L5 levels) were harvested and processed either for radioimmunoassay or immunocytochemistry. There was a significant increase in the levels of NPY and CGRP in both the DRG and ScN, ipsilateral to the ligature. These changes correlated with behavioral indices and were most marked at 14 days. For NPY (n=6), DRG 12.5 ± 2 vs. 1.5 ± 1 pg/ml; $p=0.0005$, and ScN 20 ± 1 vs. 9.5 ± 2 pg/ml; n=6; $p=0.0015$). NPY was expressed in large DRG neurons on the ligature side. Increase of CGRP (n=6) was less dramatic in the DRG (30 ± 2 vs. 20 ± 2; $p=0.001$) than in ScN (39 ± 3 vs. 17 ± 1 pg/ml; $p=0.0001$). Thus, in the 4 ligature model of sciatic neuropathy, there is up-regulation of NPY expression in the corresponding DRG neurons. Local increases in CGRP may contribute to increased ScN blood flow within the lesion.

582.16

OXPHOS PROTEIN DEFECTS IN ALZHEIMER'S DISEASE, IN PARKINSON'S DISEASE AND IN MITOCHONDRIAL ENCEPHALOMYOPATHIES REVEALED BY BLUE NATIVE ELECTROPHORESIS. H. Schägger[1], S. Diekmann*[2,3], and T.G. Ohm[2,3]. Zentrum der [1]Biologischen Chemie, [2]Morphologie, Universitätsklinikum, Frankfurt am Main, [3]Institut Anatomy, Charité, Berlin, Germany.

Blue native electrophoresis is a novel method for near-quantitative isolation of native membrane protein complexes from biological membranes and for molecular mass determination of native protein complexes (H. Schägger & G. von Jagow (1991) Anal. Biochem. 199, 223-231; H. Schägger et al. (1994) Anal. Biochem. 217, 220-230). Combined with Tricine-SDS-PAGE in the second dimension a two-dimensional micro-scale technique was elaborated for the quantification of OXPHOS enzymes from 10-20 mg specimens from skeletal muscle, heart, liver and brain (H. Schägger (1995) Meth. Enzymol. 260, in press).

In Alzheimer's disease complex V amounts in hippocampus were selectively reduced to 50% of the controls. Energy shortage might be a reason for cell degeneration.

In Parkinson's disease the amounts of complex I proteins in substantia nigra were found to be normal, whereas catalytic activities were reduced. This favors models implicating endogeneous or exogeneous inhibitors like MPP$^+$.

The 2D-electrophoretic technique proved to be a highly sensitive method for the detection of single and multiple OXPHOS defects in mitochondrial encephalomyopathies. The effects of point mutations in mitochondrial DNA were demonstrated for the first time directly at the protein level. The protein analysis is rather independent of the post mortem delay in contrast to the analysis of mRNA levels.

582.17

HETEROPLASMY AND LESIONS IN THE CNS AND SYSTEMIC ORGANS OF MELAS. T. Yoshida[1], R. Fukatsu[1], Y. Takamaru[2], K. Tsuzuki[3], K. Kimura[3], Y. Hayashi[1], K. Utsumi[1], T. Teraoka[1], T. Inoue[2]*, K. Tashiro[4], N. Fujii[3], and N. Takahata[1]. [1]Dept. of Neuropsychiatry, [3]Dept. of Microbiology, Sapporo Med. Univ. Sch. of Med. [2]Dept. of Psychiatry, [4]Dept. of Neurology, Hokkaido Univ. Sch. of Med., Sapporo 060, Japan

Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episode (MELAS) is recognized as a distinctive clinical entity in mitochondrial diseases. Recent advances indicated that genetic abnormality in mitochondrial DNA is responsible for mitochondrial respiratory enzyme complexes. But it is still unanswered whether or not this genetic deficit is involved in pathogenesis, and distribution of lesions in the CNS and systemic organs.

Case; 24 year old Japanese female. She had a mild delay in psychomotor development, and experienced generalized convulsive seizure at age 17, After recurrent episodes of right hemiplegia. hemi anopsia, she began to show difficulty in understanding and expressing speech and repetitive behaviors. MRI (T2) revealed high signal areas in bi-temporal and occipital lobes. Elevated level of lactic acid and pyruvate in serum and CSF was notable. She became mutistic and terminated in Kluver-Bucy like state, and died from intractable paralytic ileus.

Neuropathological findings were consistent with those of reported cases. Multiple necrotic foci accentuated in the parietal and occipital lobes. Ragged-red fibers seen in skeletal muscle, and cardiomyopathy. Extended mitochondria were found in GI tracts. Heteroplasmy of wild type and mutant DNA were examined using PCR and Apa I digestion. The results suggest that there is a tendency in correlation between heteroplasmy and distribution of the lesion.

Present case was considered to be a typical case of MELAS both clinically and pathologically. Further studies are needed to understand the characteristic distribution.

583

SYMPOSIUM. THE ROLE OF THE CEREBELLUM IN COGNITION. T.Thach, Washington Univ. Med. Sch. (Chairperson); J.Fiez, Univ.Iowa; R.Ivry Univ. Calif. at Berkeley; P.Strick, VA Medical Center and SUNY-Health Science Center at Syracuse.

Does the cerebellum contribute to mental activity? Julie Fiez will describe mental task performances that activate the cerebellum during functional imaging studies and aspects that are impaired by cerebellar ablation. These tasks and aspects include producing verbs in response to nouns, the Tower of Toronto game, the detection of performance errors, and the improvement in performance with repeated performance. Richard Ivry will discuss the idea that the cerebellum can be conceptualized as an internal timing mechanism. He will argue that this hypothesis can account for the deficits observed in motor performance, certain perceptual tasks, and sensorimotor learning.

How might the cerebellum control mental activities? Peter Strick will discuss the new findings that the primate cerebellum projects via thalamus to areas 6, 8, 9, 46 in the frontal lobe cortex. These areas are active in certain mental operations, and these pathways provide a means whereby the cerebellum can influence them.

What specifically might the cerebellum contribute? Tom Thach will summarize the principles thought by Marr and Albus to underly the cerebellar role in movement coordination and motor learning, and the favorable evidence. These principles will then be extended to include mental operations carried on by the above areas of cerebral cortex as influenced by their cerebellar inputs. The suggestion is that many of the mental operations may be related to mental movement, which in turn is supported by these cortical areas. A specific cerebellar contribution may be the linkage of elements within these cortical areas together through trial and error learning into a synergic combination, and its linkage to a behavioral context, whose occurrence can then automatically trigger the combined response.

584

SYMPOSIUM: LIGAND-GATED ION CHANNEL SUPERFAMILY FEUD. R.J. Lukas, Barrow Neurological Inst. (Chairperson); J. Lindstrom, Univ. of Pennsylvania; R.W. Olsen, UCLA; S.F. Heinemann, Salk Inst.; David J. Julius, UCSF.

Members of the ligand-gated ion channel (LGIC) subset of neurotransmitter receptors are multi-component, cell surface complexes that directly regulate transmembrane ionic permeability and cellular electrical excitation/inhibition throughout the nervous system. These features distinguish LGIC from the metabotropic subset of monomeric/second messenger signaling-coupled neurotransmitter receptors, from voltage-gated ion channels, and from ion channels in the plasma membrane or on microsomes that respond to intracellular ligands. Recent advances have shown that LGIC exist as a much more extended and structurally diverse group of macromolecules than heretofore realized. This session will review genetic/epi-genetic bases and functional manifestations of this receptor diversity.

Ronald J. Lukas will provide a fundamental introduction to the field and will briefly discuss use of stably transfected cell lines in studies of homooligomeric nicotinic acetylcholine receptor (nAChR) subtypes. **Jon Lindstrom** will discuss the genetic basis of nAChR subunit diversity, structural and functional manifestations of variations in nAChR subunit composition, and physiological and pathological roles of different nAChR. Similarly, **Richard W. Olsen** will describe the structurally and functionally diverse family of ionotropic GABA receptors (GABA-R) and their roles in nervous system function. The presentation by **Stephen F. Heinemann** will concern the biology of ionotropic glutamate receptors (GluR), some of their roles in learning and disease, and the placement of GluR subunits into a gene superfamily distinct from that containing GABA-R, nAChR, and glycine receptor subunit genes. **David J. Julius** will discuss how identification of ionotropic receptors for ATP establishes yet another neurotransmitter receptor gene superfamily, why ionotropic serotonin receptors belong in the GABA/glycine/nicotine receptor superfamily, and roles of ionotropic serotonin or ATP receptors in neurotransmission and behavior.

586.1

NEURONAL RESPONSES RELATED TO MEMORY RETRIEVAL IN THE PRIMATE INFEROTEMPORAL CORTEX.

Y. Naya, K. Sakai* and Y. Miyashita. Dept. of Physiol., Sch. of Med., Univ. of Tokyo, Tokyo 113, Japan.

Visual long-term memory has been assessed by the pair-association (PA) task, in which a subject retrieves and chooses the paired associate of a cue picture. Our previous report on pair-recall neurons suggests that the anterior inferotemporal (AIT) cortex is involved in the processes of memory retrieval (Sakai & Miyashita, *Nature* **354**, 152). To test the possibility that the response of pair-recall neurons is triggered by the initiation of memory retrieval, we devised the pair-association with color switch (PACS) task. Twelve pairs of Fourier descriptors were used as visual stimuli, each pair containing a green picture and a cyan picture. In the PACS task, the necessity for memory retrieval and its initiation time were controlled by a color switch in the middle of the delay period. A control task, in which there is no color switch, corresponds to the conventional delayed matching-to-sample (DMS) task where the monkey chooses the same picture as the cue. After two macaque monkeys were trained in both tasks, extracellular discharges of single neurons were recorded from the AIT cortex. We found that pair-recall neurons started to respond just after the color switch in the PACS task, when the cue-optimal picture's associate was presented as a cue. However, they showed no response change in the DMS task. This effect was statistically significant among picture-responsive delay neurons (n = 29) including pair-recall neurons (n = 12). We confirmed that this effect is not due to the visual response to colors. Furthermore, when the cue-optimal picture was presented as a cue, these neurons showed suppression after the color switch in the PACS task, whereas they showed sustained activation in the DMS task. These results demonstrate that the response of AIT neurons can be selectively triggered by the retrieval of paired associates from long-term memory store.

586.2

EFFECTS OF SELECTIVE ABLATIONS OF INFERIOR TEMPORAL CORTEX OF SQUIRREL MONKEYS ON VISUAL PERCEPTION AND MEMORY.

R. E. Weller*, M. S. LeDoux, L. M. Toll, M. K. Viikinsalo, and R. A. Hicks. Dept. of Psychology, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Anatomical studies have suggested that inferior temporal (IT) cortex of squirrel monkeys consists of caudal (ITC), intermediate (ITI), and rostral (ITR) areas, with ITC containing dorsal (ITCd) and ventral (ITCv) subdivisions. We have hypothesized that ITCv is homologous to area TEO of macaque monkeys, and ITI and ITR are homologous to posterior and anterior TE of macaques (Weller and Steele, 1992; Steele and Weller, 1993). The present study compared visual memory and pattern discrimination learning in squirrel monkeys with bilateral ablations of ITCv (n=4), ITI and ITR (n=4), or ITI and ITR plus ventrally adjacent cortex in the region of perirhinal cortex ("ITR+", n=3), with unoperated animals serving as controls (n=3). Animals were tested in a reduced WGTA on two visual memory tasks, delayed non-matching to sample (DNMS) with trial-unique objects and concurrent object discriminations, and five pairs of two-dimensional pattern discriminations. Only the ITR+ animals were significantly impaired compared to the controls on difficult variations of DNMS and the concurrent object discriminations. Although all lesioned groups were significantly worse than the controls learning three of the pattern discriminations, the ITCv animals showed the greatest deficits. Thus, the behavioral results support the suggested homologies between subdivisions of IT cortex in squirrel and macaque monkeys. These results also help to define the relative contributions of rostral IT cortex and perirhinal cortex to vision.

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586.3

DIFFERENT BEHAVIORAL EFFECTS OF DAMAGE TO VISUAL AREA TE AND PERIRHINAL CORTEX.

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Previous lesion studies of visual association area TE have reported marked impairment on tests of visual perception and visual memory. However, most previous TE lesions included damage to what is now known to be perirhinal (PR) cortex, a component of the medial temporal lobe memory system important for memory. It is possible that in previous studies of TE lesions, the inclusion of PR cortex contributed to the behavioral impairment. We compared the performance of 5 monkeys with TE lesions, 5 monkeys with PR lesions, and 7 normal monkeys (N). Both the TE and PR groups were similarly impaired on the trial-unique delayed nonmatching to sample task (percent correct averaged across delays of 15 sec, 60 sec, 10 min, and 40 min: TE=74.9, PR=75.2, N=81.0; TE or PR vs N, $p < .05$). Monkeys with TE lesions were impaired on the 8-pair concurrent discrimination task (TE=933 trials, N=469 trials, $p < .05$) but were unimpaired on retaining single object discriminations (TE=89% correct, N=86% correct, $p > .10$). By contrast, monkeys with PR lesions showed an opposite pattern (concurrent score = 639 trials, object score = 82%). Analysis of variance (using standardized scores) revealed a significant group by task interaction ($p < .05$). The findings provide the first evidence that within the visual domain, the deficit following TE lesions is different from the deficit following PR lesions. This conclusion is tempered by the fact that on the concurrent task, two of the PR monkeys performed within the range of the monkeys with TE lesions.

586.4

IMPORTANCE OF THE HIPPOCAMPAL REGION AND ENTORHINAL CORTEX IN HUMAN MEMORY: NEUROPSYCHOLOGICAL AND NEUROPATHOLOGICAL FINDINGS FROM A NEW PATIENT

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Following an ischemic episode that resulted in a circumscribed, bilateral lesion of the entire CA1 field of the hippocampus, patient RB exhibited a moderately severe anterograde memory deficit. Last year we reported neuropsychological and postmortem neuropathological findings from two additional cases of amnesia resulting from ischemia or anoxia, i.e., patients GD and LM (Rempel-Clower et al., *SN Abst.*, 1994, 20:1075). Patient GD had damage limited primarily to the CA1 field of the hippocampus and, like RB, had moderately severe anterograde amnesia and little detectable retrograde amnesia. Patient LM had more extensive damage to the hippocampal region, including all the CA fields and the dentate gyrus. He also sustained slight cell loss in entorhinal cortex, likely due to retrograde degeneration. The severity of LM's anterograde amnesia was similar to that of the other patients, but he had severe, temporally graded retrograde amnesia. We now report findings from an additional patient, WH, whose amnesia resulted from a probable ischemic event and who had more severe anterograde amnesia than patients RB, GD, or LM. Patient WH, like LM, had severe, temporally graded retrograde amnesia. Postmortem analyses revealed that patient WH had cell loss in all CA fields of the hippocampus, the dentate gyrus, and the subiculum. There was also substantial cell loss bilaterally in layers III, V, and VI of entorhinal cortex. The findings from all four patients suggest that damage limited to the hippocampal region is sufficient to produce moderately severe anterograde amnesia. More extensive damage to the hippocampal region, possibly together with damage to entorhinal cortex produces severe, temporally graded retrograde amnesia.

586.5

IMPAIRED PROBABILISTIC CLASSIFICATION LEARNING IN HUNTINGTON'S DISEASE

B.J. Knowlton*, J.S. Paulsen, and L.R. Squire Depts of Psychiatry and Neurosciences, UCSD, and VA Med Center, San Diego, 92161

Damage to the hippocampus and related areas results in impaired declarative memory, while sparing a variety of other memory abilities in a range of species. An area that may be important for nondeclarative memory is the caudate nucleus. Double dissociations have been found in experimental animals between the effects on memory of hippocampal lesions and caudate lesions. Tasks largely dependent on the hippocampus and related areas include win-shift learning problems and delayed nonmatching to sample tasks. Tasks largely dependent on the caudate nucleus include win-stay learning problems and pattern discrimination tasks, or what has been termed habit learning. A difficulty in extending the dissociation between habit learning and declarative learning to humans is that many tasks learned gradually as habits by experimental animals can be acquired by humans using a declarative (memorizing) strategy in just a few trials. Recently, amnesic patients were found to perform normally on a probabilistic classification task that is analogous to a habit learning task in that information must be learned gradually across many trials. There were 4 pattern cues, each probabilistically associated with one of two outcomes. On each trial, subjects were presented with 1, 2, or 3 of these cues, and after selecting one of the 2 outcomes, they received feedback as to whether their response was correct. Patients with Huntington's disease (HD, n=12) were tested on this task because they have prominent pathology in the caudate nucleus. Unlike amnesic patients, HD patients exhibited no learning across 50 training trials, and they performed significantly worse than a matched control group (n=12). These results are consistent with the idea that probabilistic classification learning in humans is analogous to habit learning and, like habit learning, it depends on the integrity of the caudate nucleus.

586.6

DIFFERENCES IN THE FLEXIBILITY OF DECLARATIVE AND NONDECLARATIVE MEMORY.

P.J. Reber*, B.J. Knowlton and L.R. Squire. Department of Psychiatry, UCSD and VA Medical Center, San Diego, 92161.

Declarative memory is dependent on medial temporal lobe/diencephalic brain structures. In an effort to develop a property list for declarative memory, we tested the hypothesis that declarative memory is more flexible than nondeclarative memory. Amnesic patients (n=8) and control subjects (n=16) completed a probabilistic classification task (presented as weather prediction) previously shown to be intact in amnesia and impaired in patients with Huntington's disease. In this task, four cues were each probabilistically associated with one of two outcomes (rain or shine). On each of 50 training trials, subjects saw 1, 2, or 3 of the cues, they predicted the outcome, and they received feedback. Subjects were then given a 13-item test that asked about their task knowledge in ways that required, to a varying extent, flexible application of what they had learned. For example, subjects were asked to select the one cue most associated with a given outcome. Amnesic patients and control subjects exhibited equivalent, gradual learning of the task. On the test that followed, amnesic patients were impaired on some items, e.g. they had difficulty giving a numerical rating for cues that were weakly associated with the outcome in question. In a second experiment, new control subjects completed the classification task and the follow-up 13-item test, and they were additionally asked to rate to what extent each question required indirect, flexible use of task knowledge. Those items rated as requiring the most flexible use of knowledge tended to be the same items on which amnesic patients exhibited the greatest impairment. The flexibility exhibited by control subjects most likely reflects the concomitant acquisition of declarative knowledge about the task, which was epiphenomenal to acquisition performance. The performance of the amnesic patients suggests that knowledge acquired implicitly is less flexible than declarative (explicit) knowledge and is used less effectively in situations that are dissimilar to the original learning conditions.

586.7

CONSERVATION OF A PROCESS FOR SPATIAL REPRESENTATION AND REORIENTATION ACROSS HUMAN ADULTS, CHILDREN, AND ADULT RATS. L. Hermer, E. Spelke, and L. Nadel.* Department of Psychology, Cornell University, Ithaca, NY 14853

A wealth of behavioral and physiological evidence suggests that spatial representations for navigation are only formed with reference to spatial orientation and that spatial orientation is calibrated by spatial representations of the environment (e.g. see McNaughton et al., 1995). Adult rats can reorient themselves using representations of the shape of the environment with only minimal exposure the fixed positions of cues within it (Cheng, 1986; Margules & Gallistel, 1988; Biegler & Morris, 1993, ex. 2), and they fail to reorient by nongeometric information such as distinctive odors, visual patterns, or brightnesses unless they have extensive associative training (Knierim et al., 1994). We have previously shown that children aged 18-24 mos., like adult rats, reorient in a novel rectangular environment exclusively in accord with environmental shape, even when other information is perceived, is remembered, and can be used after the same exposure to solve other memory tasks (Hermer & Spelke, 1994, 1995). In contrast, human adults readily use nonspatial information (e.g. wallcolor) or conjunctions of spatial and nonspatial information to solve these reorientation tasks. We used a series of concurrent tasks designed to interfere with specific cognitive processes to probe in adults whether the geometric and nongeometric processes were separable and whether the child's geometric reorientation process was conserved in adults. Here we report that adults engaged in a verbal interference task lose their distinctive ability to use nonspatial information in reorientation tasks, and instead rely exclusively on the shape of the environment. However, adults engaged in the same interference task were able to use surface color and pattern to retrieve a movable hidden object, showing that they like young children had encoded and could use this information in another context. These results suggest biological conservation across adult rats, young children, and human adults of a process for representing the shape of the environment and for using it to recalibrate compass sense. This process appears to be relatively independent of language and attention, unlike the nongeometric process that distinguishes human adult performance in these tasks from that of young children and other adult mammals. The results are discussed with respect to psychological and physiological models of spatial information processing in these species.

586.8

MONKEYS WITH EARLY HIPPOCAMPAL FORMATION LESIONS ARE IMPAIRED ON THE TRANSVERSE PATTERNING PROBLEM. M. C. Alvarado*, A. A. Wright & J. Bachevalier. Department of Neurobiology & Anatomy, U. T. Medical School, Houston, TX 77225

The transverse patterning problem (A+ vs. B-; B+ vs. C-; C+ vs. A-) is a task that requires subjects to use configural associations and is sensitive to hippocampal formation damage in rodents [Alvarado & Rudy, 1995, Beh. Neurosci., 109(2):204-211]. To determine whether the hippocampal region in non-human primates is also important for performance on this task, we trained five adult rhesus macaques (*Macaca mulatta*) on an object-discrimination version of transverse patterning (TP). Three monkeys were normal (N) and 2 had neonatal aspiration lesions of the hippocampal region, including parahippocampal gyrus (H). Because all subjects had extensive training on nonmatching-to-sample paradigms, the TP task was designed to discourage subjects from erroneously using the nonmatching strategy. Thus, animals were trained on two sets of TP discriminations, members of which were presented on alternate trials such that a given trial contained no objects from the preceeding trial. Six objects (A-F) were used to form two sets: Set 1-- A+ vs. B-; B+ vs. C- and C+ vs. A-; Set 2-- D+ vs. E-; E+ vs. F- and F+ vs. E-. Animals received five trials of each problem daily (total: 30 trials). Training continued until animals reached a criterion of 27/30 correct for 60 consecutive trials on each problem set, or a maximum of 1000 trials on each set. Group N solved both sets in an average of 325 trials wherease Group H did not reach criterion on either set. To assess whether their impairment was due to an inability to solve six discriminations under conditions of high interference, we altered the task slightly by adding a new object to each problem set so that the task could be solved without the use of configural associations. The problems were constructed as follows: Set 1-- A+ vs. B-; B+ vs. C- and C+ vs. X-; Set 2-- D+ vs. E-; E+ vs. F- and F+ vs. Y-. Animals in Group H reached criterion on both sets in five sessions. These results suggest that the hippocampal region in primates is important for configural or relational memory.

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586.9

MAMILLARY BODY LESIONS IN RHESUS MONKEYS IMPAIR OBJECT-IN-PLACE MEMORY AND LEAVE THE FORNIX WITHOUT FUNCTION. D. Gaffan*, A. Parker and S. A. Gutnikov. Exp Psychology, Oxford University, Oxford OX1 3UD, U.K.

Six monkeys were trained pre-operatively in an automated object-in-place memory task in which they learned 20 new scenes in each daily session. Three of the six monkeys then received stereotaxically guided mamillary body lesions, leaving the fornix intact, while the other three received a control operation. Postoperatively the control animals' rate of learning new scenes was unchanged but the animals with mamillary body lesions showed a severe impairment, equal to that seen in previous experiments after fornix transection. All six animals were then given fornix transection, in addition to the existing mamillary or control lesion. The control group now showed, after fornix transection, an impairment equal to that of the animals with mamillary body lesions alone. But the animals with mamillary body lesions did not show any additional impairment following fornix transection. We conclude that (1) the role of the mamillary bodies in a model of human episodic memory is as important as the role of the fornix (2) the fornix and mamillary bodies form a single functional memory system, since the effect of lesions in both parts is no more severe than the effects of a lesion in one of the parts alone (3) the idea that the functional effects of fornix transection result from cholinergic deafferentation of the hippocampus receives no support from the present results; rather, they support the idea that in primates the fornix and mamillary bodies, together with connected structures including the subiculum, mamillothalamic tract and cingulate bundle, form a cortico-cortical association pathway for episodic memory.

586.10

THE REPRESENTATION OF SPACE IN THE PRIMATE HIPPOCAMPUS. E.T.Rolls*, R.G.Robertson and P.Georges-Francois.
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It has recently been possible to extend the analysis of hippocampal function in spatial and memory functions in primates (Rolls and O'Mara, 1993; O'Mara et al, 1994; Treves and Rolls, 1994) by making recordings in monkeys actively walking in the laboratory, so that the activity of monkey hippocampal neurons can be analysed during active spatial exploration in environments similar to those in which "place" cells are found in rats. In a sample of 250 cells recorded in this situation, no "place" cells have so far been found. Instead, we have found a considerable population of "view" cells tuned to respond when the monkey looks at small parts of the environment. We have been able to demonstrate (1) that these hippocampal neurons respond to a view of space 'out there', not to the place where the monkey is; (2) that the responses depend on where the monkey is looking, by measuring eye position; (3) that the responses in some cases depend on the location of particular cues in the environment; (4) that the responses in some cases are not affected if the view details are obscured with curtains; and (5) that the cells retain part of their "space" tuning even in complete darkness, for several minutes. These findings lead to a reconceptualization of what is represented in the primate hippocampus, and of how it functions in memory and spatial processing.

Rolls,E.T. and O'Mara,S. (1993) Ch. 17, pp. 276-300 in Brain Mechanisms of Perception and Memory: From Neuron to Behavior, eds. T.Ono, L.R.Squire, M.E.Raichle, D.I.Perrett and M.Fukuda. Oxford University Press: New York.

O'Mara,S.M., Rolls,E.T., Berthoz,A. and Kesner,R.P. (1994) Neurons responding to whole-body motion in the primate hippocampus. Journal of Neuroscience 14: 6511-6523.

Treves,A. and Rolls,E.T. (1994) A computational analysis of the role of the hippocampus in memory. Hippocampus 4: 374-391.

586.11

NECESSARY ROLE OF HIPPOCAMPUS PLUS SUBJACENT CORTEX BUT NOT AMYGDALA IN CONDITIONAL MOTOR LEARNING. E. A. Murray* and S. P. Wise, Labs. of Neuropsychology and Systems Neuroscience, NIMH, NIH, Bethesda, MD 20892

We investigated whether either the amygdala plus subjacent cortex or the hippocampus plus subjacent cortex was necessary for conditional visuomotor learning. On each behavioral trial, rhesus monkeys were presented with a single visual stimulus in the center of a videomonitor screen. The stimulus remained on the screen until the monkey responded by making one of three possible joystick movements: left, right, and down. For each stimulus, one and only one response was scored correct and reinforced. Monkeys were required to learn a total of 24 new visuomotor conditional associations (problems) in 400 trials; 8 sets of 3 novel problems each were administered for 50 trials apiece. After the monkeys had learned the task, they received bilateral aspiration lesions of either the amygdala plus subjacent cortex (N=2) or hippocampus plus subjacent cortex (N=4). To measure the effect on learning of new visuomotor conditional problems, we examined the mean percent error obtained in the last 5 days of preoperative learning with the last 5 (of 15) postoperative days. Removal of the amygdala plus subjacent cortex (N=2) had no significant effect on the rate of learning. This finding falsifies the hypothesis that the amygdala is necessary for reward-mediated learning, in general. By contrast, removal of the hippocampus plus subjacent cortex (N=4) had a substantial effect on learning in each subject (mean percent error of 37.0 compared with 11.6). We conclude that the hippocampal formation plus subjacent cortex is necessary for the efficient association of arbitrary visual stimuli with spatially-directed movements.

586.12

THE ISSUE OF PARAHIPPOCAMPAL CORTEX IN THE RAT R.D. Burwell* and D.G. Amaral. Center for Behavioral Neuroscience, State University of New York at Stony Brook, Stony Brook, NY 11794-2575.

Neuropsychological studies of amnesic patients and experimental lesion studies in monkeys indicate that the perirhinal and parahippocampal cortices are important components of the medial temporal lobe memory system. Studies of the cortical connectivity of these regions in the monkey show that they receive distinctly different complements of cortical inputs suggesting that each uniquely contributes to memory function. In support of this notion, experimental lesions of the monkey perirhinal cortex, but not the parahippocampal cortex, impair visual object recognition memory (Ramus, S.J., Zola-Morgan, S. and L.R. Squire, *Soc. Neurosci. Abstracts* 20(2): 1047, 1994). Within the framework of these studies in the monkey, it is of interest to determine whether there are distinct cortical regions in the rat that are homologous to the perirhinal and parahippocampal cortices in the monkey. Our neuroanatomical studies of perirhinal cortex (areas 35 and 36) in the rat indicate that the rostral and caudal portions exhibit distinctly different cytoarchitectonic and histochemical characteristics. Different enough, in fact, to be considered separate cytoarchitectonic regions. Moreover, these rostral and caudal portions of perirhinal cortex demonstrate different patterns of cortical and subcortical connectivity. Thus, we use the term perirhinal to encompass rostral areas 35 and 36 and the term postrhinal to designate caudal areas 35 and 36 in the rat. The connectivity of these regions suggests that the postrhinal cortex in the rat shares a number of connectional characteristics with the parahippocampal cortex in the monkey. For example, like the parahippocampal cortex in the monkey, the postrhinal cortex, but not the perirhinal cortex, in the rat receives a strong input from the retrosplenial cortex. Cytoarchitectonic and connectional characteristics of the perirhinal and postrhinal cortices in the rat will be discussed.

587.1

INVOLVEMENT OF THE HYPOTHALAMUS IN AFRICAN TRYPANOSOMIASIS: MICROGLIA INVASION AND EFFECTS OF INTERFERON-γ AND MELATONIN. **M. Bentivoglio*[1], G. Grassi-Zucconi[2], Z.-C. Peng[1]; K. Kristensson[3].** [1]Inst. Anat., Univ. Verona, [2]Dept. Cell Biol., Univ. Perugia, Italy; and [3]Dept. Neurosci., Karolinska Inst., Stockholm, Sweden

Previous experimental evidence pointed out the involvement of the hypothalamus in the experimental infection in rats with the parasite *Trypanosoma brucei* (Tb), which causes sleeping sickness in humans. Interferon-γ (IFN-γ), which is promptly induced during the infection in both experimental rats and humans, plays a crucial role in trypanosomiasis by promoting Tb proliferation. Three experimental paradigms were adopted in the present investigation. A) Recombinant IFN-γ was intracerebroventricularly administered in normal rats, and the induction of the immediate early gene c-*fos* was used as a tool to detect neuronal activation in the brain, exploiting Fos-immunocytochemistry. After IFN-γ injection, Fos-immunoreactive (ir) neurons were selectively concentrated in the hypothalamic suprachiasmatic nucleus (SCN), which plays a role of circadian pacemaker. B) In advanced stages of Tb infection in rats, glial cells were immunocytochemically investigated in the brain using anti-GFAP and anti-OX-42 antibodies. The most consistent finding was represented by a proliferation of OX-42-ir microglial cells in the hypothalamus of the infected rats. These findings suggest that IFN-γ can exert both a direct and glial-mediated effect on the hypothalamus during Tb infection. C) Previous data indicated that the infected rats display a striking sleep fragmentation, with a considerable reduction of synchronized sleep (SS). Melatonin (3 mg/kg s.c. in 50% DMSO) was acutely administered to rats in advanced stages of Tb infection. This treatment resulted in a significant increase of the length of SS episodes in the infected rats in respect to the vehicle-exposed ones. Thus, exogenous melatonin, whose receptors are normally concentrated in the SCN, restored a normal sleep pattern during the infection.

587.2

LOCAL RATES OF CEREBRAL PROTEIN SYNTHESIS MEASURED IN SLEEPING MONKEYS ARE POSITIVELY CORRELATED WITH PERCENT TIME IN DEEP SLEEP. C. Beebe Smith*, T. Dang, M. Ito, K. Mori, H. Nakanishi, R.K. Nakamura, H. Namba, F. Storch, S. Suda, Y. Sun, C. Gillin, W. Mendelson, M. Mishkin, C. Kennedy, and L. Sokoloff. NIMH, Bethesda, MD 20892.

Regional rates of cerebral protein synthesis ($lCPS_{leu}$) were determined with the autoradiographic L-[1-^{14}C]leucine method (Smith et al., *PNAS* 85:9341, 1988) in 7 awake and 7 asleep rhesus monkeys. Monkeys were previously prepared to permit monitoring of behavioral state and conditioned to sleep in a restraining chair in a darkened, ventilated chamber. Prior to injection of [^{14}C]leucine all animals slept until their first REM period (sleep duration of 1-3 h). Controls were then awakened and remained awake. Stages of sleep in experimental animals were scored by analysis of EEG, EOG and EMG. This indicated that NREM sleep occupied 71 to 99% of the experimental period, the time spent in deep sleep (stage IV) varying from 0 to 84%. Compared to awake controls, mean values for $lCPS_{leu}$ were higher in all 57 regions in the sleeping animals, but the differences reached statistical significance in only four regions. However, in all regions, $lCPS_{leu}$ was positively correlated with percent time in deep sleep, weighted for the integrated specific activity of leucine in gray matter during the periods of deep sleep. The correlation coefficient reached statistical significance in 18 of the 57 regions ($p < 0.05$) and approached this level of significance in an additional 16 regions ($0.05 < p < 0.1$). Regions in hypothalamus, brain stem, and basal ganglia were predominantly involved. These results suggest that, in the sleeping monkey, deep sleep is accompanied by widespread increases in rates of cerebral protein synthesis.

587.3

REGIONAL CEREBRAL BLOOD FLOW CHANGES DURING DIFFERENT STAGES OF SLEEP IN HUMANS STUDIED BY POSITRON EMISSION TOMOGRAPHY. N. Hofle, D. Reutens, P. Fiset, A. Alonso*, J. Gotman, A. Evans and B.E. Jones. Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada H3A 2B4.

The present study was undertaken to examine the regional cerebral blood flow (rCBF) changes that occur during different stages of sleep in humans in order to determine if specific regions are activated or inactivated in association with spindling and delta EEG activity. PET scans were performed at night on 5 normal subjects using bolus injections of $H_2{}^{15}O$, in association with on-line computer EEG recording and sleep stage determination for 1) relaxed wakefulness with eyes closed, marked by alpha activity (8.5-11.5 Hz) in the EEG, 2) stage 2 sleep, marked by spindles (12-15 Hz), and 3) stage 3-4 sleep, marked by delta waves (1.0-4 Hz). Resulting PET images were co-registered with high resolution MRI scans. A marked and significant decrease in rCBF occurred in the thalamus during both stage 2 sleep in association with spindles ($t = -8.76$) and stage 3-4 sleep in association with delta waves ($t = -8.17$), as compared by averaged image subtraction to relaxed wakefulness with alpha activity. The decrease was centered in the dorsomedial nuclei and extended out to the peripheral edge of the dorsal and ventral, lateral nuclei through the anterior to posterior extent of the dorsal thalamus. These results indicate that the dorsal thalamus, which represents the afferent gateway to the cerebral cortex, is markedly inactivated in association with both spindle and delta activity during sleep. The inactivation of the dorsal thalamus, which could result from inhibition by surrounding reticularis GABAergic neurons, may represent the primary substrate for the loss of consciousness that occurs in humans during slow wave sleep. (Supported by MRC and CONICIT.)

587.4

RE-ENTRAINMENT OF SCN CIRCADIAN RHYTHMS FOLLOWING A 12 HOUR LIGHT-DARK CYCLE PHASE SHIFT: AN *EX VIVO* STUDY IN THE RAT. Valentin K. Gribkoff*, Rick L. Pieschl, and Frank D. Yocca. Central Nervous System Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492.

Rat suprachiasmatic nucleus (SCN) neurons *in vitro* discharge in a pattern that may have a relationship to circadian rhythms in behavior observed *in vivo*. We have examined the issue of whether SCN rhythms *in vitro* reflect the behavioral history of the animal in the period prior to sacrifice by investigating (*ex vivo*) the re-entrainment of SCN rhythms of rats subjected to a 12 hr shift in the 12/12 light/dark (L/D) cycle. Previous studies have shown that behavioral rhythms require about 7 days to re-entrain following a 12 hr shift in the L/D cycle produced by coupling 2 light periods. Slices including the SCN were obtained from adult male Long-Evans rats at 1-14 days after the 12 hr phase shift and the SCN multiple-unit activity recorded for up to 72 hrs following sacrifice. The peak firing rate for unshifted control animals was observed at CT 5.8±0.3, while shifted animals phase-delayed (relative to control) by 3.2±0.5, 5.0±0.7, 8.6±0.4, 11.5±0.5, and 11.1±0.8 hrs on days 1,2,4,7 and 14, respectively, suggesting that the SCN activity *in vitro*1.) reflected the behavioral and physiological status of the animal prior to sacrifice, and 2.) was fully re-entrained to the phase shift by day 7. The pineal hormone melatonin (MEL) was given (s.c., 1 mg/kg) to animals exposed to an identical 12 hr L/D phase shift and its effects on re-entrainment were observed at days 1-4. MEL-treated animals phase delayed 5.6±1.2, 7.6±0.8, and 9.8±0.4 hrs on days 1,2 and 4, respectively, a significant decrease in the time required to re-entrain SCN circadian rhythms. There was no significant effect of MEL on control multiple-unit SCN circadian rhythms.

587.5

THE SUPRACHIASMATIC NUCLEUS (SCN) INFLUENCES CIRCADIAN BODY TEMPERATURE AND CIRCANNUAL RHYTHMS IN GROUND SQUIRRELS. N.F. Ruby[1]*, J. Dark[2], H.C. Heller[1], and I. Zucker[2]. [1]Dept. of Biological Sciences, Stanford Univ., Stanford, CA 94305; [2]Dept. of Psychology, Univ. of California, Berkeley, CA 94720.

Circadian and circannual rhythms were monitored in female ground squirrels over a 6 year interval. Animals were weighed weekly, underwent SCN ablation or a sham operation, and were monitored for 1-3 additional years (Ta=22°C). Squirrels were then implanted with transmitters, transferred to a cold room (Ta=6°C), and Tb was measured telemetrically at 10 min intervals for 2 1/2 more years.

Circadian Tb rhythms were present in sham-operated squirrels (n=6) during normothermia (Tb=37°C) and deep torpor (Tb=7°C), but were eliminated at both Tbs in animals with complete bilateral SCN ablation (SCNx; n=8). SCNx squirrels spent twice as much time torpid and had 3 times as many torpor bouts as intact animals, but their individual bouts were 40% shorter in duration. Seasonal patterns of hibernation and body mass changes were eliminated in 4 SCNx animals; the interval between torpor bouts never exceeded 10 days during a 2 year interval. Circannual rhythms were attenuated in the remaining SCNx and partial SCNx squirrels.

The SCN likely generates circadian Tb rhythms during hibernation and may control individual torpor bout duration, as well as hibernation season duration. Circannual rhythms of body mass and hibernation may be mediated by the SCN in squirrels maintained at low Tas.

587.6

SEROTONERGIC MECHANISMS IN THE AMYGDALA AND REM SLEEP REGULATION. L. D. Sanford*, S. M. Tejani-Butt, R. J. Ross, and A. R. Morrison. Lab. for Study of the Brain in Sleep. Sch. of Vet. Med. and Dept. of Psychiatry, Univ. of Penna., Philadelphia, PA 19104

Serotonin (5-HT) has an inhibitory role in REM, presumably via direct input from the dorsal raphe nucleus (DRN) into pontine regions involved in the generation of REM. DRN also projects to the amygdala, which in turn projects massively to many of these same pontine regions. We examined the effect on REM of microinjecting 5-HT into the amygdala, and we measured the density of 5-HT transport binding sites in four amygdaloid nuclei. Rats (N=7) were implanted with cannulae aimed into the amygdala and with electrodes for recording EEG, EMG and PGO waves. Saline or 5-HT (1.5 mM) was microinjected in NREM or REM. During REM, 5-HT significantly decreased ($p < .001$) the mean latency to a change of state to either waking or NREM (59.2 ± 25.8 sec) compared to saline (131.9 ± 15.5 sec). Seventy percent of microinjections of 5-HT during REM resulted in short latency arousals (mean latency 40.9 ± 43.4 sec), and 57 percent resulted in a change of state to either waking or NREM within 35 sec (mean latency 17.11 ± 11.0 sec). In contrast, microinjections of saline did not appear to affect behavioral state. Microinjections of 5-HT during NREM rarely produced arousal and did not prevent the entrance into REM. Quantitative autoradiographic analysis of ^{3}H-cyanoimipramine binding to 5-HT transporter sites indicated moderate 5-HT innervation in the central and medial nuclei of the amygdala. Relatively greater density of 5-HT transporter sites was seen in the basolateral and lateral nuclei of the amygdala suggesting possible sites of action for the microinjections of 5-HT. The present results demonstrate a role for serotonergic mechanisms in the amygdala in the control of arousal state. Supported by MH42903, MH45742, SCOR HL42236 Project 03 and Dept. of Veterans Affairs

587.7

SEROTONIN-LIGHT INTERACTIONS DURING THE DEAD ZONE OF THE PHOTIC PHASE RESPONSE CURVE. P. D. Penev*, P. C. Zee and F. W. Turek. Dept. Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

Rapidly accumulating evidence indicates the ability of serotonin to modify the responsiveness of the rodent circadian pacemaker to light. However, the interactions between serotonergic and photic stimuli in the circadian system have been studied exclusively during the subjective night. In the present study the circadian phase-resetting properties of the 5-HT agonist, 8-OH-DPAT, were used as an endpoint to examine the potential interactions between serotonergic and photic stimuli during the subjective day.

Male golden hamsters, kept in constant darkness, were exposed to combined treatments with 8(OH)DPAT (1.0 mg/kg i.p.) and light (120 min, 500-700 lux) or activity restriction (120 min restraint procedure). Vehicle treatment at CT 7 alone (-0.8 $\pm$ 3 min, n=6) or in combination with a 2-hour light pulse (5 $\pm$ 2 min, n=8) did not affect the phase of the hamster circadian pacemaker. However, the same photic stimulus, significantly attenuated the phase resetting properties of 8(OH)DPAT at CT 7 (19 $\pm$ 6 min, $p < 0.01$) in hamsters that showed consistent phase shifts following treatment with the 5-HT agonist alone (56 $\pm$ 4 min, n=8). In contrast, the 2-hour restraint procedure had no significant effect ($p > 0.1$) on the magnitude of 8(OH)DPAT-induced phase shifts in a similarly selected group of animals (45 $\pm$ 9 min vs. 60 $\pm$ 4 min, n=6). These results indicate that in the presence of increased serotonergic activity, exposure to light during the dead zone of the photic PRC can play an important role in circadian phase control. The existence of such widespread interactions between retinal and raphe inputs may underlie the integration of environmental and behavioral time-giving cues in the mammalian circadian system.

587.8

INDUCTION OF A CYCLIC AMP RESPONSE ELEMENT MODULATOR (CREM) SPLICE VARIANT, ICER mRNA (INDUCIBLE CYCLIC AMP EARLY REPRESSOR), IN THE RAT SUPRACHIASMATIC NUCLEUS (SCN) AFTER PHOTIC AND PHARMACOLOGICAL STIMULI.

Jens D. Mikkelsen*, Kelly C. Morgan and Philip J. Larsen. Dept Anat, Univ Copenhagen, Blegdamsvej 3, 2200 N, Denmark

The mechanisms through which photic and pharmacological stimuli entrain the circadian clock located in the SCN are poorly understood. Within neurons of the SCN, photic stimuli induce a number of transcriptional factors belonging to the fos- and jun-families, which heteromerize and bind to AP-1 binding sites in the promotor region of unidentified genes. This mechanism is considered to be important in photic entrainment. Recently another transcriptional factor, ICER, which is one of many splice variants of the CREM gene, was cloned from the rat pineal gland. Although ICER has been associated with clock function in the pinealocyte, no basal expression has been detected in the SCN. Adult male rats kept under a DD cycle were given a 20 min light pulse at CT5 or CT19, and sacrificed 60 min after the pulse. Serial sections of the SCN were hybridized for ICER, c-*fos*, and junB mRNA. At CT19 light induces increases of ICER, c-*fos*, and junB mRNA expression, while much smaller increases were observed at CT5. Even though the relative rise of transcription was rather similar for all of the transcriptional factors examined, the maximal expression of ICER mRNA observed after light was about 10-fold lower than c-*fos* mRNA. In another experiment, we administered the dopamine-1 receptor agonist, SKF 38393 into pregnant rats at E19, to determine whether SCN expression of c-*fos* mRNA associated with fetal entrainment of the clock is accompanied by induction of ICER mRNA expression. In this case, a very prominent induction of both c-*fos*, junB, and ICER was observed in the SCN. These data indicate that ICER, which is a strong repressor of c-*fos* transcription plays a role in entrainment of both the fetal and adult SCN.

587.9

ASTROCYTE GFAP EXPERSSION IN SUPRACHIASMATIC NUCLEI IS ENHANCED IN TAU MUTANT HAMSTER. J. Lu*, M. Menaker. Department of Biology, NSF Center for Biological Timing, Gilmer Hall, University of Virginia, Charlottesville, VA 22903

Astrocyte glial fiberllary acidic protein immnoreactivity (GFAP-IR) exhibits circadian variation in the suprachiasmatic nuclei (SCN) of hamsters kept in constant darkness (DD), suggesting that astrocyte may be involved in circadian rhythm generation (Lavialle and Serviere 1993). A single gene mutations (tau) in the hamster causes a shortening of circadian period from 24 hrs in the wild type to 20 hrs in homozygous mutants. The present study pursues if GFAP-IR differs between tau mutants and wild type hamsters. Homozygous tau mutants, heterozygous mutants and wild type hamsters were produced by breeding heterozygous mutants. The genotypes were determined by free running period of wheel-running activity in constant darkness (DD). The average periods of homozygous, heterozygous and wild type hamster were 20 hr, 22 hr and 24 hr respectively. At one month of DD, 4-5 animals of each genotype were anaesthetized and perfused at circadian time (CT) 04, CT10, CT16 and CT22. The brains were removed and sectioned at 25 μm through SCN. The sections were immnostained for GFAP. The density of GFAP-IR was determined using Quantex image analysis system. The results show that GFAP-IR exhibits a circadian rhythm with peak at CT10 and trough at CT22 in all three genotypes. GFAP-IR amplitude was the highest in homozygous, moderate in heterozygous mutants and the lowest in wild type hamsters. At CT22, there is no significant different in GFAP-IR among three genotypes. These results suggest that SCN astrocytes are either involved in generating circadian rhythms or that GFAP levels are affected by the clock. Supported by NIH Training Grant T32-HD07382 (JL) and Air Force Office of Scientific Research (MM).

587.10

DIFFERENTIAL DISPLAY REVEALS NEW MRNAS THAT ARE REGULATED BY A CIRCADIAN CLOCK IN *XENOPUS* RETINA. C. B. Green and J. C. Besharse*. Dept. of Anatomy and Cell Biology, The University of Kansas Medical Center, Kansas City, KS 66160.

We have shown that a circadian clock is present within photoreceptors of the *Xenopus laevis* retina (Cahill and Besharse, 1993, *Neuron*, 10: 573). This clock controls multiple aspects of retinal physiology, including transcription of the tryptophan hydroxylase gene (Green and Besharse, 1994, *J. Neurochem.* 62: 2420). To elucidate molecular mechanisms of clock control, we have conducted a screen to identify clock-regulated mRNAs. *Xenopus* eye cups were cultured in constant darkness and at 6 hour intervals for 2 days, retinas and medium samples were removed. Melatonin measurement was used as a control to insure rhythmicity in the cultures, and retinal RNA was used for differential display (Liang and Pardee, 1992, *Science*, 257: 967). This permitted comparison of sub-populations of mRNA from different time points. Using the criterion of rhythmic peaks on two consecutive days, the majority of mRNAs in the retina (>99%) were not rhythmic. Lack of rhythmicity was also seen in Northern analysis of rod opsin, interphotoreceptor retinoid binding protein, actin and cfos. In contrast, a small number of displayed bands exhibited a high amplitude rhythmic pattern. Northern analysis of one of these revealed two bands (named RM1) at about 2 and 3.9 Kb. These bands had the same high amplitude rhythm seen in the display. The differential display product was used to screen a *Xenopus* retinal cDNA library resulting in isolation of 4 cDNA clones. Preliminary analysis of RM1 clones indicates that both messages are represented and may be products of differential splicing. In addition, in situ hybridization reveals that RM1 is localized to photoreceptor inner segments. Further analysis of RM1 and other rhythmic mRNAs revealed in this screen should provide insight into the molecular mechanisms of clock control.

587.11

DEFINING THE PHOTIC LIGHT INPUT PATHWAY TO THE SUPRACHIASMATIC NUCLEUS. H.M. Cooper *, R.G. Foster, J.K. Bowmaker, A. Szel, E. Nevo, J. Negroni, and A. Attar. *CERVEAU et VISION, INSERM U-371, 69675 Bron, FRANCE; Imperial College, London, UNITED KINGDOM; Inst. of Opthalmology, London, UNITED KINGDOM; Univ. Semmelweis, Budapest, HUNGARY; Inst. of Evolution, Haifa, ISRAEL.*

We have used molecular approaches, opsin immunohistochemistry, microspectrophotometry (**MSP**), retrograde tracing and viral tract tracing methods to dissect out the photic pathway regulating circadian physiology in mammals. In all species examined (sheep, primate, mouse, gerbil, mole-rat) the retinal ganglion cells (**RGC**) projecting to the suprachiasmatic nucleus are morphologically similar and resemble the *gamma* class of **RGC**'s. The cell soma is small to medium sized (9-15 μm diameter) and has 2-3 long, sparsely branched, primary dendrites with an asymmetrical spatial organization. Retinohypothalamic **RGC**'s constitute a minority (< 1%) of the total ganglion cell population in all species, except for the mole-rat in which the majority of cells projects to the **SCN**. **RGC**'s are distributed over the entire surface of the retina in nocturnal species, but in the dorso-nasal region in diurnal species. **RT-PCR** analysis of the retina in blind mammals (*rd* mice, mole-rat) which conserve circadian responses reveals that rods are not required for circadian photoreception, but that a pigment homologous to that of the mouse/human green cone opsin (spectral absorbance ≈ 505-6 nm) is involved. This is confirmed by use of anti-opsin immunohistochemistry and *in situ* **MSP** measures of photoreceptor cells. Modelling this organization illustrates how retinal and optical constraints are combined to increase the efficiency of this system for the detection of diffuse, ambient light levels.

587.12

ORGANIZATION OF THE PREGENICULATE NUCLEUS IN PRIMATES WITH SPECIAL REFERENCE TO THE INTERGENICULATE LEAFLET.

N. Chevassus Au Louis* and H.M. Cooper. *INSERM U 371, Cerveau et vision, 69 675 Bron, FRANCE.*

The mammalian ventral lateral geniculate (vLGN) nucleus is classically divided into three main parts: an external division, an internal division and the intergeniculate leaflet (IGL). The internal and external divisions are involved in visual sensory and visuomotor functions whereas the IGL plays a specific role in circadian regulation through feedback connections with the suprachiasmatic nucleus. The organization of the primate homologue of the vLGN, the pregeniculate nucleus (PGN) has received less attention. The purpose of our study was to determine the general organization of the primate PGN by assessing the anatomical and immunohistochemical speicificities of this structure in the three main primate lineages: prosimians (*Microcebus*), New World primates (*Callithrix*) and Old World primates (*Macacca*).

The distribution of calcium binding proteins, parvalbumin and calbindin, or acetylcholinesterase and cytochrome oxydase activities constitute reliable tools for identifying the PGN and for distinguishing different subdivisions within this structure. The homologue of the internal division lies adjacent to the reticular nucleus and contains few neuropeptide containing cells or fibres. The external subdivision is juxtaposed to the dorsal geniculate nucleus (dLGN) and contains fibres immunoreactive to NPY, SP and ENK. Neurons immunoreactive to NPY and ENK as well as SP fibres are present in the IGL. Both the IGL and the external division of the PGN receive a robust serotoninergic innervation. The change in position of the PGN and it's subdivisions compared to that in other mammals results from the significant expansion of the dLGN and cerebral peduncle.

587.13

CHARACTERIZATION OF THE NEURONAL CONTROL OF THE RAT PINEAL GLAND USING VIRAL TRANSNEURONAL TRACING

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Multisynaptic pathways through which the CNS modulates the activity of the rat pineal gland were characterized using a neurotrophic swine α-herpesvirus (α-HSV) that replicates within synaptically linked neurons. Following injection of an attenuated strain of swine α-HSV into the superficial pineal, the spatiotemporal passage of virus through the neuraxis was detrmined using specific antibodies recognizing PRV and other markers of viral infection. Immunohistochemical analysis revealed productive infection in spatially and temporally separated populations of neurons initially visible in the superior cervical ganglion (SCG) and subsequently apparent in the intermediolateral column (IML) of the upper thoracic spinal cord, the dorsal parvicellular subdivision of the hypothalamic paraventricular nucleus (PVN), and the suprachiasmatic nucleus (SCN). Other infected CNS cell groups known to give rise to descending projections to the IML also became infected. The multisynaptic passage of swine α-HSV through the PVN reproducibly involved subpopulations of neurons in both areas. Infected neurons first emerged in the dorsal parvicellular subdivision of the PVN followed by neurons in the lateral and medial parvicellular subfields. Infection of these neurons preceded the appearance of infected neurons in the dorsomedial SCN. Removal of the superior cervical ganglion (SCGX) prior to viral infection eliminated infection within the aforementioned CNS circuits and revealed an additional parasympathetic projection through the sphenopaltine ganglion that ultimately led to infection of neurons in the superior salivatory nucleus. The present data provide a morphological substrate for CNS control of the rhythmic secretion of melatonin from the pineal gland.

COGNITION IX

588.1

DETERMINATION OF CRITICAL BRAIN REGIONS FOR OBJECT RECOGNITION IN THE HUMAN VISUAL SYSTEM. J. Hart, Jr.*, M. Kraut, N. E. Crone, B. Soher, J. Sieracki, R.N. Bryan, & B. Gordon. Depts. of Neurology, Radiology, Biomedical Engineering, Cognitive Science, and the Zanvyl Krieger Mind/Brain Institute, The Johns Hopkins University, Baltimore, MD 21287

An important issue in both cognitive and in clinical neurosciences is whether behavioral functions are critically dependent upon the operations of focal cerebral regions, or merely associated with those operations. A patient who was being evaluated for seizure focus extirpation provided a unique opportunity to address this issue. Implanted subdural electrode arrays covered his right occipital, temporal, and parietal lobes. Behavioral testing, done across all the investigative techniques discussed here, consisted of a visual object/nonobject shape recognition task. Regional involvement was assessed using direct cortical recording and functional MRI (fMRI). Direct cortical recording showed activation of the right TPO and inferior TO regions in association with performance of the task. However, direct electrical cortical interference testing in this region failed to impair the behavior. Nor did surgical resection of this region, done for purposes of extirpation of the epileptic focus. Moreover, post-resection fMRI (10 mm thick coronal sections, imaging parameters TR/TE/α=60/40/40°) showed activation only in the left posterior middle or inferior temporal gyrus.

These findings can best be explained by: a) the left posterior middle or inferior temporal gyrus being involved in the immediate recovery of the function of object recognition after resection of the right temporo-occipital region, or more likely, b) both regions are involved in object recognition, with only the presence of one, or specifically the left middle or inferior temporal gyrus, being critically necessary to perform object recognition.

Regardless of these possibilities, these data show that regional functional activation is not necessarily a guide to whether the region is critically involved with performance of a function (either in the normal state, or following resection).

588.2

OBJECT SHAPE RECOGNITION IN THE HUMAN VISUAL SYSTEM AS SHOWN BY fMRI. M. Kraut, B. Soher, J. Sieracki, R.N. Bryan, B. Gordon,* and J. Hart, Jr. Depts. of Neurology, Radiology, Biomedical Engineering, Cognitive Science and the Zanvyl Krieger Mind/Brain Institute, The Johns Hopkins University, Baltimore, MD 21287.

We have used functional magnetic resonance imaging (fMRI) to help determine what cortical regions are activated during processing of visual object shape in humans.

We studied 8 normal young adults, 5 men and 3 women using a behavioral paradigm with a baseline condition of visual detection and an activated condition of object/nonobject shape discrimination decision. fMRI data were gathered using 10 mm thick coronally oriented sections ranging from the occipital pole to the anterior hippocampus. Scanning parameters were TR/TE/α=60/60/40°. Regions of activation in each section were detected by cross correlation analysis (Bandettini et al, J. Magn. Res. Med. 30:161), and were superimposed upon collocated T1 weighted anatomic images.

Six of the eight subjects studied exhibited significant signal changes. 4/6 showed changes in the inferior temporal gyri (3 bilateral, 1 right only), 3/6 in the occipitotemporal gyri (1 bilateral, 1 left only, 1 right only) and 2/6 at the TPO junction (1 bilateral, 1 right only). Both these last subjects also showed activation in either the inferior temporal or occipitotemporal gyri bilaterally.

These data strongly suggest that processing of object shape information in humans activates discrete cortical regions bilaterally. The cortical regions involved correspond with those regions purported to participate in the "ventral" pathway of visual object processing described in subhuman primates and are consistent with lesion studies of visual agnosia in humans.

588.3

REPEATED PRESENTATION OF OBJECTS REDUCES ACTIVITY IN VENTRAL OCCIPITOTEMPORAL CORTEX: A fMRI STUDY OF REPETITION PRIMING

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Changes in neural activity as a function of stimulus repetition were investigated with functional MRI. Line drawings of objects were presented at fixation for 180 msec, at a rate of 1 item every 2 sec, for silent naming. Subjects viewed 6 alternating blocks of objects and noise patterns (10 items per block). For 4 subjects, the same objects, in a different order, were presented on the first and second blocks, followed by a block of novel (i.e., not previously presented) objects (A, A', B), while 4 subjects viewed blocks composed of novel objects only (A, B, C). Voice on-set time data from 21 normal subjects confirmed that naming was significantly faster for blocks of repeated, compared to blocks of novel, objects (repetition priming).

Analyses of the fMRI data (echo planar imaging; TR = 3000 msec, TE = 40 msec), recorded from the posterior left hemisphere (6 contiguous 5 mm thick coronal slices) revealed an area of increased signal intensity in the ventral occipitotemporal cortex for objects relative to noise patterns. Moreover, this activity was modulated by experience. Specifically, object repetition produced a significant decrease in signal intensity that returned to its initial level when novel objects were re-introduced (A, A', B), whereas intensity in this region remained constant for subjects who viewed only novel objects (A, B, C). These data are consistent with single unit recordings from the inferior temporal lobe of monkeys [EK Miller, L Li, & R Desimone (1991). *Science* 254:1377] and suggest that increased efficiency in object processing may be mediated, at least in part, by decreased neural activity in discrete cortical regions.

588.4

SEPARATE CONCEPTS ARE RETRIEVED FROM SEPARATE NEURAL SYSTEMS: NEUROANATOMICAL AND NEUROPSYCHOLOGICAL DOUBLE DISSOCIATIONS. D. Tranel*, H. Damasio, A.R. Damasio and J.P. Brandt. Div. of Cognitive Neuroscience, Dept. of Neurology, Univ. of Iowa, Iowa City, IA 52242.

Both clinical reports and systematic neuropsychological studies have shown that patients with damage in certain brain sites develop defects in the visual recognition of non-unique concrete entities, such as animals or tools and utensils, and we have hypothesized that there are different neural systems dedicated to different domains of knowledge. In this study, we report a double dissociation relative to type of entity and site of lesion. We studied 160 subjects with focal lesions to various sectors of the telencephalon, and 150 age- and education-matched controls. All subjects performed a visual recognition task in which they had to identify entities from varied conceptual categories, including animals (n=90), fruits/vegetables (n=67), and tools/utensils (n=104). Controls performed close to 90% correct in all categories. Fourteen brain-damaged subjects had severely impaired recognition of animals (X=69.4%) and normal recognition of tools/utensils (X=93.9%); fruits/vegetables were intermediate (X=81.7%). Three brain-damaged subjects had the reverse pattern: recognition of tools/utensils was defective (X=79.3%) and recognition of animals was normal (X=91.0%); fruits/vegetables were normal (X=90.0%). Neuroanatomical analysis revealed systematic differences in the lesions of the two groups. In the animal-defective group, lesions were centered in the occipital or occipitotemporal region, unilaterally right or left, or bilaterally. In the tool/utensil-defective group, lesions were centered in the lateral aspect of the *left* occipitotemporal region. The findings suggest that different neural systems are indeed related to the reconstruction of concepts concerning different types of entities.

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588.5

SEPARATE LEXICAL CATEGORIES ARE RETRIEVED FROM SEPARATE SYSTEMS: A PET ACTIVATION STUDY. A.R. Damasio,* T.J. Grabowski, H. Damasio, D. Tranel, R.J. Frank, J. Spradling, L.L.B. Ponto, G.L. Watkins & R.D. Hichwa. Div. Cognitive Neurosci., Dept. Neurology & PET Imaging Ctr., Univ. of Iowa, Iowa City, IA 52242.

The study of subjects with lesions in left inferotemporal cortex (IT) and left temporal pole (TP) has suggested the existence of separate neural substrates for the retrieval of word forms for unique and nonunique entities. Access to proper nouns depends on the TP, whereas access to common nouns depends on IT. We have now investigated this hypothesis framework in normal subjects using positron emission tomography (PET).

Nine normal, right-handed subjects were studied with quantitative $[^{15}O]H_2O$ PET. Subjects performed three naming tasks and a control task, twice each. Stimuli were black-and-white photos of (1) familiar (famous) faces, (2) animals, and (3) tools and utensils. In the control task, subjects decided whether *un*familiar faces were presented upside down or rightside up. Oral responses were required for all tasks, and performance data were collected. The search for significant activation was directed *a priori* to TP and IT (bilaterally), defined by interactive analysis of coregistered 3D magnetic resonance images of the brains of participating subjects. PET data were analyzed with a pixelwise general linear model (Friston, 1991).

Relative to the control task, the naming tasks produced activation in left IT and both TPs. Face naming activated both TPs, but not left IT. Naming animals and tools activated left posterior IT and also left TP (but more weakly than for naming faces). Activation by naming tools was posterior and lateral to that produced by naming animals.

These data are consistent with the findings from lesion studies and further support the notion that knowledge systems are regionalized.

588.6

DIFFERENT NEURAL SYSTEMS FOR THE RECOGNITION OF ANIMALS AND MAN-MADE TOOLS.

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Several lines of evidence, ranging from anthropology through developmental psychology to clinical observation in neurological patients, suggest that human semantic knowledge of living entities is represented separately from knowledge of man-made tools. Using positron emission tomography (PET), we mapped rCBF increases in normal volunteers during the recognition of visual stimuli representing living (animals) and non-living (artefacts) entities. The subjects had to decide whether pairs of visual stimuli were different representations of the same object, or different objects: 1. animal discrimination: same-different judgement of animal pictures (same: two different pictures of the same animal, different: two pictures of different animals); 2. artefacts discrimination: same-different judgement of object pictures (same: two different pictures of the same object, different: two pictures of different objects). Two combined control conditions were used to account for the apperceptive stages of visual recognition: 1. visual texture discrimination; 2. shape discrimination: same-different judgement of meaningless shapes (modified Vanderplas figures). PET data were analysed using SPM package (MRC-CU, London). The combined control conditions were compared to each of the experimental conditions. Activations significant at $p<0.001$ were considered. Animal recognition was associated with activations in the inferior temporo-occipital areas, bilaterally, whereas artefact recognition engaged a predominantly left hemispheric network, involving also the left dorsolateral frontal cortex. These findings provide in vivo evidence for a fractionation of the neural substrates of semantic knowledge in man. Different processing requirements, preferential access to structural knowledge for living stimuli and to functional information for objects, or separate representational systems may account for this anatomical fractionation.

588.7

EVENT-RELATED POTENTIALS AND GAMMA-BAND RESPONSES (30 HZ) DISTINGUISH NOUNS FROM VERBS.

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Lesion evidence strongly suggests that the cortical counterparts of nouns and verbs are localized in different brain areas. In this study, different electrocortical responses to nouns and verbs were recorded non-invasively from the skull of healthy individuals. Differences in electrocortical responses were most pronounced close to the primary motor and visual cortices. A positive component peaking around 200 ms post stimulus onset was larger over motor cortices after verbs compared to nouns. Over visual cortices, however, nouns evoke more pronounced positivities than verbs. Analysis of spectral responses in the lower gamma-range (25-35 Hz) revealed essentially the same picture: 30-Hz responses to nouns were stronger over visual cortices and those to verbs were stronger over motor areas. Behavioral testing revealed stronger visual associations for nouns and stronger motor associations for verbs. The results are explained in the framework of a neurobiological model of language based on Hebb's concept of cell assemblies.

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588.8

OBJECT AND SPATIAL WORKING MEMORY ACTIVATES DORSOLATERAL PREFRONTAL CORTEX: A FUNCTIONAL MRI STUDY. M D'Esposito*, RK Shin, JA Detre, S Incledon, D Annis, GK Aguirre, M Grossman, DC Alsop. Departments of Neurology & Radiology, University of Pennsylvania, Philadelphia, PA 19104.

Primate studies have demonstrated that distinct neuronal populations within dorsolateral prefrontal cortex subserve working memory for objects/patterns vs spatial locations. We tested the hypothesis that a similar anatomical dissociation exists in humans by performing functional MRI studies in 8 normal subjects during tasks designed to probe object vs spatial working memory. In the memory condition, subjects attended to serially presented stimuli and determined if each stimulus or location was the same as that presented two stimuli back. In the control condition, subjects were asked to identify a single predetermined stimulus or location. Each subject was presented with up to four different stimulus sets: letters, symbols, faces and spatially-arrayed squares. Echoplanar MRI was performed at 1.5 T in 16 contiguous 5mm axial slices and regions of activation were identified by correlation with the task. Activation during the working memory condition was reliably found in dorsolateral middle frontal gyrus (Brodmann's areas 9 & 46) in all stimulus conditions. Bilateral frontal activation was seen in 7/8 subjects in all conditions, but activation was greater on the right in the spatial condition in 6/8 subjects. Thus, a similar region within dorsolateral prefrontal cortex was activated during this paradigm regardless of the type of information held in working memory (object or location). One possible explanation for this finding may be that functional MRI lacks the spatial resolution to distinguish separate neuronal populations that are dedicated to different domains of working memory. Alternatively, performance on this paradigm may require a single executive control process that functions independently of the type of information being temporarily stored.

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588.9

NEURAL SYSTEMS FOR PERCEPTION AND WORKING MEMORY ARE MORE STRONGLY COUPLED FOR LOCATIONS THAN FOR FACES Susan M. Courtney*, Leslie G. Ungerleider, Katrina Keil, James V. Haxby National Institute of Mental Health, Bethesda MD 20892-1366.

Face and location working memory activate distinct regions in extrastriate and prefrontal cortex (Courtney et al.,Soc. Neurosci. Abstr.,20:6,1994). To dissociate the roles of these regions in perception and working memory, we measured regional cerebral blood flow (rCBF) in 10 healthy volunteers using positron emission tomography (PET) while the subjects performed sensorimotor control and working memory tasks. Working memory tasks involved retention of the identity or location of a single face over intervals of 1, 5, or 9 sec. Stimuli for face and location working memory tasks were identical. For face working memory, striate and ventral occipitotemporal extrastriate areas showed a decline in rCBF with longer retention intervals, indicating these areas participate principally in perceptual and encoding operations performed during visual stimulation. By contrast, inferior and orbital frontal areas activated by the face working memory tasks showed more sustained rCBF increases over longer retention intervals, indicating greater participation in maintaining a working memory representation when visual stimuli are absent. For location working memory, however, this dissociation between extrastriate and prefrontal areas was not observed. Activations in inferior and superior parietal cortex, as well as nonsignificant increases in rCBF in superior frontal cortex, all demonstrated a tendency to increase with longer delay intervals. In contrast to the results for face working memory, these findings indicate that extrastriate areas used for the perception of spatial location are also involved in maintaining an active representation of a particular location in working memory.

588.10

FUNCTIONAL MRI STUDY OF MENTAL IMAGE GENERATION. MJ Farah*, M D'Esposito, RK Shin, M Stallcup, GK Aguirre, DC Alsop, M Grossman, LJ Tippet, JA Detre. Departments of Psychology, Neurology & Radiology, University of Pennsylvania, Philadelphia, PA 19104.

In mental imagery, or "seeing with the mind's eye", visual representations are activated by an internal image generation mechanism, rather than by external stimuli. This study addressed two issues regarding mental imagery: the involvement of occipital cortex in imagery and the localization of the image generation mechanism. Subjects listened to two classes of words under alternating conditions: abstract (non-imagable) and concrete words. Subjects were instructed to listen passively to the abstract words and were told to actively generate images of presented concrete words. Echoplanar fMRI was performed in 16 contiguous 5mm axial slices. Volumes of activation were identified by correlation with task above a threshold r value. For each subject, total volume of activation within predefined volumes of interest was measured. Although area 17 was not reliably activated by imagery, area 19 of occipital cortex was activated in two out of five subjects. Four out of five subjects showed activation of area 37, which was significantly greater on the left side ($p<0.05$). The activation of area 19 during visual images evoked by auditory stimuli suggests a role for occipital association cortex in visual imagery. Left inferior temporal activity accords well with previous localizations for image generation inferred from cases of loss of mental image generation following brain damage.

588.11

LATENCY OF THE VISUAL MEMORY POTENTIAL IS RELATED TO RECOGNITION TIME FOR IDENTIFYING FACES. H. Begleiter*, B. Porjesz and A. Litke. SUNY HSCB, Dept. Psychiatry, 450 Clarkson Ave., Brooklyn, NY 11203, USA.

A number of human studies have identified an event-related potential (ERP) which appears to index memory processes involved in object recognition, including faces. These neuroelectric phenomena in humans are rather similar to single cell studies in the inferotemporal cortex of monkeys. In order to determine the coupling of recognition time and this visual memory potential (VMP) we used a delayed matching to sample paradigm with half of the S2 faces presented in an inverted position. Our results indicate that as response time increases to inverted faces so does the latency of the VMP component of the ERP. These data provide support for the coupling of VMP and behavioral recognition.

588.12

COULD TIME-SERIES PREDICTION ASSIST VISUAL PROCESSING? W. Softky* MRB/NIDDK/NIH, BSA Bldg. #350, Bethesda, MD 20892

When a brain constructs a model of the environment from its sensory input, it might need to verify the model's accuracy. One method of verification is multivariate time-series prediction: a good model could predict the near-future activity of its sensory input fibers, much as a good scientific theory predicts future data. Such a predicting model would require copious top-down connections to compare the predictions with the input. That feedback could improve the model's performance in two ways: by biasing internal activity toward expected patterns, and by generating specific error signals if the predictions fail.

I constructed an event-driven, computationally efficient network to make such predictions in a simple dynamic visual environment. Although designed to predict accurately rather than to seem biological, the network appears similar to cortex in several respects. It uses over 8000 irregularly spiking units in three layers; over 200,000 variable-delay excitatory synapses of two types—brief and prolonged—whose effects multiply; sparse, specific, local connectivity, almost all excitatory; implicit local inhibition (winner-take-all); massive feedback signals which bias lower units' activity toward predictions; local "binding" by precisely coincident spikes; and "surprise" units, driven by mismatches between predicted and actual input activity, which detect discontinuities in the stimulus. Units in the upper layer are the most coarsely tuned in space and time, but the most stimulus-specific; in principle they could both drive, and receive feedback signals from, yet higher layers. Given as training inputs a grid of noisy spike trains driven pixel-wise by a single drifting bar at various orientations, unsupervised learning produced units tuned to bar orientation, straight or curved motion, and contour discontinuity (end-stopping). The network's generality may allow it to learn more specific and abstract responses in other stimulus environments, or modalities.

BETA-AMYLOID: NEUROPATHOLOGY II

589.1

MOLECULAR MECHANISMS OF AMYLOID ß PEPTIDE INDUCED HUMAN NEURONAL DEATH. LeBlanc, A.C.*, William Chu, Cynthya Goodyer, Hélène Douillard. Depts. Neurol. & Neurosurg. and Pediatrics, McGill U. and Bloomfield Centre for Research in Aging, LDI, JGH, Montréal, Quebec.H3T1E2

Apoptosis has been proposed as the mechanism of amyloid ß peptide (Aß) neurotoxicity. However, little is known about the molecular mechanisms of Aß-induced neuronal cell death. We have established a highly purified foetal human primary neuron culture. These neurons express high levels of amyloid precursor protein (APP) mostly of the APP_{695} type. Metabolism through the secretory pathway accounts for approximately 40% of newly synthesized APP. In addition, five C-terminal peptides arising from the endosomal lysosomal pathway are similar to those previously observed in human brain. APP metabolism through the 4 kDa Aß-producing pathway is higher than in rat neurons. To evaluate the molecular mechanisms involved in the neurotoxicity of $Aß_{1-40}$ and $Aß_{1-42}$ versus a control reverse $Aß_{40-1}$ peptide, these neuron cultures are treated at various times with physiological concentrations of each Aß peptide. Total cell death is evaluated by trypan blue exclusion and Molecular Probe's live/dead cell assay. Cell death by apoptosis is assessed by the detection of condensed chromatin with Hoescht or propidium iodide staining. We are presently evaluating the expression of immediate early genes, c-fos, jun-b and c-jun by RT-PCR, immunocytochemistry and immunoblots. The results will be compared to those obtained from serum deprivation induced neuronal apoptosis. Supported by grants from NIH, Fond de Recherche en Santé du Québec and Canadian Society of Alzheimer to AL.

589.2

INTERACTIONS OF GLIAL CELLS WITH ALZHEIMER SENILE PLAQUE CORES *IN VITRO* D.A. DeWitt*, G. Perry, C. Doller, S.L. Siedlak, and J. Silver Dept. of Neuroscience, Case Western Reserve University, Cleveland, OH 44106

Senile plaques (SP) are molecularly complex structures. Reactive astrocytes and activated microglia are an invariable feature in neuritic SPs. Previous models have demonstrated that synthetic Aβ, the major protein found in SPs can induce reactive gliosis and activate microglia. However, many other proteins are also present in the SP. Our goal was to develop a model which mimicked the SP in as many aspects as possible. Isolated SP cores were bound to glass coverslips and presented to cells in culture. Rat cortical astrocytes do not phagocytose SP cores, but become reactive and surround the SP with processes. Astrocytes which were in contact with SP cores showed increased GFAP immunoreactivity, consistant with a reactive state. This response is similar to that seen in AD brain. In contrast, microglial cells could phagocytose whole SP cores (10 μm). Phagocytosis of SP cores occured as soon as 2 hours, with >90% of cores internalized by seven days. Subsequently, Aβ immunogenic material could be seen within many small vesicles. The total number of intact SP cores decreased exponentially through 30 days in culture with microglia. These results suggest that SP material can be phagocytosed and broken down by microglial cells. Cocultures with astrocytes and incubation with proteoglycans will be used to determine if SP core phagocytosis and removal can be altered. Supported by NIH grants AG 07552, and NS 25713.

589.3

OXIDATIVE STRESS, POSTTRANSLATIONAL PROTEIN MODIFICATIONS AND ALZHEIMER DISEASE M.A. Smith, P.L. Richey, P. Mulvihill, L.M. Sayre and George Perry (SPON: Society of Neuroscientists of Africa) Departments of Pathology and Chemistry, Case Western Reserve University, Cleveland, Ohio 44106, USA

Alzheimer disease (AD) is characterized by deposits of insoluble proteins that form two main distinctive lesions called senile plaques (SP) and neurofibrillary tangles (NFT). Here we propose that one mechanism by which normally soluble proteins are accummulated as insoluble aggregates of proteins in SP and NFT could be related to oxidative postranslational protein modifications. In previous studies we have shown SP and NFT have modification by the carbonyl-related modifications known as advanced glycation end products. In this study we amplified these results by determining the presence of free carbonyl groups in NFT and PHF-τ. We chemically demonstrate carbonyl-related modifications in the several protein components of NFT using 2,4 denitrophenyl hydrazine (DNP), a reagent that reacts with protein-bound carbonyl moieties.

The *in vivo* occurrence of modifications in NFT suggests that carbonyl-modification is associated with a generalized cytoskeletal abnormality that may be critical in the pathogenesis of neurofibrillary pathology. Importantly, we also found that chemical modification of τ by carbonyl reagents renders the same solubility characteristice found in the protein components of isolated NFT further supporting the idea that extensive oxidative stress-related posttranslational modifications, through the formation of crosslinks, might account for the biochemical properties of NFT and their resistance to degradation *in vivo*. Supported by NIH grant AG09287.

589.4

τ/βPP INTERACTION: THE PATHOGENIC PATHWAY OF ALZHEIMER DISEASE? G. Perry*, S.L. Siedlak, P.L. Richey, P. Mulvihill, D. Praprotnik, R.N. Kalaria and M.A. Smith. Institute of Pathology and Department of Neurology, Case Western Reserve University, Cleveland, Ohio, USA

The main protein components of neurofibrillary tangles and senile plaques, τ and amyloid-β protein, respectively, are well characterized, however the molecular mechanisms responsible for their deposition are, as yet, unknown.

The present study demonstrates by several independent techniques that τ directly interacts with a conformation-dependent membrane spanning domain of the amyloid-β protein precursor (βPP) that includes the $βPP_{717}$ mutation site associated with familial forms of AD. First, using a dot-blot assay we found that τ only bound to C-terminal βPP peptides sharing residues $βPP_{714-723}$. Second, steric hindrance of potential τ-binding sites in AD brain with sequence-specific βPP peptide antisera was only effective with antibodies against those βPP peptides containing $βPP_{714-723}$. Third, in quantitative competition experiments only those βPP peptides encompassing the $βPP_{714-723}$ sequence were able to completely block τ binding. Fourth, using an *in vitro* solid phase binding assay, we showed that iodinated τ bound in a saturable manner with high affinity to immobilized $βPP_{714-733}$ with a calculated K_D of 9.6nM.

Our findings provide clear evidence for a direct interaction between τ and βPP and suggest that neurofibrillary tangles and senile plaques, often considered separate entities, may contribute towards each others formation.

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589.5

CSF LEVELS OF Aβ42 AND TAU ARE ALTERED IN ALZHEIMER'S DISEASE C. Vigo-Pelfrey[1], R. Motter[1], D. Kholodenko[1], R. Barbour[1], K. Johnson-Wood[1], D. Galasko[2], L. Chang[3], B. Miller[3], C. Clarke[4], R. Green[5], D. Olson[5], P. Southwick[6], R. Wolfert[6], B. Munroe[6], F. Coria[7], I. Lieberburg[1], P. Seubert[1], D. Schenk[1]* [1]Athena Neurosciences, South San Francisco, CA 94080; [2]UCSD, San Diego, CA 92161; [3]UCLA, Torrance, CA 90509; [4]University of Pennsylvania, Phladelphia, PA 19146; [5]Emory University, Atlanta, Georgia 30329; [6]Hybritech, Inc, San Diego, CA 92126; [7]Segovia Hospital, Segovia, Spain.

The CSF levels of $A\beta_{42}$ and tau were measured in a well characterized group of Alzheimer's disease (AD; n=37), other neurological disease (ND; n=32) and normal control (NC; n=20) subjects. Despite the large amount of $A\beta_{42}$ present in AD brain, levels in AD CSF was significantly lower than that in the other groups ($p<0.0001$) suggesting that clearance of this protein may be impaired in AD. As previously reported, tau levels are significantly elevated in AD ($p<0.001$). SDS-PAGE characterization of the tau from AD and control CSF did not show obvious differences in either the MW or phosphorylation state. Combining these measures identifies populations with either elevated tau and low $A\beta_{42}$ (96% AD), low tau and elevated $A\beta_{42}$ (100% non-AD) or low tau and low $A\beta_{42}$ (a mixture of the 3 groups accounting for 41% of the subjects).

(mean ± SD, pg/ml)	AD	ND	NC
$A\beta_{42}$	383±76**	543±177	632±156
Tau	407±241*	168±63	212±102

**p<.001 AD vs. either control group
*p<.001 AD vs. either control group

589.6

CHOLINERGIC FIBRE LOSS ASSOCIATED WITH DIFFUSE PLAQUES IN THE NON-DEMENTED ELDERLY. T.G. Beach[1]* and W.G. Honer[2]. [1]Departments of Pathology (Neuropathology) and [2]Psychiatry, The University of British Columbia, Vancouver, British Columbia, Canada. Consecutive cases without a history of significant neurological illness were collected at autopsy. 14 cases were ≥ 60 yrs of age; 10 were younger. Of the 14 over age 60, 9 had diffuse amyloid plaques in both inferior temporal gyrus (ITG) and entorhinal cortex (ECx), as detected with immunohistochemistry (IHC) for ß-amyloid peptide (ß-AP) while 5 did not. All 14 of the over-60 cases were mildly affected with neuropil threads and/or neurofibrillary tangles, as assessed with IHC using the Alz-50 antibody and Bielschowsky silver staining; these changes were mainly confined to the parahippocampal gyrus (Braak stages I or II). Neuritic plaques were present in only four cases (all also ß-AP + ve) but were few in number and poorly developed (CERAD criteria for AD not fulfilled). Synaptic antigen density, examined with quantitative IHC using the EP10 and SP15 monoclonal antibodies, did not differ amongst groups (under-60 vs over-60, over-60/ß-AP + ve vs over-60/ß-AP-ve) in either ITG or ECx. Cholinergic (ACh) fibre densities were estimated in sections stained with acetylcholinesterase enzyme histochemistry by manually counting their intersections with an ocular reticule. Mean ACh fibre density was decreased in both ITG and ECx (30% and 40% depletion respectively) in the over-60/ß-AP + ve group compared to the over-60/ß-AP-ve group; this was statistically significant only in ECx (t-test; p = 0.03). These results suggest that: 1) ACh fibre loss is an early, preclinical event in AD 2) Diffuse plaques are associated with a significant decrease in ACh fibre density. **Supported by the British Columbia Health Research Foundation**

589.7

ß-AMYLOID ACCUMULATION WITHIN ENTORHINAL CORTEX PREDICTS SEVERITY OF DEMENTIA IN ALZHEIMER'S DISEASE. B.J. Cummings*, C.J. Pike, and C.W. Cotman Brain Aging and Dementia Institute, University of California, Irvine, CA 92717-4550

Many have argued that ß-amyloid accumulation in Alzheimer's disease (AD) is a secondary event, in part because plaque number does not correlate strongly with cognitive dysfunction. However, several conditions are necessary to adequately address this issue: i) AD tissue from a broad range of cognitively affected individuals must be included in the analysis; ii) There must be a short interval between neuropsychological testing and death; iii) A sensitive and reproducible assay for ß-amyloid must be used; and iv) The area occupied by ß-amyloid and not simply plaque number must be measured. We have recently shown that in the aged canine, an animal model which lacks neurofibrillary tangles, the extent of ß-amyloid accumulation appears to correlate with their ability to recall previously learned information (Cummings *et al.*, 1995). For the current study of ß-amyloid deposition in AD, we selected 20 individuals from mildly impaired to severely demented based on Mini-Mental Status Exam scores (MMSE) obtained, on average, 10 months prior to death. Using formic acid enhanced immunostaining for ß-amyloid and computer based image analysis of cross sectional area ("load") rather than manual counts of plaque number, we found a strong relationship between ß-amyloid "load" in entorhinal cortex and cognitive deficits in AD patients (**$r = -0.83$**). We also quantified plaque number ($r = -0.73$) and tangle number ($r = -0.64$) in the same cases. Post-mortem ß-amyloid load in entorhinal cortex accurately predicts the severity of global dementia, and thus Aß likely represents an important factor in the clinical manifestation of AD, since ß-amyloid deposition precedes NFT formation, and ß-amyloid correlates with cognitive deficits in the absence of NFTs in the canine.

589.8

DECREASED CEREBROSPINAL FLUID α-SECRETASE CLEAVED AMYLOID PRECURSOR PROTEIN (APP) SEPARATES ALZHEIMER PATIENTS WITH THE SWEDISH $APP_{670/671}$ MUTATION FROM NON-MUTATION CARRIERS L. Lannfelt*, H. Basun, L-O. Wahlund, B. A. Rowe, S. L. Wagner Karolinska Institute, Department of Clinical Neuroscience (Geriatric Medicine), Huddinge University Hospital, S-141 48 Huddinge, Sweden
The Salk Institute Biotechnology/Industrial Associates, Inc. (SIBIA), 505 Coast Blvd, So, La Jolla, CA 92037

A constituent of senile plaques in Alzheimer's disease (AD) is ß-amyloid, a proteolytic fragment of the amyloid precursor protein (APP). APP can be metabolised by at least two pathways, of which one involves generation of soluble APP by an unidentified enzyme named α-secretase. This cleavage generates soluble α-secretase cleaved APP (α-sAPP), which was measured by a new enzyme-linked immunosorbent assay in cerebrospinal fluid from members of a Swedish AD family with a pathogenic mutation at $APP_{670/671}$. Mutation-carriers with AD had low levels of α-sAPP (160±48 ng/ml), with no overlap compared to non-carriers (257±48 ng/ml). Presymptomatic mutation-carriers showed intermediate α-sAPP levels. There was a strong correlation between decreased α-sAPP and age in the mutation-carriers (R=0.94; p=0.005). Today there exists no antemortem marker in AD with sufficient sensitivity and specificity. Measurement of α-sAPP represents a promising diagnostic marker of AD.

589.9

THE INHIBITORY EFFECTS OF BAFILOMYCIN A_1 ON β-SECRETASE ACTIVITY DIFFER AMONG CELL TYPES. B.D.Greenberg*, S.Mistretta, J.T.Durkin, S.Murthy, M.Savage and R.Siman. Cephalon, Inc., 145 Brandwine Parkway., West Chester, PA 19380.

The macrolide antibiotic bafilomycin A_1 ($bafA_1$) is a potent inhibitor of the vacuolar H^+-ATPase, the activity of which neutralizes certain acidic subcellular compartments. $BafA_1$ also inhibits the production of secreted APP derivatives generated by the so-called β-secretase (Knops *et al.*, J. Biol. Chem. 270:2419-2422, 1995; Haass *et al.*, J. Biol. Chem. 270:6186-6192, 1995). In these studies, levels of Aβ and the NH_2-terminal β-secretase fragment (here termed "APPβ") were reduced by $bafA_1$ in HEK293 cells transfected with the Swedish mutant APP cDNA, and in fibroblasts obtained from patients harboring this mutation. No inhibition of Aβ or APPβ production were observed in media conditioned by wild-type fibroblasts or HEK293 cells transfected with wild-type APP. On the other hand, $bafA_1$ did inhibit formation of these APP derivatives in human mixed brain cultures (HMBC) and HS683 neuroglioma cells transfected with wild-type APP (Knops *et al.*). Here we report results of similar experiments utilizing M17 neuroblastoma cells transfected with Swedish mutant APP_{695}. Using a pool of stable transfectants that expresses roughly equivalent levels of wild-type and mutant APP, we employed ELISAs that distinguish these two APPβ forms to show that $bafA_1$ inhibits β-secretase activity with an IC_{50} of ~2nM only on the wild-type substrate. Hence, as with HMBC and HS683 cells, the effect of $bafA_1$ on β-secretase cleavage of mutant and wild-type substrates was reversed relative to HEK293 cells. Together, these results suggest that two distinct β-secretases may exist that act on either the wild-type or mutant substrates, with a cell type-specificity. Alternatively, cleavage of these substrates by a single enzyme may occur in distinct subcellular compartments that are differentially sensitive to the effects of $bafA_1$, in which case the subcellular trafficking of these two APP forms would likely differ among cell types.

589.10

FIBROBLASTS FROM CARRIERS OF FAMILIAL AD LINKED TO CHROMOSOME 14 SHOW INCREASED Aß PRODUCTION. D. Scheuner[1]*, T. Bird[2], M. Citron[3], L. Lannfelt[4], G. Schellenberg[2], D. Selkoe[3], M. Viitanen[4], and S. G. Younkin[1,5]. [1]Case Western Reserve Univ., Cleveland, OH; [2]Univ. of Washington, Seattle, WA; [3]Harvard Univ., Boston, MA; [4]Karolinska Institute, Stockholm, Sweden; [5]Mayo Clinic Jacksonville, Jacksonville, FL.

To evaluate amyloid ß protein precursor (ßAPP) processing in familial AD linked to chromosome 14, we obtained fibroblast lines from known carriers in 4 separate families (18 lines: 5, 7, 2, and 4 from the 4 families) and from appropriate controls (15 lines). ßAPP synthesis was evaluated by pulse labeling for 20 minutes and quantitating newly synthesized ßAPP by phosphorimaging after immunoprecipitation and SDS/PAGE. Medium from sister cultures maintained for 5 days was analyzed for Aß1-40 on days 2-5 and for Aß ending at Aß42(43) on day 5. The amount of ßAPP synthesized in 20 min was not significantly different in the AD and control lines. In 5 separate experiments, we assessed [Aß1-40/"ßAPP synthesis rate"] and [Aß42(43)/"ßAPP synthesis rate"] and compared the control fibroblasts with groups of fibroblasts representing the 4 families. In each of over 50 comparisons made between the AD families and controls, the values measured in the AD families exceeded those in the controls. Of the 18 lines analyzed, 4 were from one Swedish family. The increases in the Swedish lines were small compared to those in lines from the other 3 families. By themselves, these small increases were not significant, but the fibroblasts from all 4 families showed a qualitatively similar shift with Aß42(43) increasing more than Aß1-40. Analysis of the 5 day data from all 4 families showed that [Aß1-40/"ßAPP synthesis rate"] in the 18 AD lines was 2.1 times that in the 15 controls ($p<0.003$) and that [Aß42(43)/"ßAPP synthesis rate"] in the AD lines was ~8.9 times that in controls ($p<0.0003$). These data and our similar results on plasma samples (Song et al., these meetings) indicate that in FAD linked to chromosome 14 as previously shown in FAD linked to the ßAPP717 and ΔNL mutations, ßAPP processing is altered in a way that fosters amyloid deposition.

589.11

PLASMA AMYLOID ß PROTEIN (Aß) ENDING AT Aß42(43) IS INCREASED IN CARRIERS OF FAMILIAL AD (FAD) LINKED TO CHROMOSOME 14. X-H Song[1]*, N. Suzuki[2], T. Bird[3], E. Peskind[3], G. Schellenberg[3], and S. G. Younkin[1,4]. [1]Case Western Reserve Univ., Cleveland, OH; [2]Discovery Research Division, Takeda Chemical Industries Ltd., Wadai 10, Tsukuba, Ibaraki, 300-42 Japan; [3]Univ. of Washington, Seattle, WA; [4]Mayo Clinic Jacksonville, Jacksonville, FL.

Aß1-40 and Aß1-42(43) both increase significantly in the plasma of patients with the FAD-linked ßAPPΔNL mutation (Jensen et al., this meeting). To determine whether plasma Aß increases in FAD linked to chromosome 14, we obtained plasma samples from 3 families [family 1: 6 carriers (4 affected, 1 possibly affected, 1 unaffected), 7 non-carriers; family 2: 1 affected carrier; family 3: 1 affected carrier, 1 non-carrier]. In addition, we obtained plasma from 1 affected patient with the FAD-linked ßAPP717Δile mutation and their unaffected spouse. All samples were analyzed for Aß1-40 (BAN-50/BA-27 ELISA) and Aß1-42(43) (BAN-50/BC-05 ELISA). In addition, we used a BC-05/4G8 ELISA that detects not only Aß1-42(43) but also amino-terminally truncated Aßs (e.g. Aß17-42). In the plasma of the 8 chromosome 14-linked FAD carriers, Aß1-42(43) showed a highly significant ($p<0.0003$) increase to 2.17 times that observed in the 9 non-carriers, and 7 of the 8 carriers had Aß1-42 concentrations that were higher than any of the 9 non-carriers. The concentration of AßX-42 in the carriers (BC-05/4G8 ELISA) also increased significantly ($p<0.02$) to 1.36 times that observed in the non-carriers, and the concentration of Aß1-40 increased non-significantly to 1.12 times that in the non-carriers. As expected from our previous studies of transfected cells, plasma Aß1-42 in the patient with the Δile mutation was selectively elevated to 1.6 times the mean control value (20% higher than any of the controls) whereas Aß1-40 fell in the middle of the control range. These data on plasma and our data on fibroblasts (Scheuner et al., this meeting) indicate that in chromosome 14-linked FAD as in FAD linked to ßAPP717 or ΔNL mutations, Aß concentration is elevated in a way that fosters amyloid deposition.

589.12

THE $ßAPP_{670/671}$ ALZHEIMER MUTATION (SWEDISH) INCREASES PLASMA AMYLOID ß PROTEIN CONCENTRATION. M. Jensen*[1], X-H Song[2], N. Suzuki[3], L. Lannfelt[1] and S. G. Younkin[2]. [1]Karolinska Institute, Dept. of Clinical Neuroscience and Family Medicine, Huddinge University Hospital, S- 141 86 Huddinge, Sweden. [2] Division of Neuropathology, Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106. [3] Discovery Research Division, Takeda Chemical Industries Ltd., Wadai 10, Tsukuba, Ibaraki, 300-42 Japan.

The ~4 kD amyloid ß protein, which is a cleavage product of the amyloid ß protein precursor (ßAPP), forms insoluble fibrils in brains and vessels of patients with Alzheimer's disease. Using enzyme-linked immunosorbent assays (ELISAs), we developed a method for measuring Aß 1-40 and Aß 1-42(43) in human plasma. We have used the method for analyzing plasma from a large Swedish Alzheimer family in which a double mutation at codon 670/671 of the APP gene converts lysine-methionine to asparagine-leucine. A group of 12 mutation carriers (5 symptomatic, 55-68 years old, 7 pre-symptomatic, 28-51 years old) and 31 non-carriers (20-74 years old) was investigated. The carriers showed a 2.8-fold increase of Aß 1-40 concentration compared to non-carriers ($p<0.0001$), with a total separation between the groups. A similar 2.1-fold increase of Aß 1-42(43) concentration ($p<0.0001$) was seen, with only a slight overlap between the groups. No difference in Aß levels was seen between symptomatic and pre-symptomatic carriers, which shows that the increase of Aß levels is a very early event in the disease. It also indicate the importance of plasma Aß as an early marker for identifying individuals who will develop AD because of elevated Aß concentration.

589.13

HETEROGENEITY OF CALCIUM RESPONSE IN EARLY-ONSET FAMILIAL ALZHEIMER FIBROBLASTS. [1,2] Y. Tatebayashi*, [2]M. Takeda, [3]K. Kamino, [2]T. Kudo, [1]I. Yoshida and [2]T. Nishimura. [1]Dept. Neuropsychiatry, Nissei Hosp. Osaka, Japan, [2]Dept. Neuropsychiatry, Osaka Univ. Medical Sch. Osaka, Japan, and [3]Dept. Geriatric Medicine, Osaka Univ. Medical Sch. Osaka, Japan.

Changes in calcium response were studied to clarify the pathological process of Alzheimer's disease (AD). Cultured fibroblasts from patients with early-onset familial AD (FAD; n=6; 3 families; OS-1, 2 and 3), sporadic AD (SAD; n=4), and age-matched healthy control subjects (n=4) were studied with ACAS Interactive Laser Cytometer. Fibroblasts from two independent families with FAD (OS-1 and 2) showed a suppressed calcium response after stimulation by 100nM bradykinin in Ca^{2+}-free condition compared with OS-3, SAD, and control fibroblasts when they were arrested only in S phase. Since OS-1 families has APP717:Val-Ile mutation, abnormal APP processing in S phase might be responsible for the abnormal calcium response. Microsatellite DNA polymorphisms on chromosome 14q24.3 revealed that OS-2 and -3 families were different in genotype of D14S43 and the former had more possibility to be linked with the chromosome 14 rather than the latter. These results support the existance of heterogeneity in the calcium response and may suggest that the candidate gene located on the chromosome 14 affects the APP processing and results in the cell-cycle dependent abnormal calcium response.

NEURAL-IMMUNE INTERACTIONS: OTHER

590.1

CALCITONIN GENE RELATED PEPTIDE (CGRP) RELEASE IN THE HIPPOCAMPAL FORMATION (HF) OF ADRENALECTOMIZED (ADX) RATS MAY SUPPRESS INFLAMMATORY IMMUNE RESPONSES TO DYING GRANULE CELL NEURONS. K. Bulloch[1]*, T.A. Milner [2], and B.S. McEwen[1],. Lab. of Neuroend. The Rockefeller University (1), and Cornell University, Medical Col. Dept. of Neurol. and Neurosci., N. Y., New York 10021 (2).

ADX results in cell death in granule cells of the dentate gryus (DG). Cell death occurs via apoptosis, thereby preventing the HF from undergoing massive neuronal damage due to cytokine release and a consequent inflammatory immune response. Studies now show that CGRP is increased in the HF after several types of insult, and also has an effect on such HF functions as long term potentiation (LTP) and learning. CGRP also contains cellular immune responses in specific organs such as the thymus, by inducing apoptosis in thymocytes, suppresses cell-mediated immunity and blocking macrophages from presenting antigen. Given the immunological and behavioral effects of this peptide, we have begun to characterize the distribution of CGRP in the HF of ADX rats. Light microscopic analysis shows CGRP-immunoreactivity (CGRP-ir) is distributed diffusely in the inner third molecular layer (ITML) of the DG and in DG hilar and in CA3c neurons. In ADX rats, CGRP-ir is far more intense. Electron microscopic analysis of the DG shows that CGRP-ir is primarily found in large, dense core vesicles (100-120 nm) contained in axons and in small axon terminals which form asymmetrical synapses with dendritic spines in the ITML and a few cells within the granule cell layer. Fornix lesions did not reduce CGRP-ir in the HF of ADX rats indicating that the CGRP is mostly derived from intrinsic neurons rather than the subcortical pathway. Future studies may elucidate the role CGRP plays in regulating immune responses in the HF and lead to a clearer understanding of pathological and psychological effects of CNS trauma. (Supported by MH 41256, The Arthur Vining Davis Foundation (BSM,KB); MH42834 and HL18974(TAM)

590.2

IMMUNIZATION-INDUCED INFLAMMATORY INFILTRATION OF THE CNS IN TRANSGENIC MICE EXPRESSING A MICROBIAL ANTIGEN IN ASTROCYTES. P. Borrow*, J.L. Cornell, M.D. Ruppe, and L. Mucke. Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Transgenic mice expressing a defined microbial antigen from central nervous system (CNS) cell type-specific promoters can be utilized to investigate the consequences of induction of peripheral immune responses to foreign antigens produced by different CNS cell types. Following peripheral immunization with β-galactosidase (β-gal) in complete Freund's adjuvant, transgenic mice expressing this target antigen under control of the glial fibrillary acidic protein promoter in astrocytes of the CNS mounted strong β-gal-specific T and B cell responses, and developed massive mononuclear cell infiltration of the CNS. The infiltrating cells, principally $CD4^+$ T lymphocytes and monocyte/macrophages, with some $CD8^+$ T cells and very few B cells, were found both perivascularly and intraparenchymally, and were located predominantly in the hippocampal formation and cerebellum, the areas of highest β-gal expression. Resident CNS cells were activated in response to this inflammatory infiltration of the brain, as astrocytosis, microgliosis, and upregulation of MHC class I and II antigen expression were observed. These CNS changes were accompanied by a generalized increase in blood-brain barrier permeability. The resemblance of these pathologic changes to aspects of human immune inflammatory disorders, e.g., multiple sclerosis, suggest that an initiating step in such diseases could be the induction of a peripheral immune response to microbial or autoantigens expressed in astrocytes.

590.3

INDUCIBLE NITRIC OXIDE SYNTHASE mRNA LEVELS IN RAT BRAIN ARE UPREGULATED BY PERIPHERAL LIPOPOLYSACCHARIDE ADMINISTRATION. J. Licinio*, P.W. Gold, and M.-L. Wong, Clinical Neuroendocrinology Branch, National Institute of Mental Health, NIH, Bethesda, MD 20892-1284.

Nitric oxide is an important informational sustance synthesized from L-arginine by the enzyme nitric oxide synthase (NOS). There are at least two forms of NOS, constituve and inducible. The constitutive form is present in endothelium (eNOS), and in central and peripheral nervous system (nNOS). Inducible NOS (iNOS) is present in kidney, liver, macrophages, and endothelium. An iNOS was recently cloned from rat astrocytes (Galea *et al.* J. Neurosci. Res. 1994;37:406-414). We conducted an *in situ* hybridization histochemistry study using a ^{35}S-labeled antisense riboprobe to determine whether the expression of the gene encoding the astrocyte-derived iNOS isoform in brain was affected by peripheral immune mediators. Virus and antibody-free male Sprague-Dawley rats (250g) were studied 24 h after saline or high-dose intraperitoneal (ip) lipopolysaccharide (LPS) administration. We found induction of iNOS mRNA in various brain regions 24 h after ip LPS. These data indicate that peripheral immune mediators can upregulate iNOS mRNA in rat brain and provide additional evidence that nitric oxide may participate in the complex communication pathway between the immune system and the brain. Abnormalities in that pathway are known to cause susceptibility to inflammatory disease. The elements involved in communicating signals between the immune system and the brain include cytokines, the hypothalamic-pituitary-adrenal axis, and possibly nitric oxide.

590.4

THE VAGUS MEDIATES CYTOKINE STIMULATION OF PLASMA ADRENOCORTICOTROPIN PRODUCED BY INTRAPERITONEAL INTERLEUKIN-1ß AND ENDOTOXIN / LIPOPOLYSACCHARIDE. L. P. Kapcala*, J. R. He, Y. Gao, J. O. Pieper, and L. J. DeTolla. Endocrine Div. and Prog. of Comp. Med., Univ. of Maryland Sch. of Med., Baltimore, MD 21201

Although interleukin(IL)-1ß or endotoxin/lipopolysaccharide(LPS), which stimulates cytokines, activates the hypothalamic-pituitary-adrenal axis (HPAA), the mechanism(s) by which peripheral IL-1ß or LPS stimulates is not clear. Recently, the vagus has been implicated in mediating peripheral cytokine signalling of brain. To investigate a possible mechanism of peripheral cytokine stimulation of the brain, we tested the hypothesis that the vagus mediates immune activation of the HPAA by determining the effect of vagotomy on plasma adrenocorticotropin(ACTH) in rats after intraperitoneal(ip) IL-1ß and LPS. Adult male Sprague-Dawley rats underwent subdiaphragmatic vagotomy or sham surgery 1 wk prior to study. Rats were sacrificed 2 hrs after ip saline, IL-1ß(20 µg/kg) or LPS(4 mg/kg).

*p<0.05 vs Control	Control	IL-1ß	LPS
Sham Vagot. (Pl ACTH)	26 ± 5	178 ± 52*	437 ± 58*
Vagotomy (mean±SEM)	25 ± 6	59 ± 20	304 ± 47*

Vagotomy abolished IL-1ß stimulation of plasma ACTH and attenuated (~30% decrease) the LPS stimulation of plasma ACTH.

CONCLUSION : The vagus plays a critically important role in mediating peripheral (ip) activation of the HPAA by IL-1ß and at least a partial role in mediating ip HPAA activation by LPS.

590.5

PROSTAGLANDIN E_2 INDUCES EXCITATION OF MYENTERIC NEURONS IN THE GUINEA PIG ILEUM. J.A.J.M. Dekkers, A.B.A. Kroese* and L.M.A. Akkermans. Department of Human and Animal Physiology, Agricultural University, Haarweg 10, 6709 PJ Wageningen, The Netherlands.

The mode of action of the inflammatory mediator prostaglandin E_2 (PGE_2) on myenteric neurons was investigated by intracellular recordings in a conventional plexus preparation. Bath application (3-6 min) of PGE_2 (1-1000 nM) evoked a concentration dependent and reversible slow (>5 min) depolarization in 23 of 26 AH and 12 of 13 S neurons. The slow depolarization evoked by PGE_2 was TTX-insensitive. The amplitude ranged from 4±1 mV at 1 nM to 13±3 mV at 1 µM in both types of neuron. S and AH neurons showed augmentation of excitability by PGE_2, as was observed by the occurence of spontaneous action potentials and an increase in the number of action potentials evoked by depolarizing current pulses. In AH neurons, the PGE_2 induced depolarization was accompanied by an increase in membrane resistance (control 114±13 MΩ), which ranged from 8±3 MΩ at 1 nM to 73±27 MΩ at 1 µM. In AH neurons, PGE_2 (1 µM) reduced the amplitude (7±1 mV) and the duration (8±1 s) of the post-spike afterhyperpolarization by 50%. In S neurons, PGE_2 evoked either an increase (n=6) or a decrease (n=6) in membrane resistance. Moverover, PGE_2 evoked bursts of fEPSPs in S neurons, indicative for cyclical activity in the myenteric plexus.

The results show that PGE_2 in the nM concentration range induces excitation of myenteric neurons. It is concluded that the inflammmatory mediator PGE_2 can modulate gastrointestinal motility by a direct action on neurons in the myenteric plexus.

590.6

PRIMARY DEMYELINATION AND MOTOR DISEASE INDUCED BY THE CNS PRODUCTION OF INTERLEUKIN-3 (IL-3) IN TRANSGENIC MICE I. L. Campbell *, C-S. Chiang, H.C. Powell+, A. J. Roberts and L.H.Gold, Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037. #VA Research Service, VAMC and University of California San Diego, La Jolla, CA 92093.

Macrophage/microglia (MM) may play an important role in mediating tissue injury in a variety of CNS disorders. To examine this possibility, transgenic (tg) mice were developed in which the expression of the MM activation factor, IL-3, was targeted to astrocytes using a GFAP-IL3 fusion gene construct. Tg mice with low levels of IL-3 expression developed from 5 mo of age a progressive and eventually fatal motor disorder (gait abnormality, tremor, ataxia then quadraplegia). Examination of motor performance using a rotarod apparatus revealed impairment in the tg mice just prior to the onset of visible motor changes. Neurohistologic and electron microscopic analysis in symptomatic tg mice, revealed multi-focal plaque-like, white matter lesions in cerebellum and brain stem. Lesions showed extensive primary demyelination and remyelination in association with the accumulation of large numbers of MM, many of which contained numerous lipid rich vacuoles and/or crystalline 'pole-shaped' inclusions. Lymphocytes were rarely, if at all present. In addition to recruitment from the periphery and possibly other brain areas, significant proliferation of the MM cells in the white matter was also observed. Lesion-associated MM cells were shown to be activated with markedly increased expression of the complement C3 receptor and MHC class II (Ia) molecules. As with clinical signs, white matter disease in the tg mice was progressive commencing at approx. 4 mo age. Prior to this age, increased Ia expression by perivascular MM cells was the earliest detectable change in the CNS, implicating these cells in the later development of white matter disease. In conclusion, tg mice with low levels of cerebral IL-3 expression develop a progressive motor disorder as a result of a vigorous T-cell independent MM-associated demyelination process. Many of the clinicopathologic manifestations of this disorder resemble those observed in acute MS indicating the GFAP-IL3 tg mouse represents a significant new model to study the role of MM in the pathogenesis of CNS demyelinating disease. Supported by USPHS grant MH50426.

590.7

ASSOCIATION BETWEEN INCREASED SERUM SOLUBLE INTERLEUKIN-2 RECEPTORS AND DISTURBANCE IN THE CONTROL OF MOTOR MUSCLE FORCE IN SCHIZOPHRENIC PATIENTS, M.H. Rapaport, M.P. Caligiuri*, J.B. Lohr. Dept. of Psychiatry, Univ. of CA, San Diego, School of Medicine, Psychiatric Service, San Diego Veteran's Affairs Med. Ctr., La Jolla, CA 92037

It has previously been shown that serum soluble interleukin-2 receptors(SIL-2Rs), a sign of immune activation, are increased in approximately 30% of schizophrenic subjects, and that serum SIL-2Rs are particularly elevated in schizophrenic subjects with tardive dyskinesia. In this paper we extend our previous work by investigating the relationship between motor force instability and SIL-2Rs receptors in 35 schizophrenic patients. We were particularly interested in investigating this relationship because increases in muscle force instability seem to precede the development of tardive dyskinesia and also because measurement of muscle force instablility may be a more accurate way of reproducibly measuring involuntary movements. We hypothesized that there would be a positive correlation between increased levels of SIL-2Rs and muscle motor force instability. Serum SIL-2Rs and muscle force instability were positively correlated (r=0.5, p<.001), and this correlation held even for a small subset of neuroleptic naive patients (r=.53,df 9,p=.008). Further, when we dichotomized patients into high and low serum SIL-2R groups, high serum SIL-2R schizophrenics had significantly more muscle force instability than the low serum SIL-2R receptor group (U=73.0, Z=-2.64, p<.008). These findings suggest that there is an important clinical correlation between imune activation and muscle force instability.

590.8

EPINEPHRINE STIMULATES INCREASED IL-6 BLOOD LEVELS IN HEALTHY HUMAN SUBJECTS G.H. Pelton*, L.H. Price, G.R. Heninger. Dept of Psychiatry, Yale Univ. School of Med. 34 Park St., New Haven, CT 06519

Both physiologic and psychologic challenges activate several stress responsive systems, including: 1) the Sympathetic Nervous System (SNS): (eg. incr. plasma catecholamine levels), 2) the hypothalamic-pituitary-adrenal (HPA) axis: (eg. incr. serum cortisol), and 3) the immune system: (eg incr. levels of cytokines including IL-6). In order to assess the specificity of the SNS in humans on IL-6 release and the HPA axis, several doses of Epinephrine (E) were administered to healthy human subjects and changes in plasma IL-6, cortisol and SNS variables (Heart Rate (HR) and Blood Pressure (BP)) were measured. METHODS: Placebo or single subcutaneous (SQ) E doses (0.25mg/subject, or 0.5mg/subject) or a 60 min. continuous IV E infusion at one of three doses (0.05 ug/kg/min, 0.1ug/kg/min or 0.15ug/kg/min), were admin. in a double blind randomly designed manner to healthy human subjects. Before and after the E or placebo administration, IL-6, cortisol, plasma E and norepinephrine (NE), BP and HR, and subjective mood and anxiety measures were obtained. RESULTS: IL-6 levels peaked at 90 minutes after SQ E or initiating the IV infusion, with mean peak IL-6 levels of 1.5pg/ml for the placebo, 1.7pg/ml and 2.8pg/ml for the SQ, and 1.4pg/ml, 2.9pg/ml, and 6.0pg/ml for the IV, in respective stepwise increasing doses. Changes in cortisol blood levels were inconsistent. Plasma E but not NE increased in a dose related stepwise manner. BP and HR demonstrated a clear dose response relationship with a maximal mean BP of 142/70 and mean HR of 102 at the 0.15ug/kg/min dose. Changes in IL-6 positively correlated to changes in HR. No consistent increases in anxiety or mood states were observed. DISCUSSION: This is the first demonstration that E produces a dose-dependent increase in plasma IL-6 levels in humans. The E induced increase in IL-6 occured without changes in plasma cortisol indicating that the CNS can affect the immune system during stress direcly through E release, without involvement of the HPA axis. Since E is released during both psychological and physical stress, peripheral E levels can be an important and specific mediator of the effects of stress on the immune system. This has implications for the role of stress in the dysregulation of the immune system in illness.

590.9

EFFECTS OF SURGICAL STRESS AND PHARMACOLOGIC STIMULATION OF THE HPA AXIS ON INTERLUKEN-6 IN HUMANS. G. Heninger*F. Sevarino, S. Southwick, L. Karper, D. Charney and J. Krystal, Depts. of Psychiatry and Anesthesiology, Yale Univ., New Haven, Ct 06510

In rats, stress from footshock, restraint, or a conditioned aversive stimulus produces large (over 5-10 fold) increases in plasma ACTH, corticosterone and interleukin-6 (IL-6). In humans, large increases in cortisol (C) and IL-6 have been reported following surgery and myocardial infarction, but the relative role of the HPA axis and the SNS on IL-6 release is not clear. METHODS: To compare the effects of increased plasma catecholamines to the effects of HPA axis stimulation on plasma IL-6 and C in humans, IL-6 and C were repeatedly measured before and following IV infusion of placebo and 3 drugs known to increase C levels: yohimbine (Y) (.4mg/kg), M-chlorophenylpiperazine (M) (.1 mg/kg), and ketamine (K) (.9mg/kg) and before and after open heart surgery which markedly increases plasma catecholmines. IL-6 was measured with an ELISA and C with RIA. RESULTS: In comparison to placebo, all drugs produced C increases averaging near 10 ug/dL, but the IL-6 response to Y, M and K did not differ from placebo. In contrast, surgery produced mean increases of: IL-6, 40 pg/ml; epinephrine (E), 531 pg/ml; norepineprhine (NE), 187 pg/ml; and C, 1 ug/dL. Increases in IL-6 correlated to changes in E (r =.86) but not NE or C. CONCLUSION: Changes in plasma IL-6 do relate to changes in plasma E levels, but not to HPA axis stimulation. This is validated by a recent study (Pelton, et.al., this meeting) where E infusions have been shown to increase IL-6. Supported by: The Stanley Foundation and VA Awards.

590.10

DIFFERENTIAL T CELL RESPONSE TO STRESS IN PUBERTAL COMPARED TO ADULT FEMALE MONKEYS
C.J. Rogers, C.S. Brissette-Storkus, L.A. Hayes, W.H. Chambers and J.L. Cameron* Depts.of Cell Biology & Physiology, Pathology, Psychiatry, and Neuroscience, and the Pittsburgh Cancer Institute. University of Pittsburgh, Pittsburgh, PA 15261.

We have previously shown in adult female monkeys that exposure to acute stress decreases the level of expression of the adhesion molecule, CD2, on a subpopulation of cytolytic T cells. Specifically, in adults, cytolytic T cells (i.e. $CD8^+$ cells) can be divided into two populations, one that expresses more CD2 (CD^{bright} cells) than the second ($CD2^{dim}$ cells). Acute stress exposure causes a selective loss of CD2 from the $CD2^{bright}$ population,which may impair cytolytic activity of these cells. The goal of the present study was to determine if the T cell response to stress is influenced by age or pubertal status. Blood samples were collected from 23 pubertal monkeys (19-33 months old), before and after exposure to acute stress (placement in a novel room with repetitive blood sample collection) at 3 month intervals during pubertal development. When the pubertal animals were first studied, 20 monkeys had a single level of expression of CD2 on T cells that was between the levels found in the $CD2^{dim}$ and $CD2^{bright}$ populations of adults. In these animals the level of CD2 expression was unresponsive to acute stress. However, three of the eldest pubertal monkeys demonstrated an adult-like expression of CD2 with a stress-induced loss of CD2 from the $CD2^{bright}$ population. These results indicate that both the basal expression of CD2 on T cells and the stress-responsive loss in CD2 expression are associated with pubertal development. We are currently investigating whether these developmental changes in CD2 expression are linked to developmental changes in cytolytic activity of T cells.

590.11

AGE-DEPENDENT PHENOTYPIC SWITCHING OF MAST CELLS IN NGF-TRANSGENIC MICE. M.L. Getchell[*,1,4], A. Kulkarni-Narla[2], S. Takami[2], K.M. Albers[3], and T.V. Getchell[2,4]. Depts. of [1]Surgery, [2]Physiology, and [3]Pathology; [4]Sanders-Brown Center on Aging, Univ. of Kentucky Coll. of Med., Lexington, KY 40536.

Mast cells (MCs) are effector cells that are prominently involved in the immune response to allergy and inflammation. MC phenotype and numbers were compared in the nasal and oral mucosae and skin of 3- and 6-week old transgenic mice in which NGF expression in epithelial basal cells was driven by the keratin-14 promoter, and in their nontransgenic littermates. Histochemistry with Alcian blue/safranin and berberine sulfate, which stain heparin present in connective tissue MCs but not chondroitin sulfate present in mucosal MCs, was used to distinguish between MC phenotypes. All MCs in 3-week old mice were of the mucosal phenotype except in the tongue of the transgenics, where MCs exhibited primarily mixed or connective tissue phenotypes. MC numbers in the transgenics were increased an average of 3.6X over those in the nontransgenics. In 6-week old transgenics, NGF overexpression resulted in the mucosal phenotype in tissues that contained MCs of the connective tissue or mixed phenotype in nontransgenics. MC numbers in the transgenics were increased an average of 2.5X over those in the non-transgenics. These results suggest that NGF overexpression affects the synthesis of molecular markers of MC phenotype.
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590.12

MAST CELLS EXPRESS A FUNCTIONAL PERIPHERAL CANNABINOID RECEPTOR CB2: DIFFERENTIAL SENSITIVITY TO ARACHIDONYLETHANOLAMIDE AND PALMITOYLETHANOLAMIDE. L. Facci, R. Dal Toso*, S. Romanello, A. Buriani, L. Petrelli, S.D. Skaper and A. Leon. Research/*Life* S.c.p.A., 31033 Castelfranco Veneto, Italy.

Mast cells (MCs) are bone marrow-derived cells of immune lineage found in mucosal and connective tissues and in the nervous system, where they have important roles in inflammation and in neuroimmune interactions. Little is known about endogenous molecules and mechanisms capable of modulating MC activation. Palmitoylethanolamide (PEA) is found in numerous tissues, including brain, and has been proposed to behave as a local autacoid capable of down-regulating MC activation and inflammation. A cognate N-acylamide, arachidonylethanolamide (anandamide), is synthesized in brain and is a candidate endogenous agonist for the central cannabinoid receptor (CB1). As a second cannabinoid receptor (CB2) has been described in the periphery, the possible presence of CB2 on MCs and their interaction with N-acylamides was investigated. Here we report that MCs express the gene for CB2. Further, MCs have a functional CB2 receptor protein with negative regulatory effects on their activation. PEA and anandamide bind CB2, but only the former down-modulated MC activation in vitro. The functional effect of PEA, as well as that of active cannabimimetic compounds, was antagonized by anandamide. These results strongly suggest that CB2 receptors exert, upon agonist binding, a negative regulatory effect on MC activation and therefore inflammation. The demonstration that PEA, unlike anandamide, displays agonistic activity on CB2 suggests that such naturally occurring long-chain saturated fatty acid amides are potential endogenous functional ligands for CB2. Modulatory activities on MCs exerted by these molecules strengthen a proposed autacoid local inflammation antagonism (ALIA) mechanism. PEA and its derivatives ("ALIAmides") may thus provide new antiinflammatory therapeutic strategies specifically targeted to MCs, including those of an autoimmune origin like multiple sclerosis.

DEVELOPMENT OF VISUAL SYSTEM II

591.1

SHAPING OF RECEPTIVE FIELD PROPERTIES IN DEVELOPING RETINAL GANGLION CELLS IN THE ABSENCE OF EARLY CHOLINERGIC SPONTANEOUS ACTIVITY. E. Sernagor* and N.M. Grzywacz. Smith-Kettlewell Eye Research Institute, San Francisco, CA 94115.

The maturation of receptive field (RF) properties of turtle retinal ganglion cells (GCs) occurs at 2-4 weeks post-hatching (PH), and is temporally correlated to the disappearance of spontaneous bursts of activity expressed by these cells when they are immature (Sernagor & Grzywacz, *J. Neurophysiol.*, 1995).

To study the role of these bursts in shaping mature RF properties, we have investigated their development during chronic blockade of the bursts. We have used curare, a cholinergic nicotinic blocker, because it eliminates the bursts *in vitro* (Sernagor & Grzywacz, *ARVO*, 1994). Curare (1 mM) was mixed to the slow-release polymer Elvax 40W. Pieces of Elvax were implanted intraocularly at different developmental stages, and extracellular recordings of light responses were obtained at 1 month PH. Both RF areas and the incidence of GCs with concentric RFs were significantly smaller in curarized retinas than in normal ones. The earlier curare was applied, the more pronounced the effects were. When curare was applied at hatching and turtles were dark-reared, RF areas did not expand as in non-curarized dark-reared turtles (Sernagor & Grzywacz, *Neurosci. Abstr.*, 1994). The curarized dark-reared RFs resembled those of curarized GCs raised in the light. The incidence of concentric RFs was similar in curarized retinas that developed either in normal light or in the dark, and in non-curarized dark-reared retinas. We have previously reported that the incidence of orientation selectivity is lower in dark-reared retinas. However, curare had no effect on that property.

These results demonstrate that early acetylcholine-dependent spontaneous bursts, but not light, control the development of RF areas. As GCs develop more specialized functional properties, light becomes more involved in their maturation. Both spontaneous bursts and light-driven activity are required for the maturation of concentric RFs. Finally, maturation of orientation selectivity depends primarily on visual experience. Supported by NIH (EY 10600 to E.S.; EY 08921 to N.M.G.)

591.2

OPTIC NERVE STIMULATION ELICITS EVOKED RESPONSES IN SUPERIOR COLLICULUS OF EMBRYONIC RATS *IN VITRO*. L.J. Reece* and C.H. Lim. Developmental Neurobiology Group, Research School of Biological Sciences, The Australian National University, Canberra, ACT 2601

Recent experiments *in vivo* indicate there is no optic nerve conduction in the rat until P0 and no response to visual stimuli in the colliculus until P10, close to the time of eye opening (1). Our recent *in vitro* findings of early capacity for synaptic function in the superior colliculus of the wallaby well before the onset of eye opening have led us to examine similar questions in the visual system of the rat.

Evoked responses were examined in the superior colliculus of late embryonic and early postnatal rat pups (E14 - P6) in a standard perfusion chamber. The preparation consisted of a hemi-mesencephalic/diencephalic preparation with the contralateral optic nerve intact. Responses were robust, long-lasting and could be elicited with relatively low voltage low frequency bipolar stimulation. Evoked potentials were seen as early as E18; these potentials were blocked by cobalt (a calcium blocker) and by kynurenic acid (a glutamatergic antagonist). In addition, several potentials reversed with depth, another indication of synaptic activity. Replacement of sodium by choline completely blocked the responses, indicating that sodium, not calcium, is the primary ionic carrier for axonal conduction. Responses were detected in caudal colliculus as early as E18, and conduction velocity averaged around 0.1 m/s. Application of gamma-amino-butyric acid (GABA) blocked the potential in a dose-dependent manner as early as E18; this action was itself blocked by bicuculline methiodide (BMI), a GABA-A antagonist. Our studies show there is clearly conduction in the optic nerve plus a capacity for synaptic function in the colliculus at early ages in an *in vitro* preparation; we are pursuing the question of why the *in vitro* and *in vivo* results differ dramatically.

1. Molotchnikoff, S. and Itaya, S.K. (1993), *Dev. Br. Res.*, 72: 300-304.

591.3

SPONTANEOUS BURSTING OF GANGLION CELLS IN THE DEVELOPING RETINA REQUIRES SYNAPTIC INPUT. M. B. Feller*, D.P. Wellis, F.S. Werblin, C. J. Shatz. HHMI and Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

Ganglion cells in the neonatal ferret retina spontaneously fire bursts of action potentials in the form of excitatory waves that propagate across the retina. Ganglion and amacrine cells also undergo periodic spontaneous increases in intracellular calcium concentration in a correlated fashion (Wong et al., Nature 374:716 1995). How is this activity generated at these ages, when the photoreceptors and bipolar cells are immature and not yet functional?

Combined optical imaging of fura-2 stained neonatal retinas (P0-P12) and whole cell voltage clamp recordings reveal that the periodic bursts of activity recorded in the ganglion cell layer are generated synaptically. Cells undergo a barrage of inward synaptic currents that reverse near 0 mV with a period ranging from 2-4 minutes. The synaptic currents in an individual ganglion cell, as well as the correlated waves of activity in many ganglion cells, were blocked by bath application of 100μM d-tubocurarine, a nicotinic acetylcholine receptor antagonist, or 10μM nicotine, an agonist whose tonic exposure desensitizes the receptor. Propagating waves were evoked experimentally by locally puffing potassium or nicotine at ganglion and amacrine cell processes.

Since the only known cholinergic cell type in the mammalian retina is a subset of amacrine cells, these results suggest that a requirement for the spontaneous correlated activity in ganglion cells is synaptic input from cholinergic amacrine cells

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591.4

PERIODIC SYNAPTIC CURRENTS IN THE NEONATAL LGN ARE GENERATED BY RETINAL ACTIVITY. R. Mooney*, A. A. Penn, and C. J. Shatz. HHMI & Dept. Molec. & Cell Biol. UC, Berkeley, CA 94720.

Electrophysiological and optical recording techniques show that in neonatal ferrets, rats and mice, before the onset of photoreceptor function, retinal ganglion cells generate high frequency periodic bursts of action potentials that propagate in waves across the retina. Are these patterns of retinal ganglion cell activity detectable within the LGN and, if so, what effect do they have on the electrical activity of LGN neurons?

To address these questions, we developed an *in vitro* preparation of the neonatal mouse brain containing both retinae connected to the LGN. In P0-P4 mice, highly periodic barrages of postsynaptic currents (PSCs) were observed when whole cell voltage clamp techniques were used to record synaptic currents from individual LGN neurons. These PSCs had a periodicity like the bursts exhibited by the retinal ganglion cells themselves (ca. every 30-90 seconds), were glutamatergic, and were reversibly blocked by applying lidocaine to the optic nerve, indicating that they were the result of the periodic retinal activity. Although the average PSC amplitude recorded in the LGN was quite small (ca. 5-10 pA), these PSCs could strongly depolarize the postsynaptic membrane. When the postsynaptic cell was taken out of voltage clamp, the periodic barrage of synaptic activity caused the cell to undergo a prolonged depolarization, which could drive the cell to generate bursts of action potentials. Therefore, periodic waves of retinal activity strongly influence the electrical behavior of LGN neurons, and could function in activity-dependent synaptic reorganization within the LGN.

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591.5

SPONTANEOUS RETINAL INPUTS DRIVE POSTSYNAPTIC ACTION POTENTIALS IN THE LGN. A. A. Penn*, R. Gallego, R. Mooney, and C.J. Shatz. HHMI & Dept. Molec. & Cell. Biol., UC, Berkeley, CA 94720.

Correlated spontaneous activity coming from the retina during early development is thought to play a key role in guiding the formation of highly ordered connections in the visual system. But is this activity transmitted to the LGN? We developed an *in vitro* preparation in neonatal mice in which both retinas, the retinogeniculate pathway and the LGN neurons are intact. With extracellular microelectrodes placed in the LGN, we obtained multiunit and single unit recordings of periodic bursts of spikes with an interburst periodicity resembling that of the retinal signal (30s-90s). Individual units showed repetitive, signature spiking patterns, but there was a wide range of spike patterns between individual units. To demonstrate that we were recording the postsynaptic activity of the LGN neurons, we used the fact that the retinogeniculate synapse is glutamatergic, but the spontaneous activity of the RGCs is not affected by the glutamate blockade. Application of 10μM CNQX blocked all spike activity when the electrode was in the LGN, but periodic bursting activity was maintained in the optic tract. The similarity of the periodicity of the bursts to the synaptic currents which have been recorded in the postsynaptic neurons in the same preparation (R. Mooney et al., Soc. Neurosci. Ab., '95) is further evidence that the postsynaptic neurons are driven to spike by retinal inputs. These results lend strong support to the hypothesis that correlated retinal activity can drive activity-dependent synaptic reorganization within the LGN. Moreover, because LGN neurons receive sufficiently strong inputs to generate bursts of action potentials, it is possible that even at very early times in development, information from the retina is relayed all the way to the visual cortex.

Supported by NSF IBN 93-19539, March of Dimes, NIMH 48108 to C.J.S., H.A. Whitney Fellowship to R.M., Sabbatical Fellowship from DGICYT, Spain to R.G., & M.S.T.P. Trainee GM 07365 to A.A.P.

591.6

EARLY DEVELOPMENT OF RETINOFUGAL PROJECTIONS IN THE FETAL PRIMATE. C. Meissirel* and L.M. Chalupa. Dept. of Neurobiology, Sect. of Neurobiology, Physiology and Behavior, University of California, Davis, CA 95616.

A hallmark of the primate visual system is the unique segregation of the magno- and parvocellular pathways from the retina to the cortex. Since little is known about the prenatal development of such pathways we have investigated the target specificity of retinofugal projections in the developing rhesus monkey. Two carbocyanine dyes, dil and diA, were implanted in the optic nerve, or optic tract, of fixed specimens, ranging in age from embryonic (E) day 48 to E78 (gestation 165±2 days). After an appropriate storage time at 37°C coronal sections were cut with a vibratome and anterograde labeling was examined using confocal microscopy. By E48, the first retinal axons were seen to navigate through the inner part of the dLGN to their distal midbrain targets. At this age, the more numerous crossed contingent of retinal fibers establishes a few collaterals at the level of the pregeniculate and dLGN, whereas the uncrossed contingent remains unbranched. By E53, the density of collaterals increases in the contralateral pregeniculate and dLGN. Remarkably, these branches are exclusively located in the medial part of the dLGN (the presumed parvocellular anlage) and avoid its lateral counterpart, where the magnocellular layers will emerge. By E65 a dense ramification of axonal branches is still strictly confined to the inner part of the dLGN in the prospective parvocellular layers. The magnocellular layers are not innervated until E 78, some 3 weeks after retinal fibers first invade the dLGN anlage. These observations suggest that the retinogeniculate parvocellular pathway is formed much earlier than the magnocellular pathway. (Supported by NIH EY08763 and RR00169. CM supported by M.R.E.)

591.7

A LOCAL CORTICAL SOURCE OF NEUROTROPHIN RESCUES LATERAL GENICULATE NEURONS FROM THE EFFECTS OF MONOCULAR DEPRIVATION. D.R. Riddle* and L.C. Katz. Department of Neurobiology, Duke University Medical Center, Durham, NC 27710.

Monocular deprivation during the critical period leads to marked shrinkage of neurons in the deprived-eye layers of the lateral geniculate nucleus (LGN). This atrophy may result from a decreased ability of less active LGN neurons to compete for some neurotrophin. We tested this possibility directly using a novel method for *in vivo* delivery of neurotrophins. Fluorescent latex microspheres were coated with NT-4, NT-3 or BDNF. Small adjacent injections of control and neurotrophin-coated microspheres were placed in the visual cortex of ferrets (P38-P45) and the contralateral eye was sutured closed. Since microspheres are retrogradely transported, it was possible to determine precisely which neurons in the LGN were exposed to, bound and internalized the neurotrophin. After four days, 250-700 cells labeled with NT-4-coated microspheres and a similar number of cells containing control microspheres were measured in coded sections of the ipsilateral LGN of each animal. Neurons containing control microspheres were 17% smaller in the closed-eye layer than in the open-eye layer (199±9 vs 241±8 μm^2, mean±sem, N=6 animals, $p<0.001$ by paired t-test). Cells in the closed-eye layer that contained NT-4-coated microspheres, however, showed no such shrinkage (239±11 vs 241±8 μm^2, $p>0.3$). Neurons in the open-eye layer that contained NT-4-coated microspheres were no larger than control neurons (246±7 vs 241±8 μm^2, $p>0.01$). Thus, the data reflect specific rescue of cells in the closed-eye layer from the presumed effects of a competitive imbalance, rather than a general growth promoting effect. Preliminary evidence indicates that neither NT-3 nor BDNF has a similar rescue effect. These results, taken together with evidence that exogenous NT-4 blocks normal development of ocular dominance columns (Cabelli et al., 1995, *Science* 267:1662), provide evidence for a link between activity-dependent competitive interactions and the control of neuronal growth and connectivity by neurotrophins. Supported by EY06617 (DRR) and EY07690 (LCK). We thank Regeneron Pharmaceuticals Inc. for the generous gift of the neurotrophins.

591.8

PRENATAL DEVELOPMENT OF THE HUMAN RETINOGENICULATE PROJECTION STUDIED WITH DII. R. F. Hevner*. Dept. of Pathology (Neuropathology), Stanford Univ. Med. Ctr., Stanford, CA 94305-5324.

The adult mammalian lateral geniculate nucleus (LGN) is divided into histologic laminae which receive monocular afferent inputs from either the left or right retina. During fetal development, the LGN initially appears as a homogeneous cell mass, with lamination occurring later in gestation. Previous studies in animals have shown that left and right eye inputs to the developing LGN initially overlap, and are subsequently segregated into eye-specific layers which correspond to the histologic laminae of the LGN. Shatz (J. Neurosci. 3:482-499, 1983) has shown that afferent segregation occurs prior to histologic lamination of the LGN, by about 2 weeks in cats.

In the developing human LGN, early histologic lamination is observed at about 22 weeks gestational age (GA). To determine if the segregation of left and right eye afferents precedes histologic lamination in the human LGN, the lipophilic tracer dil was injected into one optic nerve ($n = 2$) or optic tract ($n = 4$) of normal 20-22 weeks GA human fetal brains, which were obtained at post mortem examination and fixed in paraformaldehyde. After 6-10 months diffusion, sections through the LGN were cut in the coronal plane on the freezing sliding microtome, counterstained with DAPI, and examined by fluorescence microscopy. Following optic nerve injections, dil fluorescence in the LGN was localized to distinct alternating bands, presumed to be the precursors of histologic laminae. In contrast, optic tract injections labeled the entire LGN diffusely (except for cell-sparse zones between developing histologic laminae in 22 weeks GA brains). In all cases, fluorescence was strictly localized to the developing retinogeniculate pathway, without labeling of adjacent nuclei or tracts.

These results indicate that the segregation of left and right eye afferents in the human LGN begins prior to 20 weeks GA, and precedes histologic lamination by at least 2 weeks.

591.9

TRANSIENT TENASCIN-IMMUNOREACTIVE BOUNDARIES DURING THE DEVELOPMENT OF FUNCTIONAL LAYERS IN FERRET LATERAL GENICULATE NUCLEUS C.M. Müller*+, A. Faissner# and S. Altrogge+; +Max-Planck-Institute for Developmental Biology, 72076 Tübingen, Germany; #Dept. Neurobiol., University Heidelberg, 69120 Heidelberg,Germany.

The thalamic dorsal lateral geniculate nucleus (dLGN) of the adult ferret visual pathway reveals distinct anatomical layers receiving selective input from the contralateral (A) and ipsilateral (A1) eye, respectively. These layers are further subdivided into sublaminae innervated by "on"- and "off"-center ganglion cells. In ferret, the parcellation of these functional units occurs postnatally under the influence of retinal activity by axonal remodeling. The astroglia derived matrix molecule tenascin has been shown to influence axonal growth and is transiently expressed at developing functional boundaries in the CNS, e.g. around the somatosensory barrels in rodent cortex (*Steindler et al., Dev. Biol. 131:243,1989*). Using immunocytochemistry we investigated the spatio-temporal expression of tenascin in the dLGN. Our data show that strong tenascin immunoreactivity (TN-IR) is uniformly distributed in the dLGN during the first postnatal week. A prominent band of TN-IR is present at the boundary between the dLGN and the perigeniculate nucleus at P7. In the second postnatal week TN-IR in dLGN becomes strongest in the region of the future interlaminar zone between A and A1. This pattern is due to a reduction of tenascin-IR within the future layers and coincides with the time of segregation of the ipsi- and contralateral retino-thalamic projections. A further distinct TN immunoreactive band occurs within lamina A during the third postnatal week, spatio-temporally coincident with the segregation of "on"- and "off"-laminae. We propose that tenascin plays a role in the formation of the functional subdivisions of the dLGN, most probably by restricting axonal growth to individual sublaminae during the process of activity-dependent reorganization of retino-thalamic projections. (Supported by the BMFT 0316902)

591.10

SELF-ORGANIZING SENSORY MAP FORMATION ACHIEVED WITH A PURELY LOCAL LEARNING RULE MAXIMIZING INFORMATION-THEORETIC ENTROPY. Marc M. Van Hulle* Laboratorium voor Neuro- en Psychofysiologie, K.U.Leuven, Faculteit Geneeskunde, Campus Gasthuisberg, Herestraat, B-3000 Leuven, Belgium.

Models accounting for the formation of sensory maps from simple principles of self-organization abound in the literature. One of the most successful models in this respect is the Kohonen rule and its many variations. A key feature of this unsupervised competitive learning rule is the neighborhood connection kernel whose range shrinks over time. There are several problems with this procedure: If the neighborhood kernel shrinks too fast, then several topological defects may occur that will not be resolved. Moreover, as long as the neighborhood is present, the correct criterion (error potential) is not minimized. Furthermore, when the neighborhood kernel has disappeared, the resulting map will undersample high probability regions and oversample low probability regions of the afferent input space. However, the biological evidence shows that the central representation simply follows the peripheral sensor density: it is not necessary to postulate an additional magnification in the lateral geniculate nucleus or the primary visual cortex (Wässle *et al.*, Nature, 1989). In addition, in the Kohonen rule, global communication between all neurons is needed to determine the unique "winner" neuron, which again is biologically implausible. We will show that it is possible to achieve sensory maps with an unsupervised learning rule performing *local* weight updates only, and thus without requiring shrinking neighborhood kernels and global connection kernels: the rule requires only communication among immediate neighbors. The rule maximizes the information transfer (information-theoretic entropy) of the map. Its convergence and speed of convergence can be shown formally. We will apply the rule to retinotopic map formation and verify that it approximates the magnification factor found in the visual cortex.

The author is a senior research associate of the National Fund for Scientific Research (Belgium).

VISUAL CORTEX: STRIATE V

592.1

SPATIOTEMPORAL ANALYSIS OF JOINT FIRING OF NEURONS IN MONKEY V1. K.P. Purpura*, E. Katz and J.D. Victor, Dept. Neurol. & Neurosci., Cornell Univ. Med. College, New York, N.Y. 10021.

We have developed a new method for analyzing the joint firing of visually-driven neural activity. This method departs from conventional methods of crosscorrelation analysis in that details of receptive field structure and dynamics are preserved in a spatiotemporal map of the joint firing. Multi- and single electrode pair recordings were made in the striate cortex of anesthetized/paralyzed macaque monkeys. Stimuli consisted of arrays of luminance checks or oriented bars driven by multi-input pseudorandom binary M-sequences. A data set consisted of the responses elicited from 16 presentations of a given M-sequence, with every other run an inverted repeat. Standard techniques were used to calculate the 1st- and 2nd-order spatiotemporal kernels. To estimate the joint firing between two channels of activity the time of occurrence of a spike on one channel (binned to 4 ms) was used as the center point for a temporal window onto the other channel. If a spike occurred in the latter channel within the temporal window a spike was added to the measure of joint firing at the time point in the M-sequence corresponding to the occurrence of the spike in the first channel. The joint firing activity was crosscorrelated against the M-sequence to produce binary spatiotemporal kernels just as was done with the individual channels. Joint activity typically appeared in regions of receptive field overlap. These maps of joint firing, however, could not determine if the joint activity was due merely to common driving by the stimulus or to interactions between the channels. To resolve the origin of joint firing a 'shuffled' measure was produced by taking one channel of activity from one M-sequence presentation and the other channel from one of the other repeats of the sequence. Many of the maps of joint V1 activity produced by the shuffle closely resembled those of the unshuffled maps indicating that many neural pairs were driven independently by the stimulus. However, we did find several pairs for which a large part of the joint firing arose from neural interactions. The magnitude, spatial distribution and time course of these interactions will be discussed.

592.2

STIMULUS-DEPENDENT NEURONAL OSCILLATIONS IN AREAS V1 AND V2 OF THE ALERT MACAQUE MONKEY. C.M.Gray*, S.R.Friedman-Hill and P.E.Maldonado, Center for Neuroscience, Univ. of Calif., Davis, CA 95616.

Although abundant in the cat, studies of the occurrence and properties of stimulus-dependent neuronal oscillations in the visual cortex of the monkey have led to conflicting results. In an effort to resolve these differences we have performed multiunit and single unit recordings in areas V1 and V2 in two macaque monkeys trained to perform a visual fixation task. Cells were activated by drifting and stationary light bars and spatial frequency gratings. The occurrence of oscillations was evaluated using correlation and spectral analyses. In V1 48% of multiunit recordings and 30% of single unit recordings exhibited statistically significant oscillatory firing. The mean frequency was 52+/-7 Hz and 50+/-7 Hz, respectively. In V2 34% of multiunit recordings were oscillatory, having a mean frequency of 49+/-8 Hz. In no instance did we observe significant oscillations in trial-shuffled correlograms (i.e. the shift predictor), nor were any significant spectral peaks encountered at 80 Hz, the refresh rate of our video monitor. These data exclude the possibility that the oscillations were driven, in a time-locked fashion, by the visual stimulus. The oscillatory modulation was greatest for the preferred stimulus and decreased at stimulus directions, spatial frequencies and luminance values away from the optimum. Little or no oscillatory modulation was present in the spontaneous activity of oscillatory cells. Stationary stimuli evoked oscillatory responses of equal magnitude, but lower frequency, than those evoked by moving stimuli (moving freq=56+/-5 Hz, static freq=42+/-9 Hz, $p<.003$). Finally, the oscillatory firing of single cells typically consisted of repetitive sequences of high frequency bursts of action potentials "chattering". The similarity of incidence, magnitude, frequency and stimulus dependence of oscillatory activity in cat and monkey suggest that this phenomenon is a general property of mammalian visual cortex. Supported by NEI (EY08686-06) and the Klingenstein Foundation.

592.3

SHORT AND LONG-RANGE RESPONSE SYNCHRONIZATION IN MACAQUE STRIATE CORTEX. S.R. Friedman-Hill*, P.E. Maldonado and C.M. Gray, Center For Neuroscience, University of California, Davis, CA 95616

We investigated the incidence and stimulus-dependence of local and distant temporal correlations in areas V1 and V2 of the awake macaque. For single units recorded from the same site, 36% exhibited significant local synchrony. The cross correlation functions (CCF) were characterized by semi-periodic oscillation with a mean frequency of 49±6 Hz, in 92% of pairs. For the group of pairs with oscillatory CCFs, 64% had periodic ACFs for both units and 36% had periodic ACFs for one member of the pair. We also examined the incidence of cross correlations between single units recorded at sites separated by 3-5mm. Only one pair demonstrated significant CCFs. However, our present sample contains only one instance where both units have rhythmic ACFs.

Cross-correlation analysis of multiunit activity (MUA) recorded at two sites separated by 3-5mm yielded significant correlations in 38% of the pairs, with a mean frequency of 49±6 Hz. In 91% of these pairs, the ACFs of the MUA at both sites showed significant oscillations. Shared orientation preference did not appear to play a strong role in determining whether two sites exhibited synchrony. Although there were no instances of correlations between sites with orientation preference more than 67° apart, there were equivalent rates of synchrony (~50%) for sites with tuning differences of 0°, 22°, and 45°.

Oscillatory acitivity is a strong predictor of short and long-range cross correlation. Although there were instances of non-significant CCFs when one or both members of a pair had rhythmic ACFs, we found no instances of significant CCFs when both sites had non-rhythmic ACFs.

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592.4

TIME COURSE AND FREQUENCY DISTRIBUTION OF OSCILLATORY FIRING PATTERNS IN THE MACAQUE PRIMARY VISUAL CORTEX. P.E. Maldonado*; S.R. Friedman-Hill and C.M. Gray. Center for Neuroscience, University of California, Davis, CA 95616.

We have demonstrated the presence of stimulus-dependent oscillatory firing patterns in single and multiunit recordings from the primary visual cortex of alert macaque, while performing a simple visual fixation task. We examined whether the frequency distribution for oscillatory firing patterns across the sample (40 - 65 Hz), was similar to that of the responses of single units across consecutive presentations of the same square-wave drifting grating. For 35 single units studied, we found that the frequency distribution across 20 to 30 trials was statistically similar ($P < 0.01$) to that across cells. This suggests that single cells do not exhibit a constant oscillation frequency, but rather fluctuate from trial to trail, within a broad range.

The typical response of oscillating single units to a square-wave drifting grating consists of an initial transient ON response of 50 msec duration, followed by a less intense, sustained response that continues as long as the stimulus is presented in the visual field. Correlation and spectral analysis of the temporal firing patterns of 25 single neurons during the ON and the sustained response revealed that during the ON response there were no significant oscillations, whereas significant oscillations are detected during the extent of the sustained response. Thus, oscillatory firing patterns do not arise immediately upon the stimulus onset, but they develop during the subsequent sustained response. A similar analysis performed in 20 cell pairs that exhibited short range synchronous oscillations resulted in a similar temporal pattern. These results suggest that adjacent cells that are locate in the same minicolumn, initially exhibit asynchronous firing that becomes synchronized at longer latencies.
Supported by NEI EY08686-06 and the Klingenstein Foundation.

592.5

WHY NEURONS MAKE BAD COINCIDENCE DETECTORS BUT GOOD PERIODICITY DETECTORS B. Mel, Dept. Biomed. Engin., USC 1451, Los Angeles, CA 90089, E. Niebur, D. W. Croft & F. Gabbiani*, CNS, Caltech 139-74, Pasadena, CA 91125.

It has been suggested that the visual system and other sensory systems use temporal codes. We study how neurons respond to temporal structure in their inputs using simulations of (1) a 164-compartment pyramidal cell with passive or active dendrites, and NMDA or non-NMDA type synaptic input, and (2) a single-compartment integrate-and-fire neuron. In each run, 100 randomly placed excitatory synapses were activated at 100 Hz and the cell's mean output firing rate was recorded. In different runs, and over a range of peak excitatory synaptic conductances, the temporal structures of input spike trains was varied along 2 continuous dimensions: synchronicity (S) and periodicity (P). S=0 meant complete asynchrony and S=1 complete synchrony among trains; P=0 meant Poisson and P=1 periodic trains. Surprisingly, only modest increases in output firing rate were seen as S increased from 0 to 1, and only for the smallest peak synaptic conductances studied. In these cases, input coincidences were required for the cell to reach firing threshold. More surprisingly, the output firing rate was dependent on P over essentially the entire range of peak synaptic conductances studied; 3-fold increases in output firing rate were common as P increased from 0 to 1. This is explained by the fact that increases in P reduced synaptic saturation (i.e. loss of driving force) due to accidental temporal overlap of EPSPs within individual input trains.

592.6

PHYSIOLOGICALLY IDENTIFIED CELL GROUPS HAVE DISTINCT RECEPTIVE FIELD AND MORPHOLOGICAL PROPERTIES IN CAT STRIATE CORTEX. D.A.McCormick* and C.M.Gray#. *Yale Univ. Med. Sch. 333 Cedar St. New Haven CT 06510 and #Center for Neurosci., Univ. Cal., Davis, CA 95616

Neurons of the visual cortex have been characterized according to their morphology, intrinsic membrane properties, and receptive field responses, while the relationships between these features have been only partially defined. Here we performed intracellular recordings from neurons in cat striate cortex with biocytin-filled microelectrodes and correlated the morphology and receptive field properties of neurons with identified intrinsic membrane properties. Four distinct electrophysiological groups were noted: 1) Regular spiking; 2) Intrinsic Burst-generating; 3) Fast Spiking; and 4) Chattering. Regular spiking neurons formed a heterogeneous group of cells, both morphologically and in visual response properties, and were typically pyramidal cells in layers II, III, V or VI exhibiting simple or complex receptive fields. Intrinsic burst-generating neurons generated single bursts of 3-5 action potentials as well as tonic, single spike activity, were large layer V pyramidal cells, and exhibited complex receptive fields. Fast spiking neurons exhibited short duration (< 1 msec) action potentials, could generate high frequency (> 350 Hz) discharges, and were local interneurons. Chattering neurons, characterized by the generation of short duration (< 1 msec duration) action potentials in high frequency (>350 Hz) bursts, were layer II-III spiny neurons, had simple receptive fields, were the most visually responsive of the 4 classes, and generated rhythmic bursts of action potentials at 20-60 Hz upon delivery of an optimal visual stimulus or with the injection of a constant current pulse. Autocorrelograms revealed that 20-60 Hz oscillations in the visual response were typical only for chattering cells, suggesting that these neurons are the pacemakers for the generation of cortical 20-60 Hz synchronized oscillations. These results indicate that distinct cell types in the primary visual cortex perform unique tasks in local circuit activity that is dependent upon their intrinsic membrane properties as well as their anatomical connections.

592.7

EEG COHERENCE IN POST-LSD PERSISTING PERCEPTUAL DISORDER. H.D. Abraham, F.H. Duffy*. Neurophysiology Lab., Children's Hospital, Boston, MA 02115.

LSD, a potent hallucinogenic drug, is capable of causing apparently permanent hallucinations and pseudohallucinations in certain vulnerable individuals. This disorder, hallucinogen persisting perceptual disorder (HPPD), has been related to disinhibition of the visual apparatus of the CNS by studies of visual psychophysics, including CFF and dark adaptation studies, and quantified electroencephalography. In the latter, we found that visual hallucinators had decreased latencies for the P200 visually evoked potential waveform, and faster alpha frequencies. Accordingly, we studied the direction, degree and regionalization of cortical EEG coherence in 38 subjects with post-LSD visual disorder and 88 LSD naive controls. We found statistical increases in regional cortical coupling in the HPPD subjects involving the primary visual, temporal and posterior parietal cortices, and decreases bifrontally. Coherence was negatively associated with P200 latency, and positively associated with alpha frequency. These data suggest that in HPPD visual disinhibition is positively associated with increases in neuronal coherence in regions of the cerebrum serving visual function. The ramifications of this finding in light of recent neurobiological models of attention are discussed.

592.8

USING CONTRAST DEPENDENT VISUAL LATENCY FOR OBJECT RECOGNITION IN REAL AND ARTIFICIAL NEURAL NETWORKS F. Wörgötter*, R. Opara, K. Funke and U.T. Eysel Inst. Neurophysiol. Univ. Bochum, 44780 Bochum, FRG.

A consistent analysis of a visual scene requires the recognition of different separated objects. In vertebrate brains it has been suggested that this is achieved by synchronization of the activity of disjunct nerve cell assemblies. During such a process cross-talk between spatially adjacent image parts occurs, preventing efficient synchronization. Temporal differences, naturally introduced by stimulus latencies in every sensory system, are utilized in this study to counteract this effect and strongly improve network performance. In our nine-layer network we use the first three layers (*"Retina+LGN"*) for the generation of contrast dependent visual latencies. In the remaining six layers (*"Cortex"*) cell activities are synchronized by a commonly used algorithm relying on spatial neighborhood relationships to achieve feature binding. The contrast dependent visual latencies result in a large temporal separation of objects with different contrast. This leads to a much faster and much better synchronization of neurons representing corresponding image parts. The network model requires a direct link between visual latencies and the onset of synchronous oscillations in cortical cells. We recorded visually evoked local field potentials in area 17 of the anesthetized cat using flashing squares or bars of different contrast as stimuli. We observed a latency range of 33 to 113 ms for contrasts between 1.0 and 0.04. Generically the initial biphasic response was immediately followed by synchronous oscillations. Thus, the oscillation onset seems to be reset by the stimulus and the latency of the oscillations is therefore directly related to the actual stimulus induced visual latency.

592.9

CORTICAL AND THALAMIC CORRELATES OF INDUCED BRIGHTNESS A.F. Rossi*, C.D. Rittenhouse, and M.A. Paradiso Department of Neuroscience, Brown University, Providence, RI, 02912

Brightness changes can be induced in a static field by modulating the luminance of surrounding areas. We used this induction phenomenon to investigate the neural representation of brightness. Extracellular recordings were made from neurons in striate cortex and LGN of anesthetized cats using stimuli that produced brightness induction. While a cell's classical receptive field (CRF) was covered by a uniform gray field, the luminance of rectangular flanking regions was modulated sinusoidally in time, inducing brightness modulation in the CRF roughly in antiphase to the luminance modulation of the flanks. While some neurons were unaffected by the flanking stimuli, many others responded in a phase-locked manner at the temporal frequency of the flank modulation. In two important respects the response of the modulated neurons was correlated with perceived brightness. First, for many neurons the response modulation induced by the flanks was phase shifted by 180 deg. compared with the response to luminance modulation in the CRF. This is consistent with the phase shift in perceived brightness seen in the central field. Second, the luminance modulation rates that produced neural modulation were similar to those that perceptually produced brightness induction. Perceptually, brightness changes can only be induced at surprisingly low temporal frequencies (below about 3 Hz), whereas brightness changes resulting from direct luminance modulation occur up to the flicker fusion rate (30-60 Hz). We found that the responses of neurons significantly decreased when the luminance modulation of the flanks was 2 Hz or higher. Importantly, this reduced response to higher frequency luminance modulation was not observed in conditions where the luminance was modulated within the CRF. These findings suggest that brightness information is explicitly represented in the responses of neurons at early stages in the visual system. (Supported by NIH grant EY09050 and a grant from the Whitehall Foundation)

592.10

TRANSFORMATIONS OF PHYSIOLOGICAL PROPERTIES BETWEEN THE LGN AND V1 OF THE BEHAVING MONKEY. M. Gur*[1,2] and D. M. Snodderly[2,3], [1]Dept. of Biomedical Engineering, Technion, Haifa; Israel; [2]Schepens Eye Research Institute, Boston; [3]Dept. of Ophthalmology and Program in Neuroscience, Harvard Medical School, Boston,.

It is usually thought that the main difference between the parvo layers of the LGN and the input layer of V1, $4C_\beta$, is manifested in the rearrangement of the layered input from the two eyes into one layer and not in the physiological properties of single cells. We investigated whether important transformations in physiological properties can be seen in the awake monkey.

Single cells were recorded from in the parvo layers of the LGN and in $4C_\beta$ using dye marks and/or physiological criteria to verify recording sites.

Width of activating regions (ARs) were measured in 15 LGN and 9 $4C_\beta$ cells. Binocularity was tested in 25 LGN and 12 $4C_\beta$ cells. In the LGN cells, visual field locations ranged from 17 to 65 deg (μ = 37 deg) with AR widths ranging from 9' to 42' (μ = 16.8'); visual field locations for the $4C_\beta$ cells ranged from 2.25 to 5.7 deg (μ = 4 deg) with AR widths ranging from to 26' to 100' (μ = 61.4'). All LGN cells were influenced by one eye only while 11/12 $4C_\beta$ cells showed overt binocular interactions.

We conclude that in the awake monkey, profound transformations, not seen in the anesthetized preparation, take place between the parvo layers of the LGN and layer $4C_\beta$ of V1. We assume that these transformations result from cortico-cortical and cortico-LGN interactions.

592.11

HOW COMPLEX CELLS USE THEIR LATENCIES TO TELL US ABOUT SHAPES DEFINED BY TEXTURE DIFFERENCES. T.J.Gawne* and B.J.Richmond. Lab of Neuropsychology, National Institute of Mental Health, Bethesda, MD 20892.

Complex cells respond to boundaries that are defined by many different cues, including texture and local motion in addition to luminance. Previously we showed that the response strength of complex cells is affected by stimulus orientation and the response latency is affected by the cue that defined the stimulus. The relationship between response strength and response latency is separable with respect to orientation and cue (Gawne et al., 1994). Existing models of complex cells have concentrated on explaining the relationship between stimulus orientation and response strength.

Here we present a model that combines previously observed properties of LGN cells to duplicate the complex cell properties that we saw in our experiments. We start by making use of our previous model of LGN neurons (Gawne et al., 1991), where the sustained component was driven by a linear center-surround spatial filter, and the transient component was driven more nonspecifically by the difference in luminance of adjacent regions of a stimulus, i.e. the contrast. We use correlation between the activities of spatially shifted populations of LGN afferents to generate orientation specificity to boundaries defined by many cues. Direct connections between LGN afferents and the complex cell allows the transient to 'prime' the complex cell, affecting the time for the sustained component to move the membrane potential to threshold. The model does not have any elements in it with the properties of simple cells. Nonetheless, this model reproduces the effects we saw in complex cells: (1) The response strength shows similar orientation tuning to stimuli defined by a variety of cues, and (2) the response latencies are instead primarily a function of the cue.

592.12

GAP JUNCTIONS MAY PROVIDE AN IMPORTANT MEANS OF COMMUNICATION BETWEEN CELLS IN THE CEREBRAL CORTEX

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Ample evidence is now available to indicate that neurons and glia in many parts of the mammalian CNS are electrically and metabolically coupled through gap junctions. Dye coupling studies have demonstrated that gap junctions between cortical neurons are particularly prominent during development but less so in the adult cerebral cortex. In the present study, we examined the frequency and distribution of gap junctions (patch-like membrane appositions with a gap of 2nm) and the differential expression of gap junction proteins (connexins; cx) in the adult rat visual cortex. Brains of rats (3 to 6 months of age) were fixed with a mixture of aldehydes and processed for conventional and freeze-fracture electron microsopy. Connexins were visualized using immunohistochemical techniques in cryostat-cut sections with antisera directed against cx26, 32 and 43. Examination of coronally-cut ultrathin sections revealed the presence of a large number of gap junctions throughout the cortical thickness. Detailed analysis in layer V showed on average 33 gap junctions/grid square in a 200 mesh grid (100 x 100μm area). The majority of these junctions involved glial cells (63% between astrocytes and 6% between astrocytes and oligodendrocytes) but about 10% involved neurons. In approximately 20% of the cases, we were unable to identify unequivocally the cell types involved in the formation of the gap junctions. This high incidence of gap junctions were confirmed by freeze-fracture analysis. Immunoreactivity for cx32 and 43, purported to be expressed by neurons and astrocytes respectively, appeared as discrete puncta throughout the cortical thickness; cx26 labelling was confined to meninges. These results suggest that gap junctions are present in very significant numbers in the adult visual cortex and may be involved in electrical and metabolic coupling between cortical cells.

592.13

PHYSIOLOGICAL COMPUTATION OF BINOCULAR DISPARITY

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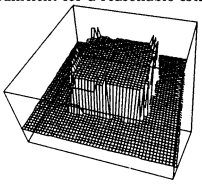
We previously proposed a physiologically realistic model for stereo vision (Qian, 1994, *Neural Computation*, 6:390–404) based on quantitative receptive field (RF) profiles mapped by Ohzawa et al. (1990, Science, 249:1037–41). Here we present several new results. First, we show that our model can be significantly improved by considering the fact that complex cell RFs are on average larger than those of simple cells. This fact is incorporated into the model by averaging *many* quadrature pairs of simple cells with nearby and overlapping RFs to construct a model complex cell. The disparity tuning curve of the resulting complex cell is much more reliable than that constructed from a *single* quadrature pair of simple cells used previously, and the computed disparity maps for random dot stereograms with a population of complex cells are very similar to human perception, with flat surfaces and sharp transitions at disparity boundaries (see figure). Second, we demonstrate through analyses that complex cells as described by Ohzawa et al. compute disparity by effectively summing up two related cross correlations between band-pass filtered left and right image patches. Third, we show that our model can be extended to a more general class of RF profiles than the commonly used Gabor functions. Finally, we demonstrate that as few as two complex cells at each spatial location are sufficient for a reasonable estimation of binocular disparity.



Computed disparity map for a 100-pixel by 100-pixel random dot stereogram with ±2 pixels of disparities on a central square and the surround respectively. Eight model complex cells were used at each location for disparity computation. Each small square of the grid represents an area of 2 × 2 pixels. Transitions at disparity boundaries occur over a distance of only 2–6 pixels.

CEREBRAL CORTEX AND LIMBIC SYSTEM III

593.1

IN VITRO PROLIFERATION OF PRIMATE CORTICAL PRECURSORS AND SPECIFICATION OF CORTICAL AREAS. H. Kennedy*, C. Dehay, M. Berland, P. Savatier° and V. Cortay. INSERM U371, 18 Av. Doyen Lépine, 69675 Bron Cedex (France), °Laboratoire de Biologie Moléculaire et Cellulaire, Ecole Normale Supérieure, 46 Allée d'Italie, 69364 LYON, (France).

Modulation of cell cycle kinetics underlies the histogenesis of areas 17 and 18 in the monkey (Dehay et al.,1993). Here we have investigated whether these regional differences in cell cycle parameters are determined by environmental factors. We have compared the proliferative characteristics of precursors cells of areas 17 and 18 in vitro. Dissociated cell cultures were prepared from the telencephalic wall of the occipital cortex of E80 and E74 monkeys and cells were grown in BHK21 culture medium. Presumptive area 17 was dissected out from the most posterior part of the occipital lobe and presumptive area 18 from the posterior bank of the lunate sulcus. Proliferation was studied by means of BrdU incorporation by cells in S phase. The total cycling population (ie the growth fraction) was revealed by PCNA (proliferating cell nuclear antigen) immunostaining. We estimated the labelling index (LI) by computing the proportion of BrdU positive cells with respect to the total cycling population. Our results show a significantly higher (LI) in area 17 precursors compared to area 18 precursors after 1, 2 , 3 and 5 days in vitro. These results show that the higher rate of proliferation that characterises A17 precursors cells in vivo, is maintained in vitro. This suggests that the proliferative characteristics of cortical precursors cells are autonomous. When ventricular zone cells from A17 are cultured alone, the LI shows similar values to those obtined when they are cultured with the immature neuroblasts of the cortical plate and intermediate zone. This suggests that the higher rate of proliferation characteristics of A17 precursors is not solely mediated by an autocrine effect.

Bibliography: Dehay et al. Nature ,366: 464-466.(1993).

593.2

MODULATION *IN VITRO* OF THE PROLIFERATION OF RODENT CORTICAL PRECURSORS BY A THALAMIC CYTOKINE. C. Dehay*, A. Guibert, P. Savatier°, V. Cortay and H. Kennedy. INSERM U371, 18 Av. Doyen Lépine, 69675 Bron Cedex (France), °Laboratoire de Biologie Moléculaire et Cellulaire, ENS, 46 Allée d'Italie, 69364 Lyon Cedex 07, (France).

In vivo results suggested that thalamic afferences may regulate cell proliferation in the ventricular zone of the monkey cortex (Dehay et al., 1991; Kennedy and Dehay, 1993). We have tested this hypothesis by studying the proliferative activity in dissociated cultures of E14 mouse cortex. The results show a significant increase of Tritiated-Thymidine incorporation in cortical precursors cultured during 48 hours in the presence of E14 dorsal thalamic explants placed in a transwell. This shows that the thalamic cells exert a mitogenic effect on cortical precursors that is mediated by a diffusible factor. By using BrdU incorporation which labells cells in S phase in combination with PCNA immunostaining which labels cycling cells, we have studied the effect of E14 thalamus conditioned medium (TCM) on the proliferation of E14 cortical precursors. Under these conditions, there is a 20% increase of the total cell number in the presence of the TCM. Further, in the presence of the TCM, there is an increase in the labelling index (LI = BrdU positive cells/ cycling cells) which reflects changes in the kinetics of the cell cycle. Higher values of LI are likely to reflect a shortening of the total duration of the cell cycle rather than a change in the absolute duration of S phase. Moreover, in the presence of TCM, there is a significant increase in the proportion of PCNA positive cells. This means that TCM induces more cell precursors to remain in the cell cycle. Therefore, the embryonic thalamus secretes a cytokine that (1) inhibits differenciation and (2) stimulates the proliferation of cortical precursors.

Bibliograhy: Dehay C. et al. Dev. Brain Res. 62:137-141.(1991)
Kennedy H. and Dehay C. Cereb. Cortex. 3: 171-186.(1993) .

593.3

RADIAL AND HORIZONTAL DEPLOYMENT OF CLONALLY RELATED CELLS IN THE RHESUS MONKEY NEOCORTEX: RELATIONSHIP TO DISTINCT MITOTIC LINEAGES. D.R. KORNACK* AND P. RAKIC. Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510.

Cells of the neocortex are organized into functionally distinct columnar and laminar domains. To examine whether cell lineage may contribute to the formation of these domains in the primate, we used recombinant retroviruses to label individual progenitor cells in the ventricular zone (VZ) of the fetal rhesus monkey in vivo, then determined histochemically the distribution of their progeny during and after the period of cortical neurogenesis. Distribution patterns of labeled cells in the VZ suggested the coexistence of asymmetrically and symmetrically dividing progenitor cells, indicating that both postmitotic and mitotic progeny are produced during cortical neurogenesis. In the cortex, several distinct patterns of clone distribution were observed and interpreted as follows: clones aligned radially suggested that asymmetrically dividing progenitors generate sequential "siblings" that migrate, in tandem, along a common radial path to the cortex. In contrast, clones oriented horizontally within a single lamina suggested that symmetric divisions produce multiple, laterally displaced progenitors which, in turn, simultaneously generate "cousin" cells that migrate, in concert, to the same cortical layer. Such simultaneous generation and migration of cousin cells may be particulary relevant in the primate cortex, in which cells generated at a particular time during neurogenesis eventually distribute as a sharp horizontal band within a lamina, as revealed in previous 3H-thymidine neuronal 'birthdating' studies in this species. Thus, we propose that different mitotic lineages, which coexist in the monkey VZ, produce distinct radial or laminar patterns of clone deployment that foreshadow the cytological organization of the adult neocortex. Supported by the U.S. Public Health Service.

593.4

DEVELOPEMENT OF CORTICAL PATHWAYS IN PRIMATES SHOW LIMITED EXUBERANT CONNECTIVITY. A. Batardière*, C. Dehay, M. Berland°, P. Barone and H. Kennedy. INSERM U371, 18 Av.Doyen Lepine, 69675 Bron Cedex, France, °Centre Hospitalier Lyon Sud, 165 Chemin du Grand Revoyet, 69495 Pierre Benite Cedex, France

A characteristic feature of the development of cortical projections to area 17 in the monkey is the prolonged reorganization of the laminar distribution of afferent neurons[1] and the absence of labeled neurons in inappropriate cortical regions (Dehay and Kennedy unpublished).To investigate whether these developmental features are unique to projections to area 17, we have injected tracers into presumptive area V4 in fetal and postnatal monkeys and analysed quatitatively the areal and laminar distribution of labeled neurons. In the fetus, injections restricted to the gray matter labeled neurons limited to cortical regions which project to area V4 in the adult. However injections which involved the white matter revealed a transient projection from the auditory cortex. Feedforeward projections from visual areas V2 and V3 showed no laminar distribution changes during development. This contrasted with feedback projections from anterior cortical regions which showed massive developmental reductions of the proportion of labeled supragranular layer neurons during fetal development.

These results suggest (1) that the laminar remodeling of connectivity is a characteristic feature of feedback connections and (2) that although transient cortical connections are rare in the developing monkey they do interconnect the auditory cortex with a visual cortical area which is implicated in color vision.

1-Barone et al. (1995) Cereb. Cortex 5: 22-38.

593.5

CORPUS CALLOSUM CELLS OF ORIGIN INCLUDE CAJAL-RETZIUS LAYER 1 CELLS IN VISUAL CORTEX: DII/DIA AND CONFOCAL LASER SCANNING MICROSCOPE (CLSM) ANALYSIS. A.J. Elberger*. Dept. of Anatomy and Neurobiology, The University of Tennessee, Memphis TN 38163.

Crystals of DiI or DiA placed in rat corpus callosum (CC) *in vitro* retrogradely label many cells throughout visual cortex via transitory CC axons which gradually disappear by postnatal day (PND) 26 (Elberger 1994). In rats with such labeling at PND 0 (day of birth) through 20, the CC cells of origin in layer 1 were examined in detail using a CLSM. The layer 1 CC cells were most abundant at PND 0-1, and absent by PND 9.

Many layer 1 CC cells were classified as Cajal-Retzius (C-R) cells because they met 2 criteria: axons and dendrites parallel to the pia for long distances, and dendrites with projections orthogonal to the pial surface. Axons of C-R cells could be followed for long distances through layer 1 and into layer 2. No cells in layers 2-6 met the C-R criteria. By PND 3 there were far fewer C-R CC cells; those present had shorter dendrites with fewer orthogonal projections. At PND 3 there were still many other layer 1 CC cells with axons and dendrites parallel to the pial surface, but they did not have dendritic projections orthogonal to the pial surface. By PND 7 there were almost no layer 1 CC cells, and by PND 9 none could be detected.

These results are almost certainly not due to transcellular DiI/DiA diffusion because: 1)many C-R CC cells were far from CC cells/processes in layers 1 and 2, 2)all C-R CC cells were as intensely labeled as other CC cells in layers 1-6, and 3)in tissue with DiI labeling cortical afferents and efferents at PND 0-20, no C-R cells were observed even when layer 1 connections were labeled. This new finding of C-R CC cells supports hypotheses about the functions of transitory CC connections, and indicates that the function of C-R cells may need to be reevaluated. Supported by NIH grant EY08466.

593.6

SHIFTS IN $GABA_A$ REVERSAL POTENTIAL DURING NEOCORTICAL DEVELOPMENT. L.H. Boyce, D.F. Owens, M.B.E. Davis, D.C. DeVivo*, A.R. Kriegstein Dept. of Neurology, Columbia University College of Physicians and Surgeons, NYC, NY 10032

In the adult CNS, GABA mediates fast inhibitory neurotransmission through activation of $GABA_A$ receptors. However, in immature neurons, activation of $GABA_A$ receptors can produce membrane depolarization presumably due to high $[Cl^-]_i$. Using *in situ* patch-clamp techniques with gramicidin perforated patches, which leave $[Cl^-]_i$ undisturbed, we were able to study the change in the GABA reversal potential (E_{GABA}) in embryonic and postnatal neocortical cells. $GABA_A$ currents are inward at resting membrane potential in ventricular zone cells throughout the embryonic period. By inhibiting Cl^- exchange with furosemide, the reversal potential can be shifted to a more negative value, consistent with Cl^- mediation of $GABA_A$ current and a high $[Cl^-]_i$. In embryonic (E19) cortical plate neurons, E_{GABA} is approximately -36 mV while resting membrane potential is approximately -65 mV. E_{GABA} becomes progressively more negative postnatally; at postnatal day 2 (P2) E_{GABA} is approximately -43 mV, and by P16 E_{GABA} is -59 mV. These data confirm a progressive shift in the $GABA_A$ reversal potential, and may reflect a changing role for GABA during early neocortical development.

593.7

Neurosteroid modulation of GABA currents and $[Ca^{++}]_i$ in embryonic neocortical cells. D.F. Owens, M.B.E. Davis, M.J.S. Heath and A.R. Kriegstein* Depts. of Neurology and Anesthesiology, Columbia Univ. Med. Center, N.Y., N.Y. 10032

Progesterone metabolites have been found to modulate $GABA_A$ receptor-mediated currents. GABA produces membrane depolarization in ventricular zone (VZ) cells of the embryonic rat cortex via activation of $GABA_A$ receptors. Since progesterone metabolites are present in the fetal circulation they could modulate $GABA_A$ currents in embryonic cells. We investigated the effects of progesterone metabolites on GABA induced currents in embryonic rat neocortex using *in situ* patch clamp recording techniques. At embryonic day 16 (E16), 5α-pregnan-3α-ol-20-one (3α-OH-DHP) at concentrations ranging from 1 nM to 1 μM was found to produce an enhancement of GABA-induced current in VZ cells. On average, 100 nM 3α-OH-DHP produced a 48% increase in the GABA current. In contrast, steroid potentiation of GABA current was not observed in embryonic cortical plate (CP) cells. In addition, using Fura-2 imaging techniques GABA was found to increase $[Ca^{++}]_i$ in both VZ and CP cells. 3α-OH-DHP (200 nM) was found to potentiate the GABA mediated Ca^{++} increase in VZ cells, but not CP cells. These differences in steroid sensitivities could reflect different subunit compositions of $GABA_A$ receptors in these two distinct regions, and could implicate a differential role for neurosteroid/GABA interactions during development.

593.8

NMDA-INDUCED $[Ca^{++}]_i$ INCREASE IN VENTRICULAR ZONE CELLS OF EMBRYONIC NEOCORTEX. A.C. Flint, M.B.E. Davis, M.J.S. Heath, J.J. LoTurco*, A.R. Kriegstein Depts. Neurology and Anesth., Columbia Physicians and Surgeons, NYC, NY 10032 and Dept. of Physiology, U of Conn., Storrs, CT 06269

Ventricular zone cells of embryonic neocortex have been previously thought to lack NMDA receptors. We found evidence of functional NMDA receptors in embryonic rat neocortical cells in the ventricular zone (VZ) and cortical plate (CP) at E16 using Ca^{++}-sensitive dyes. NMDA (10-100 μM) induced increases in $[Ca^{++}]_i$ in cells in the VZ and CP in brain slices loaded with fluo-3 and imaged using laser confocal microscopy. NMDA-responsive VZ cells included rounded cells at the ventricular surface as well as bipolar cells within the VZ. In the CP, responsive cells included more mature process-bearing cells resembling young neurons. NMDA responses were sensitive to the specific receptor antagonist AP-5 and were abolished in 0 mM $[Ca^{++}]_o$. VZ cells often responded in advance of CP cells and in the presence of TTX, bicuculline, and CNQX, arguing against indirect activation of VZ cells by NMDA-responsive CP neurons. In addition, a tissue printing technique was developed to obtain acutely isolated cells from the ventricular surface. These cells were loaded with fura 2-AM and NMDA application demonstrated a subset of responsive cells. The presence of NMDA-responsive cells in the VZ of rat embryonic cortex suggests a possible role for NMDA receptors during proliferative stages of cortical development.

593.9

ONTOGENESIS OF NADPH-DIAPHORASE EXPRESSION IN THE VISUAL CORTEX OF THE MONKEY. P. Barone*, C. Dehay, M. Berland and H. Kennedy. INSERM U371 Cerveau et Vision. BRON (France)

The free radical neurotransmitter nitric oxide (NO) is implicated in a number of developmental processes including the establishment of the adult pattern of connectivity. The presence of NO in the central nervous system can be detected by mean of histochemical visualisation of the enzyme NADPH-diaphorase (NADPH-d) activity, a NO synthase. We have investigated the distribution of NADPH-d expression during fetal and postnatal development in monkeys. During development, changes of NADPH-d expression are observed in the neuropile as well as in a sub-population of cortical and white matter cells *Neuropile*. In area V1, NADPH-d activity in the neuropile of the cortical plate is first detected at E84, where it is largely restricted to infragranular layers. Activity increases with age and adult-like levels are observed at birth where a high level of activity is located in the upper part of layer 4. Sections cut parallel to the surface reveal the presence of NADPH-d blobs in supragranular layers of the adult monkey that are co-extensive with CO blobs. *Cells*. A small population of cells express NADPH-d in area V1. They are characterized by a large cell body (15-20μ diameter), predominantly of non-pyramidal shape, and by an extensive dendritic arborization covering distances up to 1 mm or so. The size, the shape and the laminar distribution of NADPH-d positive cells changes during maturation. No labelled cells are present in the E75 fetus, and only a few labelled cells are observed in the intermediate zone and subplate of the E84 fetus. NADPH-d expression in cortical plate cells begins at E94 (i.e. at the end of neurons migration) in infragranular layers. Quantification of labelled cells in area V1 shows a continuous increase in the number of cells from E115 reaching peak values 4 months posnatally. There is a decrease in cell number between 4 months and adulthood. While this increase is moderate between birth and 4 months in infragranular layers, it is significatively higher in the supragranular layers, leading to the high proportion of NADPH-d positive cells in this compartment in the adult. White matter cells follow this developmental time course. In fetuses, NADPH-d expressing cells have a deeper distribution compared to postnatal animals where they are located just below layer 6. Our results show that NADPH-d activity maturation is reached late in postnatal life and parallels synaptogenesis. NADPH-d is found to selectively label an important sub-population of subplate neurons. These early generated neurons are known to play a crucial role in determining the precision of thalamo-cortical connections during development.

593.10

MODULATORY NEUROTRANSMITTERS REDUCE DYE-COUPLING BETWEEN DEVELOPING LAMINA II/III PYRAMIDAL NEURONS IN RAT NEOCORTEX. B. Roerig*, G. Klausa and B. Sutor. Institute of Physiology, University of Munich, Pettenkoferstrasse 12, D-80336 Munich, Germany.

During the early postnatal period neurons in rat neocortex are extensively coupled via gap junctions (Connors et al., J Neurosci 3:773, 1983; Peinado et al., Neuron 10:103, 1993). To investigate whether modulatory transmitters regulate gap junction coupling layer II/III pyramidal cells in slices from rat prefrontal and frontal cortex (P7-P19) were impaled with sharp microelectrodes and injected with the tracer neurobiotin.

On P7-P10 the average number of cells coupled to the injected neuron was 30.36 (SEM 5.72, n=14) under control conditions. Preincubation with dopamine (100 μM) or the D1 dopamine receptor agonist SKF 38393 (100 μM) significantly ($P < 0.005$, Mann-Whitney U-test) reduced the average cluster size by approximately 70 %. A 50 % reduction in the number of coupled cells per injection was produced by the D2 dopamine receptor agonist quinpirole. The transmitters serotonin (100 μM) and norepinephrine (30 μM) as well as the ß1 adrenergic agonist isoproterenol (30 μM) also induced a statistically significant reduction in tracer coupling by 60-70%. Dye-coupling was furthermore markedly suppressed by preincubation of slices with the adenylate cyclase activator forskolin (20 μM) or with the direct protein kinase A activator Sp-cAMPS (100 μM). Application of forskolin to neurons recorded in the whole cell configuration of the patch clamp technique induced a reversible increase in input resistance which was not observed in uncoupled neurons during the third postnatal week.

We conclude that modulatory afferents to the developing neocortex affect metabolic and electrical signal transfer as well as electrotonic cell properties by regulating the extent of gap junction coupling between immature neurons. Activation of adenylate cyclase and protein kinase A by G-protein coupled receptors presumably represents one major second messenger pathway involved.

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593.11

EXPRESSION OF α4-1 NICOTINIC ACETYLCHOLINE RECEPTOR mRNA IN THE RAT TELENCEPHALON DURING FETAL AND EARLY POSTNATAL DEVELOPMENT. H. Schröder*[1], N. Moser[1], J. Grunwald[1], C.-H. Ostermann[1], A. Wevers[1], D.E. Lorke[2], U. Schütz[1] and A. Maelicke[3]. [1]Inst. II für Anatomie, Univ. Köln, Köln, F.R.G., [2]Abt. Neuroanatomie, Univ. Hamburg, Hamburg, F.R.G., [3]Inst. für Physiologische Chemie und Pathobiochemie, Univ. Mainz, Mainz, F.R.G.

So far, neuronal nicotinic acetylcholine receptor (nAChR) expression has mainly been studied in adult human and rat telencephalon. In contrast, only few studies address nAChR expression in the course of embryonic and perinatal development, or under conditions of neuronal degeneration.

Gene expression of the α4-1 nAChR subunit was studied by in situ hybridization from embryonic day 14 to postnatal day 120 (E14-P120), in rat neocortex, hippocampus and olfactory bulb, using a digoxigenin-labeled α4-1 riboprobe, an alkaline phosphatase-coupled digoxigenin antibody and a color substrate reaction (Wevers et al., *Mol. Brain Res.*, 25:122, 1994). In all regions, α4-1 mRNA expression showed a transient upregulation in distinct neuronal subpopulations and preceeded cholinergic fiber ingrowth (Nyakas et al., *Neuroscience*, 59:541, 1994). Neocortical α4-1 mRNA expression was increased in layer VIb between E20 and P7. In dentate gyrus granule cells α4-1 mRNA expression was prominent about birth, still present at P25 and subsequently decreased to adult levels (P120). The olfactory bulb showed strongest expression in the mitral cell layer (MCL) at P0. During further development the number of α4-1 expressing MCL neurons decreased. Prenatally, some neurons of the external plexiform layer showed strong α4-1 expression which decreased in adult stages.

Transient mRNA expression preceeding cholinergic innervation has been reported to occur at the developing neuromuscular endplate. It needs to be elucidated whether an innervation-associated switch in non-α nAChR isoform expression as observed for the developing skeletal muscle may also occur in the central nervous system.

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AXON GUIDANCE MECHANISMS AND PATHWAYS VII

594.1

TRANSIENT INTERMEDIATE ZONE NEURONS PROJECT TO THE SPINAL CORD, THE SUPERIOL COLLICULUS, AND THE CONTRALATERAL HEMISPHERE IN RATS.
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The developing mammalian telencephalon contains many transient neurons. Transient subplate neurons have been proposed to lay down the first axonal pathways as a scaffold for the establishment of permanent cortical projections to the thalamus, the superior colliculus, and the contralateral hemisphere in cats. The questions whether there are pioneer fibers to the spinal cord and whether these pioneer neurons are prevailed in other mammalian are important issues in neocortical development. Here, I have searched for neurons giving rise pioneer fibers to the subcortical structures in rats. Tracers (DiI or BDA) were injected into the spinal cord, the superior colliculus, or the contralateral hemisphere in vivo, and a population of labeled cells were found in the lower intermediate zone (IZ) of the dorsomedial cortex. Cresyl violet staining showed an accumulation, which also corresponded to a population of GABA-immunoreactive cells in the lower intermediate zone, in a similar location. These retrogradely labeled cells were MAP2-positive (microtuble associated protein 2; neuron-specific protein), and some of them also expressed somatostatin. The IZ neurons in the dorsomedial cortex underwent their final round of cell division before embryonic day 14 (E14) and sent out fibers to the internal capsule before E16, disappearing from the IZ as the brain matured. These characteristics imply that the IZ neurons in rats pioneer the major corticofugal pathway.

594.2

DEVELOPMENT OF CHICK MOTONEURON AFFERENT AND EFFERENT CONNECTIONS. P. Quebada, M. J. Ignatius*. Div. of Neuroscience, MCB, U.C. Berkeley, Berkeley CA 94720-3200.

Motoneuron outgrowth from the vertebrate neural tube occurs at discrete ventral sites through an extracellular matrix. Sensory cells must also cross this matrix barrier from outside the neural tube, through a dorsal root entry point, then synapse on targets in both the dorsal and ventral horns. Development of these connections do not require activity or experience in the chick, suggesting pre-formed pathways rather than synaptic remodeling drive the development. We have followed the development of these motoneuron projections using several early markers of differentiation in combination with DiI tracing. By stage 15, chick motoneurons begin to project out of the ventral aspect of the neural tube. 12 to 24 hours later, subpopulations of motoneurons project dorsally and exit out the dorsal aspect of the neural tube. Their projections appear to stop in the region eventually occupied by the sensory ganglia. Sensory cell axons next enter the neural tube in close proximity to the tracts established by these motoneurons and the gamma Ia afferents send branches ventrally. During this period both population of cells express the homophillic cell adhesion molecule, DM-GRASP.

The dorsal motoneuron projection appears to be transient, but while present, may serve as a pathway used by sensory afferents to first cross this matrix barrier, then to find targets in the ventral horn of the neural tube. In addition, the breaching of the matrix may occur in concert with a population of non-neuronal cells that occupy at least the ventral site prior to axon outgrowth. Immunologically identical non-neuronal cells remain associated with the motor projections as they depart the neuronal tube, suggesting a persistent and necessary interaction. Supported by ALS Assoc. and a NIH RO1 to MJI.

594.3

DUAL FUNCTION OF CALCIUM IN LAMININ-MEDIATED GROWTH CONE GUIDANCE: I. EARLY CA2+ SIGNAL IN FILOPODIA UNDERLIES GROWTH CONE TURNING. C.V. Williams*, T.B. Kuhn, P. Dou, S.B. Kater. Anatomy & Neurobiology, Colorado State University, Fort Collins, CO 80523.

Chick drg growth cones respond to laminin (LN) model guideposts with characteristic behaviors (Kuhn et al., 1995). We assessed the role of Ca2+ in the initial response to LN guideposts; namely filopodial contact and subsequent growth cone reorientation toward the bead. In control experiments, filopodial contact with LN-coated beads resulted in 79±1% growth cones turning toward the bead (n=21). In contrast, only 19±1% of growth cones loaded with 2μM Bapta-AM (Kd=340) turned (n=21) ($p<0.01$). Similarly, neurons bathed in 2mM EGTA made filopodial contact with beads and adhered, but did not turn. Growth cones bathed in the inorganic Ca2+ channel blockers cobalt (2mM) or nickel (2mM) also failed to turn. These data suggest that an influx of Ca2+ underlies filopodial responses to LN-coated beads.

To test this, neurons were loaded with the Ca2+ indicator Fura2-AM and LN-coated beads or soluble LN were presented to growth cones. No significant changes in intracellular Ca2+ in filopodia or growth cones were observed during initial contact. It seemed likely that a rapid Ca2+-dependent exchange of information between filopodia and growth cones may exist, as supported by the pharmacological data above. We reasoned that isolation of filopodia would therefore interrupt this exchange and possibly result in a detectable Ca2+ signal. Thus we applied soluble LN to isolated filopodia and observed a rapid, sustained rise in intrafilopodial Ca2+: 80nM±12nM (n=9). These data demonstrate that growth cone turning requires an early transient Ca2+ signal restricted to filopodia contacting LN-coated beads.

594.4

DUAL FUNCTION OF CALCIUM IN LAMININ-MEDIATED GROWTH CONE GUIDANCE: II. LATE SUSTAINED CA2+-SIGNAL IN GROWTH CONES LONG AFTER TRANSIENT CONTACT IS ASSOCIATED WITH ENHANCED OUTGROWTH. T.B. Kuhn*, C.V. Williams, P. Dou, S.B. Kater. Dep. of Anatomy and Neurobiology, Colorado State University, Ft Collins, CO 80523

Laminin (LN)-coated beads provide unique guidance instructions to advancing chick DRG growth cones. Upon transient interactions, growth cones display a series of stereotype changes in their behavior and morphology, some lasting considerably beyond the time of actual contact (Kuhn et al., 1995). Among the most striking growth cone response are early turning and late, enhanced outgrowth after transient LN-contact. Growth cone turning is mediated by an influx of extracellular calcium into filopodia (Williams et al. preceding abstract).

We investigated possible targets of the early Ca2+-signal: Calmodulin (CaM), CaM-dependent kinase II (CaMK-II) and further Ca2+-signals. Inhibition of CaM completely blocked the turning response of growth cones towards LN-coated beads suggesting CaM as a primary target of the early Ca2+-signal in filopodia. In contrast, the specific CaMK-II-inhibitor, 10μM KN-62, prevented only late, enhanced outgrowth without altering growth cone turning. A late, sustained rise in intracellular Ca2+ (93+5nM, n=30) was associated with late, enhanced outgrowth as revealed by Ca2+-imaging. Importantly, this late, sustained Ca2+-signal was restricted to growth cone bodies as opposed to the early Ca2+-signal in filopodia. Inhibition of CaMK-II abolished the late,sustained Ca2+-signal and late, enhanced outgrowth.

Together with the data above this suggests the following intracellular signaling for the regulation of multiple components of growth cone behavior:

$$Ca2+\uparrow\text{(filopodia)} \longrightarrow CaM \longrightarrow CaMK\text{-}II \longrightarrow Ca2+\uparrow\text{(growth cones)}$$

594.5

REQUIREMENT OF CALCIUM IONS AND FILOPODIA ASYMMETRY IN CHEMOTROPIC TURNING OF NERVE GROWTH CONES. J-j. Wan, J.Q. Zheng, and M-m. Poo*. Dept. Biol. Sci., Columbia Univ., New York, NY 10027

Microscopic gradients of glutamate were established near the growth cone of cultured *Xenopus* spinal neurons by repetitive pulsatile ejection of picoliters of glutamate solution from a micropipette at a distance of 100 μm from the center of the growth cone. Over a restricted range of glutamate concentration (0.05 to 0.5 mM in the pipette), the growth cones exhibited positive chemotropic turning toward the source of glutamate. The filopodia appeared to be essential for the growth cone to sense and/or to respond to the glutamate gradient, since disruption of filopodia by bath-application of cytochalasin B (0.17 μg/ml) abolished the chemotropic turning response. Furthermore, time-lapse microscopy showed that a filopodia asymmetry, with more filopodia facing the glutamate source, is induced soon after the onset of the glutamate gradient, prior to the turning response. When the glutamate gradient was applied in Ca^{2+}-free medium, the turning response was abolished, and no filopodia asymmetry was induced. Although Ca^{2+} appears to be an essential second messenger for the glutamate-induced turning, robust turning response was observed over a wide range of extracellular Ca^{2+} concentration (100 nM - 2 mM). The turning response was completely blocked by AP-5, suggesting that influx of Ca^{2+} through NMDA receptors is required. Taken together, these results suggest that asymmetric influx of Ca^{2+} at the growth cone, preferential filopodia formation, and the turning of the nerve growth cone are causally-linked sequence of events.

594.6

SIGNAL TRANSDUCTION OF RECEPTOR-LINKED PROTEIN TYROSINE PHOSPHATASES. S.J. Fashena+, M. Tiemeyer*, and K. Zinn+. +Biology 216-76, Caltech, Pasadena, CA 91125 and *Cell Biology Dept. C244 SHM, Yale University School of Medicine, New Haven, CT 06510.

We are interested in the signal transduction pathways through which receptor-linked protein tyrosine phosphatases (R-PTPs) transmit extracellular cues into intracellular responses. We are investigating two R-PTPs, DPTP99A and DPTP10D, that are expressed in the CNS during *Drosophila* embryo development. To elucidate the signaling pathways utilized by these R-PTPs, we are searching for their ligands and downstream signaling molecules.

To this end, we have made receptor globulins (Rgs) containing the extracellular domains (ECD) of either DPTP99A or DPTP10D linked to an IgG Fc domain. We are using these Rgs to screen a *Drosophila* embryo cDNA library for R-PTP ligands by expression cloning in Cos cells. We are now performing secondary screens on library pools that bind Rg99A, which includes the full-length ECD of DPTP99A. We hope to identify the ligands for both R-PTPs, delineate the essential recognition domains of the R-PTPs and their ligands, and elucidate the effect of ligand binding on R-PTP activity.

We are also investigating the downstream signaling pathways of these R-PTPs. Members of the Zinn lab have identified gp150, a putative substrate and downstream signaling molecule of DPTP10D (Tian and Zinn, JBC 269: 28478). Gp150 is a transmembrane glycoprotein which includes an ECD of leucine-rich repeats and a cytoplasmic domain in which 4 tyrosine residues are present within motifs that could be recognized by SH2-domains of downstream effector molecules. In vitro, DPTP10D binds specifically to gp150 and dephosphorylates phosphorylated gp150. We have recently shown that gp150 and DPTP10D, but not DPTP99A, coimmunoprecipitate in lysates from transfected fly S2 cells. Furthermore, transfected gp150 is phosphorylated on tyrosine residues in response to a variety of treatments of whole cells. Based on these results, we can now ask whether DPTP10D dephosphorylates gp150 "in vivo" in transfected S2 cells.

594.7

PURIFICATION AND SEQUENCE ANALYSIS OF THE AXON FASCICLE SPECIFIC LAN3-2 ANTIGEN AND OF A PROTEIN WITH WHICH IT MAY INTERACT Y. Huang*, A. Dietz, J. Jellies†, K.M. Johansen, and J. Johansen. Department of Zoology & Genetics, Iowa State University, Ames, IA 50011 and †Department of Biological Sciences, Western Michigan University, Kalamazoo, MI 49008.

In leech, the mAb lan3-2 defines a small population of tightly fasciculated axon tracts in the nerve roots and interganglionic connectives which are formed by peripheral neurons. The lan3-2 antigen is a 130 kD surface glycoprotein which may be directly involved in the assembly of these axon tracts since lan3-2 antibody perturbation disrupts normal fascicle formation in embryos (Zipser et al., Neuron 3:621). To further characterize its function in specific tract formation we are molecularly identifying and cloning the lan3-2 antigen. Since the lan3-2 epitope is partly made up of sugar moieties we purified the antigen from leech CNS extracts by immunoaffinity chromatography. The purified protein was fractionated by SDS-PAGE, blotted onto PVDF membrane, and the 130 kD band cut out and trypsin digested. The sequence of seven of the resulting peptides were determined by gas-phase microsequencing. Searches of the data banks show that all seven peptides represent novel sequences; however, four (pep1-4) have limited homology to Ig- or FNIII-domains, two (pep5-6) are unique, and one (pep7) has homology to the C-terminal sequence of vertebrate thrombospondins. To test whether these peptides are indeed derived from the lan3-2 antigen we have made new antibodies to pep1 and pep7 both of which specifically recognize a 130 kD band on Western blots of leech CNS proteins. Pep1-antibody does not stain immunocytochemically so we do not yet know whether it corresponds to the lan3-2 antigen. Interestingly, while the pep7-antibody does not recognize the lan3-2 positive peripheral neurons it does label all central neurons and their peripheral axons. This suggests that we may have obtained microsequence from a copurifying protein which may interact with the lan3-2 antigen. We are screening leech cDNA expression vector libraries with the antibodies in order to obtain full length sequence of these proteins for further analysis. Supported by NIH NS28857 (JJo) and NSF 9209337 and a Sloan Fellowship (JJe).

594.8

PERTURBATION OF A GRASSHOPPER SEROTONERGIC INTERNEURON. B.G. Condron* and K. Zinn. Division of Biology, 216-76 Caltech, Pasadena, CA 91125. (condron@caltech.edu)

We are interested in how the CNS is patterned in the developing grasshopper embryo. To date, we have developed microinjection techniques to perturb specific cells. Using this approach, we have shown that the transcription factor engrailed and the signal transduction molecule protein kinase A are required to regulate the identity of cells produced by the median neuroblast. We are now examining the patterning of the lineage which produces a serotonergic interneuron. This interneuron makes very extensive connections throughout the neuropil and is thought to regulate many aspects of adult synaptic transmission. We aim to dissect the molecular and cellular cues that specify the identity of this neuron relative to its neighbors as well as those that regulate the neurite branch patterns in the CNS. We have found that neurite extension in the developing neuropil is inhibited by the neuron's own neurotransmitter serotonin as well as dopamine. Each transmitter affects outgrowth via different second messenger pathways. We have identified antagonists to both putative receptors. These specifically relieve the inhibition of neurite extension due to exogenously added neurotransmitter. When added alone antagonists increase the amount of neurite outgrowth substantially.

594.9

NEURAL RECEPTOR TYROSINE PHOSPHATASES (R-PTPs) ARE ESSENTIAL FOR MOTOR AXON GUIDANCE IN THE *DROSOPHILA* EMBRYO. Chand Desai[1], Joseph Gindhart[2], Lawrence S.B. Goldstein[2], and Kai Zinn[1]*.
1) Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125; 2) Dept. of Pharmacology, School of Medicine, University of California, San Diego, La Jolla, CA 92093.

Four R-PTPs are selectively expressed on central nervous system axons in the *Drosophila* embryo (Desai *et al.* (1994), J. Neurosci. 14, 7272). We used local P-element transposition to generate mutations in the *DPTP69D* gene. Null mutations are homozygous lethal, and cause defects in the pattern of motor axon projections to body wall muscles. The SNb branch, which contains the axons of the RP neurons and projects to ventrolateral muscles, is most strongly affected. A variety of SNb phenotypes are observed, including bypass of the entire muscle field and stalling of growth cones prior to their arrival at their appropriate muscle targets. The SNa branch also displays errors in *DPTP69D* mutants. Our previous studies had shown that null mutations in another R-PTP gene, *DPTP99A*, produce no detectable phenotypes (Hamilton et al. (1995), Roux's Arch. Dev. Biol. 204, 187). However, double mutant embryos (*DPTP69D; DPTP99A*) show a greatly increased frequency of SNb targeting errors. Similarly, the frequency of SNa branch errors also increases in double mutants. In summary, our data show that: 1) neural R-PTPs are involved in signaling events that are essential for several of the pathway choices made by SNb and SNa growth cones during outgrowth to their muscle targets; 2) the R-PTPs have partially redundant functions, so that double mutants have stronger phenotypes than single mutants.

594.10

A SUPPRESSOR SCREEN FOR ELUCIDATING THE UNC-5/UNC-6 AXON GUIDANCE SIGNALING PATHWAY IN *C. elegans*. S. Chan , J. Culotti, A. Colavita, M. Hamelin, Y. Zhou, M.-W. Su, R. Steven & I. Scott. Mt. Sinai Hosptl. Res. Inst., Toronto, Canada M5G 1X5.

In *C. elegans*, 3 genes have been identified that are required to guide pioneer growth cone and cell migrations on the basal surface of the epidermis. *unc-6* encodes a laminin-related path cue molecule that guides both dorsally-oriented and ventrally-oriented migrations. Homologues of UNC-6 have now been identified in vertebrates and in fruit flies, where they carry out similar guidance functions. *unc-5* encodes a transmembrane receptor that is activated by the UNC-6 path cue molecule and responds to it by orienting the migration towards the dorsal side. The intracellular signaling mechanisms by which UNC-5 activation is able to reorient the actin-based cytoskeleton at the leading edge of migration are unknown. We have shown that ectopic expression of UNC-5 in neurons that normally grow longitudinally on the epidermis is sufficient to steer their axons dorsally. The UNC-5-induced steering requires UNC-6, since in *unc-6* mutants ectopically expressing UNC-5, these axons have a longitudinal trajectory. This suggests a genetic paradigm for identifying additional genes that encode components of the UNC-5/UNC-6 molecular pathway for guidance, including possible accessory factors to UNC-5 or UNC-6 or components of the intracellular signaling cascade stimulated by UNC-5 activation. By looking for mutations that, like *unc-6* mutations, suppress the UNC-5-induced reorientation of these axons, we have identified several candidate genes, including *unc-40*, which has major effects on ventrally-oriented and minor effects on dorsally-oriented migrations, respectively. *unc-40* encodes a transmembrane protein related to human DCC (Deleted in Colorectal Cancer) protein.

594.11

IDENTIFICATION OF 12 GENES INVOLVED IN AXON GROWTH, DIRECTION, AND FASCICULATION, INCLUDING A FLY HOMOLOG OF THE HUMAN "DELETED IN COLON CANCER" GENE. Peter A. Kolodziej, Les Timpe, Sharon Fried, Lily Yeh Jan, and Yuh Nung Jan.* Howard Hughes Medical Institute and Depts. of Physiology and Biochemistry, Univ. of California, San Francisco, Third and Parnassus Aves., San Francisco, CA 94143.

In wild-type *Drosophila* embryos, five lateral chordotonal (lch) axons in each abdominal hemisegment originate from a midlaterally positioned cluster of neurons and grow, fasciculate, and orient ventrally as they connect with targets in the central nervous system (CNS). We have identified twenty-two recessive lethal mutations in 12 complementation groups, 8 of which are novel, that differentially affect lch axon growth, fasciculation, and ventral orientation. Mutations in 3 loci result in shorter, but fasciculated and ventrally directed axon bundles. Mutations in 5 genes cause lch axon defasciculation. Mutations in 7 genes cause some lch axon bundles to grow dorsally along a trajectory 180° from normal.

One of the genes, *frazzled*, that is required for lch axon fasciculation is generally required for axon routing and fasciculation in the embryo. *frazzled* is related to the human "deleted in colon cancer" (DCC) gene. The DCC and frazzled proteins are part of a novel subfamily of transmembrane proteins within the immunoglobulin superfamily. DCC is expressed in the vertebrate nervous system on axons, and figures prominently in colon cancer tumor progression.

594.12

MOTONEURONAL MUTANTS IN EMBRYONIC ZEBRAFISH. C.E. Beattie*, K.L. Stoesser and J.S. Eisen.
Institute of Neuroscience, University of Oregon, Eugene OR 97403.

Pathfinding is a complex process that is dependent upon both the neuron and its pathway expressing the right molecular components at the appropriate times. To identify the molecular mechanisms involved in pathfinding, we have undertaken a mutagenesis screen in zebrafish. Progeny of fish containing germline mutations induced by exposure to N-ethyl-N-nitrosourea were screened with antibodies that recognize motoneurons. Several mutations have been recovered that affect pathfinding by a small set of individually identified motoneurons. Here we focus on a dominant mutation which affects at least one primary motoneuron, CaP. Normally the axons of CaP and the other primary motoneurons leave the spinal cord, extend along a common pathway and then diverge along separate cell-specific pathways on the myotome. In this mutation, CaP is present in normal numbers and extends its axon along the common pathway but fails to extend an axon on its cell-specific pathway. We performed transplantation experiments to learn if this mutation is acting in the neuron or the myotome. Myotomes were removed from mutant embryos before axogenesis and replaced with wildtype myotomes. Preliminary results reveal that wildtype myotomes do not rescue mutant CaP axons, suggesting that the mutation is acting cell-autonomously. CaP pathfinding, therefore, appears to depend on cell intrinsic factors that differentially regulate axon outgrowth along distinct pathway regions.

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594.13

ZEBRAFISH MUTATIONS AFFECTING RETINOTECTAL AXON PATHFINDING. R. Karlstrom*, T. Trowe, H Baier, S. Klostermann, and F. Bonhoeffer. Max-Planck-Institut für Entwicklungsbiologie, 72076 Tübingen, Germany.

A fundamental issue in neurobiology is how proper neuronal connectivity is established during development. We have taken a genetic approach to this question using the zebrafish *Danio rerio*. In collaboration with the lab of Dr. Nuesslein-Volhard, we have identified mutant zebrafish that have defects in axonal connectivity between the retina and tectum. 3000 mutant families were screened by labeling retinal ganglion cells (RGCs) of 5 day fixed embryos with DiI and DiO and observing their axonal projections to and on the tectum. 154 mutants (comprising 16 complementation groups to date) were identified with defects in either the axon trajectory between eye and tectum (pathfinding mutants), or with altered trajectories on the tectum (topographic mutants).

This abstract focuses on the pathfinding mutants. The pathfinding mutants fall into four classes based on the location of pathfinding errors between eye and tectum. In class I, axons grow immediately toward the ipsilateral tectum upon leaving the eye (*belladonna*). In class II (*detour, you-too, lizard*) axons grow toward the midline before turning back to the ipsilateral tectum; in wildtype zebrafish all RGCs project to the contralateral tectum. Class III mutants (*boxer, dackel*) show defects in proper dorso-ventral axon sorting at the midline. Finally, class IV mutants (*bashful, AI72*) show rostro-caudal trajectory defects at or after the midline. In *bashful*, axons grow rostrally around the telencephalon instead of turning caudo-dorsally to the tectum. In *AI72*, axons either grow rostrally or turn caudally to grow under the tectum.

Taken together, these mutants support a model in which sequential guidance cues guide retinal axons to the contralateral tectum. Since the observed pathfinding errors are made at a limited number of distinct points along the retino-tectal pathway, the mutants may reveal the important choice points encountered by growth cones as they navigate between eye and tectum.

595.1

CHARACTERIZATION OF NEURONAL PROGENITOR CELLS ISOLATED FROM THE NEONATAL FOREBRAIN. M.B. Luskin*, R. Stewart, T. Zigova and B.J. Soteres. Dept. of Anatomy & Cell Biology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

Recent studies have shown that the postnatal subventricular zone of several mammalian species is a source of neurons as well as glia. We have shown that in vivo the anterior portion of the neonatal subventricular zone (SVZa) generates an immense number of cells which migrate to the olfactory bulb and differentiate into interneurons (Luskin, Neuron, 11:173, 1993). To investigate the properties of neonatal SVZa cells we devised a procedure to isolate and culture these cells. Ham's F10 medium, DMEM, or a 1:1 mixture of Ham's F10:DMEM supplemented with 10 % fetal calf serum was used with several different substrates. The phenotype of the cultured cells was determined 1 - 10 days later using a number of cell type-specific markers including TuJ1, an antibody recognizing neuron-specific class III β-tubulin and an antibody against GFAP to distinguish astrocytes. We demonstrated that after 1 day in culture the SVZa cells proliferated and that virtually all cells were TuJ1(+). In addition, there were substrate specific effects on neurite outgrowth. After several days in culture the vast majority of cells were TuJ1(+), while < 5% were GFAP(+). This indicates that in vitro, as in vivo, the SVZa cells and their progeny have a neuronal identity. Thus, the SVZa appears to constitute virtually a pure population of neuronal progenitor cells. Supported by the March of Dimes, NIH and the Pew Charitable Trusts.

595.2

THE EFFECT OF BULBECTOMY ON NEUROGENESIS IN THE NEONATAL FOREBRAIN. R. Betarbet*[1,2], T. Zigova[1], R.A.E. Bakay[2] and M.B. Luskin[1]. [1]Depts. of Anatomy and Cell Biol. & [2]Neurosurgery, Emory University School of Medicine, Atlanta, GA 30322.

In the rat, the anterior part of the subventricular zone (SVZa) is known to generate an immense number of neurons during the first week of postnatal life which are destined to become the interneurons of the olfactory bulb (Neuron, 11:173,1993). In this experiment we wanted to study the effect of neonatal bulbectomy on SVZa neurogenesis. Unilateral bulbectomy was performed on hypothermically anesthetized Sprague Dawley rats at postnatal day 0 (P0) or P1. One to five days after bulb ablation the pups received two intraperitoneal injections of the cell proliferation marker, BrdU (5 mg/ml; 0.3 ml/pup), which were given 6 hours apart. The pups were perfused at various times, their brains were sectioned on a cryostat in the horizontal or sagittal plane and processed for immunohistochemistry to reveal BrdU-positive cells. We compared the density and the distribution of the BrdU-positive cells on the experimental side to the control side of the forebrain. Unilateral bulbectomy resulted in an increase in the size of the subventricular zone, including the SVZa, on the experimental side. In addition, there was at least a two-fold increase in the density of BrdU-labeled cells. The increased size of the SVZa, in conjunction with the increased density of BrdU-labeled cells, indicates that the ablation leads to an increase in cell proliferation. Collectively, the results demonstrate that the proliferation of SVZa cells can be experimentally manipulated. Supported by Pew Charitable Trusts, NIH and March of Dimes.

595.3

THE PRODUCTION OF NEURONS THROUGH ASYMMETRIC CELL DIVISIONS IN THE DEVELOPING FOREBRAIN. Anjen Chenn* and Susan K. McConnell. Dept. of Biological Sciences, Stanford University, Stanford, CA 94305

We have established a system in which to observe directly the behaviors of cycling precursor cells in the mammalian central nervous system, processes which previously could be studied only indirectly. With the cellular techniques developed here, we provide evidence for the production of neurons by asymmetric division of progenitor cells. Slices through the developing cerebral wall support the normal cell movements and nuclear migrations that accompany the division of neuroepithelial progenitors. By imaging individual fluorescently-labeled progenitor cells over time, we have observed two distinct behaviors of cells following division. The first type of division is symmetric in nature, with cleavages perpendicular to the ventricular surface. These divisions are both morphologically and behaviorally symmetric: both daughter cells derived from these vertical cleavages appear to retain contact with the ventricular surface, and their subsequent movement away from the lumen is generally slow and paired. We propose that early in cortical development, these symmetric divisions are proliferative in nature and serve to maintain or expand the precursor pool. The second type of division is asymmetric, with cleavage planes parallel to ventricular surface. This type of division, observed only rarely prior to the onset of neurogenesis, becomes common as neurons are generated. Following cleavage in nearly all of these cases, the two daughters separate from one another and the basal or outer daughter moves away at rates that can be extremely rapid, approximating the rates of outward migration of young postmitotic neurons. We therefore propose that these asymmetric divisions are differentiative in nature, possibly representing a stem cell mode of division in which one daughter becomes a neuron and the other reenters the cell cycle. Supported by NIH MH51864 and GM07365.

595.4

THE ROLE OF NEUREGULINS IN OLFACTORY NEUROGENESIS. N. Mahanthappa* and G. A. Schwarting. Cambridge Neuro-Science Inc., Cambridge, MA 02139; and E. K. Shriver Center, Waltham, MA 02254.

The neuregulins are peptide growth factors of the EGF superfamily (reviewed by Peles and Yarden, BioEssays 15: 815 [1993]). They are differential splice products of a single gene and were independently cloned as neu differentiation factor, heregulin, glial growth factor, and acetylcholine receptor inducing activity. Neuregulins are known to signal via receptor tyrosine kinases of the EGF receptor family including erbB2 (c-neu), erbB3, and erbB4. Heparan sulfate proteoglycans are likely to play roles as co-receptors (Sudhalter, et al. Soc. Neurosci.Abs. 20: 1691 [1994]).

By in situ hybridization, Meyer and Birchmeier (PNAS 91: 1064 [1994]) have demonstrated that neuregulin message is highly expressed in the neuronal layer of embryonic murine olfactory epithelium (OE). In OE cultures, we have demonstrated immunocytochemically that neuregulins are expressed by olfactory receptor neurons (ORNs). In addition, erbB3 and erbB4 are expressed both by neurons and a subset of OE basal cells. These patterns of expression suggest that neuregulins may play a role in mediating ORN survival and regulating basal cell behavior. In ongoing studies, we are determining the expression of these proteins in vivo, and analyzing neuregulin actions on OE cells in culture.

595.5

BONE MORPHOGENETIC PROTEINS INDUCE ASTROGLIAL DIFFERENTIATION OF NEURAL STEM CELLS. RE Gross[1*], MF Mehler[2], T Ngo[1], L Santchi[1] and JA Kessler[2,1] Depts. of Neurosurgery[1], Neurology[2] and Neuroscience[3], Albert Einstein College of Medicine., Bronx, NY.

Bone morphogenetic proteins (BMPs) are a class of proteins within the TGFβ superfamily that were originally characterized for their osteoinductive properties. The BMPs and their relatives, including *decapentaplegic*, activin, and TGFβ1.2 &3, have a broad range of early morphogenetic (such as dorsal/ventral determination) and differentiative activities. We therefore evaluated the effects of BMPs on lineage commitment, survival and differentiation of neural stem cells. EGF-dependent neural stem cells were dissociated and cultured in serum-free medium in the presence of various BMPs, of which we will concentrate on BMP2 here. Immunocytochemistry with cell specific markers was used to define the effects on development of mature cells from stem cells. BMP2 led to a marked proliferation of GFAP+ astrocytes from stem cells. The effect was dose-dependent; all BMPs tested had similar effects, but had different dose-response curves, whereas TGFβ had no effect. Astrocytes generated in the presence of BMP2 were morphologically more highly differentiated than in its absence, and produced more GFAP/cell, based on Western blots. Dual immunofluorescence indicated that both type I (GFAP+, A2B5-) and type II (GFAP+, A2B5+) astrocytes accumulated in the presence of BMP2, whereas only type I astrocytes were present in its absence. Under conditions that lead to increased numbers of oligodendrocytes, BMP2 preferentially produced type II astrocytes over oligodendrocytes (O4+, A2B5+), presumably derived from O2A progenitors. BMP2 had similar effects on astrocytes generated from embryonic cortical cells. We conclude that BMPs are growth factors for both type I and type II astrocytes.

595.6

DYNAMICS OF MASH1 EXPRESSION IN MOUSE OLFACTORY EPITHELIUM SUGGESTS A NON-STEM CELL SITE OF MASH1 ACTION. A.L. Calof*, J.S. Mumm, R.A. Davis, J. D. Holcomb, and M.K. Gordon. Dept. of Anatomy & Neurobiology, Univ. California Col. Med., Irvine, CA 92717.

Olfactory receptor neurons (ORNs) of the mouse olfactory epithelium (OE) are produced by the terminal division of a cell called the INP (for Immediate Neuronal Precursor of ORNs). Studies indicate that INPs behave like neuronal transit amplifying cells, undergoing a limited number of amplification divisions regulated by FGFs while remaining committed to a neuronal fate (e.g. DeHamer et al., 1994, Neuron 13:1083). The cell that precedes the INP in the ORN lineage is unknown: this cell could be the stem cell thought to underlie the OE's capacity for continual neuron renewal, or it could be an earlier, distinct stage of amplifying precursor cell. Deletion by homologous recombination of the gene encoding the transcription factor MASH1 results in a profound loss of ORNs, indicating a crucial role for MASH1 action in ORN neurogenesis (Guillemot et al., 1993, Cell 75:463). To determine whether MASH1 is acting in cells of the ORN lineage, and at what stage, we investigated expression of MASH1 during OE neurogenesis, both in vitro during embryonic development, and in vivo during neurogenesis induced by ablation of one olfactory bulb (OBX) in adult mice. MASH1$^+$ cells are present in embryonic OE and form a small subset of proliferating ORN precursors in OE explant cultures. Both INPs and MASH1$^+$ cells divide rapidly and then disappear from OE cultures as neurogenesis proceeds, but disappearance of MASH1$^+$ cells precedes that of INPs, suggesting that MASH1$^+$ cells give rise to INPs. Following OBX, MASH1$^+$ cells undergo a transient increase in number in OE on the OBX side, suggesting that MASH 1$^+$ cells are not stem cells, since stem cells typically generate one copy of themselves and one other cell with each division. ^{3}H-TdR incorporation studies in OBX mice indicate that the peak of MASH1$^+$ cell proliferation precedes that of INPs, suggesting that MASH1$^+$ cells precede INPs in the ORN lineage in adult OE in vivo, as well as embryonic OE in vitro. Thus, MASH1$^+$ cells appear to be progenitors of INPs, and like them, function as neuronal transit amplifying cells in the ORN lineage. *Supported by NIH grant DC02180 to ALC.*

595.7

SPINAL CORD SUBEPENDYMAL PROGENITOR CELLS DIFFERENTIATE TO ASTROCYTES AND CONTRIBUTE TO SCAR FORMATION AFTER INJURY. Clas B. Johansson*[1, 2], Urban Lendahl[3] and Jonas Frisén[1], Dept. of Neuroscience[1], Dept. of Prosthetic Dentistry[2] and Dept. of Cell and Molecular Biology[3], Karolinska institute, S-171 77 Stockholm, Sweden.

After injuries in the central nervous system astrocytes form a dense scar which may impede axonal regeneration. Reactive astrocytes synthesize high levels of the intermediate filament GFAP. We have studied the expression of nestin, an intermediate filament protein which is highly expressed by progenitor cells for neurons and glial cells, after dorsal funiculus incision in adult rats. Nestin induction was seen within 2 days after injury in subependymal cells close to the central canal, while nestin expressing cells at later time points were found progressively further out from the central canal. From one week on nestin-immunoreactive cells were abundant in the scar tissue which formed at the injury. The nestin expressing cells in the scar tissue coexpressed GFAP. In nestin promoter/*lacZ* transgenic mice, *lacZ* expression mimicked the nestin expression pattern. These data suggested that there may exist a population of subependymal progenitor cells close to the central canal which migrate and differentiate to astro-cytes in response to injury. In order to test this cells lining the central canal were selectively labeled by injecting the fluorescent tracer DiI in the fourth ventricle in adult rats. Six days after the injection, cells lining the central canal at least 50 mm caudal to the fourth ventricle were DiI-labeled. Two days after an incision in the dorsal funiculus DiI-labeled cells could be seen dorsal to the central canal, and at later time points fluorescent cells were scattered in the scar tissue which had formed at the lesion. The DiI-labeled cells in the scar tissue showed GFAP- and nestin-immunoreactivity.

These data suggest the existence of undifferentiated progenitor cells in the subependymal zone lining the central canal which may differentiate to astrocytes and contribute to scar formation in the injured spinal cord.

595.8

DISTINCT ROLES FOR bFGF AND NT3 IN THE CONTROL OF CORTICAL NEUROGENESIS. A. Ghosh* and M. E. Greenberg. Div. of Neuroscience, Childrens Hospital and Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115

While it is generally accepted that extracellular signals can influence the development of various neural populations, the molecules that regulate the transition of dividing neuroblasts to terminally differentiated neurons have not been fully defined. Based on the effects of exogenous growth factors and neutralizing antibodies, we show here that bFGF and NT3 have striking and distinct effects on the development of cortical neurons. The proliferation of both neuronal and glial precursor cells in vitro is controlled by bFGF. The proliferative effects of bFGF do not preclude the action of endogenous differentiation promoting factors as differentiated neurons and glia are generated in these cultures in appropriate developmental sequence. Since NT3 is expressed in the cortex during embryonic development, the role of NT3 in regulating neurogenesis in these cultures was examined by direct addition to cultures or by inhibiting endogenous NT3 action using neutralizing antibodies. Whereas addition of NT3 antagonized the proliferative effects of bFGF and enhanced neuronal differentiation, blocking NT3 function led to a marked decrease in the number of differentiated neurons without affecting the proliferation of cortical precursors or the survival of postmitotic cortical neurons. These observations suggest that bFGF and NT3, by their distinct effects on cell proliferation and differentiation, are key regulators of neurogenesis in the central nervous system.

595.9

STUDY OF EARLY REGIONALISATION OF THE VENTRICULAR ZONE AND ITS ROLE IN THE PRODUCTION OF DISTINCT NEOCORTICAL AREAS.
F. Polleux*, C. Dehay and H. Kennedy. INSERM Unité 371-18 Av Doyen Lépine 69675 BRON (FRANCE).

Recently Dehay et al. [*Nature* 366: 464] showed that in primate the difference in the number of neurons per unit of surface area in area 17 and 18 is due to differences in rates of proliferation in the prospective ventricular zones. Can this principle be generalised to other cortical areas and to other species? We have taken advantage of the fact that in mouse the frontal agranular cortex contains about 27% less neurons than the adjacent parietal granular field and that this difference can be largely attributed to the absence of layer IV in the frontal cortex [Beaulieu, *Brain Research* 609: 284]. We determined the percentage of neurons heavily labelled (LI labelling index) by 11 single pulsed injections of [^{3}H] thymidine made at regular intervals throughout neurogenesis of the mouse (E11.0 to E18.5 with E1 as day of vaginal plug). This estimation has been made in adult frontal agranular area 6, frontoparietal (intermediatly granular) area 4 and parietal granular area 3 as defined by Caviness. When estimated within a single layer the LI is independant of regressive events such as naturally occuring cell death and is thought to reflect neuronal production in the ventricular zone that give rise to these cortical areas. We found that the rate and tempo of neurogenesis is characteristic for each area. Rate of production of infragranular layers of area 3 was higher than those for area 4 and 6. The switch to the production of layer IV occurs earlier in granular areas than it does in agranular ones but the rate of production of neurons destined to reside in layer IV is lower in agranular area 6 than it is in areas 4 and 3. The duration and rate of production of supragranular layers are the same in all three areas examined. Using DiI we were able to show that thalamic afferents arrive after the areal differences in neurogenesis are established.

In conclusion, we show that in mouse, the generation of cortical areas is ensured by differences in the rate of neuron production, implying an important degree of heterogeneity of the ventricular zone which is independent of thalamic innervation.

595.10

MOLECULAR GENETICS OF A DEVELOPMENTAL DISORDER OF THE CEREBRAL CORTEX ASSOCIATED WITH EPILEPSY. Y. Z. Eksioglu[1], P. Cardenas[1], K. Kamuro[2], F. J. DiMario[3], P. R. Huttenlocher[4], G. M. Duyk[5] and C. A. Walsh*[1]. [1]Lab. of Neurogenetics, Dept. of Neurology, Beth Israel Hospital/Harvard Medical School, Boston, MA 02115, [2]Dept. of Pediatrics, Kokubu Seikyo Hospital, Kagoshima 899-48, Japan, [3]Dept. of Pediatrics, Univ. of Connecticut, Farmington, CT 06030, [4]Dept. of Pediatrics, University of Chicago, Chicago, IL 60615, [5]Millenium Pharmaceuticals, Cambridge, MA 02139

Widespread use of magnetic resonance imaging in humans has revealed genetic transmission of some developmental malformations of the human cerebral cortex. Periventricular heterotopias (PH) are non cancerous nodules of neural cells in the ventricular zone beneath the cerebral cortex. Recently, a distinctive, inherited form of PH was identified that is associated with seizures, but normal intelligence and no other peripheral stigmata. Therefore, the PH gene plays a role in cerebral cortical development, as well as representing a novel epilepsy susceptibility gene.

In three multiplex pedigrees, PH was restricted exclusively to females. Males were normal and there was an excess of miscarriages suggesting that PH may be lethal to males. We tested linkage of PH to markers on the p and q arms of the X chromosome. Genetic analysis of the families revealed positive linkage to markers in distal Xq, with a maximal two-point LOD score of 3.44 for F8C (Θ=0), and a maximal multipoint LOD score of >3.7, suggesting X-linked, dominant inheritance with embryonic lethality in affected males. Thus, affected females are likely to be mosaics, since random inactivation of one X chromosome occurs early in cortical neurogenesis, and is inherited in clonal fashion.

Supported by the William Randolph Hearst Fund and the Klingenstein Foundation Fund.

595.11

A retinoic acid driven switch-like mechanism is involved in the choice between neural and mesodermal fates. D.Gottlieb*, G.Bain, M.Yao Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110

Recently we showed that genetically normal, totipotent mouse embryonic stem (ES) cells can be efficiently induced to differentiate into neuron-like cells (NLCs) in culture (Bain et al., Developmental Biology 168:342-357, 1995). Here we show that the pathway from ES cells to NLCs has two distinct steps. Efficient neural differentiation is obtained by culturing undifferentiated ES cells as aggregates for four days in standard medium (4-) and for an additional four days in medium with retinoic acid (4+). Treatment of undifferentiated ES cells with RA for four days does not induce overt neural differentiation or the expression of MASH-1 or wnt-1, 2 early neural genes. In contrast, treatment of ES cells after 4- culture induces overt neural differentiation and expression of both genes. The transition to competence is blocked if LIF is present during 4- culture. After six days in culture in standard medium, aggregates contain cells undergoing mesodermal differentiation as evidenced by expression of the brachyury, cardiac actin and ξ-globin genes. Addition of RA to 4- aggregates completely suppresses expression of these genes. In summary, ES cells cultured as aggregates in the absence of added regulatory factors make a transition to a cell type that is responsive to RA. This cell type expresses an RA-sensitive genetic pathway which simultaneously up-regulates neural gene expression and down-regulates mesodermal gene expression. Supported by NSF (IBN-08787)and NIH (NS12867).

595.12

ASCORBATE IN NEURONS AND GSH IN GLIA: EVIDENCE FROM DEVELOPING CORTEX, HIPPOCAMPUS, AND CEREBELLUM. M. E. Rice* and I. Russo-Menna, Depts. of Physiology & Neuroscience and Neurosurgery, N.Y.U. Medical Center, New York, NY 10016.

Ascorbic acid (ascorbate) and glutathione (GSH) are the most abundant low molecular weight antioxidants in the CNS, yet little is known about their cellular localization. We have proposed that ascorbate predominates in neurons and others have suggested that GSH predominates in glia. Because ascorbate and GSH are readily washed out of mammalian tissue *in vitro*, cell-specific contents have proved difficult to confirm. We addressed this question by monitoring ascorbate and GSH levels (by HPLC) during postnatal development of rat brain, when regionally distinct changes in neuron:glial ratio occur. In cortex and hippocampus, the ascorbate content and ascorbate:GSH ratio were highest shortly after birth when neuron:glial ratio was also highest. Over the next three weeks, as gliogenesis progressed, GSH rose steadily while ascorbate fell, resulting in a linear decrease in ascorbate:GSH ratio. At P3 in cortex, for example, ascorbate levels were 5 μmol g^{-1}, with an ascorbate:GSH ratio of 4. By P23, GSH had risen to adult levels (2 μmol g^{-1}), while ascorbate fell to 3.5 μmol g^{-1}, giving an ascorbate:GSH ratio less than 2. In cerebellum, by contrast, ascorbate and GSH levels were constant from P3-P9, with lower ascorbate and higher GSH than cortex or hippocampus. From P9, coincident with the birth of granule cells, ascorbate levels increased, rising to 7 μmol g^{-1} by P17, then falling again toward adult levels (3 μmol g^{-1}). Taken together, these data are consistent with preferential localization of ascorbate in neurons and GSH in glia. High levels of ascorbate in neurons (5-9 mM estimated from tissue contents and cell volume fractions) suggest that this antioxidant may provide specialized neuroprotection in developing and in adult brain.

This study was supported by NINDS grant NS-28480.

596.1

COMMISSURAL PROJECTIONS OF THE RAT PREFRONTAL CORTEX: SYNAPTIC ASSOCIATIONS WITH INTRINSIC NEURONS AND DOPAMINE AFFERENTS. D.B. Carr* and S.R. Sesack. Departments of Neuroscience and Psychiatry, University of Pittsburgh, PA 15260.

Commissural connections play an important role in the integration of complex behaviors governed by the prefrontal cortex (PFC). We sought to examine the synaptic targets of commissural terminals within the rat PFC, as well as their relation to other PFC afferents. Commissural terminals, labeled by either anterograde transport of *Phaseolus vulgaris* leucoagglutinin or anterograde degeneration following electrolytic lesion of the contralateral PFC, formed primarily asymmetric synaptic junctions on dendritic spines. Some of these spines received a second input from unlabeled terminals forming symmetric synapses. Commissural terminals were less frequently observed to form asymmetric axodendritic synapses. However, several dendrites that received such input exhibited a morphology consistent with GABAergic interneurons. These findings suggest that commissural PFC afferents utilize an excitatory neurotransmitter and the majority target spiny pyramidal neurons. A small subset of commissural projections may provide excitatory input to interneurons. Since both dopamine and GABA terminals have been reported to form symmetric synapses on dendritic spines of pyramidal cells, double-labeling studies are in progress to determine whether commissural PFC afferents converge with dopamine and/or GABA terminals onto common spines. Such a finding would suggest a role for these transmitters in modulating excitatory commissural afferents. This work is supported by USPHS grant MH50314

596.2

THE ROLE OF DOPAMINE (DA), GLUTAMATE RECEPTORS, AND OXIDATIVE STRESS IN INFLUENCING THE LONG-TERM EFFECTS OF REPEATED COCAINE ON THE DYNAMICS OF DA METABOLISM IN RAT CORTEX. F. Karoum*, J.M. Masserano and R.J. Wyatt. Neuropsychiatry Branch, NIMH Neuroscience center at St. Elizabeths, Washington, DC 20032

Repeated administration of cocaine selectively down-regulates the rate of formation of 3,4-dihydroxyphenylacetic acid (DOPAC. DOPAC T/O) in the rat frontal and cingulate cortices but not in the entorhinal cortex, the nucleus accumbens or the striatum, one week after cocaine discontiuation. To better understand how these changes occur, we have carried out several pharmacological manipulations. Cocaine was administered for one week, twice daily (10 mg/kg I.P.) alone and 30 minutes after pre-treatment with one of the following: vitamin E (15 mg/kg), haloperidol (HAL) (0.5 mg/kg); clozapine (CLZ) (20 mg/kg); or MK801 (0.3 mg/kg). Rats were sacrificed one week after termination of treatment. DOPAC T/O was assessed from the exponential decline in DOPAC concentrations 0, 20 and 40 minutes after the administration of pargyline (100 mg/kg).

Pretreatment with HAL or CLZ completely abolished the reduction in DOPAC T/O in frontal and cingulate cortex produced by cocaine. Vitamin E also protected the cingulate cortex from the long-term effects of cocaine. MK801 had no protective effect on either the frontal or cingulate cortex.

These results suggest that DA receptor activation and oxidative mechanisms may be involved in the reduction of cortical DA metabolism during repeated exposure to cocaine. NMDA receptors do not appear to be involved.

596.3

IN VIVO MAPPING OF DOPAMINE- (DA-) D4 RECEPTORS IN PRIMATE BRAIN. C. Boy[3], A. Klimke[4], M. Holschbach[2], H. Herzog[1], E. Rota-Kops[1], G. Wunderlich[3], P. Seeman[6], H. VanTol[6], R. Markstein[5], W. Gaebel[4], G. Stöcklin[2], H.W. Müller-Gärtner*[1,3], [1]Inst. Med., [2]Inst. Nucl. Chem., Res. Center Jülich, 52407 Jülich; [3]Dept. Nucl. Med., [4]Dept. Psychiatry, 40225 Düsseldorf, Germany; [5]Sandoz Pharm., Preclin. Res., 4002 Basel, Switzerland; [6]Depts. Pharm. & Psychiatry, Toronto, M5S 1A8, Canada

A significant increase of DA-D4 receptors has been reported in postmortem striatal tissue of patients with schizophrenia. This study describes the first attempt to quantify D4-like binding sites in the normal primate brain *in vivo*. Dynamic positron emission tomography- (PET-) scans were performed in baboons (n=3) using [11C] SDZ GLC 756 (GLC; K_i-values at human DA receptors *in vitro*: 1.1 nM at D1; 0.4 nM at D2; 25 nM at D3; 0.3 nM at D4.2). In PET the striatum/cerebellum-ratio (SCR) and the neocortical gray matter/cerebellum-ratio (NCR), measured 20 minutes after the injection of GLC, were used as indicators of specific DA receptor binding. The SCR and NCR were 3.4±0.4 (mean±SD) and 1.6±0.3, respectively, and increased linearly as a function of time. Blockage of D1 and D5 receptors by SCH 23390 (SCH; 0.5 mg/kg) reduced SCR and NCR by up to 40%. Following the blockage of D1, D5, D2 and D3 receptors by SCH + raclopride (RAC; 2.0 mg/kg), SCR and NCR were 1.7±0.5 and 1.5±0.3, respectively. Again, the ratios increased as function of time. Finally, blocking all known DA receptor subtypes by SCH + RAC + spiperone (1.0 mg/kg) further reduced striatal binding to an unspecific level. The data suggest a low homogeneous concentration of D4-like binding sites in striatum and neocortex. In conclusion, this differential DA receptor mapping approach indicates that D4-like binding sites, as measured *in vivo* by PET, are present at similar concentrations in striatum and neocortex of healthy primates. This new approach should therefore be useful to test the hypothesis of an increased D4 receptor density in striatum of schizophrenic patients *in vivo*.

596.4

QUANTIFICATION OF STRIATAL DOPAMINE RELEASE BY AMPHETAMINE IN LIVING HUMAN BRAIN DF Wong*, F Yokoi, C Hong, G Grunder, A Imperial, H Ravert, R Dannals, V Cunningham^x, A Gjedde^+. Johns Hopkins Medical Inst., Baltimore, MD; ^Hammersmith, London;^+Aarhus Univ Hosp, Denmark

Neuroleptics and dopamine (DA) transporter antagonists increase DA in the synaptic space. The increase affects the quantification of DA receptors using labeled neuroleptics. This hypothesis was tested with amphetamine (AMP) in five normal volunteers undergoing measurements of D_2-like DA receptor density.

To measure the density of D_2-like DA receptors, high (1.7 μCi pmol^{-1}) and low (0.019 μCi pmol^{-1}) specific activity (SA) [^{11}C]raclopride (rac) were injected i.v. on two days each before and after the i.v. administration of 0.3 mg kg^{-1} AMP, respectively. PET images of the labeled tracer in putamen (PU) and cerebellum (CB) were obtained over 90 min. Steady-state partition volumes (V_s) of [C-11]rac in CB were determined by spectral analysis (Cunningham 1993). The initial unidirectional clearances of rac into PU were used with the distribution volumes to determine free and receptor-bound contents of the tracer in PU (Blomquist et al). B_{max} and K_dV_d were determined from bound tracer content (M_b) and binding potential (M_b/M_s) at equilibrium, where V_d is volume of distribution, M_s is free tracer content and K_d is dissociation constant.

The partition volume was unaffected by the SA but declined significantly after injection of AMP. The rac B_{max} declined significantly after AMP administration (34 ± 5 vs. 21 ± 2 pmol cm^{-3}, p<.05, 1-tailed t). The K_dV_d was 8.5 ± 1.4 and 6.8 ± 0.7 pmol cm^{-3}, resp (NS). When the results were interpreted as a reflection of receptor blockade by DA induced by the IV AMP, the bound quantity of DA averaged 9 pmol cm^{-3} at high SA and 10 pmol cm^{-3} at low SA. Low SA rac alone reduced the bound quantity of DA (6 pmol cm^{-3}), suggesting a brief blockade of the receptors by unlabeled rac, the latter significant (p=0.031; 1-tailed paired *t*-test). The results support the hypothesis that AMP releases DA that blocks D_2-like DA receptors and lowers B_{max} estimates. Supported by MH42821, NARSAD, HD24061, DA09482.

596.5

AMISULPRIDE, AN ATYPICAL ANTIPSYCHOTIC, PREFERENTIALLY BLOCKS DOPAMINE D_2/D_3 AUTORECEPTORS. H. Schoemaker*, Y. Claustre, A. Oblin, L. Rouquier, R. Depoortere, D. Fage, G. Perrault, C. Carter, J. Benavides and B. Scatton. Synthélabo Recherche, 31, av. P.V. Couturier, 92220 Bagneux, France

Amisulpride, a new benzamide derivative with high affinity for both dopamine (DA) D_2 (K_i= 2.6 nM) and D_3 receptors (K_i= 3.6 nM) and a degree of limbic selectivity, shows a unique therapeutic profile against both negative, at low doses, and positive, at high doses, symptoms of schizophrenia. We sought to further define its interaction with pre- and postsynaptic dopamine receptors.

In vitro, amisulpride opposes the inhibitory effects of the D_2/D_3 agonist 7-OH-DPAT on electrically stimulated [^{3}H]DA and [^{14}C]acetylcholine release from rat striatal slices at similar concentrations.

However, in vivo and at low doses (<10 mg/kg ip) amisulpride preferentially blocks DA autoreceptors as shown by its antagonism of the inhibitory effect of 7-OH-DPAT on DA synthesis in the presence of γ-OH-butyric acid, the increase in extracellular DA levels as measured by microdialysis, and the inhibition of the hypomotility induced by low doses of apomorphine or 7-OH-DPAT. Only at higher doses (40-80 mg/kg) postsynaptic DA receptor occupancy and antagonism is apparent, as shown by a decrease in striatal acetylcholine levels and the inhibition of striatal [^{3}H]raclopride binding and apomorphine-induced hypermotility and stereotypies. In contrast, haloperidol is approximately equipotent in all of these paradigms.

The present data suggest that amisulpride is a specific DA antagonist with a preferential effect at low doses on D_2/D_3 autoreceptors in vivo and that the mechanisms governing in vitro and in vivo DA release may not be identical. These studies further demonstrate that an atypical preclinical antipsychotic profile can be obtained through selective DA (auto)receptor blockade.

596.6

DOPAMINE (D_2) RECEPTOR ACTIVATION BLOCKS SENSORY EVOKED EXCITATION OF RAT MITRAL CELLS IN VITRO. L.A. Zimmer*, S. Phillips, M. Ennis, & M.T. Shipley. Dept. Anat., Univ. Maryland, Baltimore, MD 21201

A large population of juxtaglomerular interneurons in the rat main olfactory bulb (MOB) contain dopamine (DA). DA-D_2 receptors are localized in the glomerular (GL) and olfactory nerve (ONL) layers of MOB. Destruction of the olfactory epithelium eliminates D_2 receptors in both the ONL and the GL, suggesting that D_2 receptors are present on olfactory nerve terminals. Consistent with this, olfactory receptor neurons contain abundant D_2 receptor mRNA transcripts. Together, these findings suggest that DA, released from juxtaglomerular neurons presynaptically regulates ON terminals. Mitral cells of the OB receive direct excitatory input from ON terminals. Glutamate released from ON terminals excites mitral cells via non-NMDA and NMDA receptors in the rat (Ennis, et al., this meeting). Activation of non-NMDA (AMPA/kainate) receptors evokes rapid, brief (10-40 msec) excitation of mitral cells; NMDA receptors mediate a delayed, long-lasting (100-500 msec) excitatory response. *The goal of this study was to determine if glutamate* transmission at ON->MC synapses is modulated by activation of D_2 receptors on ON terminals.

Olfactory bulbs were rapidly removed from anesthetized rats (50-125 g), 500 μm-thick horizontal slices were submerged in a recording chamber perfused with oxygenated Krebb's solution. Single (0.4 Hz), low intensity (4-40 μA) shocks were applied to the ONL while recording action potentials from mitral cells. ONL shocks evoked the short latency non-NMDA response, and the delayed, long lasting NMDA response. Bath application of DA (100 μM) decreased the response magnitude of both non-NMDA and NMDA responses by 70%. Bath application of the selective D_2 agonist, quinpirole (100 μM) also decreased the magnitude of both responses by a comparable amount. ONL-evoked excitation of mitral cells recovered 5-15 min after termination of DA or quinpirole. Bath application of the D_2 antagonist, eticlopride (200 μM) prevented DA-induced inhibition of mitral cell responses to ONL shocks. These results indicate that activation of D_2 receptors on ON terminals presynaptically inhibits glutamate release onto mitral cells. We conclude that DA release from juxtaglomerular neurons inhibits ON->MC transmission through activation of presynaptic D_2 receptors. *Support: NIH DC00347, DC02588 and NS29635.*

596.7

REGULATION OF [^{3}H]DOPAMINE ([^{3}H]DA) RELEASE FROM MESOLIMBIC AND MESOCORTICAL AREAS OF GUINEA PIG BRAIN BY SIGMA (σ) RECEPTORS. I.K. Weatherspoon*, Grace M. Gonzalez-Alvear and L.L. Werling. Dept. of Pharmacology, The George Washington University Medical Center, Washington, DC 20037.

Sigma receptors are present in nucleus accumbens (NAC) and prefrontal cortex (PFC). We have previously shown that σ receptors regulate NMDA-stimulated [^{3}H]DA release in caudate putamen (Gonzalez-Alvear and Werling, J. Pharm. Exp. Ther. 271:212-219, 1994). We sought to determine whether σ receptors might similarly regulate [^{3}H]DA release in NAC and PFC. Using a superfusion system, we tested the σ agonists (+)pentazocine ((+)pent) (1nM-10μM) and BD737 (1 nM -1 μM) alone and in the presence of the σ_1/σ_2 antagonist BD1008 (10 nM) or the σ_1-selective antagonist Dup734 (100 nM). In both areas, inhibition of [^{3}H]DA release was produced at nM concentrations consistent with a σ receptor-mediated process. BD1008 significantly reversed the inhibition in NAC mediated by 1 μM (+)Pent and 100 nM BD737. Most of the inhibition produced by 1 μM (+)Pent (40% of control-stimulated release) and all the inhibition produced by 100 nM BD737 (30% of control-stimulated release) was reversible by Dup734. Thus, in NAC, regulation of [^{3}H]DA release by (+)pent was predominantly through the σ_1 receptor. In PFC, both Dup734 and BD1008 significantly reversed the inhibition mediated by 100 nM BD737. However, the lesser ability of Dup734 to reverse (+)pent-mediated inhibition indicates a potential σ_2 receptor-mediated component of inhibition in PFC. Furthermore, in control experiments, we determined that this inhibition of release in NAC and PFC was not likely to be mediated via opioid receptors or the PCP receptor. Supported by NARSAD.

596.8

THE REGULATION OF DOPA DECARBOXYLASE IN BRAIN OF LIVING RAT BY DOPAMINE RECEPTORS. P. Cumming*, A. Ase, H. Kuwabara, and A. Gjedde. Montreal Neurological Institute, McGill University, Montreal, Que. Canada H3A 2B4

Male Wistar rats received [^{3}H]DOPA (300 μCi/kg, i.v.) 45 minutes after apomorphine (0.1 mg/kg, s.c.), or flupenthixol (1 mg/kg, i.p.) in addition to carbidopa (5 mg/kg, i.p.). After tracer circulation lasting 3-120 min, extracts of plasma and striatum were fractionated by HPLC to measure the contents of [^{3}H]DOPA, [^{3}H]DA and other metabolites. These concentrations were used to estimate the relative activity of DOPA decarboxylase (DDC) with respect to [^{3}H]DOPA and the activity of monoamine oxidase (MAO) with respect to [^{3}H]DA formed in striatum.

	DDC (min^{-1})	MAO (min^{-1})
Saline	0.26	0.013
Apomorphine	0.20	0.000
Flupenthixol	0.40	0.10

Thus, apomorphine, a D1/D2 agonist, reduced the turnover of [^{3}H]DA without altering DDC activity, while flupenthixol, a D1/D2 antagonist increased the activity of DDC by 50% with a concomitant large increase in [^{3}H]DA catabolism.

596.9

PARAQUAT-INDUCED ACTIVATION OF CENTRAL CATECHOLAMINE SYSTEMS. B.K. Edmonds* and G.L. Edwards. Dept. of Physiol. & Pharm., College of Vet. Med., Univ. of Georgia, Athens, GA 30602

Paraquat (PQ) is a toxin capable of activating neuronal and neuroendocrine systems including the hypothalamic-pituitary-adrenal (HPA) axis. The neural substrates for PQ-induced activation of the HPA axis may involve central catecholamine systems. Activation of central catecholamine systems by PQ was investigated in this study. Sprague-Dawley rats received injections (s.c.) of either PQ (25 μmol/kg) at a dose shown previously to activate the HPA axis or saline (1 ml/kg). Two hours following treatment the animals were decapitated, the brains removed, and tissue punches made through discrete brain regions, including the AV3V, the paraventricular nuclei (PVN), the ventral medial hypothothalamus (VMH), and the cortex. Tissue catecholamine levels were measured using HPLC. Norepinephrine levels were significantly lower in the region of the PVN in PQ-treated as compared to saline-treated animals; however, no difference was seen in the AV3V, the VMH and the cortex. Determination of neuronal activation using proto-oncogene c-Fos immunoreactivity (FLI) revealed increased FLI in the PVN, VMH, and AV3V of animals treated with PQ as compared to saline. Collectively, these results suggest that PQ activates central catecholamine pathways resulting in an increased release of neurotransmitter at terminal regions in the hypothalamus. This increase in catecholamine activity may be involved in PQ-induced activation the HPA axis. (Supported by UGA Physiol. & Pharm.)

596.10

IDENTIFICATION OF OOCTYES AS ONE OF THE INTRAGONADAL SITES OF NOREPINEPHRINE SYNTHESIS IN THE PRIMATE OVARY. A. Mayerhofer[1]*, G. D. Smith[2], M. Danilchik[3], J.E. Levine[4], D. Wolf[2], M. Costa[1], and S.R. Ojeda[1]. [1]Div. Neurosci., [2]Div. Reprod. Sci., OR Reg. Primate Res. Ctr, Beaverton, OR 97006, [3]SD, OHSU, Portland OR 97201, [4]Northwestern Uni., Evanston, IL 60201.

Catecholamines, which participate in gonadal development and function, are thought to derive mainly from the extrinsic innervation of the ovary. However, the primate ovary contains tyrosine-hydroxylase (TH) immunoreactive neuron-like cells (Dees et al., 74th Ann. Mtg. Endo. Soc., p. 59, 1992), and expresses the TH gene (Mayerhofer et al., Soc. Neurosci. Abstr. 20:11, 1994), suggesting an intragonadal source of catecholamines. Reverse transcription-PCR of ovarian RNA, using oligodeoxynucleotides complementary to highly conserved sequences of the human and rat dopamine-beta-hydroxylase (DBH) genes, yielded a cDNA encoding a mRNA species identical to adrenal DBH mRNA. An antisense RNA transcribed from this cDNA template was fully protected by ovarian RNA in a ribonuclease protection assay. Surprisingly, *in situ* hybridization identified oocytes as a major site of DBH mRNA expression, a localization verified by RT-PCR cloning and sequencing of a DBH cDNA from cumulus-free oocytes. Immunohistochemical localization of DBH protein by conventional and confocal laser microscopy showed that oocyte DBH mRNA is translated into its protein product. Oocytes do not contain TH mRNA and protein, but were found to express a dopamine (DA) transporter gene, similar to the one found in human brain (99% homology at the amino acid level). The biological relevance of this transporter system was suggested by the ability of isolated oocytes to metabolize DA into norepinephrine (NE), as determined by HPLC-electrochemical detection. Thus, follicular development in the primate ovary may be affected by a novel cell-cell regulatory loop involving a) DA released by nerve terminals and TH-positive neuron-like cells, b) uptake of DA by oocytes, and c) conversion of DA to NE, which may then feed back on cumulus/granulosa cells to affect their function, presumably via β-adrenergic receptors. Supported by grants from DFG (Ma 1080/4-1 to AM) and NIH (HD-24870, HD-18185 and RR-00163).

596.11

MUSCARINE AND CAFFEINE USE DIFFERENT INTERNAL Ca^{2+} POOLS TO EVOKE EXOCYTOSIS IN RAT CHROMAFFIN CELLS. X Guo*, DA Przywara, TD Wakade, and AR Wakade. Dept. of Pharmacology, Wayne State University, Detroit, MI 48201.

The effects of muscarine (Mus) and caffeine (Caf) on $[Ca^{2+}]_i$ and exocytosis were studied in cultured rat chromaffin cells. Two protocols were used to identify the contribution of internal and external Ca^{2+}. First, in a Ca^{2+} containing medium, the environment surrounding single cells was controlled by ejecting agonist with or without Ca^{2+} from an ejection pipette. Second, intracellular stores of Ca^{2+} were depleted by soaking cells in Ca^{2+}-free plus EGTA solution before transient exposure to agonist plus Ca^{2+}. Exocytosis from individual cells was measured electrochemically using a carbon fiber electrode, and $[Ca^{2+}]_i$ measured using indo-1. Caf (10 mM) and Mus (30 μM) evoked exocytosis whether or not Ca^{2+} was included in the pipette, but neither produced elevated $[Ca^{2+}]_i$ or exocytosis in cells depleted of internal Ca^{2+}. Both elevated $[Ca^{2+}]_i$ and exocytosis exhibited long latency to onset after stimulation by Caf (2.9 ± 0.38 sec) or Mus (2.2 ± 0.25 sec). However, the duration of Caf-evoked exocytosis was significantly different from that evoked by Mus (7.1 ± 0.8 and 33.1 ± 3.5 sec, respectively, 5 sec application). Even after changing Caf application between 0.5 and 30 sec, duration of exocytosis was unaffected (7.6 ± 1.1 and 7.3 ± 0.9 sec, respectively). Repeat application of Caf after the burst of exocytosis revealed an approximate 20 sec refractory period for exocytosis but not elevated $[Ca^{2+}]_i$. There was no refractory period for Mus. The refractoriness of Caf-evoked exocytosis was not due to vesicle depletion because Mus or nicotine could evoke a second round of exocytosis immediately after Caf-evoked exocytosis. Pretreatment of cells with rayanodine (0.1 to 10 μM) inhibited Caf- but not Mus-stimulated exocytosis and elevated $[Ca^{2+}]_i$. We conclude that kinetics of exocytosis by Mus and Caf are different and different pools of internal Ca^{2+} are used by these agents in rat chromaffin cells.

597.1

PENICILLIN MICROINJECTIONS IN MESENCEPHALIC RETICULAR FORMATION INDUCED CLINICAL AND EEG GENERALIZED SEIZURES. Francisco Velasco*, Marcos Velasco, Fiacro Jiménez and Francisco Estrada. Div. Neurophysiology, Nat. Med. Center, IMSS. México, D.F., MEXICO.

Fifteen adult cats had implanted electrodes to record EEG from precruciate and suprasylvian cortices and EMG from neck muscles in both sides. With the animals fully awaked, the head was fixed through a implanted head holder device in the stereotactic frame and the body in a transparent plastic cage to observe them during the experiments, while EEG and EMG were recorded. A solution containing 500U/µl of sodium penicillin (osmolarity and pH adjusted), were stereotactically injected through a Hamilton syringes along the brain stem. Three different places were injected per animal in different days. Upon completion of the experiment the places of injection were marked by the same volume of a dye (fuscina). Animals were then sacrificed and froozen sections of the brain stem obtained to determine the places of injection. 0.25 to 0.5µl of penicillin solution along mesencephalic reticular formation invariably induced EEG and clinical generalized seizures. In contrast injections on pontine reticular formation induced slow wave sleep, injections in the raphe induced paradoxycal sleep, while injection in craneal nerve nucleus induced signs of focal hyperactivity (VII= hemifacial spasm, VIII= nystagmus, X= salivation, etc) but no clinical or EEG seizures.

597.2

DECREASED SEIZURE THRESHOLD IN ADULT HIPPOCAMPAL SLICES AFTER PERINATAL HYPOXIA IN VIVO. F.E. Jensen *, C.D. Wang, and C. Geary. Div. Neurosci., Children's Hosp.[1], Harvard Med. Sch., Boston, MA 02115

We have shown that perinatal hypoxia in the rat (postnatal (P) days 10-12) acutely induces seizures and permanently decreases seizure threshold to the convulsants flurothyl and pentylenetetrazol. Depth electrode recordings during hypoxia show seizures induced in neocortex, pyriform cortex, and hippocampus. To determine whether there are long lasting changes in the hippocampus, hippocampal slices were prepared from P70-80 rats with previous exposure to a single episode of hypoxia (3% O_2) at P10. Following incubation in ACSF, extracellular recordings were made in area CA1 during 60 min incubation in Mg^{2+}-free ASCF. Slices from previously hypoxic rats were compared to litter mate controls (one slice per rat). Incubation in Mg^{2+}-free medium induced ictal discharges in 6/10 slices from rats with previous perinatal hypoxia (n=10 rats), while no ictal discharges were seen in control rats (0/10, n=10 rats) ($p<0.02$). Slices from control adults showed only interictal bursting. These data are consistent with our in vivo results suggesting that even a single episode of hypoxia can permanently decrease seizure threshold. In addition, these data suggest that hypoxic exposure during development induced hyperexcitability intrinsic to the hippocampus which is not dependent on coactivation of other structures. Supported by NS31718, NS32570 (FEJ).

597.3

EPILEPTIC SEIZURES CAUSED BY INACTIVATION OF A NOVEL GENE IN TRANSGENIC MICE. M. Toth,*[1] J. Grimsby[2], G. Buzsaki[3], and G.P. Donovan[1].[1]Department of Pharmacology, Cornell University Medical College, New York, NY 10021, [2]Department of Molecular Pharmacology and Toxicology, University of Southern California, Los Angeles, CA 90033, [3]Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102

Epidemiological data and genetic studies indicate that certain forms of epilepsy are inherited. Based on the similarity between the human and mouse genomes, mouse models of epilepsy could facilitate the discovery of genes associated with epilepsy syndromes. We have identified a line of transgenic mice that shows handling-induced behavioral seizures characterized by whole body jerks, generalized clonic seizures, and epileptic brain activity, resembling human partial epilepsy with secondary generalization. Similar to human epilepsies, the onset of seizures is age dependent, the seizure threshold is remarkably lowered, and the phenotype shows variable penetrance in the transgenic animals. Molecular and genetic studies demonstrated that the phenotype was caused by the disruption of a novel brain specific gene. Based on the characteristic phenotype, we have named the locus and the putative gene defined by the transgene insertion "*jerky*". The chromosomal localization of the *jerky* gene was mapped between D15Mit3 and the retrovirus integration site Pmv17/Tef loci on chromosome 15 by screening an interspecies cross panel. The *jerky* gene encodes a putative 41.7 kD protein displaying homology to a number of nuclear regulatory proteins.

597.4

INHIBITORY POSTSYNAPTIC CURRENTS IN DENTATE GRANULE CELLS OF HUMAN AND RAT EPILEPTIC HIPPOCAMPUS. Masako Isokawa* and Itzhak Fried. Brain Research Institute and Dept. of Neurosurgery, Sch. of Medicine, UCLA, Los Angeles, CA 90024-1761.

Previous studies indicated that 80-90% of GABA inhibition was preserved in human epileptic hippocampus (Masukawa et al., 1989; Babb et al., 1989). We have recorded IPSPs from morphologically-identified human epileptic dentate granule cells (DGCs) (Isokawa et al., 1991). The present study was initiated to further characterize $GABA_A$ receptor-mediated synaptic transmission in epileptic DGCs using whole-cell patch clamp recording. Hippocampal slices were prepared from surgically resected temporal lobe specimens of epileptic patients and from pilocarpine-treated chronically epileptic rats. Inhibitory postsynaptic currents (IPSCs) were isolated as outward currents at 0 mV, near the reversal potential of glutamate-mediated excitatory postsynaptic currents (EPSCs). IPSCs were pharmacologically confirmed as $GABA_A$ IPSCs by 10 µM bicuculline methiodide. With single orthodromic activation of DGCs, IPSC amplitude was similar in human and rat epileptic DGCs and in normal rat DGCs. However, tetanic stimulation at -30 mV, at which NMDA receptors were activated, reduced IPSC amplitude at 0 mV in all epileptic cells. In some cases, IPSCs were completely abolished. IPSC amplitude recovered in several tens of seconds to several minutes. Although the magnitude of the effect of tetanic stimulation on IPSCs varied, the effect was stronger on epileptic DGCs compared to control DGCs. An excitatory neurotransmitter glutamate may interact with inhibitory $GABA_A$ neurotransmission in epileptic hippocampus in a perverted way to give rise to epileptogenic excitation. Supported by NINDS grants NS02808 and NS31180.

597.5

NEUROSTEROID MODULATION OF BENZODIAZEPINE BINDING TO NEOCORTICAL $GABA_A$ RECEPTORS IN HUMAN FOCAL EPILEPSY VARIES WITH PATHOLOGY. P.C. Van Ness, I.A. Awad, M. Estes, Cleveland Clinic, Cleveland, OH 44195; Q. Nguyen, R.W. Olsen,* UCLA School of Medicine, Los Angeles, CA 90024

Different pathologies cause similar epilepsies. Epilepsy pathology and localization may alter neurotransmitter receptor subunits. $GABA_A$ receptors have multiple possible subunit combinations which may play a role in some human epilepsies. Excessive subcortical heterotopias (which are associated with mesial temporal sclerosis (MTS)) and cortical dysplasia are both developmental disorders that could have altered receptors.

Oriented neocortical tissue obtained at time of surgery in 34 intractable partial epilepsy patients was sliced into 5 mm slabs. Assayed tissue was frozen in isopentane cooled with liquid nitrogen; alternate sections had routine histopathology. Temporal and frontal neocortex with tumor, heterotopias and cortical dysplasia were studied; cases with multiple pathologies were excluded. Tumoral epilepsy tissue was intentionally from uninvolved regions. $GABA_A$ receptor binding was assayed autoradiographically on frozen, unfixed specimens using [^{3}H]flunitrazepam (FLU) for the benzodiazepine (BZ) site (Nguyen et al., 1995), with and without 10 µM alphaxalone. An investigator, blind to pathology, quantitated FLU binding to cortical laminae I-III, IV, and V-VI.

Regional FLU binding without alphaxalone was similar for all pathologies. Temporal neocortex with subcortical heterotopia (N=17) had increased laminar FLU binding of 10.4-12.8% with alphaxalone compared to decreases of 6.4-8.8% in tumor (N=8) and 1.9-5.6% in cortical dysplasia (N=4). In frontal neocortex, alphaxalone enhanced FLU binding by 12.9-18.2% in heterotopias (N=2) compared to 5.8-9.8% for cortical dysplasia (N=3).

The pathology-specific neurosteroid modulation of BZ binding suggests possible $GABA_A$ receptor subunit alterations such as increased numbers of steroid-sensitive subunits or decreased steroid-insensitive subunits. Temporal lobe receptor changes possibly cause or result from MTS. Other non-mesial temporal lesions have been associated with hippocampal cell loss. Understanding pathology specific receptor differences could lead to more specific pharmacologic and preventive interventions for epilepsies. Supported by NS22071

597.6

METHYLATION OF NUCLEIC ACID DURING NEUROBLASTIC DIVISION INDUCES EXPERIMENTAL NEURONAL MIGRATION DISORDERS. I.M. Germano*, MD and E.F.Sperber, PhD. Department of Neurosurgery, Mount Sinai School of Medicine and Department of Neurology and Neuroscience, Albert Einstein College of Medicine, New York, NY

Neuronal migration disorders (NMD) are often found in patients undergoing evaluation for epilepsy. Although recent data suggest that some types of NMD may be genetically inherited, the mechanisms leading to NMD have not been fully investigated yet. The aim of the present study is to assess the effects of alteration to nucleic acids during the neuroblastic division on NMD. We exposed pregnant rats to the alkylating neurotoxin methylaxozymethanol (MAM; 25 mg/kg in saline i.p.) at pregnancy day (PG) 13, 14, 15, and 16. In the rat, neuroblastic division occurs at PG 15.

Histological examination of 150 14 day-old rat pup belonging to X litters showed that all rats (100%) exposed to MAM at PG 15 had NMD. The incidence of NMD in the other groups was as following: 63% in PG 13, 70% in PG 14, 85% in PG 16. Histological features of experimentally-induced NMD included neuronal dysplasia of the cortex and hippocampus. In the neocortex, we found cortical laminar disorganization, ectopic neurons in the subcortical white matter, ectopic neurons in cortical layer I, persistent granular layer and marginal glial neuronal heterotopia. Hippocampal features included discrete areas of neuronal ectopia in the CA1 subfield. Based on histological criteria, NMD were divided into 3 groups: mild, moderate, severe. Moderate and severe NMD were found only in PG 15 rats.

These data shows that methylation of the nucleic acid at the time of neuroblastic division induces NMD. In our study the methylation was "environmentally" acquired, however, a similar mechanism may be genetically inherited. MAM-induced experimental NMD have histological characteristics similar to those observed in patients with NMD and epilepsy. Ongoing studies in our laboratory are using this model to bring insights on the link between NMD and epilepsy.

597.7

OPPOSITE CHANGES OF "CENTRAL" AND "PERIPHERAL" BENZODIAZEPINE RECEPTORS IN HIPPOCAMPUS OF ELECTRICALLY KINDLED RATS.

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Modifications of glutamate and GABA-A receptor subunits have been described in animal models of kindling epileptogenesis.

To investigate possible mechanisms of GABA-A receptor modifications, central (CBR) and peripheral (PBR) benzodiazepine binding sites and the levels of their endogenous ligand diazepam binding inhibitor (DBI) were studied in ventral and dorsal hippocampus of rats electrically kindled in amygdala or dorsal hippocampus. CBR density and affinity were studied by the specific binding of ^{3}H-Flumazenil, PBR by the specific binding of ^{3}H-PK-11195 and DBI levels were assayed by RIA using polyclonal rabbit antibodies raised against rat DBI.

CBR density (Bmax) was decreased ($p < 0.05$) in ventral hippocampus of rats fully kindled in amygdala and hippocampus, whereas PBR density was significantly increased in the same areas. Binding affinity did not change in any investigated area. DBI levels were increased, although not significantly, in ventral and dorsal hippocampus of rats kindled in amygdala and hippocampus.

These data support a possible role of DBI and its receptors in kindling epileptogenesis; PBR might induce CBR-GABA-A receptor modifications through neurosteroids active at this receptor complex.

597.8

METABOLIC MARKERS OF SEIZURES AND SEIZURE-INDUCED NEURONAL DAMAGE AS ASSESSED BY MAGNETIC RESONANCE SPECTROSCOPY. Imad Najm*, Youssef Comair, Yang Wang, Thian Ng, and Hans Lüders, Depts of Neurology, Neurosurgery and Radiology, Cleveland Clinic, Cleveland, OH 44195

Kainic acid (KA)-induced seizures lead to a cascade of molecular and metabolic changes associated with neuronal loss in the hippocampus. Pretreatment with cycloheximide, a protein synthesis inhibitor, protects against KA-induced cell damage. This model represents a unique way to dissociate status epilepticus from seizure-induced neuronal damage. We used ^{1}H-MR Spectroscopy to identify specific metabolic markers of seizure activity without cell damage as compared to markers of seizure-induced neuronal loss. Rats were pretreated with normal saline or cycloheximide (s.c. injection, 2mg/Kg) one hour before KA injection (10 mg/Kg). Rat brains (n=10) were scanned at the hippocampal level before and 24 hours after the seizures. Spectra were recorded and the relative ratios of N-acetylaspartate (NAA), choline, and lactate (Lac) over creatine (Cr) were calculated and compared between the 2 groups. Cresyl violet staining of 30 um sections showed a lack of significant neuronal loss in CA3 and CA1 areas of the hippocampus in cycloheximide pretreated rats. A significant increase in lactate ratios was observed in KA-treated rats 24 hrs after the seizure onset but no significant lactate increase was found in cyclo-pretreated rats. NAA ratios were significantly lower in KA-treated rats as compared to control rats. Cyclo-pretreatment prevented any change in NAA ratios 24 hrs after the seizures. No change in choline ratios was found in any of the 2 treatment groups as compared to control values.

Our results show that a prolonged increase in lactate after status epilepticus is associated with neuronal damage and confirm the hypothesis that NAA is a neuronal density marker.

597.9

EPILEPTIFORM DISCHARGES AND THEIR RELATION TO FOCAL CEREBRAL HEMODYNAMIC ABNORMALITIES: AN EEG TRIGGERED FUNCTIONAL MRI STUDY. G. Schlaug*, J.R. Ives, M. Patel, V. Thangaraj, R.R. Edelman, D.L. Schomer and S. Warach. Depts. of Neurology and Radiology, Beth Israel Hospital, Boston, MA 02215.

Localization of the epileptogenic zone in patients with medically intractable epilepsy depends on the detection of structurally and functionally abnormal brain areas. However, their relationships to the patient's regional epileptiform discharges are difficult to assess using current imaging techniques such as PET and SPECT and often require invasive procedures. We have implemented a system that allows us to monitor the patient's electroencephalogram (EEG) in the bore of a 1.5 Tesla magnet using cable telemetry techniques and fiber-optical isolation. Echo planar fMRI scans using blood oxygen level dependent (BOLD) contrast as well as MR-perfusion techniques (EPISTAR) can be manually triggered using various time delays after epileptiform discharges are detected on EEG recordings. Images during the patient's subclinical electrographic epileptic events are compared to baseline scans without discharges. Functional MR images are coregistered with high resolution anatomical images allowing direct structural-functional comparisons.

Thresholded and spatially filtered percentage change maps and cross-correlational analysis of MR signal changes comparing fMR images with and without epileptiform discharges revealed focal MR signal increases in patients with subclinical epileptiform discharges of presumably temporal and extra-temporal origin.

Time-locking epileptiform discharges obtained from EEG recordings while the patient is in the MRI scanner offers a new approach to the study of hemodynamic/metabolic changes during focal and even generalized epileptic discharges, and may be sensitive for non-invasive localization of epileptic foci in human epilepsy.

597.10

Temporal Alteration of Sodium Content and Apparent Diffusion Coefficient in Epileptic Rat Brain Induced by Kainic Acid

Y. Wang, A. Majors, T. Ng, J. Najm, M. Xue, H. Lüders, M. Modic, Y. Comair*, The Cleveland Clinic Foundation, Cleveland, OH 44195

In situ 3-dimensional sodium magnetic resonance imaging (MRI) technique was used to study temporal changes in cellular sodium in various regions (hippocampal formation, pyriform cortex and amygdala) of seizing rat brains induced by kainic acid (KA) in the ictal phase and many time points postictally. We concomitantly measured the ADC changes. Adult Sprague Dawley rats (n=12), weighing 230-280g, were used. MRI of their brains were scanned 5 hrs, 24 hrs and 7 days post KA. The apparent sodium concentration calculated from the known capillary reference was about 50 mM in control rat brain. During the early postictal phase, no significant changes of sodium concentration in hippocampus, pyriform cortex and amygdala were observed while ADC were decreased in pyriform cortex and amygdala. At 24 hours postictally, sodium increased to 62.1±12.0 mM in hippocampus, and 89.5±11.3 mM in pyriform cortex and 88.3±12.5 in amygdala, while ADC were decreased in pyriform cortex, amygdala, and hippocampus. High sodium level persisted till 7 days later as ADC were back to normal. Our *in situ* sodium measurements showed a pronounced elevation of sodium signal intensity in areas (90%, $p<0.01$ in pynform cortex) susceptible to neuronal damage, e.g. the hippocampus and pyriform cortex, which signifies the ionic steady states being perturbed by KA induced seizure. The ADC decreased at 24 hr postictally changes could be due to increase of intracellular space due to edema and increased permeability of cell to the water. The ADC increased in these area 7 days later implies that the dead neurons were replaced by water and glial cells.

597.11

EQUIVALENT PROTECTION AGAINST NMDA SEIZURES BY MK801, PHENYTOIN AND A DC ELECTROMAGNETIC FIELD. J.Eric Piña-Garza, Robert R. Holcomb, Michael J. McLean* . Department of Neurology, Vanderbilt University, Nashville TN 37212.

We evaluated a DC electromagnetic (DCEM) device against seizures induced by intracerebroventricular (i.c.v.) injection of N-methyl-D-aspartate (NMDA) in mice, a model used to evaluate novel antiepileptic drugs (AEDs). The DCEM device consists of four coils of alternating polarities connected to a DC power supply. Soft iron cores in the centers of the coils were located at the corners of a 14 cm. square. This array produced a time invariant electromagnetic field of ±60 mT. Seizures produced by i.c.v injection of 1 nmol of NMDA consisted of wild running, loss of righting reflex, myoclonus, clonus, tonus and rarely death within 2 min. Seizure stages were characterized electrographically. MK-801 (2 mg/Kg intraperitoneally, 30 min. before NMDA) and phenytoin (40 mg/Kg intraperitoneally, 2 h before NMDA) pretreatment blocked all but the wild running phase. For up to 2.5 h before NMDA injection, a single 15 min. treatment with DCEM in positions of steep gradients had similar efficacy. No protection was afforded by (1) an AC electromagnetic device; (2) interposing a flux return ring between the DCEM device and the mice; or, (3) positioning the mice over a single pole of the DCEM (no gradient).

DCEM fields with steep gradients merit further evaluation as a non-pharmacological adjunct for the treatment of seizures.

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597.12

ALTERED GABA-A RECEPTOR SUBTYPE-SPECIFIC AND ALLOSTERIC BINDING PROPERTIES IN NEOCORTEX RESECTED FROM HUMAN FOCAL EPILEPSY TISSUE ASSOCIATED WITH SEVERE HIPPOCAMPAL PATHOLOGY AND SPROUTING. R.W. Olsen, C.R. Houser[1,2], R. Makela, and A.V. Delgado-Escueta*[2]. Depts. of Pharmacology, Neurobiology[1], UCLA, and Neurology[2], West LA VAMC, and Cal. Comprehensive Epilepsy Program, Los Angeles, CA 90024.

$GABA_A$ receptor (GABAR) binding was altered in temporal neocortex of surgically resected tissue from human focal epilepsy patients with severe hippocampal pathology. Frozen unfixed temporal cortex sections were assayed for a battery of GABAR ligands and allosteric modulators using semi-quantitative autoradiography. The density and distribution of three sites on the GABAR complex (GABA, benzodiazepine [BZ], and picrotoxin/TBPS) were measured, as well as allosteric interactions between sites. Two parameters differed from normal autopsy tissue: modulation of TBPS binding by the anesthetic neuroactive steroid alphaxalone (increased), and the levels of diazepam-insensitive binding of the BZ ligand Ro15-4513, indicative of the α4 subunit (also increased). Alterations were significant only in patients with severe hippocampal sclerosis, neuronal cell loss, and sprouting, compared to epilepsy patients with less severe hippocampal involvement. Further, changes were more marked in the anterior or posterior regions presenting 'more spiking' activity in intraoperative corticography recordings relative to nearby tissue. These GABAR changes in neocortex of patients with severe hippocampal involvement are likely to be representative of several altered neurochemical anatomy markers, suggestive of incorrect cell positioning, synaptic organization, and/or circuitry in cortex, presumably related to comparable pathophysiology in the hippocampus. Supported by NS21908 and NS22071.

598.1

NERVE GROWTH FACTOR NEUROPROTECTION IS DEPENDENT UPON INDUCTION OF CATALASE.

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Understanding the potential neuroprotective role of neuronal growth factors requires development of relevant cellular and molecular models. When tissue culture medium is exposed to hemodialyzers it causes beading and degeneration of dorsal root ganglion (DRG) axons *in vitro*. Toxic activity was dependent upon ethylene oxide (EO) sterilization of the dialyzer. In the present study, we found that increasing the concentration of NGF prevented this toxicity. Cells were exposed to dialyzer treated medium with either 3.5, 5, or 10 ng/ml NGF. Beading of neurites was seen at 3.5 and 5 ng/ml, but was completely inhibited at 10 ng/ml NGF. DRG neurons were exposed to dialyzer treated medium with various combinations of catalase (100 u/ml), glutathione peroxidase (GSH-PX 1u/ml) or superoxide dismutase (SOD 35 u/ml) together with the minimum level of NGF needed to give sustainable neurite outgrowth (3.5 ng/ml). The toxic beading was completely inhibited by catalase and GSH-PX but not by SOD. The protective effect of both NGF and catalase were abolished by combination with the specific catalase inhibitor 3-amino 1,2,4-triazole. Excess peroxides could be eluted from dialyzers and NGF was shown to increase catalase activity by 55-80% in DRG neurons.

Conclusion. EO used for sterilization of hemodialyzers reacts with a plasticizer in the dialyzer or a component of serum in the medium to form peroxides that causes oxidative stress in DRG neurons. NGF protects the DRG specifically by upregulating catalase or GSH-PX activity. This provides a new cellular model of NGF neuroprotection, the components of which are testable in animals and humans (supported by NS14304).

598.2

IMMUNOSUPPRESSANT, FK506, PROTECTS AGAINST METHAMPHETAMINE NEUROTOXICITY

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Besides their roles in the immune system, the immunophilins cyclophilin and FK506 binding protein (FKBP) are highly concentrated in the brain in discrete neuronal structures where they are colocalized with the Ca^{2+}-activated phosphatase, calcineurin. We recently showed that cyclosporin A and FK506 block the neurotoxicity elicited by glutamate acting at N-methyl-D-aspartate (NMDA) receptors in cerebral cortical cultures. The mechanism for the neuroprotective effects of these drugs appears to be inhibition of calcineurin. Methamphetamine is neurotoxic to dopamine containing neurons as indicated by permanent depletions in striatal dopamine (DA) after methamphetamine administration. Depletions in striatal DA following methamphetamine is blocked by antagonists of the NMDA receptor, thus suggesting that NMDA receptor activation is some how involved in methamphetamine neurotoxicity. Based on these findings we examined the effects of FK506 and cyclosporin A on methamphetamine neurotoxicity. Methamphetamine (10 mg/kg sc) was given every 2 hrs X 4 doses to 8 week and 24 week old C57B6J mice who were pretreated with varying doses of either FK506, cyclosporin A or vehicle. Mice were killed 4 days later and DA and its metabolites were measured by HPLC. FK506 and cyclosporin A prevented the depletions in DA due to methamphetamine toxicity. These data suggest that immunophilins and calcineurin play a role in methamphetamine neurotoxicity.

598.3

NEUROTOXIC AND NEUROPROTECTIVE EFFECTS OF POLY-CHLORINATED BIPHENYLS AND ADENOSINE LIGANDS IN RAT BRAIN

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Loss of dopaminergic cells in the substantia nigra decreases dopamine (DA) concentration in the striatum and produces the main symptoms of Parkinson's disease. Although the etiology of Parkinson's disease is unknown, environmental factors are proposed to play a role in development of the disease. Polychlorinated biphenyls (PCBs) are widespread in the environment and have been shown to cause central and peripheral toxic effects in humans and animals. We report that several PCBs *inhibit* tyrosine hydroxylase (TH) activity and DA synthesis in rat striatum *in vitro* (IC_{50} ~ 150-200 μM). In contrast to the inhibition of TH activity by PCBs, we have observed that TH activity and DA synthesis in striatal tissue is stimulated by activation of adenosine A_2 receptors. We report that 2-phenylamino-adenosine (2-PAD) stimulates TH activity and DA synthesis in rat striatum *in vitro* (EC_{50} = 10 μM) through an A_2 receptor mechanism. 2-PAD also stimulates DA synthesis in rat striatum *in vivo* (ED_{50} ~ 0.6 μmoles/kg) after intracerebroventricular injection. These results suggest that A_2 ligands may attenuate PCB induced inhibition of TH activity and DA synthesis. We propose that PCBs, present as contaminants in the environment, may be dopaminergic neurotoxicants and may play a role in the etiology of Parkinson's disease. Furthermore, we propose that adenosine receptor mechanisms may play a neuroprotective role to modulate PCB-induced dopaminergic neurotoxicity. [Support: US-EPA and UNC-CH #544786]

598.4

BCL-2 INHIBITS APOPTOSIS INDUCED BY THAPSIGARGIN OR CAFFEINE IN GT1-7 NEURONAL CELLS.

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Cell death induced by thapsigargin (TG) or caffeine was investigated in GT1-7 cells, an immortalized mouse hypothalamic neurosecretory line. Cells overexpressing the protooncogene *bcl-2* were compared with control cells; trypan blue exclusion was used to determine cell viability. Apoptosis was determined by nuclear condensation and margination seen after acridine orange staining, and by regular DNA fragmentation ("laddering") seen with agarose gel electrophoresis. After 24 hr treatment, TG (50 nM) caused 45% death in control cells, but only 2% death in bcl-2 cells ($P<0.01$), while caffeine (10 mM) caused 41% death in control cells, but only 2% in bcl-2 cells ($P<0.01$). After 48 hours treatment, TG caused 95% death in control cells, but only 16% death in bcl-2 cells ($P<0.01$), while caffeine caused 76% death in control cells, but only 4% in bcl-2 cells ($P<0.01$). After 24 hr or 48 hr treatment with 50 nM TG or 10 mM caffeine, control cells showed a significantly higher percentage of nuclear condensation and margination compared to bcl-2 cells with same treatment. DNA laddering was also detected in drug-treated control cells. TG and caffeine cause release of intracellular Ca^{2+} stores, so we tested whether drugs that inhibit such release would protect against TG- or caffeine-induced death. Dantrolene (120 μM) and TMB-8 (100 μM) gave significant protection against both drugs at 24 hr. These results indicate that TG or caffeine cause death in GT1-7 cells at least in part through an apoptotic mechanism, and that this death is likely precipitated by release of intracellular Ca^{2+} stores. Overexpression of *bcl-2* protects against cell death initiated by intracellular calcium release in these neuronal cells. Supported by the Souer Stroke Fund and NIH AG12282.

598.5

WALLERIAN DEGENERATION AND ACRYLAMIDE NEUROPATHY ARE INHIBITED BY CALCIUM ANTAGONISTS IN CULTURED MOUSE SENSORY AXONS.

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The axonal neuropathies produce an axonal degeneration which is similar in appearance to Wallerian degeneration. The distal axonal degeneration produced by axonopathies is often called "Wallerian-like degeneration" because of the morphologic similarities, but the degree to which these two forms of axonal degeneration share a similar underlying mechanism remains uncertain. Using a cultured murine dorsal root ganglion model, we have previously demonstrated that axonal degeneration after axotomy can be inhibited by calcium channel and calpain inhibitors, and is delayed in cultures prepared from C57BL/Ola mice. We now extend these results to the toxic axonopathy produced in established axons by acrylamide.

We have developed a tissue culture model of axonal neuropathy using low dose acrylamide to produce distal axonal degeneration in cultured murine dorsal root ganglia. The axonal degeneration produced in this tissue culture model is comparable to *in vivo* axonopathy in several key respects. These cultured axons show initial changes in the distal portions of the axons, with subsequent involvement of the proximal axon and progression to axonal loss. The morphologic appearance of the acrylamide-injured axons is similar to the morphology of axotomy-induced axonal degeneration in these cultures. The neurons are able to regenerate axons once acrylamide is withdrawn.

The progression of the acrylamide-induced changes is delayed in cultures exposed to calcium antagonists at doses which inhibit axotomy-induced axonal degeneration. Cultures can also be prepared with ganglia from C57BL/Ola mice, which show delayed Wallerian degeneration after axon transection *in vivo*. Cultures prepared using ganglia taken from C57BL/Ola mice are less susceptible to acrylamide-induced axonal toxicity than cultures prepared from normal (C57BL/6) mice. These observations are consistent with a common mechanism for axonal degeneration induced by either axotomy or acrylamide toxicity.

598.6

IN VITRO AND *IN VIVO* NEUROPROTECTION WITH MELATONIN AGAINST THE TOXICITY OF SINGLET OXYGEN.

H. Manev*, C.M. Cagnoli, A. Kharlamov, C. Atabay and E. Kharlamov, ASRI, Medical College of Pennsylvania and Hahnemann University, Allegheny Campus, Pittsburgh, PA, 15212.

Singlet oxygen [$O_2(^1\Delta g)$] is a very reactive molecule that can be produced by living cells and may contribute to cytotoxicity. The pineal hormone melatonin has been reported to possess potent antiexcitotoxic activity (Exp. Neurol. 1995, 131:39), and to be capable of scavenging free radicals and $O_2(^1\Delta g)$. We investigated whether melatonin might reduce the neurotoxic action of $O_2(^1\Delta g)$ that was generated by exposing a photosensitive dye, rose bengal, to light. In a cell-free system, melatonin protected the enzyme creatine kinase (EC 2.7.3.2) from the rose bengal-induced injury. The cytotoxic effect of singlet oxygen was studied *in vitro* in primary cultures of rat cerebellar granule neurons pretreated with a photosensitive dye, rose bengal, and exposed to light. We found that this procedure triggers neuronal death which is preceded by mitochondrial impairment (assayed by the rate of the reduction of MTT into formazan), and by DNA fragmentation – a marker of apoptosis (determined *in situ* by terminal deoxynucleotidyl transferase assay). Cell death was assayed with trypan blue. In this model of apoptotic neuronal death protection was obtained with melatonin. *In vivo*, unilateral cortical thrombosis was induced by illuminating the skull of rose bengal-treated rats for 10 min with a focused beam of light (A. Kharlamov *et al.*, this meeting). The core of the thrombotic lesion was associated with a rapid cell degeneration. The extent of the lesion was assayed 24 h after injury. Brain slices were incubated with 2% triphenyltetrazolium chloride for 15 min at 37°C, and the size of the infarcted area was measured. Melatonin (3 × 2.5 mg/kg; intraperitoneally) significantly reduced the lesion size. The results suggest that melatonin counteracts the cytotoxic action of singlet oxygen *in vitro* and *in vivo*. Further studies should define the role singlet oxygen and melatonin play in neurodegenerative pathologies.

598.7

DIRECT CYTOTOXICITY OF HIV COAT PROTEIN GP120 ON HUMAN NT NEURONS. P. Wu, B. Du, W. Hatch* and E.F. Terwilliger. Divisions of Infectious Disease and Hematology/ Oncology, New England Deaconess Hospital, Harvard Medical School, Boston, MA 02215

HIV-1-induced neurotoxicity is currently believed to be mediated through an indirect mechanism involving activation of macrophages/ microglia by its coat protein gp120. Neurotoxins released from these cells then trigger neuronal death through a final common pathway containing the NMDA receptor and its Ca^{++} channel. We report here, for the first time, a moderate yet direct toxic effect of gp120 on human NT neurons. NT neurons were obtained, as a pure population (>95%) of cells with neuronal phenotype, from retinoic acid-induced terminal differentiation of NT2/D1 precursor cells, a subline of human teratocarcinoma cells. These cells were treated with recombinant gp120 in the presence or absence of Ca^{++} or MK801. The viability of the neurons was monitored by both morphological appearance and the quantitative MTT assay. Treatment of mature NT neurons at 6 weeks of age with 1 nM gp120 for 24 h caused a 25% loss in cell number. However, no toxic effect was detectable on cultures at only 4 weeks of age, at which time neuronal maturation has not been completed. Furthermore, the gp120-mediated neurotoxicity was abolished either by a NMDA receptor open-channel blocker, MK801, or by culturing in a Ca^{++}-free environment. Together these data indicate that the HIV-1 gp120 protein can cause a direct neurotoxicity on mature human NT neurons by acting through the NMDA-receptor pathway. NT neurons may be a useful model for future studies to understand mechanisms underlying the gp120-mediated neurotoxicity.

598.8

LEAD (Pb^{2+}) FACILITATES THE ONSET OF APOPTOSIS IN NEURONS. A. Oberto, N. Marks*, H. Evans† and A. Guidotti
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Pb^{2+} exposure has devastating neurotoxic effects on the developing nervous system. In the present study, we examined in primary cultures of cerebellar granule cells prepared from 6 day old rats whether Pb^{2+} neurotoxicity is mediated by necrosis or apoptosis (programmed cell death).

Pb^{2+} (10 to 100 μM) failed to potentiate neuronal necrosis induced by 10 to 100 μM glutamate in presence of 1 μM glycine, 25 mM K^+ and nominal absence of Mg^{2+} in 8 DIV cerebellar cultures. Moreover, 100 μM Pb^{2+} added to 8 DIV cultures induced death in 20% of neurons even when Pb^{2+} was applied to the cultures together with 5 μM MK 801 (to block NMDA receptors) or 10 μM NBQX (to block AMPA/kainate receptors).

Since this effect of Pb^{2+} was abolished by treating the cultures with actinomycin D, we studied whether Pb^{2+} potentiates neuronal apoptosis in 8 DIV cerebellar neurons deprived of serum in presence of 5 mM K^+, 5 μM MK 801 and 10 μM NBQX. In this model of apoptosis Pb^{2+} treatment facilitated apoptosis in a dose dependent manner. A two fold increase of apoptotic neuronal death was induced by 100 μM Pb^{2+} whereas, a 50% increase in neuronal death was observed with 25 μM Pb^{2+}. Apoptotic cell death was revealed morphologically by cell shrinking and chromatin condensation, and biochemically by the typical DNA fragmentation pattern. Lead potentiated apoptosis was completely blocked by 1 μg/ml of cycloheximide and 1 μg/ml of actinomycin D.
Supported by Grant NS28130-04.

598.9

NO· INDUCES CYTOSKELETAL CHANGES DURING APOPTOSIS IN CEREBELLAR GRANULE CELLS. E. Bonfoco*, B. Zhivotovsky, S.A. Lipton† and P. Nicotera§. Inst. of Environmental Med., Box 210, Karolinska Institutet, 17177 Stockholm, Sweden,†Laboratory of Cellular and Molec. Neurosci., Children's Hosp., Harvard Med. School, Boston 02115 USA and § Faculty of Biology, Univ. of Konstanz, Box 5560, Konstanz, Germany.

We have recently reported that NO· and $O_2^{\cdot-}$, which react to form peroxynitrite, can induce either necrosis or apoptosis in cortical cultures depending on the intensity of the insult. Here, we further investigate the mechanisms by which treatement with NO· donors elicits apoptosis in a virtually pure neuronal population, cerebellar granule cells. We found that the NO·-donor, SNOC (S-nitrosocysteine), causes early cytoskeletal alterations which precede formation of high molecular weight DNA fragments and apoptotic body formation. Actin fragmentation occurred 1h after exposure to 300 µM SNOC. At 6 h, α and β-tubulines were also fragmented. Concomitantly, formation of 200 kbp-sized DNA fragments and initial chromatin condensation became appreciable. Later, at 12 h, formation of 50 Kbp DNA fragments and DNA laddering appeared on agarose gels. Pretreatment with agents that stabilize the cytoskeleton partially prevented the formation of high molecular weight DNA fragments and apoptotic bodies. This suggest that the early cytoskeletal changes induced by NO· treatement, are a critical step in the progression of neuronal apoptosis.

598.10

MARINE BIOMOLECULES INHIBIT RAT BRAIN NITRIC OXIDE SYNTHASE. V. R. Janapala, D. Desaiah*, P. J. S. Vig, Y. Venkateswarlu and A. V. Rama Rao. Dept. Neurology, Univ. Miss. Med. Ctr., Jackson MS 39216; Org. Chem., Indian Inst. Chem. Technol., Hyderabad, India.

A large number of substances of medical importance have been isolated from marine flora and fauna and their chemical structures were elucidated. Among the many compounds isolated in our laboratories only two compounds were identified as neurotoxicants. The pure (100%) compounds Xestospongin D and Araguspongin C isolated from sponge *Haliclona exigue* were tested for their effects on rat brain nitric oxide synthase (NOS) activity *in vitro*. The results showed that NOS was inhibited in a dose and time dependent manner with an estimated I_{50} of 31.5 µM, Xestospongin D and 46.5 µM, Araguspongin C and the maximum inhibition occured within 3 minutes. To explore the mechanism of action of these compounds on NOS, we have conducted kinetic studies with L-arginine, NADPH and Ca^{2+} in the presence of I_{50} concentration of the two compounds. The V_{max} and K_m values were calculated using the Michaele's Menton equation. The results show that both the compounds are competitive inhibitors of NOS with the substrate, L-arginine and uncompetitive with NADPH and free Ca^{2+}. The NOS inhibition by these two compounds was similar to L-NAME a known inhibitor of NOS. These results suggest that the marine biomolecules are modulators of neuronal NOS.

598.11

INHIBITION OF NITRIC OXIDE SYNTHASE IN RAT BRAIN BY AROCLOR 1242. K. L. Chandna, P. J. S. Vig*, M. Pande, V. R. Janapala and D. Desaiah. Dept. Neurol., Univ. Miss. Med. Ctr., Jackson, MS 39216.

Polychlorinated biphenyls (PCBs), called Aroclors, are industrial chemicals, and cause persistent environmental contamination. PCBs exist as 209 different congeners depending on the chlorine substitution patterns on the biphenyl rings. Exposure to high concentrations of PCBs results in adverse health effects involving many organ toxicity. Nitric Oxide (NO) is a known messenger molecule and is synthesized from L-arginine by Nitric Oxide Synthase (NOS). The aim of this study was to investigat the effect of Aroclor 1242 on brain NOS. The crude NOS was prepared from normal rat brain and its activity was determined by enzymatic conversion of [^{3}H] arginine to [^{3}H] citrulline. NOS activity was quantified by measuring the radioactivity of the flow through fraction containing [^{3}H] citrulline. Nitric Oxide Synthase activity was significantly inhibited *in vitro* by Aroclor 1242 in a concentration (100-250µg/ml) dependent manner. A 50% inhibition of the enzyme was obtained at 150µg/ml concentration of Aroclor 1242. These results indicate that Aroclor 1242 is a potent inhibitor of neuronal NOS which may be related to the neurotoxic action of PCBs.

598.12

INHIBITION OF NEURONAL NITRIC OXIDE SYNTHASE BY 3,3'-IMINODIPROPIONITRILE *IN VIVO*. P.J.S. Vig, V. V. Vedanarayanan, D. Desaiah, V.R. Janapala, M. Pande and S. H. Subramony* Depts of Neurology and Pediatrics, Univ. of Miss. Med. Ctr. Jackson, MS. 39216.

3,3,'-Iminodipropionitrile(IDPN) is a well known neurotoxin and affects peripheral and central nervous system. Nitric oxide has been described as a novel neurotransmitter and a retrograde modulator of synaptic transmission. Therefore, the present study was initiated to determine the *in vivo* effects of IDPN on neuronal nitric oxide synthase (nNOS) in rats treated with a single IP injection of 0.5, 1.0, 1.5 and 2.0g IDPN/kg body weight. The animals were sacrificed after 72 hrs. post-treatment. A dose-dependent and significant inhibition of nNOS was observed in the spinal cord and frontal cortex of IDPN treated rats. Whereas, in temporal cortex and brain stem the decrease of nNOS was not significant as compared to the control group. These data suggest that IDPN neurotoxicity is associated with the decrease in nNOS activity in discrete brain regions.

598.13

INDUCTION OF IMMUNOLOGIC NITRIC OXIDE SYNTHASE IN AIDS DEMENTIA. B. Wildemann[1], M. Sasaski[1], J.D. Glass[1], J.C. McArthur[1], V.I. Christov[1*], T.M. Dawson[1,2], V.L. Dawson[1,3]. Departments of Neurology[1], Neuroscience[2] and Physiology[3], Johns Hopkins University School of Medicine, Baltimore, MD 21287

AIDS is often associated with multiple neurological abnormalities including deficits in cognitive and motor functions. Progressive dementia develops in 15-20% of adults with AIDS, and less severe cognitive impairment is even more frequent. Although evidence of productive HIV replication is nearly universal in the brain at autopsy, the pathogenesis of HIV dementia remains elusive. The principal targets for HIV replication within the CNS are macrophages and microglia, and infection is rarely identified within glial cells or neurons. The clinical severity of dementia correlates strongly with macrophage activation in autopsy tissue (J.D. Glass, unpublished). Thus, neuronal cell injury and death may occur through indirect mechanisms from the release of toxic substances from adjacent HIV-infected cells. HIV-1 and its associated coat proteins can induce immunologic nitric oxide synthase (iNOS) in *in vitro* culture systems and we have shown that activation of iNOS can cause neuronal injury. We therefore speculate that activation of iNOS may be linked to the development or progression of HIV dementia. Using semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) for human iNOS mRNA expression we analyzed cortical brain tissue from HIV positive (AIDS) demented and non-demented patients, and seronegative controls and compared it to the expression for human β-actin. Human iNOS is markedly elevated in AIDS with severe dementia and modestly elevated in AIDS with mild dementia and in AIDS without dementia compared to seronegative controls. These data indicate that induction of iNOS may play a role in severe AIDS dementia.

PRESYNAPTIC MECHANISMS IX

599.1

SYNAPTIC DEPRESSION INDUCED BY POST-SYNAPTIC ELEVATION OF CALCIUM AT DEVELOPING NEUROMUSCULAR JUNCTIONS. S. Casb[1*], M-m. Poo[1], and R.S. Zucker[2]. [1]Dept. Biol. Sci., Columbia Univ., N.Y., N.Y. 10027. [2]Neurobiology Div., Univ. of Calif., Berkeley, CA. 94720.

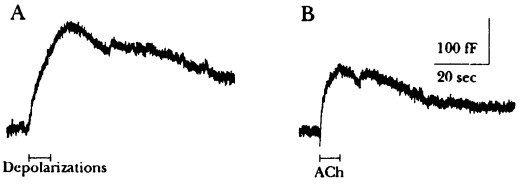
Previous studies have shown that persistent synaptic depression can be induced by brief repetitive activation of postsynaptic acetylcholine receptors in *Xenopus* nerve-muscle co-cultures. Increased postsynaptic Ca^{2+} may be required. Using photolysis of caged Ca^{2+} compounds loaded into the postsynaptic muscle cell we examined whether Ca^{2+} elevation is sufficient to induce synaptic depression. Postsynaptic myocytes were loaded with DM-Nitrophen (NP) or NITR-5 (N5) through a whole-cell patch pipette. Brief illumination of the myocyte resulted in a significant reduction (69 ± 17%, and 55 ± 13%, for NP and N5 respectively, 5 min post-illumination) in the amplitude of evoked postsynaptic currents (EPCs). Similar illumination of unloaded myocytes was without significant effect. We also examined whether postsynaptic elevation of Ca^{2+} may result in depression of synapses made by the presynaptic neuron on another muscle cell. Myocytes loaded with N5 were manipulated close to or in direct contact with a neurite innervating another myocyte. Illumination of the loaded myocyte resulted in a significant reduction of EPC amplitudes in the innervated myocyte. The effect appeared to be larger if the loaded myocyte was in contact with the neurite (59 ± 5% in contact, vs. 36 ± 9% not in contact). These results suggest that postsynaptic elevation of Ca^{2+} may induce depression mediated by a retrograde signal that propagates to adjacent presynaptic sites.

599.2

CAPACITANCE CHANGES CAN BE ELICITED BY ACTIVATION OF VOLTAGE-DEPENDENT CA^{2+} CHANNELS OR NICOTINIC ACh RECEPTORS. A. B. Harkins* and A. P. Fox. Dept. of Pharmacological and Physiological Sciences. Univ. of Chicago. Chicago, IL 60637.

Ca^{2+}-dependent secretion may be regulated by multiple Ca^{2+} influx pathways. Differentiated PC-12 cells were voltage-clamped at -80 mV in the whole-cell recording configuration. Membrane capacitance and conductance changes were measured simultaneously with solutions that optimized both Ca^{2+} currents and secretion. Activation of voltage-dependent Ca^{2+} channels with a depolarizing train of twenty voltage steps to 20 mV (each step, 200 ms duration and 200 ms interpulse) evoked an increase in membrane capacitance that corresponds to the secretion of ~220 vesicles (Fig. A). Similarly, while the cell was voltage-clamped at -80 mV, activation of nicotinic ACh receptors with a solution that contained 100 μM ACh and 1 μM atropine evoked an increase in membrane capacitance[1] that corresponds to the secretion of ~140 vesicles (Fig. B). These results suggest that Ca^{2+} influx through multiple pathways may each be important for secretion.

[1]Mollard, P., *et al.* (1995) Biophysical J. Abstracts. 68:A395.



599.3

PRE- VS. POSTSYNAPTIC MODULATION OF CORTICAL SYNAPTIC ACTIVITY BY PHOSPHATASE 2B VS. PHOSPHATASES 1 AND 2A. G.D. Thomas*, R.G. Victor, E. Marban, and B. O'Rourke. UT Southwestern Medical Center, Dallas, TX 75235 and Johns Hopkins University, Baltimore, MD 21205.

Reversible protein phosphorylation is thought to play an important regulatory role in synaptic neurotransmission. We recently have shown in cultured rat cortical neurons that inhibition of phosphatase (PP) 2B increases the frequency, but not the amplitude, of postsynaptic glutamatergic currents, implicating a presynaptic site of action for PP2B. To determine the role of other phosphatases in synaptic neurotransmission, we used whole-cell voltage clamp to record spontaneous glutamatergic currents in the presence of calyculin A (1 μM in bath solution), a membrane permeant inhibitor of PP1 and 2A which has no effect on PP2B. In 9 neurons, calyculin increased postsynaptic current amplitude (-155±16 pA before vs. -211±32 pA after, $p<0.05$) without changing current frequency. In these same neurons, subsequent inhibition of PP2B with cyclosporine A or FK506 had no further effect on current amplitude, but increased current frequency (0.80±0.06 Hz before vs. 1.03±0.07 Hz after, $p<0.05$). The increased current amplitude seen with calyculin involved a postsynaptic mechanism, since the effect was reproduced by microcystin (10 μM in pipette solution, n=5), which is a membrane impermeant inhibitor of PP1 and 2A. Thus, in rat cortical neurons, glutamatergic neurotransmission appears to be frequency-modulated through a presynaptic mechanism by PP2B, and amplitude-modulated through a postsynaptic mechanism by PP1 and 2A.

599.4

A PRESYNAPTIC MECHANISM FOR SHORT-TERM DEPRESSION B.A. Armitage* and S. A. Siegelbaum. Columbia University, New York, New York, 10032.

Homosynaptic depression (HSD) is a steady decrease in the amplitude of the excitatory postsynaptic potential (EPSP) in response to successive stimuli. Previous work showed that HSD is due to a decrease in transmitter release (Castellucci & Kandel, 1974). We have used the glutaminergic synapse between an *Aplysia* pleural sensory neuron and the L7 motor neuron to study the mechanisms of induction and expression of HSD.

We first asked whether this induction occurs as a result of activity in the presynaptic cell, in the postsynaptic cell, or of simultaneous activity in both cells. Pressure applications of glutamate to the synapse (100 msec, 5X, ISI=1 min.) activated the postsynaptic cell but did not cause HSD (n=5). Stimulation of the sensory cell in the presence of DNQX (8X, ISI=1 min.), which blocked the EPSP, produced HSD (n=5). These two results show that presynaptic activity alone is necessary and sufficient to induce depression.

We next asked whether induction of HSD is due to calcium influx during the action potential or to vesicle fusion/transmitter release. In order to differentiate these two events, we used high osmotic solution to trigger release in the absence of calcium influx. Although this massive release transiently depleted vesicles, the synapse recovered from depletion within 5 minutes at which point the evoked release had not changed, indicating that vesicle fusion/transmitter release did not induce HSD. It is therefore likely that calcium influx during an action potential is necessary for induction of HSD.

We also investigated the mechanism of expression of HSD. We tested the hypothesis that the decrease in release was due to a decrease in calcium influx using fluorescence imaging and video microscopy. The calcium transient during a single action potential at presynaptic release sites was quantified. This transient showed no significant change (<5%) during HSD, (6 stim., ISI=20 sec, n=18). The mechanism of expression of HSD must therefore involve a change in the release apparatus itself. An intriguing possibility is that this change is mediated by a calcium dependent enzyme, such as calcineurin.

599.5

DEPOLARIZATION-EVOKED CALCIUM-DEPENDENT SECRETION OF TRANSMITTER FROM FIBROBLASTS.

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Within minutes following loading of acetylcholine (ACh) into Swiss 3T3 fibroblasts with a whole-cell recording pipette, a brief (5 msec) step-depolarization of the fibroblast frequently evoked a pulse of ACh secretion, as revealed by the appearance of endplate current (EPC)-like events in a *Xenopus* myocyte manipulated into contact with the fibroblast. The failure rate of evoked ACh secretion decreased and the mean amplitude of EPC-like events increased with time, with a characteristic time of about 10 min. The evoked secretion appears to result from Ca^{2+}-dependent exocytosis of vesicles that had accumulated cytosolic ACh, since (1) it was blocked by extracellular application of either Co^{2+} (10 mM) or Cd^{2+} (1 mM), or by cytosolic loading of BAPTA (5 mM), a Ca^{2+} buffer with fast binding kinetics, (2) it was significantly reduced by the treatment of FCCP, an ionophore that dissipates the proton gradient across the vesicular membrane, and (3) it exhibited paired pulse facilitation in a Ca^{2+}-dependent manner. Spontaneous quantal ACh secretion also occurred in the absence of depolarization, resulting in miniature endplate current-like events in the myocyte of similar time course as that induced by the evoked release. These results suggest that the basic machinery for packaging and Ca^{2+}-dependent secretion of neurotransmitter exists in the fibroblast, and the exocytotic machinery at the nerve terminal differs from that in non-neuronal cells only in a quantitative manner.

599.6

IMAGING ZINC RELEASE FROM MOSSY FIBER TERMINALS.

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Zinc is released synaptically by the stimulation of some excitatory pathways in the mammalian cortex. In spite of zinc's action upon a number of ion channels *in vitro*, the precise role of the zinc release remains uncertain. In addition, little is known about the conditions for zinc release. We have used a zinc specific probe TFLZn (TeFLabs) to image zinc release from mossy fiber terminals of live rat hippocampal slices.

Slices (350 μm) were loaded with TFLZn (K^+ Salt, 0.25 mM, 1 hr.). The slices were imaged with an intensified CCD camera, the illumination being provided by a pulsed laser (337 nm, 3 ns). Mossy fibers terminals with high levels of TFLZn fluorescence could be clearly discerned in live slices and on mossy fiber boutons on acutely isolated CA3 neurons.

Electrical stimulation of the mossy fiber pathway led to a rapid decline in the mossy fiber fluoresence, that was blocked by TTX and low extracellular Ca^{2+}, consistent with the synaptic release of zinc. Zinc release could only be elicited by stimulating the mossy fiber pathway at a rate greater than 5 Hz. We present evidence that electrical stimulation triggers the replenishment of synaptic vesicles with zinc from an intracellular source.

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599.7

LACK OF SATURATION OF POSTSYNAPTIC RECEPTORS BY SINGLE TRANSMITTER PACKETS AT EXCITATORY HIPPOCAMPAL SYNAPSES.

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It has often been suggested that a single packet of neurotransmitter is sufficient to saturate postsynaptic receptors at excitatory glutamatergic synapses between CNS neurons. To test this saturation hypothesis, we used the styryl dye FM1-43 to visualize individual synapses between cultured hippocampal neurons, and focally stimulated them with a puffer pipette while recording EPSCs under whole-cell voltage clamp (*Nature* in press). The amplitude distribution of single bouton EPSCs was broad and skewed, with a coefficent of variation (C.V.) averaging ~0.45. The wide variability of EPSC size at individual synapses would be inconsistent with the saturation hypothesis, unless one invokes some mechanism of trial-to-trial variation of postsynaptic responsivity due to desensitization or concerted regulation (e.g. by phosphorylation). Such a scenario was not supported by analysis of single bouton EPSC amplitude as a function of latency from the immediately preceding synaptic event. Over a range of 20-140 ms, there was no significant correlation between EPSC amplitude and preceding interval. As a direct measure of postsynaptic variability, we recorded responses to focal iontophoretic application of glutamate at single synapses. The response to direct glutamate application (1 ms pulse) was very fast, with rise and decay characteristics approaching the kinetics of EPSCs themselves. The maximal peak inward current produced by a saturating glutamate application was about twice as big as the median size of spontaneous minis in the same neurons, suggesting that the concentration of endogenous release transmitter generally falls short of that needed to saturate all postsynaptic receptors. Furthermore, with submaximal glutamate application, responses averaging ~30 or ~70% of maximal showed little variability (CV ~ 0.1). This provides strong evidence that the large variation in the amplitude of single bouton EPSCs must originate from presynaptic factors.

599.8

PRESYNAPTIC INHIBITION AT HIPPOCAMPAL SYNAPSES: DEVELOPMENT AND ROLE OF PRESYNAPTIC Ca^{2+} CHANNELS.

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Previous results revealed a developmental change in the presynaptic Ca^{2+} channels coupled to glutamate release in cultured rat hippocampal pyramidal neurons (J. Neurosci. in press). This preparation has now been used to examine whether such a switch in presynaptic Ca^{2+} channels is manifest as a change in the ability of the synapse to undergo presynaptic inhibition. Experiments entailed whole-cell voltage clamp recordings of monosynaptic or autaptic excitatory postsynaptic currents (EPSCs) in cultured pyramidal neurons. Application of a maximal concentration of the A1 adenosine receptor agonist N^6-cyclopentyladenosine (CPA) inhibited EPSCs by 74 ± 2% in immature cells (<15 days in culture). The inhibition induced by CPA was significantly less in mature cells (>20 days in culture; 47 ± 3%). Thus, presynaptic inhibition became weaker during synapse maturation. This change correlates with the developmental reduction in the role of N-type Ca^{2+} channels in transmitter release reported earlier. A second series of experiments examined the role of N-type Ca^{2+} channels more directly. In mature cells, the application of the N-type Ca^{2+} channel blocker ω-conotoxin GVIA (ω-CTx GVIA; 2.5 μM) between two successive applications of CPA caused a reduction in the percentage inhibition induced by CPA. In contrast, application of the P/Q-type Ca^{2+} channel blocker ω-Aga IVA (1 μM) led to an enhancement of the percentage inhibition induced by CPA (confirming results of Wu and Saggau, Neuron 12:1139). A third series of experiments examined the stoichiometry of N-type verses P/Q-type channels for triggering transmitter release under control conditions and during presynaptic inhibition. Under control conditions, ω-CTx GVIA blocked EPSCs by 41 ± 4%. During maximal inhibition by CPA, subsequent application of ω-CTx GVIA blocked only 19 ± 8% of the remaining EPSC. In contrast, the percentage block by ω-Aga-IVA was increased from 47 ± 5% under control conditions to 76 ± 4% in the presence of CPA. Together, the results indicate that CPA inhibited N-type but not P/Q-type Ca^{2+} channels at the presynaptic terminal.

599.9

RETROGRADE SIGNALLING AND POSSIBLE AXONAL CONDUCTION BLOCK IN DEPOLARIZATION-INDUCED SUPPRESSION OF INHIBITION IN RAT HIPPOCAMPAL CELLS.

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Transient depolarization of pyramidal cells, via action potentials or brief voltage steps, suppresses spontaneous $GABA_A$ergic inhibitory postsynaptic currents (sIPSCs) for 1-2 min. Depolarization-induced suppression of inhibition (DSI) is induced by events in the postsynaptic cell (e.g., postsynaptic BAPTA inhibits DSI) but does not involve decreases in $GABA_A$ receptor sensitivity assessed with iontophoretic GABA or mIPSC analysis. DSI could therefore involve depression of GABA via a retrograde mechanism.

We investigated DSI expression following 1-s, 60-mV voltage steps (V_H = -60 mV) with whole-cell voltage clamp of pyramidal cells in the rat hippocampal slice. Normal mIPSC frequency is low, and an effect on mIPSCs could have been missed, so we increased mIPSC frequency by bath applying 15 mM KCl. Although numerous Ca^{2+}-sensitive mIPSCs appeared in high $[K^+]$, DSI was not seen, further arguing against a reduction of postsynaptic $GABA_A$ receptor sensitivity in DSI. On the other hand, 50-100 μM 4-aminopyridine or 250 nM veratridine blocked DSI presynaptically. We observed an increase in failures of evoked monosynaptic IPSCs during DSI, which also supports a presynaptic locus of expression. Interestingly, paired-pulse depression of IPSCs, which is mediated by presynaptic processes, did not change during DSI, indicating that DSI expression may involve presynaptic axonal conduction block rather than alteration of the GABA release mechanism per se. Supported by NS30219 and NS22010 to B.E.A.

599.10

NOREPINEPHRINE HYPERPOLARIZES A SUBSET OF HIPPOCAMPAL INTERNEURONS LOCATED IN STRATUM LACUNOSUM-MOLECULARE.

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Norepinephrine (NE) has potent inhibitory effects in the *in vivo* hippocampus, a structure which contains a high density of noradrenergic terminals. We previously demonstrated that NE caused an increase in tonic inhibition in rat hippocampal slices, visible as an increase in the frequency of spontaneous IPSCs recorded from CA1 pyramidal cells. Whole-cell recordings from visualized interneurons in different strata of area CA1 confirmed that NE has a direct depolarizing action on most interneurons, which is primarily mediated by an α_1 adrenoceptor-dependent reduction in a resting K^+ conductance.

We have now identified a subset of interneurons located in distal stratum radiatum and stratum lacunosum-moleculare (L-M) which are hyperpolarized (~ 8 mV) by NE (10 μM). This response appears to be mediated by an α_2 adrenoceptor, as it was mimicked by α-methylnorepinephrine, and blocked by RX-821002. The hyperpolarization was associated with a large decrease (> 25%) in input resistance, and an outward current (~ 25 pA) at the resting potential. This current reversed at ~ E_K, and was unaffected by changes in $[Cl^-]_i$, suggesting that the NE-induced hyperpolarization is mediated by an increase in K^+ conductance. These interneurons had electrophysiological and morphological properties distinct from those which were depolarized by NE, with axonal ramifications found primarily in stratum L-M. The amplitude of isolated late-IPSPs evoked through distal L-M stimulation was reduced in CA1 pyramidal cells by α adrenoceptor agonists, suggesting that these interneurons may contribute to the activation of $GABA_B$ receptors on pyramidal cells. These results suggest an important role for NE in modulating inhibitory circuits in the hippocampus through its divergent actions on inhibitory interneurons.

599.11

PRESYNAPTIC INHIBITION OF EVOKED NEUROTRANSMISSION BY REDOX CONGENERS OF NITRIC OXIDE. Zhuo-Hua Pan*, Michael M. Segal, and Stuart A. Lipton. Dept. of Neurology, Children's Hospital and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

Nitric oxide (NO·) reacts only very slowly with thiol (-SH) groups under physiological conditions, while other endogenous redox-related species of the NO group, such as nitrosonium ion (NO^+, with one less electron than NO·), react rapidly with thiol and thus can regulate protein function (Lipton et al., *Nature* 1993). Here, we found that nitroso-compounds, such as nitroglycerin (NTG) and S-nitrosocysteine (75-1000 μM each), acting as nitrosonium donors, decreased the efficacy of *evoked* neurotransmission 20-80% in a dose-dependent manner by a presynaptic, thiol-related mechanism (n = 26). Nitrosonium donors inhibited both excitatory and inhibitory synaptic activity in cortical neuronal cultures recorded with patch electrodes in the absence of TTX. In contrast, NO· donors, such as DEA/NO (≤1000 μM), did not inhibit synaptic activity. The NO moiety may exert its effects by stimulating guanylate cyclase; however, the addition of 1 mM 8-bromo-cGMP to the extracellular solution did not decrease synaptic activity in this preparation. Recording evoked autaptic excitatory postsynaptic currents from isolated hippocampal neurons in the presence of TTX (Segal, *J. Neurophysiol.* 1991), we found similar inhibitory effects of nitrosonium donors (n = 6). In contrast, *spontaneous* miniature excitatory postsynaptic currents under these conditions were enhanced 42% by NTG in perforated patch recordings ($P < 0.001$, n = 4). These findings may help explain otherwise puzzling observations that the NO moiety can either increase, decrease, or have no net effect on synaptic activity.

599.12

FUNCTIONAL DIFFERENTIATION OF PRESYNAPTIC COMPARTMENT DURING SYNAPTOGENESIS IN CULTURED HIPPOCAMPAL NEURONS
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Hippocampal neurons maintained in primary culture recycle synaptic vesicles (Matteoli et al., 1992) and express functional glutamate receptors (Verderio et al., 1994) since very early stages of neuronal development. The establishment of neuronal polarity and the formation of synaptic contacts is accompanied by a regional redistribution of presynaptic and postsynaptic components. It has been found recently that mechanisms for calcium-dependent recycling of synaptic vesicles (Kraszewski et al., 1995) and glutamate release (Verderio et al., 1995) are activated already before synaptogenesis. However, formation of synaptic contacts coincides with a change in the subtype of calcium channels primarily involved in mediating neurotrasmitter release, ω-CTx-GVIA sensitive channels being primarily involved in mediating glutamate release at early developmental stages and ω-Aga-IVA sensitive channels playing a prominent role in controlling neurotransmitter secretion at mature synaptic contacts. Moreover, formation of synaptic contacts coincides with the activation of a low-affinity calcium sensor for exocytosis. These results indicate that the secretory and transducing neuronal machinery is already functional before synaptogenesis. On the other hand, specific components of the exocytotic machinery appear to be selectively activated only after the maturation of synaptic contacts.

Kraszewski K. et al. (1995) J. Neurosci., in press.
Matteoli, M. et al. (1992) J. Cell Biol. 117, 849-861.
Verderio, C. et al. (1994) J. Cell Biol. 126, 1527-1536.
Verderio C. et al. (1995) Proc.Natl.Acad.Sci., in press.

GENESIS OF NEURONS AND GLIA V

600.1

IDENTIFICATION OF CIS-ELEMENTS IN THE Tα1 PROMOTER RESPONSIBLE FOR NEURON-SPECIFIC GENE EXPRESSION IN TRANSGENIC MICE D.Rogers*, A.Gloster, N.Laferriere+, D.Brown+, A.Peterson#, F.D.Miller; + Dept. of Biology, University of Ottawa; # Dept. of Molecular Oncology , Royal Victoria Hospital, Montreal; Centre For Neuronal Survival, Montreal Neurological Institute, Montreal, Canada, H3A 2B4

The rat Tα1 gene belongs to the α-tubulin gene family. Expression of the Tα1 gene is restricted to neurons. In the developing embryo, Tα1 is initially expressed in populations of cells undergoing neuronal commitment and differentiation, and is maintained during the period of elaboration of neuronal processes. A 1.1kb stretch of the Tα1 5' upstream promoter sequence is sufficient to confer this pattern of expression to a lacZ reporter gene in transgenic mice (Gloster et al 1994.) In vitro, we have used the P19 embryonal carcinoma (EC) cell line to assay promoter activity. This same 1.1kb promoter construct is turned on in stably transfected P19 EC cells coincident with neuronal differentiation and neurite extension. Deletion of a specific 66bp region from this 1.1kb fragment altered the activity of the promoter both in vivo and in vitro. In differentiated P19 EC cells, the activity of the mutant construct preceded the activity of the wild type construct, and the activity of the mutant construct was not restricted to neurons. In transgenic mice, expression in both the developing embryo and the adult was perturbed in a manner consistent with the P19 EC cell data. Thus, the deleted 66bp sequence contains one or more functional cis elements. which are involved in specification of gene expression in early developing neurons.

600.2

REGULATORY ELEMENTS IN THE MURINE NEUROFILAMENT LIGHT CHAIN GENE THAT ARE ACTIVE DURING DEVELOPMENT IN A BINARY TRANSGENIC MOUSE SYSTEM. P. J. Yaworsky* and C. Kappen, S.C. Johnson Med. Res. Center, Mayo Clinic, 13400 E. Shea Blvd., Scottsdale, AZ 85259.

We report here on the developmental activity of regulatory elements that direct transgene expression specifically to neurons in our binary transgenic mouse system. In this system, the transgene of interest (transresponder) is transcriptionally active only in the presence of the viral transactivator VP16. The transactivator is supplied by a second strain of transgenic mice (transactivator) harboring the VP16 gene under control of specific regulatory elements. Using a LacZ transresponder gene, we here show transactivation of LacZ specifically in developing neurons.

To direct VP16 expression to differentiating neurons, we have initially used 1.7 kb upstream sequence of the murine neurofilament light chain (NF-L) gene promoter. Here we report that this fragment reflects the activity of the endogenous NF-L promoter only at certain stages of development. Thus, additional elements appear to control NF-L gene expression. Based on the evolutionary conservation to the intermediate filament gene nestin and on published reports implicating intragenic sequences in tissue-specific expression, we have isolated intron sequences from the murine NF-L gene. These putative regulatory elements were linked to the VP16 gene and injected into transgenic eggs harboring a LacZ transresponder gene. At different stages of development, embryos were isolated, screened for the presence of the transgene, and stained for ß-galactosidase activity. We will present the results of our characterization of the activity of the NF-gene promoter as well as our progress in identifying additional neuron-specific regulatory elements in the NF-L gene.

Our transactivators with neurons-specific VP16 expression will allow us to target transresponder gene expression specifically to the developing nervous system. These transgenic mice will be useful to study the function of various transgenes in neuronal development.

600.3

EXPRESSION OF mRNA FOR GDNF AND THE NEUROTROPHINS, NGF, BDNF AND NT-3, BY ADULT HUMAN ASTROCYTES IN CULTURE G. Moretto*[1], B. Kirschenbaum[2] and S. A. Goldman[2] Depts. of [1]Neurol. Science, Univ. of Verona, Italy, and [2]Neurology, Cornell Univ. Medical Col., NYC, 10021.

The adult mammalian forebrain harbors neuronal precursor cells throughout an extensive area of the subependymal zone (SZ). However, in vivo neurogenesis is limited to a few sites in the adult brain, such as the olfactory bulb. The limited occurrence of neurogenesis in vivo contrasts to the wide distribution of SZ neuronal precursor cells found in vitro, in human as well as rat brain (Cereb. Cortex 6:576-89, 1994). Neurons arising from these cells express *trkB*, and their survival is specifically promoted by BDNF (PNAS 92:210-4, 1995). This suggests that the permissiveness of adult brain to neurogenesis might depend in part upon the local availability of post-mitotic trophic support in vivo. In fetal rats and humans, the production of BDNF has been noted by astrocytes as well as by neurons (J. Neurop. Exp. Neur. 53:78-85, 1994). We thus asked whether adult human astrocytes could also express BDNF. We further asked if adult glia could express the neurotrophin (NT) family members NGF and NT-3, and 2 other neurotrophic agents, CNTF and GDNF. We used RT-PCR to detect their mRNAs in highly enriched, multiply-passaged and neuron-free cultures of astrocytes, derived from fresh resections of adult human temporal cortex. We found that mRNA for NGF, BDNF and NT-3 were each expressed in these cultures, appearing as PCR products of 189, 296 and 176 base pairs, respectively. Stimulation for 3 d with 1 mM dibutyryl-cAMP, with resultant astrocytic stellation, generally up-regulated the mRNA of each NT. GDNF mRNA was also detected, as possible splice variants of 197 and 272 bp, but at lower levels than the NTs; GDNF was also up-regulated by dbcAMP. The astrocytes never expressed CNTF mRNA, despite high levels of G6PDH mRNA, a positive control, under the conditions. Thus, adult human cortical astrocytes can express mRNAs for members of the NT family, including NGF, BDNF and NT-3, as well as for GDNF.

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600.4

SPATIO-TEMPORALLY DIFFERENTIAL EXPRESSION AND KINATE-INDUCIBILITY OF GENE FOR THREE SPECIES OF FATTY ACID BINDING PROTEINS IN DEVELOPING AND MATURE RAT BRAIN. Y.Owada* and H.Kondo, Dept. of Anatomy, Tohoku Univ. Sch. of Med., Sendai 980, Japan.

By in situ hybridization, the expression signals for heart type (H-) FABP mRNA in the brain were under the detection levels at embryonic stages and they gradually increased after birth and localized in neuronal cells in the gray matters including the cerebral cortex, thalamus and cerebellar granule cell layer at postnatal stages. In contrast, brain type (B-) and cutaneous type (C-) FABP mRNA levels were very high at embryonic stages and decreased dramatically as the postnatal development proceeded. B- and C-FABP mRNA were expressed intensely in the ventricular germinal zones, whereas the cerebellar external granule cells expressed C-FABP mRNA intensely but not B-FABP mRNA. B-FABP mRNAs was expressed in glial cells, such as cells in the olfactory nerve fiber layer and cerebellar medulla and radial glial cells in the Purkinje cell layer at embryonic and early postnatal stages, while C-FABP mRNA was detected in both glial and neuronal cells. In addition, while the expression of B- and C-FABP mRNAs was markedly elevated in the hippocampus, corpus callosum and cerebral cortex 48 hr after KA treatment, no such expressional change was observed for H-FABP mRNA. These differential expression patterns suggest that H-, B- and C-FABP may play different roles in neuronal and glial cells during their proliferation, differentiation and reaction to pathological lesions.

600.5

DEVELOPMENTAL MECHANISMS RESPONSIBLE FOR STRAIN DIFFERENCES IN THE RETINAL GANGLION CELL POPULATION

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We have found substantial strain variation in the retinal ganglion cell population among inbred strains of mice. These differences may be caused by variation in the magnitude of cell production or the severity of cell death. To assess mechanisms producing these differences we estimated the number of ganglion cells in several strains of mice at birth (P0), before the onset of naturally occurring ganglion cell loss in this species (Williams et al., 1990, Exp Brain Res 82:393). Therefore, the population of cells at P0 provides a good estimate of total ganglion cell production. The cell population was estimated from electron microscopic analysis of axons in optic nerve cross-sections. Four strains of mice have been examined: two with high populations at maturity (BALB/c: 64,400 ± 2,200; DBA/2: 62,400 ± 1,100) and two with low populations (LP: 52,200 ± 1,900; C57BL/6: 53,900 ± 1,400) at maturity. At birth, one low strain, C57BL/6, has a population of 175,000 ± 12,000 cells (n=10). A high strain, BALB/c, has 237,000 ± 17,000 cells (n=4). The increased cell production in BALB/c relative to C57BL/6 can account for the adult differences. The decrease in the population from P0 to maturity in both strains amounts to 70%. However, LP neonates have an average of 210,000 ± 22,000 cells (n=4), well above the neonatal average of the other low strain. In the LP strain, 75% of the neonatal population dies. Preliminary analysis of the DBA/2 strain suggests that only 60% of the neonatal population is eliminated. These results indicate that strain differences in the ganglion cell population at maturity are due both to variation in ganglion cell production and death.

600.6

MESENCEPHALIC PROGENITOR CELLS: ENDOGENOUS AMINO ACID PROFILE DURING *IN VITRO* DIFFERENTIATION.

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Multipotential progenitor cells obtained from embryonic or adult CNS can be grown as spheric aggregates (neurospheres) in medium containing EGF. Plating these cells on an adhesive substrate induces phenotypic differentiation, thus allowing us to characterize this process. Endogenous amino acids (AAs) can play a role as neurotransmitters, metabolic precursors or even as differentiating agents. Here we correlate the presence of the different cell types with their content of free AAs. Neurospheres, obtained from dissociated E15 rat embryo ventral mesencephalon, were grown in DMEM:F-12 (1:1) supplemented with glucose, N2 components and 20 ng/ml EGF for a minimum of 5 passages, and then plated on poli-L-ornithine coated dishes or coverslips. Immunocytochemical and biochemical analyses were performed at 2 hours, 1, 3, 6, 10 or 20 days post plating (dpp). AA contents were analyzed by HPLC (OPA-precolumn, fluorimetric detection). During the initial 6 dpp, the cultures contained mostly undifferentiated cells (nestin$^+$), and a minority of oligodendrocytes (O1$^+$); neurons (ß-tubulin III$^+$) peaked at 10 dpp, and astrocytes (GFAP$^+$) were majority at 20 dpp. The levels of all AA underwent a sharp decrease after cell plating. Most of them increased gradually during the following 10 or 20 days. Gln was the majoritary AA at 20 dpp (more than 300 µmol/mg cell protein). Taurine also accumulated in cells, peaking (125 µmol/mg protein) at 10 dpp; taurine is probably synthesized *de novo*, since it was not included in the feeding medium. GABA is present in small quantities in neurospheres and disappears after plating, but its synthesis is active between 10 and 20 dpp. The serotonine precursor Trp is only detectable at 10 dpp. Putative neurotransmitters Glu and Asp reach high levels by 10 dpp. The time course of appearance of some AAs aggree with their role as neurottransmitters or suggest their implication in CNS differentiation processes.

600.7

THE SEQUENCE OF EXPRESSION OF THE α7 NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNIT IN THE EMBRYONIC CHICK BRAIN.

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The neuronal nicotinic acetylcholine receptor (nAChR) is composed of two α (ligand binding) and three β (structural) subunits that assemble to form an ion channel. Nine α and three β subunits have been identified. Therefore, depending on how the different αs and βs are combined, there is potentially a large number of nAChR subtypes that may differ in their ligand affinity and physiological characteristics. Initial attempts to identify cholinoceptive cells in the brain used α-bungarotoxin (αBgt), a protein from snake toxin, which binds with high affinity to muscle nAChRs. It was first believed that the high affinity αBgt binding in the brain represented all functional nAChRs. However, more recently, it has been demonstrated that there are neuronal nAChRs that do not bind αBgt and that αBgt binds selectively to nAChRs consisting of α7 and/or α8 subunits. The α7 subunit is the major αBgt binding protein in the chick brain and has been found to form functional nAChRs. In the present study, the mouse monoclonal antibody 306 (kindly provided by Jon Lindstrom) was used to detect α7 subunits in the developing brain of chick embryos from embryonic day 4 (E4) to hatchling (E21). The purpose of this study was to determine the timing of the first appearance of α7 subunits in various brain areas. A high density of cells containing α7 subunits were detected in the nucleus isthmi, pars magnocellularis as early as E12. Alpha-7 immunoreactive cells in other structures did not appear until later during development: in the nucleus spiriformis lateralis at E13; in the nucleus isthmi, pars parvocellularis at E14; in the central nucleus of the inferior colliculus and stratum griseum centrale at E16. The present study demonstrates that α7 subunits are expressed in various brain regions at different times during embryogenesis. These studies will characterize the normal developmental pattern of nAChRs and aid in determining the effects of early exposure to nicotine and various cholinergic ligands. Supported by NIH EY 06890 (HJK) and NIH AG 00216 (FHGage).

600.8

CHANGES IN THE EXPRESSION OF GAP JUNCTIONS DURING NEURONAL DIFFERENTIATION OF NTERA-2 CLONAL HUMAN EMBRYONAL CARCINOMA CELLS

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Gap junctions are intercellular membrane channels which provide for the transfer of low molecular weight molecules and ions between the cytoplasms of adjacent cells. In the central nervous system, gap junctions have been detected in a variety of cell types including neurons, astrocytes and oligodendrocytes. During development, there is extensive regulation of the spatial and temporal expression of gap junctions which controls differentiation and growth. The function of gap junctions in neurons and other neural cell types can be investigated by using a human teratocarcinoma cell line, NTera-2/clone D1 (NT2), which exhibits properties characteristic of a committed neuronal precursor at an early stage of development. NT2 cells can differentiate into postmitotic CNS neurons (NT2-N) upon addition of retinoic acid (RA). For differentiation studies, cells were treated with $1X10^{-5}$M RA for 4 weeks and then incubated in mitotic inhibitors for 3 weeks. NT2 cells expressed connexin 43 when they were not treated with RA and within their first 3 weeks of RA-treatment. The level of connexin 43 immunoreactivity decreased during RA-treatment. There was no connexin 43 immunoreactivity in NT2-N cells. The microtubule associated proteins (MAP2 and MAP5) immunoreactivity started at the end of first week of RA-treatment and became much stronger in the following stages. The differentiated NT2-N cells showed a strong immunoreactivity for MAP2 and MAP5 but they were negative for glial fibrillary acidic proteins (GFAP). The dye-coupling techniques showed that the gap junctional communication in NT2 cells decreased when the cells were treated with RA for 10 days. (Supported by MRC of Canada, CCGN)

600.9

ONTOGENY OF MITOCHONDRIAL BENZODIAZEPINE RECEPTOR AND ITS PUTATIVE ENDOGENOUS LIGAND DIAZEPAM BINDING INHIBITOR IN RAT BRAIN AND PERIPHERAL TISSUES.

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A recognition site for benzodiazepines (BD) structurally different from that linked to the "central type" BD-receptor has been designated as mitochondrial BD-receptor (MBR), on the basis of its localization. One putative endogenous ligand for MBR is the diazepam binding inhibitor (DBI) peptide. It has been suggested that this peptide is involved in the regulation of steroidogenesis, metabolism of acyl-CoA, modulation of GABA-A receptor and several other biological processes.

The expression of MBR and DBI was studied during rat embryonic development by immunohistochemistry and *in situ* hybridization. Both methods demonstrated that the expression of DBI in brain, dorsal root and trigeminal ganglion was detectable as early as E13-14. In brain DBI expression was most abundant in ependymal cells. The staining intensity of DBI increased during gestation. In the peripheral tissues, such as kidney, pancreas, heart, skin, thymus, intestine, adrenal gland, lung, salivary gland and thyroid gland, the expression of DBI occurred at later embryonic stages, being detectable after E16-19; BDI was present at E14 only in liver. Simultaneous expression of DBI and MBR occurred e.g. in brown adipose tissue and liver. In nervous system DBI is expressed in early embryonic life, suggesting that it may play an important role during ontogenesis.

600.10

EXPRESSION OF TRYPTAMINE IN RAT BRAIN EMBRYOS

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Tryptamine (T) is present in the rat central nervous system in very low concentration. The maximun of its brain concentration is in the first postnatal day. In a previous work we have demonstrated the localization of tryptamine in the adult rat brain (Brusco et al, 1994, NeuroReport 5).

In this work we have studied the immunocytochemical expression of T in brains of rat embryos at different developmental stages. Rat embryos from E12 to E20 obtained by caesarea operation (E1: day 1 of embryo development) and newborns (within the first day of life) were fixed in 5 % glutaraldehyde by intracardiac perfusion or by immersion of the brains in the fixative solution. After 2 hs of fixation the brains were immersed in 5% sacarose and then embeded in 30% sacarose previous freezing at -60 °C. Serial cryostat sections of 15-20 µm thick were mounted in gelatinated slides. The immunocytochemical peroxidase anti-peroxidase reaction was carried out using anti-T as the primary antibody.

The results showed that T is present in the rat brain starting the 14 th day of embryonary development. The localization of T is restricted to neurons and fibers. A network of thin tryptaminergic fibers in the ependymal epithelium of the aqueduct was found in the ventral zone.

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600.11

PRODUCTION AND DIAGNOSTIC VALIDATION OF TIMED PREGNANCIES IN THE BRAZILIAN OPOSSUM. J. Sonea*, S. Di, J. Iqbal, K. Miles, E. Riedesel and C. D. Jacobson. Departments of Veterinary Anatomy and Veterinary Clinical Sciences and the Neuroscience Program, Iowa State University, Ames, Iowa 50011

The Brazilian Opossum, *Monodelphis domestica*, is a small marsupial whose young are born in an extremely immature state with a protracted period of development. Studies utilizing postnatal administration of tritiated thymidine and Bromodeoxyuridine immunohistochemistry have indicated that morphogenesis and neurogenesis of the forebrain continue during the early postnatal life of the opossum. However, these studies also indicate that the hindbrain is significantly more developed at birth than that of the forebrain. In order to determine the patterns of neuronal differentiation, neurogenesis and patterns of connectivity during early development it is necessary to look at hindbrain development during the prenatal period. Thus, we have devised a technique to produce timed pregnancies. This procedure will be described. Further, in order to verify the time of conception, we have devised a scheme to accurately characterize the sexual behaviors of adult male and female opossums. These data will also be presented. Once a timed pregnancy has occurred, utilizing diagnostic ultrasound evaluation on day 12 of gestation, we have successfully demonstrated the presence of multiple amniotic sacs in the gravid uterus. This procedure has allowed the creation of a protocol to administer bromodeoxyuridine to the pregnant female and thereby determine the neurogenesis of hindbrain structures. Preliminary results of this technique will be demonstrated. In summary, we have successfully created a technique to accurately determine the time of conception in *Monodelphis*. This technique will allow studies to determine the complete developmental history of hindbrain structures. In combination, with the ability to study development of forebrain structures postnatally, studies can now be conducted to relate the neurogenesis, morphogenesis and connectivity patterns of structures in the forebrain and hindbrain.

600.12

POSTLARVAL NEUROGENESIS IN THE OLFACTORY MIDBRAIN OF A DECAPOD CRUSTACEAN. M. Schmidt*. Institut für Biologie, TU Berlin, 10587 Berlin, Germany.

After a short larval development decapod crustaceans obtain adult morphology and by successive molts grow in size continuously up to 1000 times throughout their entire, often very long life-span. This poses the question by which adaptive mechanisms the CNS copes with such a dramatic growth of body size, specifically whether neurogenesis may be involved. To address this question, the number of olfactory projection neurons was quantified by two independent measures in the brains of shore crabs, *Carcinus maenas*, ranging from 100 mg to 50 g in weight. Firstly, the number of somata, which form a compact and isolated cluster (lateral cluster), was determined by dividing the total cluster volume obtained from serial sections by the average soma volume ($n \geq 100$) measured with an image analysis system (SIS). Secondly, the number of axons arising from the olfactory projection neurons was counted on TEM-micrographs of cross-sections through the easily identifiable olfactory globular tract which is constituted by these extremely thin axons (diameter ≤ 0.1 µm). Both measures yielded very similar results. From the smallest to the largest animal the number of olfactory projection neurons doubled (from 15.000 to 30.000 in one hemibrain) and the increase in neuron number was continuous over the entire size range. In brains of small crabs, several somata in the lateral cluster showed nuclei in different stages of mitosis (metaphase, anaphase), providing direct evidence for ongoing cell divisions. Together these data demonstrate that among olfactory projection neurons of the shore crab neurogenesis prevails throughout the entire life-span. Future investigations will show whether neurogenesis in the decapod brain is a common feature or a specialization of the central olfactory system.

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CELL DIFFERENTIATION AND MIGRATION VI

601.1

HUMAN LIGANDS OF THE NOTCH RECEPTOR G. E. Gray*#, R. Mann#, D. Henrique+, D. Ish-Horowitz+, and S. Artavanis-Tsakonas# Dept. of Cell Biology and HHMI, Yale University Medical School, New Haven, CT#, Oxford University, Oxford, UK+.

Intercellular signaling through the Notch pathway affects cell-fate decisions in several Drosophila tissues, including the CNS. In vertebrates, a set of studies by a number of different groups has established the importance of Notch signaling in normal development and in cell-fate decisions, in the nervous system and elsewhere. To study the intercellular interactions that regulate vertebrate Notch activity, we have cloned and sequenced two candidate human Notch ligands. These genes code for putative transmembrane proteins bearing extracellular EGF repeats, including the cryptic EGF repeat (the "DSL domain") that has been found in all identified ligands of Notch and its homologues. The number and organization of EGF repeats in these ligands suggests they are homologues of the Drosophila Notch ligand *Serrate*, which functions in wing development. Both ligands have substantial similarity to Jagged , the rat Notch ligand recently identified by Lindsell et al. (1995, Cell, 909-917). We are currently investigating the expression patterns of these ligands in normal developing tissues and in human cancers.

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601.2

A POU TRANSCRIPTION FACTOR, BRN-2, IS REQUIRED FOR THE EARLY DEVELOPMENT OF HYPOTHALAMIC NEUROSECRETORY NEURONS. H. Kawano[1], K. Kawamura[1]*, S. Nakai[2], T. Noda[2] [1]Dept of Anatomy, Sch. of Med., Keio Univ., Tokyo 160 and [2]Dept. of Cell Biology, Cancer Institute, Tokyo 170, Japan

The POU gene family is a large group of transcription factors sharing a conserved bipartite DNA-binding domain. They have been shown to serve as developmental transactivators of genes that define and maintain specific cell phenotypes. Brn-2 is a member of POU transcription factors and is expressed in the nervous system in a region-specific manner. In the present study, we created a null mutation in mouse Brn-2 gene by homologous recombination and analyzed the phenotypes of Brn-2-deficient mice. All homozygous mice died in a few days after birth and histological examination of the central nervous system of neonatal homozygotes revealed severe defects in the hypothalamo-hypophysial neuroendocrine system. By immunohistochemistry, vasopressin neurons in the paraventricular and supraoptic nuclei, as well as TRH and CRF neurons in the paraventricular nucleus, and somatostatin neurons in the periventricular area were not found in the homozygotes. Hypothalamic magnocellular neurosecretory neurons immunoreactive for spot 35, a calcium-binding protein, were detected from embryonic day 11 (E11) in the wild-type. In homozygotes, these neurons were gradually eliminated between E12 and E14 during the migration.

Since the paraventricular and supraoptic nuclei specifically express Brn-2 mRNA signals in normal animals, Brn-2 is likely to transactivate the genes prerequisite to survival of certain hypothalamic neurosecretory neurons in the early developmental stage.

601.3

DEVELOPMENTAL EXPRESSION OF SCIP AND KROX-20 IN RODENT SCIATIC NERVE: IMPLICATIONS FOR THE TRANSCRIPTIONAL CONTROL OF MYELIN GENE EXPRESSION.

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Preliminary data have indicated that non-myelinating Schwann cells are differentiated from myelinating Schwann cells by only a few transcription factors that may differ between the two states; in particular, myelinating Schwann cells seem to express SCIP and Krox-20, while non-myelinating Schwann cells express Pax-3. We report here a study of the developmental expression of SCIP and Krox-20 in Schwann cells of Rat sciatic nerves from the time of birth to adulthood. SCIP and Krox-20 both are detectable by immunohistochemistry at postnatal day 2, and are expressed at a high level in virtually every myelinating Schwann cell nucleus by day 4. Krox-20 expression is then maintained in myelinating Schwann cell nuclei, whereas the number of nuclei expressing SCIP declines exponentially, with less than 1% of myelinating Schwann cell nuclei being SCIP+ by postnatal day 13. SCIP and Krox-20 expression is inversely related to the expression of the p75 low-affinity NGF receptor, a marker of immature and nonmyelinating Schwann cells, and coincident with myelination and the high level of expression of myelin gene products, such as P_0. The results of the present study lead to further questions about the possible interaction between SCIP and Krox-20 in the elaboration of myelin in peripheral nerve, and serve to precisely define the spatial and temporal expression of these transcription factors.

601.4

CHARACTERIZATION OF THE PAX 2 AND EN-1 EXPRESSING CELLS IN THE EMBRYONIC CHICK SPINAL CORD. J. D. Burrill*, S. E. Koester, and M. Goulding, MNL-G, Salk Institute, La Jolla, CA. 92037

To identify the cells that express the transcription factors Pax 2 and En-1 wholemounts or vibratome sections of st 15-21 chick neural tubes were labeled with antibodies against Pax 2 and En-1. Pax 2 and En-1 are both expressed in a band of cells in the intermediate third of the spinal cord. Pax 2+ cells were located between the dorsal root entry and the ventral root exit zones. En-1+ cells were located immediately dorsal to the ventral root exit zone, corresponding to the ventral third of the Pax 2+ band.

We have used two approaches to morphologically identify the Pax 2+ and En-1+ cells. First, we have double labeled neural tubes with either the antibody against Pax 2 or En-1 and an antibody against neuron specific ß-tubulin (TUJ1). Second, we have used a replication competent retroviral vector, RCAS(A), to express a fusion of the microtubule associated protein tau and the myc epitope in a random subset of cells. The fusion protein is transported in the processes of infected cells enabling their morphological characterization by staining with an antibody against myc. These embryos were then labeled with an antibody against either Pax 2 or En-1.

Both Pax 2+ and En-1+ cells extend a process ventrally toward the floor plate. In most cases, the process of Pax 2+ cells continues ventrally past the lateral longitudinal fasciculus (LLF) toward the midline, but occasionally it entered the ipsilateral LLF and turned rostrally. Dorsal commissural neurons and association interneurons were never Pax 2+ or En-1+. Examination of caudal portions of neural tube revealed that some neuroepithelial shaped cells were Pax 2+ or En-1+, suggesting that Pax 2 and En-1 are expressed in cells before differentiation, and therefore may play a role in controlling cell fate.

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601.5

EXPRESSION OF ETS1 GENE IN THE HYPOTHALAMUS AND PITUITARY DURING RAT DEVELOPMENT. F.M. Laurent-Huck*, C. Egles, P. Kienlen, C. Nelson, M.E. Stoeckel and J.M. Félix. IPCB, Univ. Louis Pasteur, 67084 Strasbourg, France.

The family of "ets" genes is coding for transcription factors ; these proteins contain a conserved DNA binding domain : the Ets binding domain. Ets-1 is a member of this family, essentially expressed in the thymus, but also in endothelial cells during vessel formation (angiogenesis).

We study Ets-1 gene expression in the developing rat hypothalamo-hypophysial system, using in situ hybridization on paraformaldehyde-fixed coronal and sagittal brain sections.

At Embryonic day 12 (E12) and E13, Ets-1 mRNA is already synthetized in the neural tube. Cells synthetizing Ets-1 mRNA form small and heavily labeled cord-like or round structures. Mesenchymatous cells surrounding the brain and hypophysis are also heavily labeled. In the hypophysis, cells synthetize Ets-1 mRNA in the neural lobe from E15, and in the anterior lobe from E16, slightly preceding the establishment of vascularization ; no Ets-1 mRNA is detected in the avascular intermediate lobe of the hypophysis, at any stage. From E14, cord-like Ets-1 mRNA labeling is very intense in the whole diencephalon. During perinatal stages, it is detected in several hypothalamic nuclei, especially in the supraoptic and paraventricular nuclei. In both hypothalamus and hypophysis, Ets-1 gene expression is maximal during fetal and perinatal stages, and progressively decreases thereafter until adulthood.

A spatiotemporal correlation is observed between Ets-1 gene expression and blood vessel formation in the hypothalamus and pituitary of the Rat, suggesting a role for Ets-1 in angiogenesis, in this system.

601.6

ISOLATION AND CHARACTERIZATION OF 118 C2H2-TYPE ZINC FINGER cDNAS FROM HUMAN BRAIN. K. G. Becker[1], J. W. Nagle[2], M. E. Franko[1], J.Joy *[1], A. Gado[1], K. Ozato[3], W. E. Biddison[1], and P. D. Drew[1]. [1]Molecular Immunology Section, Neuroimmunlogy Branch; [2]Neurogenetics Section, NINDS; [3]Lab. of Molecular Growth Regulation, NICHD; NIH, Bethesda, MD 20892.

Transcription factors have been shown to exist in gene families, with similar structural and functional characteristics. We have recently isolated 118 novel cDNAs from the C2H2-type zinc finger family of DNA binding proteins[1]. These clones may represent a large percentage of the zinc finger genes expressed in the human brain. This was done using family specific degenerate oligonucleotide hybridization followed by rapid domain specific partial sequence tagging. We are also in the process of isolating members of the steroid-thyroid hormone receptor and forkhead families, as well as cDNAs positive for CAG trinucleotide repeats.

We are currently involved in the biological characterization of many of the novel clones found to date. We have developed a large scale quantitative differential screening assay using over 600 known purified phage isolates corresponding to each novel clone. These phage are gridded onto phage plates, transferred to filters, and hybridized with differential cDNA probes from different neural cell types and regions of the brain. Using this approach, we have screened for relative RNA expression patterns of a large number of C2H2-type zinc finger genes in human neuroblastomas, astrocytomas, glioblastomas, as well as in the human hippocampus, cerebral cortex, and cerebellum.

We have also obtained northern blot data, full length cDNA sequence, and chromosomal localization on a subset of novel clones. The novel zinc finger clone, C2H2-150, maps to 7q35-36.1 and is currently a candidate for a developmental disorder.

The zinc finger cDNA clone, C2H2-34.10, is the human equivalent of the *Xenopus* Pol III basal transcription factor TFIIIA[2]. We show that this gene has nine fingers corresponding to the frog cDNA organization, is expressed in neuronal cells, and has at least one splice variant with specific excision of the fifth finger unit.

601.7

ECTOPIC EXPRESSION OF A FOREBRAIN-SPECIFIC TRANSCRIPTION FACTOR INTO THE MID/HIND-BRAIN OF TRANSGENIC MICE: THE ROLE OF BF-1 IN MOUSE BRAIN DEVELOPMENT. S.O. Huh* and E. Lai. Laboratory of Molecular Developmental Biology, Cell Biology and Genetics Program, Division of Endocrinology, Memorial Sloan-Kettering Cancer Center, New York., NY 10021.

Mouse Brain Factor-1 (BF-1) gene is a member of winged-helix (or fork-head) transcription factor gene family with an important function in the development of forebrain. In developing rodent brain, BF-1 gene is specifically expressed in telencephalon, neural retina, and olfactory neuroepithelium.

From the study of microcephalic-phenotype of the BF-1 knock-out mice generated in our laboratory (Xuan et al., Neuron, 1995 in press), we have learned that BF-1 might play an important role in determination of the forebrain structure. To study more about the function of the BF-1 gene in brain development, we produced transgenic mice expressing BF-1 in the mid/hind brain region. About 400 fertilized eggs were microinjected with En2-BF-1 transgene. So far, 28 offspring have been analyzed and two of them carried the En-2-BF-1 transgene in the genome, as shown by genomic southern blot analyses. Preliminary observation of the hematoxylin/eosin-stained brain sections from transgenic E15.5 embryo reveals some phenotypic abnormally when compared to the wild-type embryo. The periaqueductal gray and future colliculi, where the endogenous En-2 gene is expressed, is enlarged to about two-fold thicker layer compared to that of the control mouse brain. In addition, the cerebral aqueduct expanded to a round tube-like structure with its diameter about 5 fold larger than that of wild-type aqueduct. To identify potential targets of BF-1 that are ectopically induced in the mid/hind brain region of the En-2-BF-1 transgenic mice, we are carrying out in-situ hybridization analyses using probes from candidate regulatory genes, such as dlx and emx.

601.8

PRECURSOR CELL MIGRATION AND DIFFERENTIATION IN EMBRYONIC MOUSE-RAT BRAIN CHIMAERAS. O. Brüstle, U. Maskos, and R.D.G. McKay*. Lab. of Molecular Biology, NINDS, NIH, Bethesda, MD 20892

To test whether the developmental potential of a neuronal progenitor in a given part of the neuroepithelium is restricted to a local fate, precursor cells derived from distinct aspects of the developing mouse brain were distributed over a large area of the embryonic rat neuroepithelium, using intrauterine transplantation. The transplanted cells were traced with two independent genetic marking methods, i.e., *in situ* hybridization with a probe to mouse satellite DNA, and X-Gal histochemistry following implantation of *lacZ*-expressing donor cells. Single cell suspensions prepared from E14 mouse cortex or ganglionic eminences were deposited in the telencephalic vesicles of E16 - E18 rat fetuses. The implanted cells entered the host neuroepithelium, and neurons generated by both donor cell populations migrated with high efficiency and along legitimate pathways into the developing cerebral cortex and the hippocampal formation. The young neurons acquired region-specific morphologies and expressed antigens appropriate for their final location. For example, cortical interneurons and cells integrated into the dentate granule cell layer displayed immunoreactivity for calbindin, a calcium-binding protein typically expressed in these neuronal subpopulations. The limbic system-associated membrane protein (LAMP; Levitt P, *Science* 223:299-301, 1984) was detected in donor-derived neurons in hippocampus and cingulate cortex but not in those which had settled in the sensorimotor cortex. Transplanted precursors were found to migrate into various other areas undergoing neurogenesis at or after the timepoint of implantation, i.e., septum, striatum, and late developing nuclei of thalamus, hypothalamus, midbrain, and pons. These observations show that heterotopically positioned E14 mouse neuroepithelial precursors adopt the migration behavior of neighboring host cells and can be recruited to a variety of local fates.

601.9

REGIONAL INTEGRATION OF NEURAL PRECURSORS DERIVED FROM SELECTED REGIONS OF THE FETAL MOUSE BRAIN AFTER GRAFTING INTO THE DEVELOPING RAT FOREBRAIN M. Olsson*, K. Campbell, K. Bjerregaard, C. Winkler and A. Björklund Dept. of Medical Cell Research, Univ. of Lund, S-223 62 Lund, Sweden

We have previously reported (Soc. Neurosci. Abstr. 205.10, 1994) that embryonic neural precursors derived from the E14 mouse lateral ganglionic eminence (LGE) injected isochronically into the developing E15 rat forebrain ventricle are capable of incorporating into the host striatum (in contrast to precursors derived from the medial ganglionic eminence (MGE)) and to differentiate like their host counterparts with respect to phenotypical expression and axonal projections. The same *in utero* cross-species transplantation model was used here in order to examine the influence of donor and host age as well as the origin of the progenitor pool on the ability of the progenitors to integrate regionally and develop in a site-specific manner in the developing brain. Precursors derived from the mouse embryonic day (E) 11.5-12 or 13.5-14 LGE, MGE and cortical ventricular zone (CVZ) were injected into the E15, E17 or E19 rat cerebral ventricle. At 0 to 42 days posnatal age, brains were fixed and processed for M6 (a mouse-specific neuronal marker) immunoreactivity and regional brain markers. M6-positive cellular profiles were observed in numerous forebrain nuclei after grafts of either LGE, MGE or CVZ tissue. Isochronically grafted CVZ precursors were distributed in a distinct pattern different from that previously observed for the LGE derived progenitors. Thus the CVZ cells integrated less frequently into the striatum and they were more frequently observed to integrate into the frontal and parietal cortex extending appropriate cortical projections. The degree of parenchymal integration appeared to be less extensive in older recipients (E17 and E19), with a larger proportion of M6-positive clusters remaining attached to the ventricular walls. Nevertheless, the specificity of the integration and projection patterns was maintained in the older recipients. Furthermore, progenitors derived from E11.5-12 appear to follow similar patterns as those from the E13.5-14 brains. Preliminary results indicate that mouse progenitors derived from mid- and hindbrain regions such as the ventral mesencephalon and rhombencephalic lip also display distinct and different patterns of integration and axonal projections after grafting into the forebrain ventricle. These results suggest that intraventricularly injected neural progenitors obtained from diverse brain regions possess the ability to integrate homotypically and undergo site-specific differentiation into the host brain during an extended period (E15-19) of fetal rat brain development.

601.10

ADULT RAT HIPPOCAMPAL PROGENITOR CELLS GRAFTED INTO THE OLFACTORY BULB SURVIVE AND EXPRESS A NEURONAL PHENOTYPE

J.O. Suhonen*, D. A. Peterson, J. Ray and F.H. Gage. Laboratory of Genetics, The Salk Institute, La Jolla, CA 92037-1099 U.S.A.

Although most neurons in adult CNS are postmitotic and terminally differentiated, neurogenesis does occur in certain regions of the adult CNS such as ventricular zone, hippocampus and olfactory bulb (OLB). Recently, we have shown that adult hippocampal cells can be cultured for over one year in the presence of basic fibroblast growth factor (FGF-2). When grafted back to adult rat hippocampus, these progenitor cells differentiate into neurons or glial cells.

To determine whether these cultured adult neuronal progenitor cells would survive and differentiate into neurons after grafting into olfactory bulb, bromodeoxyuridine (BrdU) labeled cells were grafted into the intact olfactory bulb of adult rats at the base of the olfactory tubercle. Eight weeks post-grafting, transplanted cells migrated up to 6 mm rostral into the OLB, but only 1.0 - 1.5 mm caudal to the site of injection. After migration, grafted cells integrated into the cytoarchitecture of the OLB. Most of the cells were observed in the granule (GR) and glomerular (GLM) cell layers or in the subependymal zone of OLB. Some BrdU-positive cells in the GR and GLM cell layers showed co-localization with NeuN, a marker for differentiated neurons.

These findings indicate that adult neuronal progenitor cells survive, migrate several millimeters, integrate, differentiate and express neuronal marker after grafting in the olfactory bulb. Although the cells are derived from hippocampus, they survive engraftment in a different target zone, the OLB, where they respond to local environmental cues resulting in integration into the target cytoarchitecture and differentiation.

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601.11

GENETICALLY MARKED CLONES OF ADULT RAT PROGENITORS RESPOND TO CHANGES IN EXOGENOUS CUES BY DIFFERENTIATING INTO NEURONS OR GLIA. T.D. Palmer*, H.K. Raymon, J. Takahashi, J. Ray and F. H. Gage, Laboratory of Genetics, The Salk Institute, La Jolla, CA 92037

Basic fibroblast growth factor-responsive progenitors from the adult rat hippocampus were retrovirally marked and clonal populations were evaluated for their ability to differentiate into glia or neurons *in vitro*. Each clone gave rise to cells expressing markers characteristic of astrocytes (GFAP), oligodendrocytes (galactocerebroside, myelin basic protein) or neurons (Map5, NeuN, Tau, and high molecular weight neurofilament). Differentiation was strongly influenced by the culture environment with oligodendrocyte differentiation favored in the presence of FGF-2 and astrocyte and neuronal differentiation favored by withdrawal of FGF-2. Serum stimulation yielded monolayers of protoplasmic astrocytes upon which neurons were observed. Further treatment of the serum stimulated cultures with retinoic acid induced immature NeuN, Map5-positive neurons to differentiate into mature NF200 and Tau-positive neurons. Populations enriched for oligodendrocytes (<80%) or mixtures of astrocytes (40%) and immature neurons (30%) were evaluated by southern blot. The integrated retroviral genome in all clones confirmed that each population was derived from a single marked cell. Two of these multipotent clones were grafted into the adult rat hippocampus where cells expressing the retrovirally transferred alkaline phosphatase gene were readily detected after one week. Comparison of progenitor differentiation *in vitro* and *in vivo* will provide insights into the local cues which regulate neurogenesis and gliogenesis in the adult mammalian brain.

601.12

IN VITRO AND IN VIVO CHARACTERIZATION OF A NEURAL PROGENITOR POPULATION GENETICALLY MODIFIED TO EXPRESS HUMAN TYROSINE HYDROXYLASE H.K. Raymon*, S. Thode, J. Winkler, J. Ray, D.E. Michalik, L.J. Thal, L.J. Fisher, and F.H. Gage, Laboratory of Genetics, Salk Institute, La Jolla, CA 92037-1099 and Dept. of Neurosciences, UCSD, La Jolla, CA 92093.

The adult rodent hippocampus harbors progenitor cells which have the ability to proliferate and differentiate into neurons. Using the mitogen, basic fibroblast growth factor (FGF-2), we have been able to isolate neural progenitors *in vitro* from the hippocampus of adult rats. These cells retain the capacity to differentiate into both neurons and glia in culture and when implanted into the adult brain. The purpose of the present study was to assess the potential of neural progenitors for gene delivery to the CNS. In addition, the effect of foreign transgene expression on the fate determination of hippocampal neural progenitors is currently being determined.

Cells from the adult hippocampus were cultured in the presence of 20 ng/ml FGF-2 for several months through 18 passages. Neural progenitors were infected via retroviral-mediated gene transfer to express human tyrosine hydroxylase (hTH). Immunohistochemical and Western blot analysis of infected cell cultures revealed TH immunoreactivity (TH-IR) while uninfected parent cultures did not exhibit any staining. Infected cells were assayed for the production and release of L-DOPA in the presence of the co-factor, tetrahydrobiopterin (BH_4). Following a 16 hr incubation with 100 μM BH_4, detectable levels of L-DOPA were noted in the medium and in the cell pellet, indicating that not only is the transgene expressed *in vitro* but that it is functional. In order to determine whether the transgene functions *in vivo*, infected cells have been grafted into the dopamine-depleted striatum of adult rats. The rotational behavior of the animals is currently being analyzed. At one week, infected hippocampal neural progenitors still expressed the transgene as evidenced by TH-IR providing preliminary support for using this cell type for gene delivery to the brain. *Supported by NS09627-01 and the Alliance for Research on Aging.*

601.13

ORIGIN OF RHOMBOMERE DETERMINES AUTONOMOUS VERSUS ENVIRONMENTALLY REGULATED EXPRESSION OF *HOXa3* IN THE AVIAN EMBRYO. J. R. Saldivar[1], C. E. Krull[1], R. Krumlauf[2], and M. Bronner-Fraser*[1]. [1]Dev. Biol. Ctr., UC.-Irvine, Irvine,CA 92717. [2]Lab. of Dev. Neurobiol., Natl. Inst. for Med. Res., The Ridgeway, Mill Hill, London NW7 1AA.

The spatiotemporal patterns of *Hoxa3* expression in the neuroepithelium and associated neural crest cells of the chick hindbrain was examined using *in situ* hybridization in conjunction with DiI labeling. Initial expression of *Hoxa3* is diffuse in the neural plate; later expression becomes restricted rostrally to the r4/5 border. Neural crest cells from r5 contribute to both the r4 and r6 streams that eventually populate branchial arches 2 and 3 respectively. Neural crest from r5 that migrate rostrally into the r4 stream down-regulate expression of *Hoxa3* while neural crest cells that migrate caudally into the r6 stream maintain gene expression. Transposition of r4 plus r5 by grafting results in *Hoxa3* expression in the neural tube according to the rhombomere's original location. While neural crest cells migrate laterally from r5 into branchial arch 2, *Hoxa3* expression is down-regulated as they emigrate from the neural tube. Rotations of r4 to r6 performed prior to rhombomere formation also show autonomous expression of *Hoxa3* in the neural tube, while migrating neural crest cells from r6 into branchial arch 2 also down-regulate *Hoxa3* expression. Conversely, rotations of r4 to r6 after rhombomere formation reveals autonomous expression of Hoxa3 both in the neural tube and neural crest from r6 migrating into branchial arch 2. Our results suggest that neural crest cells expressing the same Hox gene may respond differently to environmental signals depending on their rhombomeric origin. Supported by DE 10066.

601.14

ORIGIN AND SPECIFICATION OF SPINAL CORD OLIGODENDROCYTES Pringle[1], N.P., [2]Guthrie,S., [1]Yu,W.-P., [2]Lumsden,A. [3]Harper*,S & [1]Richardson, W.D. [1]MRC Laboratory for Molecular Cell Biology and Department of Biology, University College London, Gower Street, London WC1E 6BT. [2]Department of Anatomy, Guy's Hospital Medical School, UMDS, London Bridge, London SE1 9RT. [3]Merck Sharp & Dohme, Neuroscience Research Centre, Terlings Park, Harlow, Essex CM20 2QR.

We generated chick-quail chimeras in which dorsal or ventral segments of the developing chick spinal cord were replaced by homotypic segments of quail spinal cord. When quail ventral spinal cord was grafted into a ventral position, quail oligodendrocytes and their precursors were found throughout the operated side of the cord including the most dorsal white matter. They were also found up to 1mm away from the grafts in both rostral and caudal directions. Thus, oligodendrocyte precursors migrate both radially and longitudinally from their site of origin during normal development. These *in vivo* results confirm that oligodendrocyte precursors originate in the ventral part of the cord, in accord with previous circumstantial evidence[1-4]. Preliminary results from experiments in which dorsal quail tissue was grafted into a dorsal position in the chicken recipient indicate that no oligodendrocytes arise from the dorsal half of the cord.

In the absence of a notochord (in ultraviolet-irradiated *Xenopus* embryos or in caudal regions of the Danforth's Short Tail mouse mutant), cells of the ventrally-derived oligodendrocyte lineage fail to develop. When a second notochord was grafted into a dorsolateral position relative to the spinal cord of a chick embryo, a second floor plate was induced along with a new source of PDGFRα$^+$ oligodendrocyte precursors, displaced dorsally with respect to the original source. Thus, development of ventrally-derived glia, like ventral neurons, relies on signals from the notochord/floor plate complex. These signals appear to act early, by influencing the specification of neuroepithelial precursor cells in the ventricular zone.

1. Warf, B.C., Fok-Seang, J., Miller, R.H. (1991) J. Neurosci. **11**, 2477-88
2. Pringle, N.P., Richardson, W.D. (1993) Development **117**, 525-33
3. Noll, E., Miller, R.H. (1993) Development **118**, 563-73
4. Yu, W.-P., Collarini,E.J., Pringle,N.P.,Richardson,W.D.(1994) Neuron **12**, 1353-62

CELL DIFFERENTIATION AND MIGRATION VII

602.1

IDENTIFICATION OF NEURONAL PRECURSORS IN THE DEVELOPING OLFACTORY SYSTEM BY HU ANTIGEN EXPRESSION

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Recent evidence suggests that pioneer olfactory axons modulate the proliferation and differentiation of telencephalic precursor cells to induce the morphogenesis of the olfactory bulb. If true, the region of the telencephalon targeted by pioneer axons should have more cells differentiating into neurons than the rest of the telencephalon. Hu proteins (Hu D, Hu C and Hel N-1) are the earliest known proteins expressed exclusively by mammalian neurons and are the human homologues of Elav, which is required for neurogenesis in Drosophila. Mab 16A11 which binds specifically to an epitope present in all known Hu proteins was used to investigate neuronal development in the olfactory system of E12-20 rats.

At E12-13, Hu+ neurons were located in the basal half of the olfactory epithelium. From E14 to E20 Hu+ cells increased dramatically and were present throughout the basal two-thirds of the epithelium. Numerous globular-shaped cells expressed Hu in the deepest part of the epithelium. From E12-15, numerous Hu+ cells were intimately associated with growing olfactory axons. These cells may be migrating from the olfactory epithelium. At E12, Hu+ cells comprised a single layer located just below the surface of the entire telencephalic vesicle. At E13-14 Hu+ cells were still limited to the superficial layer of the telencephalon except in a small ventral region corresponding to the site containing pioneer olfactory axons. Double labeling with GAP43 showed that Hu+ cells were closely associated with pioneer olfactory axons in the olfactory bulb primordium. At E15-16 the olfactory bulb appeared and pioneer axons were no longer present in the ventricular zone. A thick layer of Hu+ cells was present throughout the olfactory bulb but none were present in ventricular zone.

These findings indicate that: (1) Hu is expressed by very immature ORNs and possibly ORN precursors; (2) neurons are present along the olfactory pathway from its earliest stages of development (3) the arrival of pioneer olfactory axons is associated with the differentiation of neuronal precursors in the telencephalon. Supported by NIH DC00347, NS29218 (MTS) & NS29682 (HF).

602.2

IN VIVO FATES OF THE CONSTITUTIVELY PROLIFERATING SUBEPENDYMAL POPULATION IN ADULT MAMMALIAN FOREBRAIN. D. van der Kooy*, C.G. Craig, R. D'Sa, C. Morshead, A. Roach[1]. Depts. of Anatomy and [1]Molecular and Medical Genetics, Univ. of Toronto and [1]Samuel Lunenfeld Research Institute, Toronto, Ontario M5S 1A8.

In adult mouse brain, the subependymal region lining the forebrain lateral ventricles contains at least two kinetically distinct cell populations-a relatively quiescent neural stem cell population and a constitutively proliferating neural progenitor population. Experiments utilizing retroviral (RV) labeling of the constitutively proliferating adult mouse population *in vivo*, determined there to be a progressive loss of β-gal$^+$ cells with increasing survival times. Relative to 1 day survival animals, 6, 12 and 28 days following RV injection there were 61, 95 and 100% loss of β-gal$^+$ cells in the lateral ventricle subependyma, respectively. This temporal loss of β-gal$^+$ cells could be due to a progressive loss of RV reporter gene expression, the migration of β-gal$^+$ cells away from the lateral ventricle, or cell death. If the loss of β-gal$^+$ cells is due to a loss of RV gene expression, then a progressive increase in β-gal$^+$/RV-DNA$^+$ cells would correlate with increased survival time. Regions of RV infected brain sections containing β-gal$^+$ cells and nearby negative regions were assayed for RV-DNA by nested-PCR. Of the β-gal$^+$ regions tested, 22% from day 1 sections resulted in positive PCR signals (probably due to non-integrated transient RV-DNA), whereas 6 and 28 days following infection this percentage decreased to 1.2 and 0.8%, respectively. Thus, loss of RV gene expression does not contribute to the temporal loss of lateral ventricle β-gal$^+$ cells. To determine if β-gal$^+$ cells migrate away from the lateral ventricle, larger brain regions were sectioned and assayed for β-gal$^+$ cells. Correlating with increased survival times was a progressive rostral migration of β-gal$^+$ cells to the olfactory bulb (OB). The total number of β-gal$^+$ cells (including the OB) increased approximately 2.5-fold over 28 days. These results were reproduced with BrdU-labeling of constitutively proliferating cells, and suggest that rostrally migrating cells continue to proliferate en route to the OB. Thus, a major *in vivo* fate of some of the progeny of constitutively proliferating adult lateral ventricle subependymal cells is rostral migration to the OB. However, the lack of a larger temporal increase (it was only 2.5-fold) in the β-gal$^+$ cell population, even though migrating cells continue to proliferate on the way to the OB, suggests that a major fate of other progeny of the β-gal$^+$ lateral ventricle cells is cell death.

602.3

KALLMANN PROTEIN EXPRESSION IN THE DEVELOPING CHICK BRAIN. R.B. Norgren*, Dept. Cell Biol. & Anat., Univ. Nebraska Med. Ctr. Omaha, NE. 68198.

LHRH neurons originate in the olfactory placode and migrate to the brain during embryonic development. Some of the symptoms associated with X-linked Kallmann's disease are due to a failure of the olfactory nerve to enter the telencephalon and LHRH neurons to migrate into the brain. The gene involved in this disease, *KAL*, has been identified in humans and chick. Based on the predicted protein sequence of chick KAL, we chose three peptides for production of polyclonal antibodies. Using immunocytochemistry, we examined the distribution of KAL-like immunostaining in the E16 chick nervous system. In the telencephalon, there was strong staining in what appear to be mitral cells of the olfactory bulb. The lamina medullaris dorsalis demarcated a region of strong staining (including the ectostriatum, neostriatum, hyperstriatum ventralis and hyperstriatum accessorium) from a region of weak staining (including the lobus parolfactorius). In the diencephalon, dorsal structures (thalamic nuclei) were more intensely stained than ventral structures (preoptic area, ventral hypothalamus). Several nuclei in the mesencephalon were labeled. A distinct band of immunostaining was observed in the stratum griseum centrale of the optic tectum. Strong immunostaining was also observed in purkinje cells of the cerebellum. Immunostaining in alternate sections was blocked when the antibody was preabsorbed with the synthetic peptide used to make the antibody. It will be necessary to perform a western blot in order to confirm that the staining we observed represents only KAL immunostaining. In general, our immunocytochemistry results are in agreement with previous reports of KAL message in the chick brain. The pattern of KAL protein expression may help explain some of the symptoms of Kallmann's syndrome. This work was funded by NIH grant R01 NS30047 (RBN).

602.4

CARNOSINE-LIKE IMMUNOREACTIVITY IN THE SUBEPENDYMAL LAYER AND IN THE ROSTRAL MIGRATORY STREAM OF THE ADULT RAT. L. Bonfanti, P. Peretto, A. Merighi and A. Fasolo*. Dip. di Morfofisiologia Veterinaria and Dip. di Biologia Animale, University of Turin, Turin, Italy.

The mammalian subependymal layer (SEL) of the lateral ventricles and its rostral extension in the olfactory bulb are a site of continued cell proliferation and migration during adulthood (Lois & Alvarez-Buylla, *Science* 264 : 1145: 1994). Cells migrate to the superficial layers of the olfactory bulb following a characteristic pattern commonly referred to as the "rostral migratory stream". The whole system is specifically marked by antibodies against the highly sialylated, weakly adhesive isoform of the neural cell adhesion molecule NCAM (PSA-NCAM, Bonfanti & Theodosis, *Neuroscience* 62 : 291, 1994), a molecule related to structural plasticity. Carnosine and other related aminoacylhistidine dipeptides are considered to play a role in the plasticity of some peripheral sensory cells, i.e. the primary olfactory neurons and the hair cells of the inner ear (in frogs), whereas in the brain the molecule is present in glial elements. In this study, a strong immunoreactivity (IR) for carnosine was found in the SEL of the adult rat brain. At the ventricular level, carnosine-IR structures included the ependyma and the SEL; more anteriorly they were associated with the rostral extension of the SEL occupied by the migratory stream. Double labellings in combination with GFAP and vimentin revealed a high density of glial cells and processes throughout the SEL, although there was no co-localization with carnosine. The latter filled the spaces between glial processes, which also contained PSA-NCAM-positive structures. In the olfactory bulb, the staining for carnosine was limited to the SEL. After systemic injections of 5-bromo-2'-deoxyuridine (BrdU), at different survival times, the BrdU-positive nuclei appeared broadly distributed with carnosine-IR in the SEL. Our observations show that carnosine is present in the SEL and in the rostral migratory stream of the adult rat, but does not appear to be associated with glial structures.

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602.5

SUBPOPULATIONS OF MIGRATING NEURONS EXPRESS DIFFERENT LEVELS OF LHRH IN QUAIL AND CHICK EMBRYOS. C. Gao*, R. Abou-Nasr and R.B. Norgren. Dept. of Cell Biol. and Anat., Univ. of Nebraska Med. Ctr., Omaha, NE 68198.

LHRH neurons of the septal-preoptic area originate in the olfactory placode and migrate in the olfactory nerve into the brain during embryonic development. In adult birds, LHRH neurons have been found in the septal-preoptic area, mesencephalon and more recently in the lateral anterior nucleus of thalamus (LA) (Kuenzel & Blahser, '91). LHRH neurons of the LA do not originate in the olfactory placode (Norgren & Gao, '94). Using immunocytochemistry, we examined the distribution of LHRH neurons in the embryonic and adult quail nervous system. The pattern of LHRH immunostaining in quail embryos was similar to chick. However, there were many fewer neurons immuonstained for LHRH from the olfactory placode to the septal-preoptic area in quail than in chick embryos. In contrast, there were more labeled neurons and more intense LHRH immunostaining in the thalamus of the quail than in the thalamus of chick embryos. Several results suggest that our immuno-staining reflects differences in LHRH expression in quail and chick embryos rather than differences in antibody binding or in the number of migrating LHRH neurons. 1. The antibodies we used worked well on all LHRH neurons in adult quail. 2. Species differences in LHRH expression during development have also been found in rats and mice. 3. *In situ* hybridization experiments indicate LHRH message is not expressed in the developing olfactory system of amphibian embryos although ablation studies show LHRH neurons must be migrating in the olfactory system. Therefore, it is likely that there are species differences in LHRH expression in migrating neurons. The current results should also be considered for quail-chick chimeras involving the olfactory placode. This work was funded by NIH grants R01 NS30047 (RBN) and DE06632 (D. Noden).

602.6

NEURAL CELL ADHESION MOLECULE (N-CAM)-,L1-AND S100-IMMUNOREACTIVITY ON THE MIGRATION ROUTE OF LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) NEURONS ORIGINATING FROM THE OLFACTORY PLACODE OF THE EARLY HUMAN EMBRYO. M. Schwanzel-Fukuda* and D.W. Pfaff, The Rockefeller University, New York, NY.; J. J. Hemperly, Becton Dickinson and Co., Research Triangle Park, NC; K.L. Crossin, Scripps Research Institute, La Jolla, CA; P.M.G. Bouloux, Royal Free Hospital, London, UK; J.-P. Hardelin and C. Petit, Institut Pasteur, Paris, France.

LHRH-immunoreactive (ir) neurons are present in the epithelium of the medial olfactory pit on day 42 of embryogenesis (E) and migrate into the brain along branches of the terminal and vomeronasal nerves. By 28 E neural cell adhesion molecule (N-CAM)-ir cells are seen only in the epithelium of the medial olfactory pit. These "pioneer" N-CAM-ir cells migrate out of the epithelium of the medial olfactory pit, form an aggregate in the nasal mesenchyme between the olfactory pit and the developing forebrain and receive the ingrowing N-CAM-ir central processes of the olfactory, vomeronasal and terminal nerves. The N-CAM-ir cellular aggregate contacts the rostral forebrain and N-CAM-ir central processes now form a scaffold along which the LHRH neurons migrate into the brain. Numerous blood vessels develop in this part of the nasal mesenchyme at this time and may assist in formation of the migration route into the brain. In a search for guiding principles in addition to N-CAM adjacent sections were treated with antibodies to L1 and S100. L1-ir was seen on cell bodies and axons of the migration route (similar to N-CAM) and in the olfactory bulb itself (in contrast to N-CAM). Antibody to S100 shows a later-appearing (by 42 E) population of glial cells which also originate in the epithelium of the olfactory pit and closely follow the axons of the migration route. These cells form the sheath cells of the olfactory, vomeronasal and terminal nerves, and do not appear to lead the migration of LHRH cells into the brain. Supported by NIH grant DC 00880 (M.S.-F.)

602.7

MIGRATION OF GnRH AND NEUROPEPTIDE Y (NPY) NEURONS FROM THE OLFACTORY PLACODE TO THE CNS. E.M. Hilal,* P. Gandhi and A.J. Silverman. Dept. Anat.&Cell Biol., Columbia Univ., NY NY.

The olfactory epithelium (OE) of the vertebrate embryo gives rise to sensory neurons and several populations of migratory cells. Prominent among the latter are GnRH neurons which differentiate within the OE and migrate along the olfactory nerve (ON) to the CNS. Evidence from several laboratories has suggested that there are non-GnRH neurons which follow this pathway. We have initiated studies to characterize the phenotype of these migratory cells. NPY neurons are functionally related to the reproductive axis, modulating the release of GnRH and directly enhancing GnRH-induced LH secretion from gonadotropes. NPY terminals innervate the hypothalamic and basal forebrain regions and are closely associated with the cell bodies and fibers of GnRH neurons. Based on the functional and anatomical relationship of GnRH and NPY, we asked if a population of NPY neurons originates within the OE. Embryonic chicks were prepared for immunocytochemical detection of either peptide. At stages 28-30, NPY-positive neurons and processes were present in the OE, ON and at the junction of the ON and forebrain. There was also a "stream" of NPY cells within the telencephalon that appeared to be an extension of those in the ON. The number of NPY positive cells in the OE/ON increased between stages 28 (39-81) and 30 (110-227). At the earlier stage NPY cells were relatively more numerous in the OE than at the later stage. The NPY cells in OE/ON are 1/10th as numerous as those containing GnRH. By stage 42, there were neither NPY-positive neurons nor neurites remaining in the periphery. This suggests that all NPY immunoreactivity in the OE and ON is due to the migratory population. These data further suggest that, similar to GnRH neurons, a NPY population originates in the OE and migrates into the CNS during embryogenesis. Experiments are now in progress to determine the earliest appearance of NPY immunoreactivity. The possibility that some GnRH neurons express NPY will be tested. HD 10665.

602.8

DISRUPTION OF N-GLYCOSYLATION IN OLFACTORY EXPLANTS: EFFECTS ON PERIPHERIN FIBER OUTGROWTH AND LHRH NEURONAL MIGRATION. S. Fueshko* and S. Wray. Lab of Neurochemistry, NINDS, NIH, Bethesda, MD 20892.

During development, luteinizing hormone releasing hormone (LHRH) neurons arise from progenitor cells in the olfactory placode, and migrate into the CNS along a subset of peripherin positive axons. Utilizing an *in vitro* explant system we are examining the mechanisms involved in directing the outgrowth of the peripherin fibers, and the subsequent neurophilic migration of LHRH neurons. We have previously shown that these processes do not appear to be dependent on spontaneous electrical activity and/or depolarization. We have therefore begun to examine the role of glycosylation in the movement of LHRH neurons. Inhibition of N-glycosylation was accomplished by treatment of cultures with tunicamycin. A time course experiment using 1.25 µg/ml tunicamycin indicated a critical period between 24 and 36 hrs *in vitro*, roughly equivalent to E12-E12.5 *in vivo*. Surprisingly, during this period, an exposure to tunicamycin for as few as 12 hrs abolished the olfactory epithelium, including LHRH neurons and peripherin fibers. This phenomenon was not observed at later timepoints. These data suggest that N-glycosylation may be involved in the early stages of peripherin fiber outgrowth, but is not involved in the association of LHRH neurons with the peripherin fibers. This does not rule out a role for other forms of glycosylation in these processes. Studies utilizing lectins are currently underway to determine what specific carbohydrate moieties are involved in LHRH neuronal migration and/or the outgrowth of olfactory axons.

602.9

MECHANISMS OF MIGRATION OF OLFACTORY INTERNEURON PRECURSORS IN THE SUBVENTRICULAR ZONE. H. HU, H. TOMASIEWICZ, T. MAGNUSON AND U. RUTISHAUSER*. Department of Genetics, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

Olfactory interneuron precursors migrate to the olfactory bulb through a specific tangential pathway within the subventricular zone (SVZ). This migration process is retarded in NCAM-mutant mice, a defect reflecting their loss of NCAM polysialic acid (PSA). To study the mechanism of this migration, we have carried out transplantations of SVZ cells of NCAM mutant and wild type newborn mice. Surprisingly, mutant cells were found to migrate normally when transplanted to the SVZa of a wild type host, whereas wild type cells did not migrate in a mutant host. These findings suggest that the host SVZ environment, and not the individual transplanted cells, is the determining factor in generation of the mutant phenotype. However, migration of cells from SVZ explants into a collagen matrix was also found to be PSA-dependent, suggesting that a specific SVZ environment is not required. A resolution of this apparent paradox was suggested by observations of the behavior of cells during migration. That is, the migration in vitro occurred as streams or chains of closely-apposed cells, suggesting that SVZ cells may serve as their own migration environment. Such chains of migrating SVZ cells have also been observed in the adult animal (Rousselot et al, J. Comp. Neurol. 351:51-61, 1995). Additional transplantation studies indicate a strong rostral attraction of these cells toward but not within the bulb, and that PSA is not required for radial migration of cells within the bulb.

602.10

LHRH NEURONAL MIGRATION IS DISRUPTED BY ENZYMATIC REMOVAL OF POLYSIALIC ACID IN EMBRYONIC MICE. K.Yoshida[1], G.A. Schwarting*[1], U. Rutishauser[2], and J.E. Crandall[1]. [1]The Shriver Center, Waltham, MA 02254 & Program in Neuroscience, Harvard Med. Sch., Boston, MA 02115 and [2]Dept. Neuroscience, Case Western Res. Med. Sch. Cleveland, OH 44106.

The unique migrational pathway of LHRH neurons begins in the olfactory placode, crosses the cribiform plate, continues into the basal forebrain and reaches the hypothalamus. Experiments in chick suggest that the polysialated form of N-CAM (PSA-NCAM) is involved in the selective movement of these cells. In embryonic mice and rats PSA-NCAM is associated with the caudal branch of the vomeronasal nerve which may act as a selective guidance pathway. To directly test the notion that PSA-NCAM is involved in LHRH cell migration, we micro-injected the enzyme, endoneuroaminidase N, into embryonic day 12 (E12) and E13 mice. Three days later, the heads were processed for immuno-cytochemistry for PSA-NCAM, NCAM and LHRH. PSA-NCAM was not detectable in the nasal cavity, olfactory bulb or forebrain of endo N-treated embryos whereas NCAM immunoreactivity was similar to controls. The distribution of LHRH cells was clearly altered in animals where there was no detectable PSA-NCAM. More LHRH cells remained in groups along branches of the vomeronasal nerve in the nose compared to the controls. Conversely, significantly fewer LHRH cells were present in the basal forebrain. Thus, PSA may be required to aid the movement of LHRH cells in their migration from the nasal cavity to the forebrain. Supported by HD05515.

602.11

IMMATURE NEURONS PROLIFERATE AND MIGRATE ALONG AN ASTROCYTE/TENASCIN-RICH PATHWAY FROM THE ADULT SUBEPENDYMAL ZONE TO THE OLFACTORY BULB L.B. Thomas*, M.A. Gates, and D.A. Steindler Dept. of Anat. & Neurobiol. and Neurosurg., Univ. of Tenn., Memphis, TN 38163

The rodent subependymal zone (SEZ) has recently received much attention as it has been demonstrated that stem cells persist in this area in adult mammals which can be manipulated to produce neurons *in vitro* in the presence of selected growth factors. Furthermore, it has been demonstrated that some of the progeny of these stem cells migrate to the olfactory bulb *in vivo* and differentiate into mature neurons. A recent study has examined the developmental expression of certain astrocyte-derived, extracellular matrix (ECM) molecules such as tenascin (TN) and chondroitin sulfate containing proteoglycans (CSPG) in the SEZ (Gates et al., J Comp Neurol, in press). These molecules form boundaries around developing functional units in the brain. They are, for the most part, expressed in very low levels in the adult brain. Interestingly, the expression of these molecules remains high in the SEZ throughout adulthood. Using the neuronal marker TuJ1 (which stains class III β-tubulin expressed only in immature and mature neurons), we demonstrate that the path along which these neurons migrate is distinctly rich in TN and CSPG. Furthermore, this path contains a high number of glial fibrillary acidic protein (GFAP) + astrocytes in the normal adult brain. Using immunohistochemistry for bromodeoxyuridine (BrdU, a thymidine analog incorporated into the DNA of dividing cells during the S phase), we demonstrate that cell proliferation occurs along the entire course of this pathway from the SEZ to the olfactory bulb. This study has important implications concerning the migration of neurons in the mature CNS. It appears that these neuronal stem cells are born and migrate preferentially along this ECM-rich pathway. Preliminary studies suggest that these ECM molecules may also be expressed in the human SEZ. These findings may explain why young neurons migrate only towards the olfactory bulb and not into other CNS regions such as the overlying cortex or striatum that are normally ECM- and growth factor-poor. Supported by NIH/NINDS grant 29225.

602.12

A NOVEL METHOD TO CULTURE THE SUBEPENDYMAL ZONE OF THE ADULT RODENT REVEALS IMMATURE NEURONS THAT PREFER AN ENVIRONMENT RICH IN EXTRACELLULAR MATRIX MOLECULES H. Fillmore, M.A. Gates, L.B. Thomas, J.B. Schweitzer* and D.A. Steindler. Dept. of Anat. & Neurobiology and Neurosurgery, Univ. of Tenn., Memphis, TN 38163

The adult rodent subependymal zone (SEZ) has been an area of intense study since several laboratories have recently reported that SEZ stem cells can generate neurons in vitro in the presence of certain growth factors. Studies of the SEZ to date have predominantly utilized dissociated cell suspensions to produce these neuronal cells. We report a novel method in which the SEZ is cultured as an explant on a filter. Using this technique, spheres of cells immunoreactive for neuronal markers are detected similar to those seen in dissociated culture paradigms. We demonstrate that cells in the SEZ explants are immunoreactive for the neuronal markers neuron specific enolase and TuJ1 (which recognizes class III β-tubulin found only in immature or mature neurons), as well as for the cell proliferation marker BrdU. Using this culture system allows detailed studies which more accurately reflect the in vivo environment. We have recently demonstrated that the extracellular matrix (ECM) molecules tenascin and chondroitin sulfate containing proteoglycans (CSPG) persists in the SEZ throughout adulthood. Furthermore, it has been demonstrated that cells born in the SEZ migrate to the olfactory bulb where they differentiate into neurons. This migratory path is rich in ECM molecules. We therefore hypothesize that the stem cells migrate freely to the olfactory bulb along this path, but they do not migrate into the overlying cortex and striatum because of a lack of ECM and perhaps certain growth factors in these adult regions. This explant culture method has allowed us to examine the role of ECM molecules in and around the SEZ as well as the effect of such ECM molecules on SEZ stem cells. Preliminary studies suggest that different dissociated cells selectively adhere to the ECM-rich SEZ, and perturbing these cultures with, for example, tenascin and CSPG antibodies, affects cell attachment and subsequent neurite growth. Supported by NIH/NINDS grant 29225.

602.13

MIGRATION OF LHRH NEURONS IN EXPLANT CULTURES OF EMBRYONIC CHICK OLFACTORY PLACODE. M. R. L. Maas*, P. F. Gadson, Jr. and R. B. Norgren, Dept. of Cell Biol. and Anat., Univ. of Nebraska Med. Ctr., Omaha, NE 68198

During embryonic development LHRH neurons originate in olfactory epithelium (OE) and migrate to the telencephalon within the olfactory nerve. Apparent migration of LHRH neurons has been observed in mammalian explant systems but has not been previously reported in the chick. In this study, we examined the effect of age of OE on LHRH neuron organization, the possible role of soluble factors in LHRH neuron differentiation, and the early migratory behavior of LHRH neurons. Explants from early (HH Stages 14-19) and later embryos (HH Stage 20 and older) contained intensely immunostained LHRH neurons. LHRH neurons were closely associated with each other and organized in cell streams in explants from the later but not the earlier stage embryos. Large numbers of LHRH neurons were observed in 30.9% of OE explants which received either conditioned media or were co-cultures but in only 14.8% of untreated OE explants indicating a soluble factor from brain explants may enhance LHRH production either directly or by blocking an inhibitor to LHRH differentiation. Untreated OE explants which were disturbed contained large numbers of LHRH neurons in 28.6% of the cultures, compared to only 11.7% in undisturbed untreated OE cultures. Although most LHRH neurons *in vivo* originate from rostral-medial OE, LHRH neurons were found in explant cultures of dissected medial, lateral, rostral, and caudal halves. Thus, disruption of the organization of the OE appeared to stimulate LHRH proliferation or expression, indicating a possible role of the local environment in the regulation of LHRH expression. This work was funded by NIH grant R01 NS30047 (RBN).

602.14

IN VITRO IMMUNOCYTOCHEMICAL STUDIES OF NEURONAL MIGRATION IN EMBRYONIC RODENT FOREBRAIN. S.A. Tobet*, I.K. Hanna, T.W. Chickering, & G.A. Schwarting, Prog. Neuroscience, The Shriver Center, Waltham, MA 02254 & Harvard Medical School, Boston, MA 02115.

During development neurons migrate across diverse terrains that range from crossing the cribriform plate into the brain, to moving through a maze of radial glial or axonal fibers, to traversing fiber bundles such as the anterior commissure (AC). To study the migration of neurons in development we are utilizing an approach wherein cell migration is visualized in vitro, in tissue slices that maintain the structure of the forebrain. On days 15 or 18 of gestation rat embryos were dissected, and the heads embedded in agarose and cut by Vibratome into 300-350 micron slices. In some slices individual cells were randomly labelled by brief immersion in the carbocyanine dye, DiI and followed by fluorescence video microscopy (e.g., Tobet et al., Horm Behav (1994) 28:320-327). Alternatively, similarly cut sections were fixed by acrolein or formaldehyde on days 0 to 3 after preparation. Sections taken from rats at E15 were sagittal and processed for gonadotropin releasing hormone (GnRH) immunocytochemistry (ICC). Sections taken from rats at E18 were coronal and processed for tyrosine hydroxylase (TH) ICC. In each case, immunoreactive cells occupied stereotyped positions on the day of preparation - GnRH cells close to the cribriform plate and TH cells under and within the AC. By examining slices by ICC after varying days in vitro, cell migration can be assessed from the changes in cell orientations and positions. The data reveal patterns and rates of cell movements that are complementary to studies utilizing fluorescence video microscopy.

603.1

LONGITUDINAL AXON TRAJECTORIES CORRELATE WITH HOMEOBOX GENE EXPRESSION DOMAINS IN MOUSE EMBRYOS. G.S. Mastick* and S.S. Easter, Jr. Dept.Biology, U.Michigan, Ann Arbor, MI 48109

We previously described the formation of a continuous longitudinal tract through the early brain: axons of the tract of the postoptic commissure (tpoc) project caudally from the most anterior neuromere of the prosencephalon, prosomere 6 (p6), to merge with the pre-existing descending tract of the mesencephalic nucleus of the trigeminal nerve (dtmesV) in p1 and mesencephalon (mes), into the rhombencephalon. The tracts thus appear to course along the dorsal side of the alar-basal boundary, projecting through several neuromeres and intervening transverse boundaries.

We demonstrate that dtmesV and tpoc axon trajectories, and subsequent shapes of the tracts, vary in a neuromere-specific manner that correlates with gene expression domains. Axons were labelled with diI on embryonic days 9.5 and 10.5, then photoconverted; subsequent whole mount in situ hybridization revealed expression of the homeobox genes Nkx-2.2, Pax-6, and Pax-3. The dtmesV axons initially project ventrally, then turn caudally into a narrow path, passing ventral to a dorsal Pax-3 domain, and dorsal to a longitudinal Nkx-2.2 stripe. The tract widens, as its axons make loops and errors at a gap in the Pax-3 domain and Nkx-2.2 stripe coinciding with the mes/rhombomere 1 border. The tpoc is initially narrow, just dorsal to the Nkx-2.2 stripe in p6 and p5, widens upon entering Pax-6 and Nkx-2.2 zones in dorsal p4 and p3, narrows again upon diving through a dorsal-ward deflection of the Nkx-2.2 stripe, and widens in a Pax-6$^-$ zone in p2. Our results imply that the navigation of these longitudinal axons is regulated by gene-specific cellular domains, corresponding to neuromeres, interneuromeric boundaries, and the longitudinal alar/basal boundary. In the basal plate, axons of the medial longitudinal fasciculus (mlf) have relatively uniform caudal trajectories, suggesting that these domain-specific effects on axon growth are limited to the alar plate.

603.2

cDNA SUBTRACTION AND ANALYSIS OF THE GENES EXPRESSED IN MES-METENCEPHALIC BOUNDARY OF CHICK EMBRYO. J.Funahashi*, and H.Nakamura. Inst. Development, Aging and Cancer, Tohoku Univ., Sendai 980-77, Japan.

The optic tectum is a primary visual center of avian brain that differentiates from mesencephalon and receives retinal fibers. The projection is organized in a topographically ordered fashion such that nasal retinal fibers project to caudal part of the tectum, and temporal fibers to the rostral. We are interested in the molecular basis for positonal specification in the tectum along the rostrocaudal axis. Recently we have shown that the ectopic expression of *En*, the chick homologue of the Drosophila *engrailed*, induced branching of the nasal fiber in rostral part of mesencephalon. This suggests that *En* play an important roll in determination of positional specificity in mesencephalon. On attempt to clone genes which expression is regulated by *En*, or which regulate the expression pattern of *En*, we constructed subtracted cDNA libraries; rostral minus caudal and caudal minus rostral. Some of the isolated clones exhibit spatially and temporarily restricted expression pattern in mesencephalon. Functional analysis of these clones using retrovirus infection technique and embryonic manipulation is under way.

603.3

THE MURINE *ENGRAILED* GENES ARE REQUIRED FOR THE FORMATION OF DEEP MESENCEPHALIC NUCLEI. H.H. Simon* W. Wurst# and D.D.M. O'Leary. The Salk Institute, La Jolla, CA #GSF-Research Center, Oberschleissheim, Germany.

The embryonic expression domains of *En-1* and *En-2* in the murine brain overlap to a large extent. They are expressed in the dorsal aspect of the most rostral hindbrain and throughout the neuroepithelium in caudal midbrain. These expression domains coincide with the embryonic regions which give rise to the cerebellum, the inferior and superior colliculi, and more ventrally located mesencephalic nuclei. Homozygous mice for the *En-2* mutation show only a small change in the organization of the cerebellum. The *En-1* mutant mice, which die at P0, are more severely effected; dorsally, the entire cerebellum, inferior colliculus and the caudal superior colliculus are absent; ventrally, only the mesencephalic cranial nerves are effected.

Here we describe the phenotype of the *En-1/En-2* double mutant. The double mutant phenotype reflects the actual expression patterns of *En-1* and *En-2* to a greater extent than either of the single mutants. In addition to the already described deficiencies in the cerebellum and colliculi, these mice are deprived of deeper located mesencephalic neuronal cell types. The largest source of noradrenergic neurons in the central nervous system, the locus coeruleus, cannot be detected immunohistochemically using antibodies to tyrosine hydroxylase. The mesencephalic dopaminergic cell groups A8, A9 and A10 are reduced to a few TH positive neurons. Furthermore, the midbrain serotonergic cell groups, B7 and B8, the dorsal and median raphe nuclei, respectively, are not detectable using antibodies to serotonin. Although these populations of TH and serotonin positive cells can be detected as early as E11/12 in normal mice, they were not detected in the double mutant mice. Our results suggest that the normal induction of these chemically-defined neuronal cell types does not occur. Furthermore, the phenotype of the double mutant strongly supports the hypothesis of functional redundancy of *En-1* and *En-2* during embryogenesis.

603.4

CELLULAR PROCESSES DURING THE ONTOGENY OF A TRANSIENT DRG IN THE CHICK EMBRYO. R.S. Goldstein,*, O. Rosen, R.Gefen, C. Avivi and R. Freidman. Dept. Life Science, Bar-Ilan Univ. 52900 Ramat-Gan, ISRAEL

A striking example of axial variation or patterning in nervous system development is the disappearance of the DRG that develop adjacent to the occipital and the first cervical segments of the CNS of the amniote embryo. It was recently shown that the overexpression of a single *Hox* gene can "rescue" one of these "transient" DRG. We have now characterized the cellular processes taking place during the ontogeny and demise of the 2nd cervical DRG (C2) in the chick embryo, to serve as a basis for examining the roles of *Hox* genes in pattern formation of the nervous system of the trunk. At St. 18 (E2.5), the C2 DRG is indistinguishable from permanent DRG C5 and C6 in whole-mount and in cross-sections stained with the HNK-1 antibody. A few hours later at E3 (H&H St. 20), it first appears different from conventional neighboring DRG. At this stage, C2 has half the volume and number of cells as ganglia C5 and C6, has a distinctive shape and location, and its peripheral nerve root begins to degenerate. The early difference in size/cell number appears to be caused by both differential rates of proliferation and apoptosis. At St. 20 50% fewer cells in C2 are in S-phase than in neighboring ganglia, and apoptotic cells are more abundant in C2 than in conventional DRG.. C2 grows at a slower pace than do neighboring ganglia, from St 18 through St. 32. At the height of normal DRG apoptosis in cervical ganglia at St. 28, 3-4 four times more pycnotic nuclei are present in C2, resulting in the elimination of this ganglion in 80% of St. 36 embryos. In spite of the programmed removal of C2, neurons differentiate successfully there, and are present at least from St 20 to St. 32. Our results suggest that *Hox* genes may produce the difference in fate between C2 and other cervical DRG by modifying the expression of trophic factor(s) and/or their receptors involved in DRG cell survival and proliferation, starting at St.18-20.

603.5

EVIDENCE FOR PRENATAL DETERMINATION OF CONE OPSIN PHENOTYPES AND POSITION IN THE MOUSE RETINA. K. C. Wikler*, A. Szel, and A-L. Jacobsen. Sect. of Neurobiol., Yale U. Sch. Medicine, New Haven, CT.; Dept. Anatomy, Semmelweis U. Med. Sch, Budapest, Hungary.

We proposed that cone: cone interactions may underlie the specification of the wavelength- sensitive cone subtypes in the fetal monkey retina. To explore this issue we developed an organotypic culture system to exploit the topographic separation of red/green (R/G) and blue (B) cones into respective dorsal and ventral fields in the mouse retina. Dorsal (D) and ventral (V) hemi-retinae from postnatal (P) day 3 mice, prior to opsin expression, were flatmounted onto collagen-coated porous membranes, incubated for 8 to 10 days, fixed, and processed for immunocytochemical identification of the R/G and B opsins.

The distribution of cone subtypes in vitro paralleled that seen in vivo: R/G cones were found exclusively in dorsal retina and B cones were abundant in ventral retina, decreasing in density dorsal to the optic disc. The emergence of the asymmetric D- V distribution of B cones was also evident in explants taken from P0 retinae; however, dorsal cones failed to express the red/green opsin in these cultures.

To test whether immature cones can be induced to express an ectopic opsin, we exposed prospective R/G or B cones to putative locally restricted diffusable factors. Separate D and V retinal pieces from P0 or P3 retinae were co- cultured with D or V hemi-retinae from either same age or P14 tissue. The emergence of the D- V gradient of B cones was unperturbed in all co- culture conditions and the expression of the R/G opsin remained restricted to incubated P3 explants. These results suggest that the regional segregation of cone subtypes is intrinsically determined prior to birth, however, the completion of R/G cone differentiation appears to be dependent on events occurring during the first two postnatal days.

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603.6

RETINOIDS AS CANDIDATE MOLECULES FOR THE EMERGENCE OF SEGREGATED COMPARTMENTS OF CONE SUBTYPES IN THE MOUSE RETINA. D. L. Stull* and K. C. Wikler. Section of Neurobiology, Yale Sch. Medicine, New Haven, CT, 06510.

We are interested in the cellular and molecular mechanisms underlying the emergence of separate fields of red/green and blue cone subtypes in the mouse retina. In this study, we selected the retinoids as candidate molecules since they appear to regulate pattern formation in the developing central nervous system. To determine if neuroblasts in the retinal neuroepithelium are responsive to endogenous retinoids, we examined embryonic mice possessing a retinoid-dependent LacZ reporter transgene (Balkan et al, 1992).

Transgenic mice at embryonic (E) day E10, E11, E12, or E14 were sectioned, immunoreacted for β- galactosidase (β-gal), and counterstained with Bis- benzadine. A dynamic pattern in the cellular response to retinoids during this embryonic period was revealed by reconstruction of the distribution of β-gal positive cells from immunoreacted serial sections. Retinoid- responsive neuroblasts were found throughout the optic vesicle at E10. However, we observed the emergence of a striking dorsal- ventral asymmetry in the distribution of immunoreactive dividing cells in the neuroepithelium by E11 and E12, coincident with the formation of the retinal pigment epithelium and neural retina. Neuroblasts located dorsal to the optic disc demonstrated a functional response to endogenous retinoids, while the majority of dividing cells in ventral retina remained quiescent. By E14, neuroblasts located in ventral and peripheral retina showed an increased response to these molecules.

These results indicate retinal neuroblasts respond to endogenous retinoids prior to (E10 and E11) and coincident with (E12 and E14) cone genesis. In addition, the asymmetric response of the neuroepithelium reveals an early parcellation of the retina into dorsal and ventral compartments. Together these results suggest that the retinoids regulate the specification of early- generated retinal cell types and contribute to the emergence of the dorsal-ventral separation of red/green and blue cone subtypes. Supported by EY09917 (KCW).

603.7

PCR-CLONING AND EXPRESSION PATTERN OF COLLAPSIN IN THE EMBRYONIC RAT. R.J. Giger, G. de Wit, J. Verhaagen*. Netherlands Institute for Brain Research, Amsterdam, NL.

The establishement of specific connections in the developing nervous system depends on the capability of axons to reach distant target areas. In addition to neurite outgrowth promoting molecules, repulsive factors, which induce growth cone collapse and paralysis, have been shown to be important cues for axon guidance. Recently a chicken growth cone-collapsing protein, named collapsin, has been cloned and shown to induce the collapse of chicken DRG growth cones (Luo et al., 1993; Cell 75, 217-227). To investigate the expression pattern of collapsin in mammals, the rat homologue was cloned from adult rat brain material by RT-PCR using primers based on the human semaphorin sequence. The PCR product covers the entire translated part of the collapsin mRNA and shows a sequence identity of ~82% to chicken collapsin and ~93% to human semaphorin. Cryostat sections of embryonic rat were analyzed for expression of collapsin by in situ hybridization, using an antisense cRNA probe labeled with digoxigenin-11-UTP. In sagital sections of embryonic day 15 rats, collapsin was found to be expressed in the basal telencephalon; the vestibular ganglion; in close contact with the olfactory epithelium; in the pons; the medulla; and the spinal cord. In transversal sections the ventral horn of the spinal cord and cells surrounding the central canal were stained. Collapsin shows a dynamic expression pattern in the nervous system, but is also expressed in non-neuronal tissue like intestine and muscle.

603.8

ROBUST OUTGROWTH AND PATTERNING OF AXONS FROM EMBRYONIC MOUSE XENOGRAFTS INTO NEONATAL RAT CORTEX. S.Jhaveri*[1] & M.H.Hankin[2]. [1]Deptartment of Brain & Cognitive Sciences, Mass. Institute of Technology, Cambridge, MA 02139; [2]Departments of Anatomy & Neurobiology, Medical College of Ohio, Toledo, OH 43614.

The capacity of homotopic and heterotopic cortical grafts to form patterned connections in the host brain was explored using a xenografting paradigm. Cortical tissue from embryonic (E15-18) mouse donors was transplanted to parietal or occipital cortex of neonatal rats. After 1-8 weeks, graft outgrowth into the recipient brain was visualized immunohistochemically, using the mouse-specific antibody M6 (Neurosci.Letts. 61:221, 1985).

Despite considerable variability in the extent of M6 immunoreactivity, some general observations can be made regarding donor tissue outgrowth. Invasion of neonatal rat cortex by transplant-derived fibers was extensive and frequently showed growth into the white matter; in some brains M6-positive axons could be traced rostrally from donor tissue in occipital cortex. Axons preferred to travel in supra- and infra-granular layers, avoiding layer IV. In fortuitous cases with grafts placed in the barrelfield cortex, M6-positive axons surrounded large clusters of host cells, reminiscent of the callosal projection to the barrel septae. Widespread migration of donor glia (assessed using the mouse glia-specific antibody M2) into the host tissue was evident. Fiber outgrowth was usually more widespread than that seen for glia; the fiber and glial outgrowth did not show a tight spatial relationship.

Based on results obtained so far, our working hypothesis is that projections from cortical xenografts grow out in a manner similar to that seen for cortical callosal afferents in normal animals.

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603.9

SYNAPTIC COMPETITION OBSERVED DURING THE REFORMATION OF A NEUROMUSCULAR TOPOGRAPHIC MAP. M.B. Laskowski*, H. Colman and J.W. Lichtman. WAMI Medical Program, University of Idaho, Moscow, ID 83844 and Dept. of Anatomy and Neurobiology, Washington University, St. Louis, MO 63110.

We have been studying the mechanisms whereby regenerating motoneurons reestablish a rostrocaudal bias on the surface of some skeletal muscles. The serratus anterior (SA) muscle of the rat displays a topographic map prior to birth, and the map is reestablished after denervation of the long thoracic nerve (LTN)(J. Neurosci. 8:3094, 1988). Here we explored the progress of the formation of this map under conditions of potentially enhanced synaptic competition. The SA muscles of 3-day old neonatal rat pups were denervated by crushing the LTN. Two days later the rostral (C6) branch to the LTN was crushed, and the caudal half of the muscle removed. This experimental approach allowed unopposed access of caudal (C7) motoneurons to vacated synaptic sites on the remaining rostral half of the SA. Intracellular recording revealed that 2 days after the second denervation, 62% of the reinnervated end-plates contained only axons from the C7 branch; 18% from C6; and 18% from both C6 and C7 branches. Four days after the second denervation only 14% of the reinnervated end-plates retained input solely by C7 axons; 52% by C6 only; and 33% by both C6 and C7. This progressive loss of input by C7 continued, so that after 6 days, axons from C6 were the sole input to reinnervated end-plates. During the transition from C7-dominated to C6-dominated input, we recorded from selected end-plates with inputs from both C6 and C7 axons. At end-plates co-innervated by C6 and C7 axons, the average quantal content from C6 was greater than C7. In some of these cases, we also noted a difference in quantal size. We have thus developed an experimental situation where axons are forced to compete with each other, and where the outcome is predictable: inputs from C6 axons will suppress inputs from C7 axons. Further, we have observed evidence of synaptic competition during this process, with significant differences in synaptic efficacy between the two inputs to an end-plate. Supported by NIH.

603.10

EARLY BORN CHOLINERGIC NEURONS HAVE A PREFERENCE FOR THE STRIATAL PATCH COMPARTMENT, WHILE LATE BORN CHOLINERGIC NEURONS ARE MORE LIKELY TO BE LOCATED IN THE MATRIX COMPARTMENT. E.H.S. van Vulpen* and D. van der Kooy. Neurobiology Research Group, Dept Anatomy, University of Toronto, Toronto, Ontario, M5S 1A8.

The striatal patch and matrix compartment neurons are born at different times during rat development. The majority of the early born neurons (embryonic (E) days 13-17) preferentially end up in the patch compartment, while the majority of the later born neurons (E18-postnatal (P) day 2) end up in the matrix compartment. Although the cholinergic interneurons are born early in neurogenesis (between E12 and E17), and we would therefore expect them to be located mainly in the patches, they are relatively homogeneously distributed in the adult, with a preference for the matrix area just outside the patches (intermediate zone-IZ). To ask if birthdate can predict the localization of cholinergic neurons in the striatum, we marked new postmitotic neurons in the embryo with a maternal injection of bromodeoxyuridine (BRDU) on E13, E15 or E17. The pups were sacrificed at P40 and the tissue was processed for BRDU and choline acetyltransferase double labelling. Cholinergic neurons that became postmitotic at E13, had a significantly higher chance of ending up in the patch compartment compared to either IZ or matrix. On the other hand cholinergic neurons that became postmitotic at E17 had a higher chance of ending up in either the IZ or the rest of the matrix compartment. We conclude that birthdate can predict compartmental localization, with cholinergic neurons in the intermediate zone following the same pattern as the cholinergic neurons in the rest of the matrix compartment. Thus, cholinergic neurons show the same relative birthdate/compartment relationship as to other striatal neurons, although the absolute birthdates of cholinergic neurons are shifted earlier in neurogenesis. Because selective adhesiveness of the early born patch neurons is thought to be important in the formation of patches, we predict that only the earliest born cholinergic neurons in the patches will show adhesive properties.

603.11

NEURON-SPECIFIC GENE EXPRESSION DURING DEVELOPMENT IN A BINARY TRANSGENIC MOUSE SYSTEM. C. Kappen*, D. P. Gardner, M. P. Macias and P. J. Yaworsky. S. C. Johnson Medical Research Center, Mayo Clinic Scottsdale, Scottsdale, AZ, 85259.

Transgenic mouse technology has been widely used to study gene expression and function in the developing embryo and in adult tissues. Many transgene products, however, interfere with embryonic development precluding the establishment of permanent transgenic mouse lines. To overcome these limitations, we utilize a binary system of gene regulation in transgenic mice. This system consists of two components in independently transgenic mouse lines. One transgenic mouse strain carries a silent transgene (transresponder) that is activated only when a transcriptional activator is supplied by a second transgenic mouse line (transactivator). This transgenic mouse line harbors the gene for the potent viral transcriptional activator VP16, under the control of a neuron-specific promoter. The parental strains can be maintained independently without phenotypic effects, and double transgenic embryos and offspring can be generated reproducibly simply by breeding. This system affords the ability to study genes that could disrupt normal embryonic development.

We here report transactivation of transgenes in this system specifically in the developing nervous system. We have created transgenic mice in which VP16 expression is controlled by the promoter of the murine neurofilament light subunit (NF-L) gene that directs transgene expression in differentiating neurons. Additional transactivator mice have been generated that express VP16 under control of the rat nestin gene enhancer. This regulatory element directs transgene expression to proliferating neuroepithelial precursor cells. We have crossed these transactivator strains to transresponder mice carrying a LacZ reporter gene and examined embryos at various stages for β-galactosidase activity. The patterns of LacZ activity indicate that transactivation is specific to precursors and early differentiation stages of neurons in the developing brain and spinal cord. Our goal is to apply the VP16-based binary transgenic system to investigate the role of homeobox genes for regional and cell-type specification in the developing nervous system.

603.12

Regulation of ventral polarity in the vertebrate CNS by a 19 kiloDalton form of Sonic hedgehog.

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Patterning of the vertebrate central nervous system (CNS) depends on cell interactions. For example, induction of a specialized population of ventral midline cells, the floor plate require signaling from the underlying notochord, whereas signals from the notochord and floor plate induce motor neuron differentiation. Experiments in a variety of vertebrate embryos and in neural explants in vitro indicate that Sonic hedgehog (Shh) can mediate floor plate induction. Shh encodes a signal which is implicated in both short and long range interactions that pattern the CNS, somite and limb. Shh is processed to generate two peptides (19 kiloDalton and 26/27 kiloDalton) both of which are secreted. However, the in vivo distribution of Shh peptides in relation with these patterning events, has not been reported. To investigate Shh distribution in vivo we have generated peptide specific antibodies. Immunostaining of chick and mouse embryos indicates that Shh peptides are expressed in the notochord and floor plate at the appropriate time for their postulated patterning functions. To further investigate Shh inductive abilities we have applied recombinant Shh peptides to neural explant in serum free medium.

High concentrations of Shh bound to a matrix induce floor plate and motor neurons, whilst addition of Shh to the medium leads to a dose dependent induction of motor neurons. All inducing activity resides in a highly conserved 19 kiloDalton amino terminal peptide. Moreover, antibodies which specifically recognize this peptide block induction of motor neurons by the notochord. We propose that Shh acts as a morphogen to induce distinct ventral cell types in the vertebrate central nervous system.

*non contributing author

603.13

TROPOMYOSIN EXPRESSION MARKS SEGMENT BOUNDARIES AND EARLY SEGREGATION OF NEUROEPITHELIUM IN THE DEVELOPING RAT BRAIN.

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Segmentation is important in the patterning of the vertebrate hindbrain. These segments, rhombomeres, are morphologically similar units which eventually develop characteristic, segment-specific structures. It has been proposed recently that similar segmentation exists in the developing prosencephalon, dividing it into prosomeres. While numerous genes have been identified that specify individual segments, the cellular and molecular nature of the boundaries between adjacent segments is not clear. Immunocytochemical mapping in early embryos was performed with TM311, a monclonal antibody recognizing an epitope encoded by exon 1a of the rat α-tropomyosin (TM) gene, found in all 284 residue (long) TMs. At embryonic day (E)11/12, TM specifically marks boundaries between rhombomeres in the hindbrain. At E12, TM is expressed in neuroepithelial cells at the: 1) caudal boundary of P1, between the pretectum and tectum; 2) P2-P3 boundary including the zona limitans intrathalamica; 3) P3-P4 boundary between the ventral thalamus and hypothalamus; and 4) P5-P6 boundary between the preoptic areas and presumptive basal ganglia. Interestingly, TM immunoreactivity at boundaries is usually accompanied by morphological distinctions, suggesting a structural role for TM in shaping the neuroepithelium. Furthermore, TM is expressed at E11 in cells that appear to serve as forerunners of the choroid plexus (CP), almost 3 days prior to CP expansion into the lateral ventricle. At E14, TM marks the ventro-medial wall of the dorsal telencephalon, an area thought to be presumptive hippocampus. These cells are not immunoreactive for a neuron-specific marker, microtubule associated protein 1B (MAP1B), indicating that the neuroepithelium that forms the choroid plexus is functionally segregated early from neuronal precursors. The specific TM expression patterns may reflect a role for the protein in dynamic regulation of cell shape in specific brain regions during embryogenesis. Supported by NIMH45507 (PL) and HL35726 (SEHD).

603.14

RE-CONSTRUCTION OF THE MOUSE *Hox-b3* GENE EXPRESSION PATTERN IN TRANSGENIC Mice. C.T.Kwan, P.M.Chiu, [a]R.Krumlauf and M.H.Sham. (SPON: The Hong Kong Society of Neurosciences). Department of Biochemistry, University of Hong Kong, Sassoon Road, Hong Kong. [a]National Institute for Medical Research, Mill Hill, London, NW7 1AA, U.K..

Developing hindbrain are subdivided into repeated morphological units, known as rhombomere in which each of them can establish a distinct phenotype. *Hox-b3* gene belongs to a class of gene, which is involved in pattern formation, expresses in the developing hindbrain with an anterior boundary in rhombomere 5 (r5). To identify the regulatory elements in the large genomic region of nearly 30 kb of the *Hox-b* cluster, we generated *lacZ* reporter gene constructs with a series of genomic sequences flanking the *Hox-b3* gene. Studies of the whole mount *lacZ* expression patterns revealed that different genomic sequences can specifically direct expression in wide range of tissues including hindbrain, spinal cord and mesodermal derivatives in transgenic embryos. Each of these elements can impose a subset of *Hoxb-3* expression patterns in transgenic mice. Of these, two neural specific elements can direct *lacZ* expression in (r5) while another one in r6, r7 and the spinal cord. Recombinant reporter gene construct containing both of these two elements direct *lacZ* expression from the spinal cord, extending to the hindbrain with a limit of expression at r5. Interestingly the expression level in both r5 and r6 were higher than the remaining CNS regions. We have also isolated mesodermal specific element which direct *lacZ* expression in the somites of the 9.5 dpc embryo. Histological studies of the 12.5 dpc transgenic embryo revealed that expression were also found in the fore- and hindlimb buds, stomach, heart, pancreas and muscle surrounding the vertebrae. When genomic regions containing the tissue specific regulatory elements were introduced into transgenic mice, the expression of the reporter gene was detected in all the endogenous expression domains. Moreover, higher level of *lacZ* expression was detected in r5. Our results suggest that the cis-acting elements identified by transgenic mice analyses are able to direct spatially-specific expression of *Hoxb-3*, and when recombined, could re-construct the endogenous pattern.

603.15

A NOVEL PREPARATION FOR INVESTIGATING TRIGEMINAL CONNECTIONS IN FETAL RATS. P.M.E. Waite*, S. Ho and T. A. Henderson, Anatomy, UNSW, Sydney; Devel. Neurobiol, ANU, Canberra, Australia; Neurology, Washington Univ., St Louis, MO 63110.

Sensory connections from trigeminal ganglion cells innervating rat facial skin and whiskers grow into the brainstem from embryonic day (E) 13 and are topographically organised (Erzurumlu & Jhaveri '92, J. Neurosci. 12:3946). However, it is not known whether individual arbours are initially circumscribed and precisely localised or are initially exuberant and later pruned back. We utilised a novel method to investigate this early arbour development. The preparation consists of a horizontal 'slice' through the head, containing the base of the skull, the maxillary and mandibular processes and the brainstem, maintained in oxygenated Kreb's solution at 27°C. The trigeminal ganglion and its peripheral and central connections are preserved intact and are accessible for recording and labelling. Injections of 6% neurobiotin have been made into the trigeminal ganglion of rat pups from E14-E21. At E14 axons are present throughout the trigeminal tract from the pons to upper medulla but no collaterals are seen in the trigeminal nuclei. By E15, simple collaterals are present in the trigeminal nuclei at all levels. Two days later individual collaterals are relatively widespread and overlapping, with arbours covering, on average, 2.1% of the cross-sectional area of the principal nucleus. By E19, single collaterals are more densely branched and restricted in size, with the cross-sectional area reduced to 1.2% of the total nuclear area. This compares with the value of 0.75% for the cross-sectional areas of individual arbours, relative to the total area of the principal nucleus, in adult rats (Shang et al. '93, Soc Neurosci Abs. 19: 326). These results indicate that the central arbours of trigeminal afferents are relatively more widespread during an early stage of development than in mature animals. Supported by NH&MRC, (PW) and DE07734 (TH).

603.16

REGULATION OF COLLAGEN TYPE XIV EXPRESSION IN THE EARLY CHICK EMBRYO AND ITS FUNCTION. S. Tono-oka[1,3], M. Fukushima[1], S. Tanase[2], K. Ohta[1], T. Miike[3], and H. Tanaka[1]*, [1]Div. Dev. Neurobiol., Kumamoto Univ. Graduate Sch. Med. Sciences, Kuhonji 4, Kumamoto 862, [2]Dept. of Biochemistry, [3]Dept. of Child Development, Kumamoto Univ. Sch. Med., Honjo 2, Kumamoto 860, Japan

We isolated a monoclonal antibody, called DBM, that uniquely stained dorsal aspect of the spinal cord basement membrane. Asymmetric antibody staining on the spinal cord basement membrane was detected just after neural tube closure. DBM antigen was restricted to only on the ectoderm, but not on the endoderm nor notochord basement membrane at early developmental stages and distributed with the dorsoventral gradient at the entire body. DBM staining in the main muscle connective tissue was developmentally transient and reappeared after denervation. Furthermore, since notochord basement membrane became to express DBM antigen after neural tube removal or transplantation under the skin ectoderm, DBM expression on the neural tube basement membrane was appeared to be regulated by skin ectoderm.

DBM antigenic molecule was purified from E 10-12 whole chik embryos and found to be FACIT (fibril associated collagens with interrupted triple helices) collagen type XIV. It seems to control the temporal and spatial arrangement of collagen fibrils that are specific to the tissues. These unique expression and regulation of collagen type XIV in the basement membrane suggest its importance of body development. We are now examining the roles of collagen type XIV on cell adhesion and neurite outgrowth of neural tube cells in cell culture.

603.17

FORMATION OF THE TAIL SPINAL CORD *IN VITRO*. R.D. Heathcote*, N. Vali, and A. Chen, Department of Biological Sciences, University of Wisconsin-Milwaukee, Box 413, Milwaukee, WI 53201.

In the frog *Xenopus laevis*, new spinal cord forms as the tail elongates. Recent work in other laboratories suggests that the "tail organizer" derives from the dorsal lip of the blastopore, or Spemann's organizer. Studies in our laboratory showed that neural plate explants containing the tail organizer differentiated tail-like structures, including notochord and neural tube. To determine whether these tail structures resulted from the differentiation of new tissue, we surgically removed the notochord from neural plate explants and tested for notochord differentiation in the resulting tails. At the time of surgery (stage 14/15), notochord is immunoreactive for the notochord marker keratan sulphate (monoclonal antibody MZ-15; courtesy of Dr. F. Watt). Removal of the notochord eliminated staining; however, after two to three days in culture, neural plates had tails that contained differentiated notochord immunoreactive for MZ-15. Thus notochord and presumably other tissue in the growing tail must come from new differentiation.

Neural tissue resembling spinal cord associated with notochord in the tail structures formed *in vitro*. We used the organization of catecholaminergic neurons to test whether normal spinal cord morphogenesis occurred *in vitro*. In the spinal cord, catecholaminergic neurons form two longitudinal columns of dispersed cells. In neural plate explants without tail organizer, catecholaminergic neurons differentiated in a dispersed pattern, but lacked longitudinal columns. In explants with tail organizer, catecholaminergic neurons associated with new notochord and formed longitudinal columns of dispersed cells. These results indicate that differentiation and morphogenesis of some populations of tail spinal cord neurons occur *in vitro*.

603.18

SECONDARY NEURULATION IN TELEOSTS OCCURS BY APOPTOSIS.

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In most vertebrates, primary neurulation occurs by elevation and medial folding of the neural plate resulting in the neural plate. In teleost fishes however, the neural tube forms as a solid mass of cells, the medullary cord, that opens by a process referred to as secondary neurulation or cavitation. The mechanism of cavitation has been in question, but one hypothesis is that programmed cell death ablates cells centrally to open the medullary cord. Apoptosis, a type of programmed cell death involves the cleavage of genomic DNA. Cells undergoing apoptosis can be identified in tissue sections by performing an *in situ* nick translation reaction. In this study, catfish embryos (*Ictalurus punctatus*) were collected and fixed during the time of cavitation. Apoptotic cells were detected in the developing neural tube, particularly within the center region prior to apparent cavitation. These results suggest that programmed cell death is partly responsible for the establishment of early CNS of teleosts.

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604.1

DISTRIBUTION OF INJECTED ^{59}Fe AND ^{54}Mn IN THE HYPOTRANSFERRINEMIC (Hp) MOUSE. T.K. Dickinson, A.G. Devenyi, & J.R. Connor*, Penn State University, Hershey Medical Center, Hershey, PA 17033.

The hypotransferrinemic (Hp) mouse has a mutation in the transferrin (Tf) gene causing defective splicing of a Tf precursor mRNA. This results in production of <1% of the normal circulating level of Tf. Homozygous mutant animals require supplemental injections of mouse serum of purified Tf. Heterozygote (Hh) Hp animals have approximately 50% of normal circulating Tf levels require no supplemental Tf and live a normal life span. Hh animals are, however, subject to abnormal iron (Fe) deposition with age. For this study, adult wild type (WT), Hh and mutant Hp mice were injected with ^{59}Fe or manganese (^{54}Mn) to determine their distribu-tion in Tf-deficient animals. Our goal is to utilize the mutant and Hh Hp mice to further our understanding of Tf's role in normal Fe and Mn mobilization and diseases in which brain Fe (Parkinson's, Alzheimer's) or brain Mn (chronic liver disease) are altered. Animals received a single injection and were sacrificed at 24 hours, 7 days and 4 weeks. Analysis included brain, liver, spleen, heart, plasma and blood formed elements. Over the 4 week period, plasma, brain and heart levels of Fe and Mn remained constant whereas liver and spleen levels fell. WT and Hh animals had similar Fe and Mn arguing that 50% Tf levels are sufficient for normal Fe and Mn delivery to tissue. Alterations in both Fe and Mn delivery to liver and spleen in mutant animals is evidence for Tf's role in proper tissue targeting. Similarity in brain levels of Fe and Mn in all three animals argues for the existence of a significant, non-Tf mediated, Fe and Mn delivery to brain.

604.2

S100 AND CALMODULIN EXPRESSION IN DIABETES. J. Chessher, N. Gardner, S. Schaffer, G. Wilson, W. E. Zimmer, and D. B. Zimmer.* Departments of Pharmacology and Structural and Cellular Biology, University of South Alabama, Mobile, AL 36688.

The calcium-modulated proteins S100A1, S100B, and calmodulin are expressed at high levels in the nervous system. Altered expression of these proteins has been documented in many disease states in which intracellular calcium levels are altered, including some neurodegenerative disorders and diabetes. In order to better understand the mechanisms which regulate calcium-modulated protein expression in diabetes, we have examined the steady-state mRNA levels for S100A1, S100B, and calmodulin in Type I and Type II diabetic rats and age-matched controls. Total RNA from aorta, fast and slow-twitch muscle fibers, liver, heart, brain, large intestine, kidney, white and brown fat, and spleen was blotted onto nylon membranes. The resulting filters were hybridized with probes specific for the single rat S100A1 or S100B mRNAs or the multiple rat calmodulin mRNAs produced by each of the three rat calmodulin genes. In the brain, S100A1 and S100B mRNA levels were lower in animals with Type I diabetes when compared to control animals. However, S100A1 levels were decreased and S100B levels unaltered in animals with Type II diabetes. The levels of the three calmodulin mRNAs were unaltered in Type I diabetes while the level of two calmodulin mRNAs decreased in Type II diabetes. The mRNA levels for these proteins also changed in the other tissues examined, but these changes did not always parallel those observed in brain. These results demonstrate that the diabetic state does not coordinately regulate the expression of these calcium-binding proteins. Thus, the contribution of these gene products to the expression of the diabetic phenotype will be specific for the diabetic state and will vary from tissue to tissue.
Supported by grants from the NIH (30660 to DBZ and ES-03456 to GW)

604.3

IMMUNOHISTCHEMICAL LOCALIZATION OF GLIAL CELL-LINE DERIVED NEUROTROPHIC FACTOR IN THE HUMAN BRAINS OF NORMAL AND PARKINSONIAN CASES. A. Matsuo, D.G. Walker, P. L. McGeer and E. G. McGeer*. Kinsmen Lab. of Neurol. Res., Univ. of British Columbia, Vancouver, B.C., Canada, V6T 1Z3.

Glial cell-line derived neurotrophic factor (GDNF) is a recently identified protein, known to be produced by astrocytes, and known to have protective effects on dopamine neurons. We employed immunohistochemistry to study the cellular localization of GDNF in normal and Parkinson's disease (PD) cases. In normal brains, GDNF was found as granular deposits on woolly fibers in the substantia nigra (SN) and globus pallidus, especially in the internal segment. It had a more distributed granular localization in the neuropil of the caudate and putamen. Double immunostaining established that the woolly fibers were substance-P positive. Some neuronal nuclei in the SN, caudate, putamen and thalamus were more smoothly stained, suggesting a translocation to nuclei. In PD SN, GDNF staining was less intense than in controls. We detected GDNF mRNA expression in the SN of normal and PD cases using the reverse transcriptase-polymerase chain reaction technique, establishing local production. This may be from astrocytes, since occasional astrocytes in the area were GDNF positive.

The present study supports a role for GDNF in basal ganglia function. Changes of GDNF expression in Parkinsonian brains could be related to the pathology of the disease. (Supported by a grant from the Parkinson Foundation of Canada.)

604.4

THYROID HORMONE DEFICIENCY DISRUPTS THE DEVELOPMENTAL PATTERN OF EXPRESSION OF NEUROTROPHINS AND THEIR RECEPTORS IN THE CEREBELLUM.
Isabelle Neveu*, Hiroshi Funakoshi and Ernest Arenas. Lab of Molecular Neurobiology, Karolinska Institute, S-17177 Stockholm, Sweden.

Neurotrophic factors play important roles in neuronal development and are likely to participate in the development of the cerebellum. Alteration in the expression of neurotrophins and/or their receptors may contribute to the impairment observed in the development of the cerebellum in case of thyroid hormone (T3) deficiency. Therefore, we have compared the developmental expression of neurotrophins and their receptors in the cerebellum of normal and hypothyroid rats. RNAse protection assay did not show any significant changes in the levels of the high-affinity receptors such as full length trk B and trk C mRNAs. However, we have observed a retardation in the downregulation of the low-affinity receptor, P75^{NGFR}, which resulted in 2-3 times higher levels of P75^{NGFR}mRNA at postnatal day 10. T3 deficiency also disrupted the developmental pattern of neurotrophins expression. In normal rats, the levels of BDNF, which were relatively low during the first two postnatal weeks, dramatically increased from P15. This upregulation appeared to be strongly impaired in hypothyroid rats since the BDNF mRNA levels was 4-fold lower in treated rats compared to controls. T3 deficiency also disrupted the transient increase of NT-3 mRNA levels which occurs in normal rats during the second postnatal week. These results suggest that alterations in BDNF and NT-3 expression may contribute to the impairments observed in the development of the cerebellum in hypothyroid rats. To test this hypothesis, we have grafted in the cerebellum of newborn rats, genetically-modified cell lines producing NT-3 or BDNF and we are currently studying if supplying neurotrophins would prevent some impairments occuring during the development of the cerebellum in hypothyroid rats.

604.5

NEUROTROPHIC FACTOR EXPRESSION IN CULTURED ASTROCYTES FROM NEONATAL, ADULT AND INJURED RAT BRAIN. Vivian W. Wu and Joan P. Schwartz*. Clinical Neuroscience Branch, NINDS, NIH, Bethesda, MD 20892

Much interest has focused on the role of cytokines as modulators of neurotrophic factor production by astrocytes in brain, particularly after injury when cytokine levels are increased. Astrocytes cultured from 6-hydroxydopamine (6-OHDA) lesioned brain can be distinguished from normal adult astrocytes on the basis of enhanced staining with either of two monoclonal antibodies (13A11 and 01E4; gift of Dennis Landis) which bind to an epitope present only in reactive astrocytes in vivo. Cultured astrocytes from neonatal animals show an intermediate degree of staining. Comparable differences are seen in GFAP staining. Levels of NGF mRNA were compared in astrocytes cultured from postnatal day 2 rats with those from adult rats with or without 6-OHDA lesion. Interleukin-1β (IL-1ß), tumor necrosis factor-α (TNF-α) and interferon -γ (IFN-γ) increase NGF mRNA expression in neonatal striatal and cortical astrocytes, but had only minimal effect in quiescent adult striatal and cortical astrocytes. IL-1β treatment also caused a significant increase in NGF mRNA in reactive striatal astrocytes prepared 7 days after 6-OHDA injection relative to those prepared from saline-injected brain. The effects of TNF-α and IFN-γ are now being tested. These cultures will allow us to study the processes by which quiescent astrocytes in adult brain convert to reactive astrocytes following injury.

604.6

REGULATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) PROTEIN LEVELS IN RAT BRAIN FOLLOWING GLOBAL FOREBRAIN ISCHEMIA Zaal Kokaia[1]*, Hiroyuki Nawa[2], Hiroyuki Uchino[3], Eskil Elmér[1], Merab Kokaia[1], Maj-Lis Smith[3], Josette Carnahan[4], Bo K. Siesjö[3] and Olle Lindvall[1]
[1] Restorative Neurology Unit, Department of Neurology, University Hospital, S-221 85 Lund, Sweden; [2] Beckman Neuroscience Center, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 11724, USA; [3] Laboratory of Experimental Brain Research, University Hospital, S-221 85 Lund, Sweden; [4] Amgen Center, Thousand Oaks, California 91320, USA.

In previous studies, we have demonstrated differential and insult-specific changes of BDNF mRNA in different structures of the brain after kindling-evoked seizures, global forebrain ischemia, cortical spreading depression and hypoglycemic coma. Whether these changes of mRNA levels lead to the presumed alterations of BDNF protein levels has been unclear. Here we show differential changes of BDNF protein levels in the hippocampus and cortex of animals subjected to forebrain ischemia.

Male Wistar rats (n=24) were anesthetized with halothane and global forebrain ischemia was then induced for 10 min by bilateral occlusion of the common carotid arteries combined with hypotension. The animals were sacrificed after 6 h, 12 h, 24 h and 1 week of recovery. After decapitation the brains were immediately removed and dentate gyrus, hippocampal CA1 and CA3 regions and parietal cortex were dissected bilaterally. BDNF protein levels were measured using a two-site enzymatic immuno-assay which detects trace amounts of BDNF protein (>1 pg/assay) (Nawa *et al.*, 1995).

Following global forebrain ischemia there was an increase of BDNF protein levels in the dentate gyrus and the CA3 region (6 h and 1 week after the ischemic insult, respectively). In contrast, in the CA1 region and the parietal cortex, which show selective neuronal damage after the forebrain ischemia, reduced levels of BDNF protein were detected 24 h after the insult. These data show that insult-induced changes of BDNF gene expression are followed by alterations of BDNF protein levels. Our findings are in good agreement with the hypothesis that BDNF has a neuroprotective role after brain insults.

604.7

INCREASED EXPRESSION OF CNTF mRNA IN RAT HIPPOCAMPUS FOLLOWING ENTORHINAL CORTEX ABLATION A. G. Woods*, K. M. Guthrie and C. M. Gall, Departments of Anatomy and Neurobiology, and Psychobiology, University of California, Irvine, CA 92717.

Ciliary neurotrophic factor (CNTF) has been demonstrated to promote neuronal survival both *in vivo* and *in vitro*, and to induce the differentiation of both neurons and astrocytes. In the peripheral nervous system, CNTF mRNA is primarily localized to Schwann cells. In the normal adult central nervous system, the highest levels CNTF mRNA are found in the olfactory bulb and in areas of white matter, such as the optic nerve and spinal cord. Application of CNTF induces axonal sprouting and reinnervation of muscle in the periphery, raising the possibility that it may also facilitate sprouting in brain. We have recently shown that the mRNA for insulin-like growth factor-1 (IGF-1) increases with a temporal and spatial correspondence to reactive hippocampal sprouting which occurs following entorhinal cortex lesion. Using *in situ* hybridization we examined levels of CNTF mRNA in the brains of rats sacrificed at 3, 5, 7, 10 and 14 days following unilateral lesion of entorhinal cortex. CNTF mRNA was increased with a similar time course and distribution to IGF-1 mRNA, although CNTF mRNA appeared to be expressed slightly earlier and was primarily concentrated in areas of white matter. At 3 days postlesion, substantial increases in CNTF cRNA hybridization occurred in the dentate gyrus outer molecular layer and hilus, stratum lacunosum moleculare, fimbria and alveus. This effect occurred bilaterally, although hybridization density was greater in the hippocampus ipsilateral to the lesion. By 10 days postlesion, hybridization in most areas had returned to control levels, although labeling was still evident in corpus callosum at 14 days. Dense hybridization was also observed at the site of the lesion beginning at three days, and remained elevated through 14 days. These data indicate that CNTF mRNA expression by oligodendroglia is increased in regions containing degenerating axons and that this factor may play a role in reactive axonal growth in brain. Supported by NIA grant AG 00538.

604.8

NEUROTROPHIN EXPRESSION IN RAT BRAIN AFTER IMMUNOLESION. J.Yu* and R. Perez-Polo, Dept. Of Human Biological Chemistry and Genetics, Univ. of Texas Medical Branch, Galveston, Texas 77555-0652.

Neurotrophin is expressed at high levels in adult hippocampus, and to a lesser extent, in cerebral cortex, two principal targets of the ascending basal forebrain cholinergic projection systems. Since neuronal activity imposes a regulatory influence over the expression of NGF and BDNF genes in adult hippocampus, the topographical correlation of neurotrophin expression with cholinergic terminal distribution from septal cholinergic neurons raises the question as to wether the cholinergic system regulates the synthesis of neurotrophins. Studies using fimbria-fornix transection or pharmacological manipulations have been controversial, since they cause confounding changes to the cholinergic and other neurotransmitter systems. Our aim was to examine the importance of cholinergic regulation of neurotrophin synthesis by intraventricular injections of 192 IgG-saporin, a cytotoxin to septal cholinergic neurons that extensively and selectively kills $p75^{NGFR}$-bearing neurons in basal forebrain. We showed that 192 IgG-saporin resulted in profound decreases in $p75^{NGFR}$ and *trkA* mRNA levels in the basal forebrain at 1 week and up to 5 months after injection, but there were no significant decreases of $p75^{NGFR}$ and/or *trkA* expressions in striatum and cerebellum. Ribonuclease protection assays showed that the cholinergic deafferentation of the hippocampus, cortex, and olfactory bulb-the three main targets of basal forebrain cholinergic neurons-failed to produce any significant changes in NGF, BDNF and/or NT-3 mRNA levels there. Thus, the cholinergic system in the adult rat brain does not influence neurotrophin expression in a significant way and the reported effects of fimbria-fornix transection and pharmacological manipulations on neurotrophin expression in adult hippocampus may be mediated via other neurotransmitter or signalling mechanisms. Supported in part by NINDS NS18708. This is publication #28A and supported by USPHS grant P01AG10514 awarded by NIA.

604.9

NEUROTROPHIC FACTOR EXPRESSION IN THE RAT BRAIN FOLLOWING CYTOTOXIC LESIONS TO THE HIPPOCAMPAL DENTATE GYRUS GRANULAR CELLS. C.R. Breese*, J. Logel, C. Drebing, C.E. Adams, Y. Rollins, S. Leonard, Department of Pharmacology, University of Colorado Health Sciences Center, and Veterans Administration Medical Center, Denver, CO 80262.

Previous studies from our laboratory have shown that intradentate cytotoxic colchicine injections induces a substantial increase in IGF-1 gene expression in lesioned dentate gyrus and the damaged cortex (Neuroscience Abstracts 541.1, 1994). In the present study, the temporal and cellular expression of neurotrophic factors from several different families were examined in normal and lesioned rat brains, including, BDNF, CNTF, aFGF, bFGF, FGF-5, GDNF, NGF, NT-3, and TGFβ1. In comparison, IGF-1 demonstrated the highest levels of expression in the lesioned hippocampus and damaged cortex. A large, temporally dependent increase was observed for BDNF in both ipsilateral and contralateral hippocampal CA3 neurons and dentate gyrus granular cells. There was only a negligible increase in bFGF, NGF, and TGFβ1 gene expression in the lesioned hippocampus; with expressional changes primarily restricted to later post-lesion time points (3-10 days). The other neurotrophic factors examined were either not expressed at detectable levels in the hippocampus (CNTF, aFGF, and GDNF), or demonstrated little or no change as a result of the lesion (FGF-5 and NT-3). While the present results demonstrate potential role for different neurotrophic factors in coordinating the post-injury response in the brain, particularly that of IGF-1. Compared to IGF-1, the lesion induced expressional changes in the neurotrophic factors examined were temporally correlated, and therefore, probably not directly involved with the temporal coordination of IGF-1 expression, but may provide for other important aspects of the post-traumatic injury response in the CNS.

604.10

EXPRESSION OF NEUROTROPHIC FACTORS IN A DEMYELINATING NEUROPATHY. H. Hyman Friedman, T.N. Jelsma*, Y.-C. Wang, G.M. Bray, and A.J. Aguayo, The Montreal General Hospital Research Institute, 1650 Cedar Ave., Montreal, P.Q., H3G 1A4.

Peripheral nerve transection is known to result in a strong upregulation of NGF and BDNF mRNA expression in the degenerating distal stump. Concomitantly, CNTF expression is reduced more than ten fold. On the other hand, the patterns of neurotrophin expression in demyelinating diseases of the peripheral nervous system have not been investigated. Trembler (Tr) mice have a dominantly inherited demyelinating neuropathy due to a point mutation in the pmp22 gene. In this neuropathy, there is incomplete ensheathment of axons by myelin-forming Schwann cells, myelin breakdown, and Schwann cell proliferation throughout the life of the animal. To determine if the abnormal Schwann cell phenotype affects the expression of neurotrophic factors, we compared mRNA levels of BDNF, NGF, and CNTF in Tr and control peripheral nerves. Using a sensitive RT-PCR quantitation technique, the levels of BDNF mRNA and of NGF mRNA were similar in intact and Tr nerves. In contrast, the level of CNTF mRNA was dramatically reduced in the Tr nerve. The levels of expression of neurotrophic factors in sciatic nerves following transection were also tested to determine if Tr myelin-forming Schwann cells maintain the ability to upregulate BDNF and NGF. Two weeks after transection, the levels of BDNF and NGF mRNAs were increased in the distal stumps of both intact and Tr nerves. These results indicate that demyelination and Schwann cell proliferation in the Trembler mouse are not accompanied by an increase in neurotrophin expression as seen after axonal breakdown; however, both cause a downregulation of CNTF mRNA. The effects of such changes on neuronal function have not been clarified.

604.11

EXPRESSION OF P75 (LOW AFFINITY NGF RECEPTOR) IN SCHWANN CELLS (SC) FOLLOWING LONG TERM DENERVATION. S. You, Th. Petrov, P.H. Chung, R.S. Smith* and T. Gordon, Dept. of Pharmacology and Div. of Neuroscience, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2S2.

p75 is synthesized in SC in the distal stump of injured peripheral nerves. We have used short term (1 week) and long term (1- 12 months) transected distal sciatic nerves of the rat to determine the variations in the expression of p75 by using immunocytochemistry and *in situ* hybridization.

Semi-quantitative analysis of the intensity of the immunocytochemical reaction revealed that the synthesis of the p75 protein is rapidly enhanced to reach a peak at 1 month after denervation. Subsequently it decreased and was almost undetectable 6 months following denervation. Double immunocytochemistry for p75 and the S-100 protein (an excellent marker for SC) confirmed that the p75 immunoreactivity is confined to the SC. In our *in situ* hybridization experiments the mRNA was detected by autoradiography. Quantitative analysis revealed that the upregulation of the p75 mRNA coincides in time with the enhanced synthesis of the p75 protein and reaches a peak at 1 month after denervation. However, the downregulation at later stages is more rapid and after the 4 months p75 mRNA was undetectable.

The presence of p75 in the SC is necessary for regeneration, and our results suggest that, the SC's will ensure support for regeneration primarily in the 1st month after the denervation. The lack of p75 in long-term denervated distal nerve stumps may be one of the factors that contribute for unsatisfactory functional recovery after prolonged denervation.

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604.12

BDNF GENE EXPRESSION IN CULTURED RAT ASTROCYTE IS ENHANCED UPON GLIA DAMAGE M.J.Tsai*, W.Y.Lee, H.C.Hung, A.M.Huang and E.H.Y.Lee Institute of Biomedical Sci., Academia Sinica, Taipei 115, Taiwan, R.O.C.

Brain-derived neurotrophic factor (BDNF) has been shown to be a neurotrophic factor for several types of neurons. Recent study indicates the presence of BDNF gene also in astrocytes. The present work examined the possible role of BDNF also as a trophic factor for glial cells. Cultured rat and mouse astrocytes were used. 1-Methyl-4-phenylpyridinium (MPP^+) and α-amino-adipate (α-AA) were adopted as pharmacological tools to destroy glial cells and the quantitative RT-PCR method was used to assess BDNF gene expression. Results indicated that MPP^+ (10-75 μM) produced a dose-dependent loss of cell viability as judged by the amount of lactate dehydrogenase (LDH) released into the incubation medium in rat astrocyte, while it also markedly elevated BDNF gene expression in a dose-response fashion. However, MPP^+ (5-40 μM) also produced a dose-dependent cell damage in mouse astrocyte, while BDNF gene expression was not increased accordingly. The gliotoxin α-AA yielded similar results. These results together partially explain the differential vulnerability of mouse and rat to MPP^+ toxicity and suggest that BDNF may serve as a trophic factor for rat astrocyte cells.

604.13

EXPERIMENTALLY INDUCED DIABETES ALTERS TrK A GENE EXPRESSION IN PRIMARY SENSORY NEURONS OF RATS Alison S. Depto, Francis J. Liuzzi* and Aaron I. Vinik. The Diabetic Neuropathy Study Group, The Diabetes Institutes and Departments of Internal Medicine, and Anatomy and Neurobiology, Eastern Virginia Med. Sch. Norfolk, VA 23501

Diabetic neuropathy has significant sensory and autonomic components as well as impaired nerve regenerative capacity. Abnormalities in expression of nerve growth factor (NGF) or its receptors may be involved. NGF regulates expression of the preprotachykinin gene products which are decreased in diabetes. Tyrosine receptor kinase (trk) A, the high affinity receptor for NGF, is necessary for survival, maintenance, and growth of a subset of sensory neurons. In this study, we used in situ hybridization and Northern blot analyses to examine expression of trk A in L4 and L5 dorsal root ganglion (DRG) neurons from normal and streptozotocin-induced diabetic rats. Overall levels of trk A was approximately two fold higher in DRG neurons from diabetic animals. TrkA mRNA were expressed by 33% of neurons in both groups, To examine expression of trk A during nerve regeneration, normal and diabetic animals were subjected to sciatic nerve transection, and silicon tube implantation, followed by 4 weeks of nerve regeneration. Axotomy decreased expression of trk A in both regenerative groups by approximately half compared to DRGs from non-axotomized counterparts. In normal regenerative animals, trk A was expressed by 15 % of neurons. DRGs from diabetic regenerative animals demonstrated 24% trk A positive neurons. These data demonstrate that trk A mRNA expression increases in diabetes. It is unimpaired in diabetic sensory neurons undergoing regeneration. The cause for sensory neuropathy in diabetes may not reside in defective Trk A gene expression. It may yet be related to abnormalities in neurotropins and their receptors.

604.14

NEUROTROPHIC ACTIVITY FOR BRAINSTEM CHOLINERGIC NEURONS IN CYTOKINE-ACTIVATED ASTROCYTES. M. Sagoh*, K. Yoshida, H. Kamiguchi, M. Inaba, H. Sasaki, M. Otani, S. Toya. Dept. of Neurosurg., Sch. of Med., Keio Univ., Shinjuku-ku, Tokyo 160, Japan

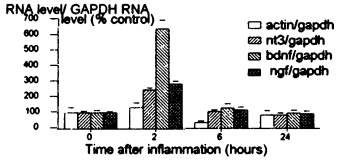
Astrocytes has various types of neurotrophic activity. We have previously demonstrated that some cytokines, including fibroblast growth factor (FGF), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) increase choline acetyltransferase (ChAT) activity of the septal cholinergic neurons via nerve growth factor produced by astrocytes. In the present study, neurotrophic activity for NGF-insensitive brainstem cholinergic neurons in astrocytes was investigated. The effect of astrocyte-conditioned medium (ACM) on selective neuronal cultures obtained from E-16 rat brainstem was examined. ChAT activity of the brainstem neurons was enhanced by the addition of ACM. The effect of ACM obtained from astrocytes stimulated by acidic FGF, IL-1β and TNF-α was more potent than that of unstimulated ACM. Subsequently, partial purification of the neurotrophic molecules for brainstem cholinergic neurons in cytokine-stimulated ACM was performed by using a heparin-affinity column and a gel-filtration column. Neurotrophic factor for brainstem cholinergic neurons was identified as a heparin binding protein, which molecular weight was 40 - 60 kD.

604.15

INFLAMMATION PRODUCES UP-REGULATION OF NEUROTROPHIN MESSENGER RNA LEVELS IN BLADDER

Daniela Oddiah[1], Steve B McMahon[2] and Marcus Rattray[1]* [1]Division of Biochemistry and Molecular Biology and [2]Division of Physiology, UMDS, University of London, Guy's Hospital, London SE1 9RT, U.K.

There is increasing evidence that neurotrophins can influence the activity of sensory neurones after acute inflammation. Using reverse-transcriptase PCR and in situ hybridisation, we have measured changes in levels of NGF, BDNF and NT3 mRNAs in bladder after inflammation. Inflammation was induced by administration of 0.5 ml 25% turpentine oil into the bladder for one hour. RNA was extracted from bladder and used for RT-PCR using primers 5'-end labelled with ^{32}P. Quantification of gel autoradiograms showed that inflammation produced a marked up-regulation of BDNF mRNA with a smaller increase in the levels of NGF and NT3 mRNA (see figure). The maximum effect occurred two hours after inflammation, and had disappeared 6 h and 24 h after inflammation. In situ hybridisation analysis suggested that NGF mRNA was expressed by epithelial cells of the bladder, whereas BDNF mRNA was mostly expressed in the muscular layer. Our results support the hypothesis that production of neurotrophins in target tissues is a component of the pronociceptive response to inflammation. Research supported by MRC



NEUROTROPHIC FACTORS: BIOLOGIC EFFECTS X

605.1

THE ROLE OF SECONDARY MUSCLE FIBER AND MUSCLE-DERIVED NT-4/5 FOR MOTORNEURON SURVIVAL IN TRANSGENIC MICE. T.J. Brennan, E.N.Olson, W.H. Klein, H.S. Phillips*, J.W. Winslow. Genentech, Inc., S. San Francisco, CA 94080 and M.D. Anderson Cancer Center, Houston, TX 77030.

We have generated transgenic mouse models to determine aspects of the muscle target which may regulate motorneuron number during programmed cell death. The role of target size and stage of muscle development on motor neuron survival was studied in mice in which the muscle regulatory gene myogenin was mutated, resulting in the loss of secondary myofiber formation. Over 60% of lumbar spinal motorneurons, and 100% of facial motorneurons survive at E18 relative to wild-type controls despite the loss of approximately 90% of hindlimb and facial muscle mass, respectively. DiI-retrograde labelling, cholinesterase and bungarotoxin staining of motor endplates and clustered nicotinic acetylcholine receptors, respectively, demonstrate that the surviving motorneurons have normal projection characteristics into the hindlimb, and establish synaptic contact into the remaining rudimentary fibers. These findings indicate that primary muscle fibers, representing only a fraction of normal muscle mass, contribute significantly to the control of motorneuron survival during development. To determine whether an increased level of a muscle-derived, motorneuron trophic factor is sufficient to promote survival during programmed cell death, 2 lines of transgenic mice were generated overexpressing NT4/5 mRNA in skeletal muscle. Normal numbers of facial motorneurons in postnatal NT4/5 transgenic mice were observed, suggesting limited availability of NT4/5 protein, or the limited responsivenes of motorneurons to ectopic trophic factor expression in vivo.

605.2

EFFECT OF NEUROTROPHINS ON mRNA LEVELS IN AXOTOMIZED ADULT RAT FACIAL MOTONEURONS. K.Fernandes, B.J.Jasmin, W.Tetzlaff*. Department of Physiology, Faculty of Medicine, University of Ottawa, Ottawa, Canada.

We have previously reported that direct osmotic minipump application of NT-4 to the proximal stump of axotomized adult rat facial motoneurons increases the axotomy-induced expression of GAP-43 and Tα1-tubulin mRNA over contralateral vehicle-treated axotomized facial motoneurons, while preventing the axotomy-induced decline in AChE mRNA. In the present study we further describe the ability of the other neurotrophin family members, BDNF, NT-3 and NGF, to regulate these genes associated with regeneration or neurotransmission. *In-situ* hybridization reveals that, in comparison to contralateral vehicle treated axotomized facial motoneurons, AChE mRNA expression after axotomy is significantly elevated by BDNF (195%, n=8), NT-4 (202%, n=4), and NT-3 (146%, n=6), while NGF had no effect. The axotomy-induced increase in GAP-43 mRNA (33X) is further increased with NT-4 (211%, n=4), and to a lesser extent by BDNF (120%, n=9), but not by NT-3 (99%, n=6). Similarly, the axotomy-induced stimulation of Tα1-tubulin (17X) is increased by BDNF (166%, n=4), NT-4 (170%, n=4), and NT-3 (124%, n=6).

These results support the hypothesis that the axotomy-induced decrease in neurotransmission associated gene expression, but not the accompanying increase in regeneration associated genes (GAP-43,Tα1-tubulin), may be triggered by the loss of target-derived neurotrophins.

605.3

Expression of neurotrophic molecules under control of muscle specific promoters M. Willem, A. Kawata, P. Carroll, M. Meyer* and H. Thoenen Department of Neurochemistry, Max-Planck-Institut for Psychiatry, Am Klopferspitz 18a, D-82152 Martinsried, Germany.

Several molecules exhibit trophic effects on motoneurons either in vivo or in vitro or both. CNTF(Ciliary Neurotrophic Factor) and BDNF (Brain Derived Neurotrophic Factor) represent two of these factors and it is the goal of our investigations to elucidate, in a mouse model system, their suitability for the treatment of degenerative diseases of motoneurons. Muscle fibres have the special ability to take up and express intramuscularly injected DNA. Our studies evaluate muscle as a delivery system to introduce neurotrophin molecules postnatally into mice. CNTF and BDNF genes under the control of various viral and muscle specific promoters were introduced into muscles by direct plasmid injection. Muscle tissues from such mice are being analysed to examine the extent and length of time of the expression of the transfected genes. We are analysing the effect of an unilateral injection of these plasmids into the gastrognemius- and soleus-muscle of pmn/pmn mice, an autosomal recessive mutant leading to caudo-cranial motor neuron degeneration. In parallel we are establishing transgenic mice overexpressing BDNF or a secretable form of CNTF driven by a muscle creatine kinase promoter in skeletal muscle. Five founder animals expressing BDNF were obtained by pro nucleus injection. 3 lines showed specific expression of the transgene in skeletal muscle. The consequences of the muscle specific BDNF-overexpression especially on the motor nervous system are under investigation. The mice carrying the transgene have been crossed with a BDNF mutant mouse line to achieve a rescue effect on the PNS in the BDNF -/- mice, which die within the first three postnatal weeks.

605.4

TrkB Neurotrophic Factor Receptor Agonists Prevent Loss of the Cholinergic Motor Neuronal Phenotype after Injury in the Adult Rat. A. Blesch[1,*], D. O'Keefe[2], E. Mafong[2], M.H. Tuszynski[1,2]. [1]Dept Neurosciences, University of California-San Diego, La Jolla, CA. 92093; [2]VA Medical Center, San Diego, CA.

CNS neurons demonstrate differential responses to neurotrophic factors depending upon phenotype, age, and presence of appropriate neurotrophin receptors. Injured neonatal motor neurons respond to BDNF, NT-3, CNTF and GDNF, but injured adult motor neuron responses to neurotrophic factors have not been fully characterized. In the present study, the ability of various neurotrophins to prevent injury-induced downregulation of the cholinergic neuronal phenotype in injured adult motor neurons was assessed.

Adult Fischer 344 rats underwent unilateral hypoglossal nerve transections, followed by intracerebroventricular infusions of either human NGF, BDNF, NT-3, NT-4/5, CNTF, GDNF or artificial CSF continuously for two weeks (1.4 ug/d). 2 weeks later, ChAT immunoreactivity could be detected in only 26.5±4.1% of hypoglossal neurons in control animals that received artificial CSF infusions. In contrast, the trkB agonists BDNF and NT-4/5 prevented the loss of ChAT labelling in 97±11% and 99±5% of neurons, respectively. NGF and NT-3 exerted no protective effect; CNTF and GDNF effects are under assessment. In all cases, injured hypoglossal neurons upregulated expression of the p75 low affinity neurotrophin receptor.

Thus, injured adult motor neurons remain responsive to trkB but not to trkA or trkC agonists. ICV delivery of neurotrophins is an effective means of eliciting reponses from motor neurons.

605.5

CNTF POTENTIATES THE EFFECTS OF BDNF, GDNF, OR HGF IN CULTURED MOTOR NEURONS. V. Wong*, Y. Song, R. Arriaga, and R.M. Lindsay. Regeneron Pharmaceuticals, Tarrytown, NY 10591.

CNTF, BDNF, GDNF, and hepatocyte growth factor (HGF) have been shown to promote survival and/or differentiation of motor neurons in vitro and in vivo. In this study, we show that CNTF synergizes with BDNF, GDNF, or HGF, but not NT-3, to enhance motor neuron survival and/or differentiation in vitro. When added alone to motor neuron cultures, CNTF, BDNF, GDNF, or HGF all stimulated choline acetyltransferase (ChAT) activity. However, when CNTF was added in combination with BDNF, GDNF, or HGF, ChAT activity was elevated to levels above the sum of each of the two factors alone. For example, when saturating concentrations of HGF (50ng/ml) and CNTF (1ng/ml) were added simultaneously to motor neuron cultures, ChAT activity was elevated 14-fold after 48 hr, almost doubling the sum of the 2 factors alone. Matrix studies showed that much lower concentrations of these factors can be used in combination to achieve effects equivalent to that of high doses of a single factor. In the presence of 1ng/ml GDNF, <0.1ng/ml of CNTF was required to achieve its maximal stimulation of ChAT activity, as compared to 1ng/ml when administered alone. The cellular mechanisms that mediate these synergies are currently being investigated. Thus, we show that the combination of neurotrophic factors at low doses results in equivalent or better effects on motor neuron survival and/or differentiation compared to the individual factors alone. These findings may be relevant to the treatment of human motor neuron diseases where previous studies have shown that high doses of neurotrophic factors can result in deleterious effects.

605.6

COMPARISON OF THE EFFECTS OF CNTF, BDNF AND GDNF ON CULTURED MOTOR NEURONS. R. Y. Xu*, S. Kahn and J. Lile. Dept. of Neuroscience, Amgen Inc. Thousand Oaks, CA 91320

CNTF, BDNF, and GDNF have been shown in vitro and in vivo to support the survival and differentiation of developing and adult chick or rat motoneurons. Two of these factors currently in clinical trial for potential treatment of ALS. Using partial purified rat and chick motor neurons, we examined the effects of these factors or the combinations of these factors in cultures grown for 3 days. The level of choline acetyltransferase(Chat) and 3-<4,5-Dimethylthiazol-2-yl>-2,5-diphenyltetrazolium bromide(MTT) uptake was determined in the presence of CNTF, BDNF, or GDNF or with the combination of these factors at concentration from 0.01 ng/ml to 10 ng/ml. Each of these factor alone increased Chat activity by 30% to 200% over the control, and MTT by 30% to 150% over the control. The combination of CNTF and GDNF, CNTF and BDNF, or GDNF and BDNF were greater than either alone measured by Chat and MTT. Combinations with GDNF achieved greater Chat activity than other combinations. The findings indicate some overlapping effects of these factors and some intriguing functions of each factor, which may be useful when the combinations are tested in animal models of moroneuron diseases.

605.7

LOCAL ADDITION OF NEUROTROPHINS NGF, BDNF AND NT-3 ENHANCES PERIPHERAL NERVE REGENERATION IN ADULT ANIMALS. R.D. Lainetti[1], R.L. Sidman[2], R.M. Lindsay[3] and C.F. Da-Silva[1]*. [1]Dept. of Histology, University of Sao Paulo, SP 05508-900, Brazil, [2]New England Reg. Primate Res. Ctr., Harvard Med. Sch., Southborough, MA 01772-9102, [3]Regeneron Pharm., Tarrytown, NY 10591.

Neurotrophins were tested for their effects on regenerating peripheral neurons in adult animals. In 12 male adult C57BL/6J mice the left sciatic nerve was cut and both proximal and distal stumps were sutured into a polyethylene tube (PT) (0.76 mm ID) leaving a 4 mm gap. The animals were divided into 4 groups of 3 animals each and implanted with PTs filled with one of the following solutions: (1) purified preparation of collagen (Vitrogen, 2.4 mg/ml) plus cytochrome C (a protein used as a control for neurotrophins); (2) Vitrogen + β-NGF; (3) Vitrogen + BDNF and (4) Vitrogen + NT-3. All solutions (2 µl/tube) were made up in 1:1 volume ratio, with 100 µg/ml of neurotrophin in the tubes. After a survival time of 4 weeks, the PTs with the regenerated nerve cables were processed for Epon embedding. Myelinated axons were counted from the mid-portion of the cables with a computer-controlled system (Biographics). The results showed a significant difference ($p<0.05$) in the number of axons between the control cyt. C group (1338±200, mean±SEM) and the NGF (1982±115), BDNF (2282±114) and NT-3 (2671±245) groups. These data demonstrate that local administration of neurotrophins increases peripheral axon regeneration in adult animals. Sensory DRG and spinal motor neuron counts as well as axon counts in the L_5 spinal roots are in progress and should determine whether this stimulatory effect of neurotrophins is due to a neurite-promoting activity or a survival action on axotomized neurons or both.

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605.8

EFFECTS OF BDNF OR CNTF TREATMENT ON GALACTOSE NEUROPATHY. A.P. Mizisin*, M. Bache, P. DiStefano, R.M. Lindsay, N.A. Calcutt. University of California, San Diego, La Jolla, CA and Regeneron Inc., Tarrytown, NY.

Amelioration of Schwann cell injury and axonal dwindling in galactose intoxication by aldose reductase inhibitors prompted speculation that exaggerated sugar metabolism in Schwann cells and skeletal muscle disrupts synthesis and release of neurotrophic factors that influence maintenance of axonal structure and function. The present study investigated the effects of BDNF or CNTF, neurotrophic factors normally localized to skeletal muscle and Schwann cells respectively, on myelinated fiber disorders of galactose-fed rats. Adult, female Sprague-Dawley rats were fed diets containing complete micronutrient supplements and either 0% D-galactose (control) or 40% D-galactose. Groups of control or galactose-fed rats received either BDNF (15 mg/kg) or CNTF (1 mg/kg) by thrice weekly subcutaneous injections. The aldose reductase inhibitor (ARI), Ponalrestat (50 mg/kg/d), was administered to another group by oral gavage. After two months, motor and sensory nerve conduction velocity (MNCV and SNCV) were measured and the sciatic nerves processed for light microscopy. The distal sciatic nerve and soleus and extensor digitorum longus muscles were analyzed for polyol content. In galactose-fed animals, Ponalrestat reduced dulcitol accumulation by 80% in nerve and by >50% in muscle while BDNF and CNTF were without effect. Galactose significantly reduced MNCV by 13% and SNCV by 17% compared to untreated or BDNF-, CNTF- and ARI-treated controls. ARI-treatment of galactose-fed animals resulted in MNCV and SNCV that were 93% and 99% of control values. Treatment with neurotrophic factors resulted in differential effects on CV. MNCV in the BDNF-treated galactose-fed group was increased to 92% of control values while SNCV was not affected. In contrast, SNCV in the CNTF-treated galactose animals was increased to 96% of control values while MNCV was not affected. Ponalrestat, but not BDNF or CNTF, prevented the observed 19% decrease in mean axonal diameter and a shift in axonal size-frequency histograms towards smaller fibers apparent in the sciatic nerve of galactose-fed rats. These observations indicate that BDNF and CNTF have differential effects on motor and sensory nerve function by mechanisms not obviously dependent on restoration of axonal caliber.

605.9

PERIPHERAL NERVE REGENERATION AND BDNF. D.M.Shirley, S.A.Williams, P.M.Santos*, Southern IL Sch. of Med., Div. of Otolaryngology, Springfield, IL 62794

Brain derived neurotrophic factor (BDNF) has demonstrated trophic activities in the developing and adult rat and a rescue effect following axotomy-induced cell death in the rat embryo. No previous studies have evaluated *functional* nerve regeneration following supraphysiologic concentration of BDNF exposure. We studied the potential effects of BDNF on motor nerve regeneration following transection injury in adult rats. Gait analysis and the tension transduction device (TTD) for neuromuscular evaluation allowed for sensitive and *functional* evaluation of peroneal nerve regeneration. Twenty four rats underwent entubulation repair. BDNF, (100mg/uL) or control (phosphate buffered solution) was injected into a Silastic channel holding the transected nerve ends. Gait analysis, measuring the ankle angle, was performed preinjury and 2, 4, 6, 10, and 12 weeks postinjury and demonstrated a significant difference between uninjured and injured legs, 19 degrees and 64 degrees, respectively ($p< 0.001$, ANOVA). The TTD measured elicited force development of dorsiflexion at 13 weeks postinjury and demonstrated a significant difference in force development between injured and uninjured legs, 148 grams and 58 grams, respectively ($p<0.001$). No difference was demonstrated between BDNF versus control solution with either gait analysis or the TTD.

605.10

ADDITIVE RESCUE EFFECTS OF GDNF AND BDNF ON AXOTOMIZED SCIATIC MOTONEURONS IN NEWBORN RATS. R.Vejsada[1], R.M.Lindsay[2] and A.C.Kato[1]* [1] Dept. Pharmacology and Div. Clinical Neuromuscular Research, CMU, University of Geneva, Switzerland; [2]Regeneron Pharmaceuticals Inc., Tarrytown, N.Y., USA

Both GDNF and BDNF, members of distant families of trophic factors, promote survival of motoneurons *in vitro* and *in vivo*. We have compared the effects of GDNF and BDNF, and of co-treatment with these factors, on axotomized sciatic motoneurons in neonatal rats. Motoneurons were quantified using the fluorescent retrograde tracer Fluoro-Gold to specifically label the sciatic pool. Application of GDNF directly to the transected nerve significantly enhanced motoneuron survival, as determined 7 days later, in comparison to controls treated with BSA. Dose-response studies showed that the effects of GDNF were superior or equal to BDNF within the concentration range tested (0.01-1.6 mg/ml). However, the number of surviving cells dramatically decreased at 2 weeks post-lesion; the rescue effects of a single dose of GDNF were thus transient, as we have previously shown for several other neurotrophic factors (Eur. J. Neurosci. 7, 108-115, 1995). When both GDNF and BDNF were applied simultaneously, motoneuron survival was better than with each of these factors alone, suggesting that their effects may be additive. Furthermore, when a single dose of GDNF to the nerve stump was combined with BDNF injections (5 mg/kg, s.c.) at 3-day intervals for 2 weeks, motoneuron loss during the later post-lesion period could be partially arrested. These results show that combinations of neurotrophic factors from different families may be more effective than administration of single factors for rescuing lesioned motoneurons.

605.11

INVOLVEMENT OF THE MUSCLE-DERIVED NEUROTROPHIC FACTOR IN THE MATURATION OF NERVE TERMINAL AT THE DEVELOPING NEUROMUSCULAR SYNAPSES. J. C. Liou, W. M. Fu*. Pharmacological Institute, College of Medicine, National Taiwan University, Taipei, Taiwan.

Although the effects of neurotrophins on neuronal survival and differentiation have been extensively studied, little is known about their effects in synaptic development and function. Here we reported the chronic effects of neurotrophin-3 (NT-3) in the maturation of neuromuscular synapse in *Xenopus* nerve-muscle co-cultures. Compared with the myocyte-contacted neuron, nerve terminals which were not contacted by the myocyte released ACh with a smaller quantal size detection by manipulated myoball in 3-day-old culture implicating an indispensable role of muscle in the maturation and/or survival of neuromuscular synapses. Chronic treatment with d-tubocurarine (d-Tc) or tyrosine kinase inhibitor k252a for 2 days then washout before the measurement of synaptic currents, spontaneous synaptic currents (SSCs) and impulse-evoked synaptic currents (ESCs) occurred with much smaller amplitude at natural synapses. However, postsynaptic iontophoretic ACh-induced currents were not greatly affected by these treatments. Simultaneous chronic treatment with NT-3 and d-Tc partially reversed the suppression effect of d-Tc and such reversal effect could be further abolished when k252a was applied concomitantly. In addition, treatment non-myocyte-contacted neurons with NT-3 for 2 days increased the size of SSCs which was detected by manipulated myoball. Taken together, these results suggest that muscle-derived NT-3 may participate in the functional maturation of synapses and the secretion of NT-3 is activity-dependent.

605.12

BDNF AND GDNF BUT NOT CNTF INHIBIT AXOTOMY-INDUCED NITRIC OXIDE SYNTHASE (NOS) EXPRESSION IN MOTOR NEURONS OF CRANIAL NERVES. W.H.A. Yu*. Department of Cell Biology & Anatomical Sciences, City University of New York Medical School, NY, NY 10031.

We have shown previously that following unilateral transection of the hypoglossal, vagus, and facial nerves of adult rats, a number of neurons in the ipsilateral hypoglossal nucleus, dorsal motor nucleus of the vagus (DMV), and facial motor nucleus exhibited NADPH-diaphorase activity coincident with neuronal NOS immunoreactivity. Target reinnervation down-regulated axotomy-induced NOS expression. When the nerves failed to regenerate or were prevented from target contact, increasing numbers of axotomized neurons became intensely NADPH-diaphorase positive with time, suggesting that targets may contain "signals" which normally suppress NOS expression. Soluble factors synthesized in targets and taken up by neurons via retrograde axonal transport are likely candidates for such "signals". The present study tested the possibility of BDNF, CNTF, and GDNF acting as such "signals". Following unilateral transection of the adult rat hypoglossal and vagus nerves, gelfoams (5 mm^3) containing either BDNF, CNTF, GDNF (30 ug each), or vehicle (0.1 M PBS, 20 ul) were applied to the proximal nerve stumps. BDNF partially and GDNF nearly completely suppressed NOS expression 4 days after axotomy in hypoglossal and DMV neurons. CNTF by itself was ineffective but potentiated the BDNF effect in hypoglossal neurons. The inhibitory effects of BDNF and GDNF on NOS expression were, however, diminished 7 days after axotomy. Infusion of GDNF (1 ug/h) or BDNF combined with CNTF (0.5 ug each/h) to the hypoglossal nerve stump over a period of 7 days by a catheter connected to an osmotic pump (Alzet, model 2001) was effective in keeping NOS expression near zero for 7 days after axotomy. These results are consistent with the hypothesis that axotomy interrupts and target reinnervation restores target-derived "signals" to motor neurons for inhibiting NOS expression.

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605.13

THE EFFECTS OF NEUROTROPHINS ON MUSCLE AFFERENTS AND EFFERENTS IN ADULT RATS. John B. Munson*, David L. Shelton+ and Stephen B. McMahon. Dept Physiol, St Thomas Hospital Medical School, London, UK, and Dept Neurosci+, Genentech, San Francisco, USA.

Axotomy of muscle afferents and efferents alters many functional properties, including slowing of axonal conduction velocity (CV: Titmus & Faber, Prog in Neurobiol 35). These alterations might result from deprivation of specific neurotrophins (NTs) normally obtained from target tissue. We tested this in adult Wistar rats by providing to intact or axotomised gastrocnemius muscle nerves NTs or molecules capable of sequestering particular NTs (trk-IgG fusion molecules, Shelton et al, J Neurosci 15) chronically via osmotic pumps. We also studied unoperated rats and rats with axotomised muscle nerves only. We determined the CV distribution of myelinated afferent and efferent fibres by single unit recording from dorsal and ventral root filaments. Results are based on experimental times of 2 weeks.

We first confirmed that CV of muscle afferents and efferents slows progressively for at least 4 weeks following chronic axotomy. Infusion of trkB-IgG (at 0.5 µg/hr) into the popliteal fossa significantly slowed CV of intact gastrocnemius efferents but not afferents, explainable if either BDNF or NT4/5 were essential to efferent (but not afferent) function. Infusion of trkA-IgG at 0.5 µg/hr was without effect on CV of intact myelinated afferents or efferents, suggesting these neurones are unresponsive to NGF and providing also a control for non-specific effects of the procedures. NT5 provided to axotomised nerves at 125 ng/hour, partially restored CV for efferents but not afferents.

These experiments suggest that endogenous NTs have a physiological role in the maintenance of muscle nerve properties in adult mammals, and that exogenous NTs may rescue neurones from some of the effects of nerve injury. Supported by the MRC (SBM) and USPHS (JBM).

605.14

EFFECTS OF EXCESS BDNF ON THE DEVELOPMENT OF MOTONEURONS AND THE Ia AFFERENT-MOTONEURON SYNAPSE IN THE NEONATAL RAT SPINAL CORD. B.S. Seebach* & L.M. Mendell, Dept. of Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

The "neurotrophic hypothesis" predicts that the level of available neurotrophin during periods of rapid development should influence cell and synaptic properties of neurons expressing the appropriate *trk* receptor. Because motoneurons express *trk*B receptors, the amount of available BDNF, a neurotrophin that binds to and activates *trk*B receptors, may be important during their development. The present study examined the effect of excess BDNF on the postnatal development of motoneuron and synaptic properties by comparison with results from previous observations of normal development (Seebach & Mendell, Neurosci. Abst. 1994). Rats (n=7) unselected as to sex received daily injections of BDNF from birth through 7 d. At postnatal day 8, the excised spinal cord was hemisected, L5 dorsal and ventral roots were stimulated via suction electrodes, and evoked responses were recorded intracellularly from motoneurons. Rheobase and input resistance of motoneurons and amplitude of monosynaptic epsps evoked by dorsal root stimulation were examined in animals receiving 2, 5, or 10 µg/g doses of BDNF and were compared with normals. BDNF injections did not affect the average weight of animals. However, average motoneuron rheobase increased steadily with larger doses of BDNF (from 2.5 nA in normals to 4.0 nA at 10 µg/g BDNF, $p<0.05$), while input resistance decreased (from 21.0 MΩ in normals to 12.9 MΩ at 10 µg/g BDNF, $p<0.05$), indicating that excess BDNF may increase motoneuron size. NT-3 injections were without effect on these measures. Although epsp amplitude bears an irregular relationship to cell resistance for individual motoneurons in the immature spinal cord, the average amplitude of monosynaptic epsps was inversely correlated with BDNF dose, consistent with the idea that motoneuron size increases with larger doses of BDNF. We conclude that the postnatal development of motoneurons in the rat is influenced by the level of available BDNF.

Supported by NIH. BDNF courtesy of Regeneron Pharmaceuticals.

605.15

NEUROTROPHIC REGULATION OF EXCITABILITY IN ADULT RAT MOTONEURONS. M. Gonzalez* and W.F. Collins, III. Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

While neurotrophins have profound effects on developing neurons, little is known about possible effects on adult neurons. The observations of BDNF and trkB receptor m-RNAs in hindlimb muscle and motoneurons, respectively, suggest that muscle-derived BDNF is important in the maintenance of motoneuron properties.

To investigate this possibility, male Sprague-Dawley rats (300-450 g) were anesthetized with a mixture of ketamine and xylazine and a 3x3x5 mm piece of gelfoam, saturated with BDNF (16 mg/ml in phosphate buffered saline, pH 7.4), or saline was inserted into the left popliteal fossa (6 rats per treatment group). After 5 days survival, the animals were prepared for in vivo intracellular recording and the electrical properties of medial gastrocnemius (MG) motoneurons in the BDNF (n=49) and saline treated (n=45) rats were measured. Data were analyzed using ANOVAs.

BDNF produced significant decreases in mean MG motoneuron rheobase and total cell capacitance that were associated with a tendency towards increased mean input resistance. The significant increase in excitability along with the other observed changes are indicative of a decrease in motoneuron size. No significant treatment effect was observed in conduction velocity, membrane and equalizing time constants, afterhyperpolarization amplitude and duration, membrane potential sag, action potential height, and electrotonic length.

One functional implication of these results is that motoneurons innervating BDNF-producing muscle fibers would be expected to exhibit increased excitability and be recruited earlier. Furthermore, differential expression of BDNF by different muscle units could underlie motor unit type-specific differences in motoneuron excitability and recruitment order.

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605.16

NGF-INDUCED INCREASE IN THERMAL SENSITIVITY OF NOCICEPTIVE FIBERS *IN VITRO*. A. Rueff* & L. M. Mendell, Dept. Neurobiol. & Behavior, SUNY, Stony Brook, NY 11794.

Injection of NGF into adult rats induces a prolonged hyperalgesia with hypersensitivity to thermal and mechanical stimulation (Lewin *et al.*, 1993, J. Neurosci. 13, 2136-2148). Previous studies have suggested a peripheral component in the mechanism responsible for NGF-induced thermal hyperalgesia whereas mechanical hyperalgesia appeared to be centrally mediated (Lewin *et al.*, 1994, Eur. J. Neurosci. 6, 1903-1912; Rueff & Mendell, 1994, Soc. Neurosci. Abstr. 20, 287.2).

Here we used a previously described *in vitro* skin-saphenous nerve preparation that allowed us to study isolated cutaneous receptive fields (Reeh, 1986, Neurosci. Lett. 66, 141-146). After having established the conduction velocity of clearly identified single units with unmyelinated (C-fibers) or myelinated (Aδ- and Aβ-fibers) axons, we studied the sensitivity to noxious heat and mechanical thresholds of each unit under control conditions. Subsequently, we superfused the receptive field, that had been isolated with a metal cylinder, for 15 min. with up to 400ng/ml NGF. No spontaneous activity was observed in any fiber class while applying NGF. Following exposure to NGF mechanical thresholds as well as receptive field sizes of either nociceptive (C- and Aδ-fibers) or non-nociceptive (Aβ-fibers) units remained unchanged. However, we observed a clear increase in noxious heat evoked discharge in about 50% of all heat sensitive units following NGF. This result confirms a possible peripheral site for NGF-induced thermal sensitization. Possible mechanisms will be discussed. [Supported by NIH-NS14899 and NS32264; Swiss National Science Foundation Grant 823A-040158 (AR)/ NGF provided by Genentech Inc.]

605.17

IMMUNOGLOBIN LOCALIZATION IN RAT SKIN, DRG, AND SPINAL CORD AFTER RABBIT SERA INJECTIONS. J.R. Tonra*[1], L.M. Mendell[2]. [1]Dept. Physiol. & Biophys. and [2]Dept. Neurobiol & Behav., SUNY Stony Brook, Stony Brook, NY 11794.

Rabbit sera raised against antigens (e.g. NGF) are injected into rats to elucidate antigenic function *in vivo*. In order to localize structures to which antibodies have access, we examined rabbit IgG IR in rat tissues after normal rabbit serum (NRS) injections. NRS was administered daily to female Sprague Dawley rats at embryonic days (E) 20-22, postnatal days (PND) 2-4, and PND 15-17 (3 rats/ group). For the embryonic treatment the mother was injected with 2 ml NRS s.c. Postnatal treatments were 5 µl/gram s.c. Negative controls included staining of tissue from treated rats with primary antibody against rabbit IgG preabsorbed with rabbit IgG, and staining of untreated rat tissue. Rats were anaesthetized one day after the last treatment and perfused transcardially with 2 ml saline followed by 20-30 ml fixative. Lumbo-sacral spinal cord, L5 DRGs and glabrous skin from the foot pad were analyzed. In skin, rabbit IgG IR is intense in dermis but barely detectable in epidermis, suggesting a dermal-epidermal barrier to rabbit IgG in this region of skin. The spinal cord exhibited little rabbit IgG IR and IgG permeability appeared to decrease with age (Hulsebosch & Fabian, Neurosci. Lett. 98). DRG cells from the two older groups displayed very few IgG IR cells but large clusters of IR cells were found in rats treated from E20-22, often in the same section as large clusters of negative cells. Preliminary results with longer treatments starting on PND 2 suggest strong IR develops in a subset of DRG cells after extended treatments with NRS or rabbit serum raised against NGF. Supported by NIH.

605.18

FUNCTIONAL ROLES OF NEUROTROPHIC FACTORS DURING SYNAPTOGENESIS IN A NEURON-MUSCLE CO-CULTURE SYSTEM. A. K. Y. Fu*, K. M. Chan, K. W. K. Tsim and N. Y. Ip. Department of Biology, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong.

The formation of the neuromuscular junction involves the co-ordination and expression of pre- and post-synaptic proteins. Although a number of such proteins have been identified, their precise functional roles remain to be elucidated. One approach to study this problem is to manipulate the level of pre- and/or post-synaptic proteins, and examine the functional consequence on synapse formation. Previous studies suggest that neurotrophic factors may enhance the activity and efficacy of developing neuromuscular synapse. Consistent with this finding, transcripts encoding for specific neurotrophic factors in muscle were found to be developmentally regulated during the critical period for the formation of neuromuscular junctions. We have set up a co-culture system of neurons and muscles, i.e. neuroblastoma NG108-15 and C2C12 myoblasts, to examine the functional consequence of overexpressing neurotrophic factors in muscle cells. The C2C12 cells were stably transfected with expression constructs of neurotrophic factors tagged with the green fluorescent protein. These transfected C2C12 cells were co-cultured with NG108-15 cells, and their ability in inducing the formation of acetylcholine receptor (AChR) aggregates was compared with the untransfected C2C12 cells. In addition, the transfected C2C12 cells were fused with untransfected C2C12 cells, and co-cultured with NG108-15 cells. The extent and localization of AChR aggregates were analyzed in the vicinity of neurotrophic factors (as indicated by the green fluorescent signal) expressed in the C2C12 myotubes.

605.19

THE EFFECTS OF CILIARY NEUROTROPHIC FACTOR ON GAP-43 mRNA EXPRESSION IN THE PERIPHERAL NERVOUS SYSTEM. S.G. Siegel*, A.W. English, and G.K. Pavlath. Depts. of Anatomy & Cell Biology and Pharmacology, Emory University, Atlanta, GA 30322

The growth associated protein GAP-43 has been localized to neurons and glial cells of the peripheral and central nervous systems. Motoneurons express GAP-43 during development, after which GAP-43 levels decline substantially. However, significant upregulation of GAP-43 is observed following peripheral nerve injury and has been associated with regeneration. In partially denervated muscle, GAP-43 immunoreactivity is found in Schwann cells as well as in axons near the denervated zone, suggesting a role in neuronal sprouting. Neuronal sprouting from axons following partial denervation is known to be accompanied by extension of processes from axon sheath (Schwann) cells. Ciliary neurotrophic factor (CNTF) induces sprouting in intact motor axons, but it is not known whether CNTF acts directly on neurons or indirectly via Schwann cells. Since GAP-43 mRNA from sciatic nerve and muscle is exclusively of Schwann cell origin, any upregulation of GAP-43 mRNA in these tissues indicates that Schwann cells are responding to CNTF. Adult rats were injected with CNTF either intramuscularly or into the sciatic nerves. Control animals received similar injections of saline. The triceps surae muscles, sciatic nerves, and lumbo-sacral spinal cords were removed from the animals and analyzed for GAP-43 mRNA by Northern analysis. Intramuscular injections of CNTF resulted in an increase in muscle GAP-43 mRNA, suggesting a local response by the Schwann cells within the muscle. These data are consistent with the hypothesis that Schwann cells, which produce CNTF, are also responsive to CNTF.

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This work was supported by the Foundation for Physical Therapy.

605.20

***IN VIVO* EFFECTS OF PUTATIVE NEUROTROPHIC FACTORS CEP 1347, CNTF, AND IGF-I IN RESCUING MAMMALIAN MOTONEURONS FROM NATURALLY OCCURRING DEATH.** M.W. Harty[1*], M.A. Glicksman[2], N.T. Neff[2], C. Murakata[3] and D.R. Sengelaub[1]. [1]Program in Neural Science, Indiana University, Bloomington, IN 47405; [2]Cephalon, Inc., West Chester, PA 19380; [3]Kyowa-Hakko Kogyo, Tokyo, Japan.

The spinal nucleus of the bulbocavernosus (SNB) of male rats contains about three times as many motoneurons as the SNB of female rats, the result of an androgen-induced rescue of motoneurons during development. Several trophic factors have been identified which influence neuronal survival, and it is likely that androgen's trophic effects on the SNB are mediated through the action of such factors. To assess the efficacy of compounds demonstrated to have trophic effects on motoneuron survival *in vivo*, developing females were treated with either insulin-like growth factor (IGF-I), ciliary neurotrophic factor (CNTF), or CEP 1347 (a K-252a analogue).

Female rats were injected (s.c., over the SNB target muscles) from postnatal (P) day 1 (day of birth) through P5 with either IGF-I, CNTF, CEP 1347, or vehicle. During this period, SNB motoneurons normally die in females, but can be rescued with androgen treatment. Counts of SNB motoneurons at P10 in pups treated with IGF-I (1 mg/kg) did not differ from those of normal or vehicle-treated pups. However, CEP 1347 (0.1 or 1 mg/kg) significantly reduced motoneuron death; SNB motoneuron numbers were equivalent to those of androgen-treated females. As previously reported (Forger *et al.*, '93), CNTF (0.5 µg) also significantly masculinized SNB motoneuron number. Thus, like CNTF, CEP 1347 can rescue SNB motoneurons from naturally occurring death. (Supported by Cephalon)

605.21

TrkB RECEPTORS MEDIATE SURVIVAL OF FACIAL MOTONEURONS AFTER AXOTOMY. J. Frisén*, M. Barbacid and I. Silos-Santiago. Department of Molecular Oncology, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08540.

Brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4) are known to support motoneuron survival in vitro. BDNF has also been shown to prevent the death of motoneurons after neonatal axotomy. Expression of BDNF and NT-4 increase after peripheral nerve lesion in Schwann cells distal to the injury and BDNF expression increases in denervated muscle. Furthermore, the expression of TrkB, the signaling receptor for BDNF and NT-4, also increases in motoneurons after axotomy. These data, taken together, suggest that TrkB receptors may mediate signals that support regeneration and/or survival of motoneurons after axotomy. We have studied the role of TrkB in motoneuron survival after axotomy in postnatal mice that lack the TrkB tyrosine kinase receptor. The facial nerve was cut unilaterally at postnatal day 6 (P6) in wild type and trkB (-/-) knock out mice. Animals were allowed to survive until P10 and their motoneurons were counted in the injured and uninjured facial motor nucleus. In wild type mice the number of facial motoneurons decreased by about 40% in the axotomized side when compared with the unoperated control side. In the trkB (-/-) mutant mice, the reduction of neurons in the injured facial motor nucleus was 65% compared with the uninjured side. These results suggest that neurotrophin signaling through TrkB receptors has an important role in the survival of at least certain injured neurons.

605.22

BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) SPARES CHOLINE ACETYLTRANSFERASE mRNA FOLLOWING AXOTOMY OF MOTOR NEURONS *IN VIVO*. W. Wang, P. Salvaterra*, S. Loera, A. Rosenthal[1], M. Glicksman[2], and A.Y. Chiu. Beckman Res. Inst., City of Hope, Duarte, CA, [1] Genentech, S. San Francisco, CA,[2] Cephalon, West Chester, PA.

Choline acetyltransferase (ChAT, EC2.3.1.6) is a specific marker gene for neurons that use acetylcholine as a neurotransmitter. Using a quantitative RNase protection assay, we have investigated the dynamic changes in ChAT mRNA levels following axotomy and with application of trophic molecules to the proximal nerve stump. A week after unilateral transection of the hypoglossal nerve in adult rats, the levels of ChAT mRNA in the axotomized neurons decreased dramatically to around 15% of the uninjured, control half of the motor nucleus. When the cut end of the nerve was chronically exposed to BDNF for a week, ChAT mRNA levels were 60% of control. BDNF thus prevented the injury-induced loss of ChAT mRNA. NT4/5 also partially prevented this decrease, although not as effectively as BDNF. NT4/5-treated neurons expressed 30% of normal levels of ChAT mRNA. However, if the nerve stump was exposed to PBS-BSA, ChAT mRNA levels fell to 15% of normal, indicating that the trophic molecules acted with some specificity. These effects of BDNF and NT4/5 on ChAT mRNA levels following motor neuron injury suggest the existence of regulatory pathways responsive to these neurotrophic factors that can "rescue" cholinergic gene expression. The effect of neurotrophins, and BDNF in particular, on motor neurons after lesion raises the possibility that they may be useful in treating patients with motor neuron and neuromuscular disorders, such as motor neuropathies and amyotrophic lateral sclerosis. *Supported by the Council for Tobacco Research (PMS) and NICHHD (AYC).*

605.23

EVALUATION OF PEG-BDNF IN AXOTOMIZED FACIAL MOTOR NEURONS OF ADULT RATS. O. T. Lopez, O. Kinstler, E. Cheung, A. A. Welcher*, Q. Yan Amgen, Inc, Amgen center, Thousand Oaks, CA 91320

Brain derived neurotrophic factor (BDNF), a member of the NGF family of neurotrophins, is involved in survival and maintenance of function of motor neurons. Systemically administered BDNF has been shown to attenuate the decrease of choline acetyltransferase (ChAT) immunoreactivity in axotomized facial motor neurons of adult rats. We have compared the effects of BDNF with a polyethylene glycol conjugated BDNF analog (PEG-BDNF) in this model by reducing the dose and administration frequency of the analog while still producing the attenuating effects on ChAT following motor neuron axotomy. Our results show that due to its short half-life and rapid clearance, it requires a BDNF dose of 10mg/kg (s.c., q.d.) to attenuate (by 66±1%) the decrease of ChAT immunoreactivity, while the PEG-BDNF at a dose of 0.3mg/kg (s.c., q.d.) produced superior effects (attenuated decrease by 83±3%). Furthermore, when injected every other day, the PEG-BDNF (0.3mg/kg) produced similar effects to those seen with daily treatments of 10mg/kg BDNF (68±2% vs 66±1%). The pharmacokinetics study indicates that there is a 2x increase in bioavailability, a 15x decrease in absorption, a 10x decrease in protein clearance, and a 10x increase in the terminal half-life with PEG-BDNF compared to the native form. The results of this study suggest that PEG-BDNF may provide an alternative for in vivo applications without compromising the overall effectiveness of the BDNF protein as a therapeutic agent for adult motor neuron disease.

605.24

CILIARY NEUROTROPHIC FACTOR REGULATES MULTIPLE INNERVATION ONLY DURING NEUROMUSCULAR SYNAPSE ELIMINATION. C. L. Jordan* Dept. Psych., UC Berkeley, CA 94720.

Recent demonstrations suggest that exogenous ciliary neurotrophic factor (CNTF) can prevent the normal loss of synapses during developmental synapse elimination. However, because CNTF can also induce motor axons in adult muscle to sprout, which might lead to the formation of multiple innervation, the present study sought to determine whether the effects of CNTF on multiple innervation in development are restricted to the period of synapse elimination. Human recombinant CNTF (1 μg/g body weight) or vehicle was delivered daily to the hind limbs of male rat pups. Animals received bilateral s.c. injections of vehicle only or CNTF on one side and vehicle on the other. Treatment occurred from postnatal day 7 (P7) to P14 or from P14 to P21. The earlier treatment period (P7-P14) overlaps with synapse elimination in the extensor digitorum longus (EDL). Based on the number of stained motor axons innervating individual EDL muscle fibers, I found that CNTF influenced the level of multiple innervation during only the first treatment period and only in EDL muscles from the CNTF-treated limb. Thus, CNTF probably regulates the level of multiple innervation in the EDL by controlling synapse elimination and not by an effect on axonal sprouting. Because CNTF had only unilateral effects on synapse elimination in the EDL, skeletal muscles are probably an important site for the direct action of CNTF on synapse elimination or for the directed transport of CNTF to motoneuronal cell bodies. Supported by IBN-9210229, IBN-9309856 and Regeneron Pharmaceuticals.

605.25

MOTOR ENDPLATE PHOSPHOTYROSINE LOSS FOLLOWING DENERVATION. S.R. Songer*, E.A. Lassiter, and A.W. English. Dept. of Anatomy and Cell Biology, Emory Univ., Atlanta, GA 30322.

Several muscle proteins are phosphorylated on tyrosine residues. Tyrosine phosphorylation of muscle acetylcholine receptors (AChRs), which are densely aggregated at motor endplates, is innervation-dependent. Longitudinal sections of adult rat gastrocnemius muscle were stained to separately label AChRs and tyrosine-phosphate residues. The only detectable phosphotyrosine (PY) was localized to the motor endplate and corresponded to labeling for AChRs. Endplates viewed at high magnification were scored as either PY-positive or -negative based on visible PY labeling. In intact control muscle and in reinnervated muscle, 100% of the endplates were PY-positive. The medial gastrocnemius muscle was permanently denervated by nerve transection in different subjects for varying periods of time. The percentage of PY-positive endplates underwent a sharp decline during the first 1-2 days after denervation, but returned to a normal level by days 7-8. From this point, PY underwent a long-term decline. An examination of time intervals immediately following denervation revealed that at 6 and 12 hours most endplates were PY-positive. However, by 18 and 24 hours the percentage of PY-positive endplates was markedly decreased. This post-denervation pattern of PY loss, return, and decline may be a consequence of the absence of neuromuscular and/or neurotrophic activity. In support of the role of neurotrophic action, the length of the distal nerve stump following denervation may influence the time course of endplate PY loss. Longer stump-lengths promote the retention of PY at times when it would be expected to be reduced. In conclusion, these results are consistent with the innervation-dependence of endplate PY; however, it is possible that some aspect of innervation other than activity is involved.

606.1

LUMBAR DRGs OF *trk*A(-/-) MICE EXHIBIT MASSIVE APOPTOSIS PRIOR TO INNERVATION OF DISTAL HINDLIMB TARGETS. F.A. White*[1], I. Silos-Santiago[2], D.C. Molliver[1], J.C. Harding[1], M.T. Saxena[1], M. Barbacid[2], and W.D. Snider[1]. [1]Dept. of Neurology, CSNSI, Washington University, St. Louis, Mo, 63110, [2]Dept. of Molecular Biology, Bristol-Myers Squibb, Princeton, NJ 08543.
Recently reported differences in the magnitude of DRG neuron loss in *trk*C(-/-) and *NT-3* (-/-) mutant mice have lead to the idea that NT-3 may transiently support sensory neurons by acting on the NGF signaling receptor TrkA. However, temporal relationships between the onset of TrkA expression by sensory neurons, the arrival of TrkA (+) sensory axons in the proximity to sources of NGF and NT-3, and the onset of neuronal dependence on TrkA-signaling for survival have not been determined. We show here that trkA is expressed in DRGs at E10.0, the earliest day at which a collection of ganglion cells is apparent. Neuronal apoptosis is detected in DRGs as early as E11.5 in wild type mice. Analysis of DRGs in *trk*A (-/-) mice shows no increase in this early death suggesting that survival of neurons at E11.5 is independent of trkA signaling. However by E13.5, we find that most lumbar DRG neurons are dependent on TrkA-signaling as evidenced by massive apoptosis in lumbar DRGs in *trk*A(-/-) animals. In order to examine relationships between onset of TrkA signalling and access to sources of neurotrophin support, we stained sensory axons with an antibody to TrkA and studied neurotrophin expression in the hindlimb bud at these same ages. NT-3 mRNA is present in hindlimb as early as E11.5. In contrast, NGF mRNA does not appear until 24-48 hours later. These neurotrophin mRNA distributions are different in that NT-3 is expressed in muscle precursors in the interior of the limb bud, whereas NGF expression is superficial and restricted to surface ectoderm. TrkA+ sensory axons innervate surface ectoderm between E11-13 in proximal hindlimb regions, but do not reach the surface of the distal hindlimb until after E15. Our results demonstrate that many DRG axons are in proximity to sources of both NT-3 and NGF at the onset of TrkA dependence. However, many DRG neurons in lower lumbar ganglia are TrkA-dependent before their axons are in proximity to a source of NGF in the surface ectoderm.

606.2

LACK OF COLLATERAL SPROUTING OF NOCICEPTIVE NERVES IN ADULT p75 KNOCK-OUT MICE. J. Diamond, S. Lourenssen, E. Pertens, and B. Urschel*. Dept. of Biomedical Sciences, McMaster University, Hamilton, Ontario. Canada. L8N 3Z5

While the high-affinity trkA receptor seems essential for survival and neuritogenesis in NGF-responsive neurons during development and *in vitro*, the function of the low-affinity p75 receptor is less clear. We have now studied sensory neurons in adult mice with a targeted mutation in the p75 receptor; the results provide new insight into the function of this transmembrane molecule.

Undamaged mechano-nociceptive (Aδ) and heat-nociceptive (C) fibers (but not the mechanosensory Aα fibers) normally undergo vigorous collateral sprouting into adjacent denervated skin; this is a totally NGF-dependent nerve growth. In contrast, after nerve crush all three classes of sensory axons regenerate independently of NGF. Nevertheless, both regenerating and collaterally sprouting fibers use the same dermal perineurial pathways to reinnervate denervated skin. Interestingly, both p75 and trk expression increase in DRG neurons prior to and during the collateral sprouting of their axons, but not during their regeneration.

In adult p75 knock-out mice the collateral sprouting of both classes of nociceptive fibres appears to be totally absent. Thus the utilisation of NGF for this type of growth appears to be entirely dependent on the presence of the p75 receptor. In contrast, regeneration of all sensory nerves appears perfectly normal, and is clearly independent of the p75 receptor. Preliminary nerve counts show reductions of almost 40% in both large and small myelinated sensory axons, and about 60% in the unmyelinated population. Thus during development of these mice a significant fraction of differing DRG sensory neuron subgroups survives the absence of the p75 receptor, including a subpopulation of the NGF-sensitive neurons whose collateral sprouting in the adult continues to be totally dependent on this neurotrophin, and on the presence of the p75 receptor.

606.3

IMPAIRED REGENERATION OF NOCICEPTIVE FIBRES IN STEEL/C-KIT MUTANT MICE. S. Lourenssen[1], M. Kluppel[2], A. Bernstein[2] and J. Diamond[1]*. [1]Dept. of Biomedical Sciences, McMaster University, Hamiltion, Ontario, Canada, L8N 3Z5 [2]Program in Molecular Biology and Cancer, Samuel Lunenfeld Research Institute, Toronto, Ontario, Canada, M5G 1X5

The *steel* locus in the mouse encodes steel factor, the ligand for the c-kit tyrosine kinase receptor encoded by the *W* locus. Mice with mutations in the the *Sl* or *W* loci are characterized by defects in melanogenesis, gametogenesis, and hematopoiesis. Interestingly, steel and c-kit are expressed in cell lineages that appear not to be affected by mutations in *Sl* or *W* loci, including peripheral and central nervous systems; one of the highest levels of expression occurrs in dorsal root ganglia (DRG). Steel factor, which is expressed in Schwann cells, has been shown to induce neurite outgrowth from a sub-population of NGF-responsive embryonic DRG neurons *in vitro*, and also to act as a survival factor for these neurons.

We have now examined cutaneous mechano-nociceptive (Aδ) and heat-nociceptive (C) fibres in adult mice with mutations in *Sl* or *W* loci. The regeneration of these axons after nerve crush occurs entirely independently of NGF, while the collateral sprouting of the undamaged fibres into adjacent denervated skin is totally dependent on the availability of NGF. In the mutant animals collateral sprouting was perfectly normal. Unexpectedly, however, the regeneration of the same sensory axons was significantly impaired. The growth processes underlying regenerative neurite extension are likely to be similar, perhaps identical, to those underlying collateral sprouting. If so, then the activation of c-kit signalling pathways is probably an important event in the triggering of the regenerative program by nerve damage, while the initiation and maintenance of collateral sprouting by NGF must utilise other signalling pathways. We hope also to present results on changes in c-kit and steel expression in the nerves and dorsal root ganglia of normal animals during these two growth processes.

606.4

NGF DEPRIVATION DURING EARLY LIFE PERMANENTLY IMPAIRS COLLATERAL SPROUTING BUT NOT AXONAL REGENERATION. Y. Kril*, J. Fawcett, K.M. Mearow, E. Pertens, J. Diamond. Dept of Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada. L8N 3Z5.

A chance observation prompted us to examine the possibility that a brief interference in the availability of endogenous NGF during early postnatal life might permanently compromise the reparative capacity of mature NGF-sensitive sensory and sympathetic neurons. We have focused our attention on the ability of these neurons to functionally reinnervate denervated skin by (1) collateral sprouting of undamaged neurons, and (2) by the regeneration of damaged axons. Neonatal rats were treated with antibodies to NGF (anti-NGF) during various critical periods of neuronal development; non-immune serum-treated littermates served as controls. Aside from a slight ptosis in the earliest treated groups, the adults in the anti-NGF treated rats showed no obvious behavioral differences from their control littermates. After nerve crush, all classes of sensory axons regenerated normally in all animals (such growth, we have shown, is NGF-independent). However, in the adults that had received daily anti-NGF injections during the first two weeks of life, the collateral sprouting of nociceptive Aδ and C fibres into adjacent denervated skin was markedly reduced, and in some instances was absent altogether. Such sprouting is entirely NGF-dependent This result could not be explained by the reported phenotypic switching of nociceptive neurons.

Thus trophic deprivation in early life can permanently impair reconstructive mechanisms in the peripheral sensory system. Since regeneration was unaffected, the impairment probably centres on the regulation of neuritic outgrowth, rather than the growth process itself. We are now examining whether the normal upregulation of p75 and trkA receptor mRNA that precedes, and accompanies, collateral sprouting (but is unchanged during regeneration) is affected in these sprouting-compromised rats, and are also extending the study to sympathetic neurons.

606.5

DEVELOPMENTAL DISTRIBUTION OF ECTOPIC SUBSTANCE P FIBERS IN THE CNS OF TRANSGENIC MICE OVER-EXPRESSING NERVE GROWTH FACTOR IN MYELINATING OLIGODENDROCYTES. Weiya Ma, A. Ribeiro-da-Silva*, J.-P. Julien and A. C. Cuello. Dept. of Pharmacology & Therapeutics and Centre for Research in Neurosciences, McGill University, Montréal, Québec, Canada We have generated transgenic mice which over-express NGF driven by myelin basic protein promoter. In an early study (Ma et al., Eur.J.Neurosci., in press), we showed NGF immunoreactive (IR) oligodendrocytes and substance P (SP)-IR ectopic fibers of sensory origin in the white matter of the spinal cord of adult transgenic mice. In the present study, we investigated the postnatal distributions of NGF-IR oligodendrocytes and ectopic SP-IR fibers in the CNS of transgenic mice. NGF-IR oligodendrocytes were found in the white matter of CNS from postnatal day (PD) 0 to 2 months. Unexpectedly, SP-IR fibers were found in the white matter of the spinal cord, medulla, pons and cerebellum of both transgenic mice and controls from PD 0 to 2. By PD 5, these SP-IR fibers had decreased dramatically in controls, but increased markedly in transgenic mice. From PD 5 on, the ectopic SP-IR fibers in transgenic mice increased in numbers to reach the adult level by PD 20 and remained throughout adulthood. The percentages of SP-IR cells in sensory ganglia were significantly higher than in controls. We conclude that the myelinating oligodendrocytes in the CNS of this transgenic mice creates an environmental in which the over-expression of NGF induce SP-IR fibers normally occurring in the white matter of CNS during early postnatal period to sprout in an abnormal territory. In adulthood, the maintenance of the ectopic fibers is likely independent of NGF expression. (Supported by Neuroscience Network of Canadian Centres of Excellence)

606.6

OVEREXPRESSION OF NGF IN SKIN OF TRANSGENIC MICE INCREASES TRKA AND P75 EXPRESSION AND SIZE AND NUMBER OF SYMPATHETIC NEURONS. Isaac B. Caton*, David M. McKinnon[1], Hong-Seng Wang[1], Thomas P. Goodness, Frankie E. Davis, Kathryn M. Albers, and Brian M. Davis. Depts. of Anatomy & Neurobiology and Pathology, Univ. of Kentucky College of Med., Lexington, KY; [1] Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY.

Recently, our laboratories isolated transgenic mice (K14-NGF mice) that express elevated levels of NGF mRNA in stratified epithelium including skin (J. Neurosci.14:1422). Morphometric analysis shows that NGF increases the number (ca. 2.5 fold) and size of neurons in sympathetic ganglia. The neuron size difference between control and transgenic mice is greatest at PN6 but is still significant in adults. In addition, electrophysiological analysis revealed that male mouse superior cervical ganglia has two physiological distinct types of sympathetic neurons and that these are differentially affected by the increased level of NGF. The distribution of sympathetic fibers in several tissues was also examined by tyrosine hydroxylase immunocytochemistry. Despite the dramatic hypertrophy of sympathetic ganglia, hyperinnervation was confined to structures known to either express the NGF transgene (e.g skin) or contain supernormal levels of NGF peptide (e.g. lymph nodes, dorsal root ganglia) as demonstrated by ELISA. RT-PCR analysis also showed an increase in both trkA and p75 NGF receptor mRNA expression in sympathetic ganglia but no change for either NGF receptor in sensory ganglia from the same animals. Funded by NS 31825 (BMD) and NS-29755 (DMM).

606.7

EFFECTS OF NGF AND NT-3 OVEREXPRESSION IN SKIN OF TRANSGENIC MICE ON INNERVATION AND CGRP AND OPIATE RECEPTOR IMMUNOLABELING OF DORSAL HORN.
Brian M. Davis*, Kathryn M. Albers, Bruce E. Maley, Robert P. Elde[2], Thomas P. Goodness and Bruce Mendelson[1]. Depts. of Anatomy & Neurobiology and Pathology, Univ. of Kentucky College of Med., Lexington, KY; [1]Anatomy, Univ. of Arkansas Medical Sch., Little Rock, AR; [2] Cell Biology & Neuroanatomy, Univ. of Minnesota, Minneapolis, MN.

To determine if the somatotopic organization of cutaneous nerve projections was altered in transgenic animals overexpressing NGF in epithelial structures (J. Neurosci.14:1422), dorsal primary rami were labeled with DiI at the level of thoracic segment 10 in neonatal mice. In control and transgenic animals, the central processes of the labeled primary afferents projected to the lateral portions of the ipsilateral and contralateral dorsal horns. There were no apparent differences in the spatial patterns of cutaneous nerve projections between transgenic and control mice. However, the density of the labeled fibers and boutons was significantly greater in transgenic mice. Characterization of primary sensory fiber projections using CGRP immunocytochemistry revealed similar hyperinnervation that was apparent as early as postnatal day 7, and maintained in adults (but decreased compared to earlier time points). Adult transgenic mice were also examined for distribution of μ and ∂ opiate receptors using immunocytochemistry. Preliminary results indicate a significant increase in μ opiate receptor immunoreactivity in both NGF and NT-3 transgenic mice particularly in ventral horn and in ependymal cells. Funded by NS 31825 (BMD) and AA 09205 (BM).

606.8

DIFFERENTIAL EFFECTS OF VARIOUS NEUROTROPHIN AND *TRK* RECEPTOR DELETIONS ON MECHANORECEPTORS IN THE MESENCHYMAL SHEATH AFFILIATED WITH MOUSE WHISKER FOLLICLES.
B.T.Fundin*, F.L.Rice, I.Silos-Santiago, A.M.Fagan, H.Aldskogius, M.Barbacid, P.J.Ernfors. Dept. of Neuroscience and Lab. of Molecular Neurobiology, Karolinska Institute, Stockholm, Sweden S0104 01; Dept. of Pharmacology and Neuroscience, Albany Medical College, Albany, NY 12208; Dept. of Molecular Biology, Bristol-Myers Squibb, Princeton, NJ 08543.

Three types of mechanoreceptors arising from large myelinated axons are in the mesenchymal sheath that encases each whisker follicle in the mouse: straight lanceolate endings at the level of the ring sinus as well as irregular lanceolate endings and reticular endings at the level of the cavernous sinus. Anti-PGP 9.5 and the antibody RT97 were used to examine these endings by immunofluorescence in mice, ranging in age from P0 (i.e. day of birth) to P25, that had selective homozygous deletions of either a neurotrophin gene (BDNF or NT3) or a receptor gene (*trk*A, *trk*B or *trk*C). The amount of straight lanceolate endings was reduced although not eliminated by the absence of *trk*A, *trk*B and BDNF. Deletions of *trk*C resulted in a slight but distinct increase, while the loss of NT3 had no obvious effect. In contrast, reticular endings were totally lacking after *trk*A deletions, and substantially increased by the absence of *trk*C. No obvious effect were obtained by the loss of *trk*B, BDNF and NT3. Finally, deletions of *trk*B and BDNF resulted in a complete loss of irregular lanceolate endings, and only NT3 deletions resulted in a noticeable increase. Unlike with the two other sets of endings, *trk*A and *trk*C deletions had no obvious effects on the irregular lanceolate endings. These results indicate that the development or maintenance of each of these three presumptive mechanoreceptors is regulated by positive and negative interactions of different sets of neurotrophins and receptors. Moreover, each of these neurotrophins and *trk* receptors, including *trk*A, has an affect on at least two types of mechanoreceptors.

606.9

DIFFERENTIAL EFFECTS OF VARIOUS NEUROTROPHIN AND *TRK* RECEPTOR DELETIONS ON THE UNMYELINATED INNERVATION OF THE EPIDERMIS IN THE MOUSE WHISKER PAD.
G.A.Wilkinson, F.L.Rice, B.T.Fundin, I. Silos-Santiago, A.M.Fagan, R.J.Smeyne, S.D.Glick*, P.J.Ernfors, L.F.Reichardt, M.Barbacid. Program in Neurosci., Howard Hughes Med. Center, Univ. of California, San Francisco, CA 94143; Dept. of Pharmacol. & Neurosci., Albany Medical College; Albany, NY 12208; Dept. of Molecular Biology, Bristol-Myers Squibb, Princeton, NJ 08543; Dept. of Neurosci. and Lab. of Molecular Neurobiology, Karolinska Institute, Stockholm, Sweden S0104 01.

Fine caliber unmyelinated innervation of the epidermis in the whisker pad was analyzed by immunofluorescence in mice, ranging in age from P0 (i.e. day of birth) to P25, that had selective homozygous deletions of either a neurotrophin gene (BDNF, NT3) or a receptor gene (*trk*A, *trk*B, *trk*C). This innervation was revealed by using antibodies against PGP 9.5 (a neuronal cytoplasmic protein) and CGRP as well as antibody RT97 against a phosphorylated component of neurofilament protein. *Trk*A deletions resulted in the permanent absence of all the innervation which normally consists of numerous fine profiles labeled only with anti-PGP 9.5 and a smaller contingent that also labels with anti-CGRP. *Trk*B deletions resulted in a surprisingly massive increase in both components during the first postnatal week that was partially maintained at enhanced levels during subsequent maturation. *Trk*C deletions resulted in a transient increase only of anti-PGP 9.5 profiles but actually reduced the presence of profiles colabeled with anti-CGRP. The epidermal innervation was slightly increased at birth in BDNF and NT3 knockouts but the loss of BDNF eventually resulted in normal appearing innervation whereas the loss of NT3 resulted in a drastic reduction of the CGRP component. These results indicate that the development or maintenance of the unmyelinated epidermal innervation is regulated by positive and negative interactions of several neurotrophins and *trk* receptors.

606.10

EFFECTS OF NT3 AND TRKC MANIPULATIONS ON DEVELOPING MERKEL INNERVATION IN THE MYSTACIAL PAD OF THE MOUSE
J.Arvidsson, F.L.Rice*, B.T.Fundin, K.M.Albers, I.Silos-Santiago, A.M.Fagan, M.Barbacid, P.J.Ernfors, B.M.Davis. Dept. of Neuroscience and Lab. of Molecular Neurobiology, Karolinska Institute, Stockholm, Sweden S0104 01; Dept. of Pharmacology and Neuroscience, Albany Medical College, Albany, NY 12208; Dept. of Pathology and Dept. of Anatomy and Neurobiology, Un. of Kentucky, Lexington, KY 40536; Dept. of Molecular Biology, Bristol-Myers Squibb, Princeton, NJ 08543.

The Merkel innervation in the whisker pad was analyzed by immunocytochemistry in mice, ranging in age from P0 to P25, that had selective homozygous deletions of either a neurotrophin gene (BDNF or NT3) or a receptor gene (*trk*A, *trk*B, *trk*C). In other mice, NGF or NT3 was hyperexpressed through a transgene construct containing an epidermal-specific promotor gene. This innervation was revealed by antibodies against PGP 9.5 (which label all innervation and Merkel cells) and the antibody RT97 which labels myelinated axons and their endings. Normally, all Merkel innervation in the whisker pad is well developed at birth and is located at the mouth of guard hair and whisker follicles and in the outer root sheath of whisker follicles. In contrast to other vibrissa related mechanoreceptors, the Merkel innervation was only affected by manipulations of NT3 and trkC. In newborn mice lacking NT3 or *trk*C, many Merkel cells were present, however, few if any were obviously innervated. By two weeks, virtually all of the Merkel cells had disappeared and no Merkel endings were evident. Consistent with this observation, the hyperexpression of NT3 resulted in a dramatic increase in the presence of Merkel cells and endings at least at the mouth of guard hair and whisker follicles. Consistent with preferential high affinity binding found in previous studies, these results indicate that the development or maintenance of Merkel endings is dependent on NT3 acting through the trkC receptor.

606.11

COMPOSITION OF THE DEEP NERVES INNERVATING THE POSTERORBITAL SINUS HAIR IN TRANSGENIC MICE THAT OVEREXPRESS NERVE GROWTH FACTOR IN THE EPIDERMIS.
Thomas P. Goodness*, Bruce E. Maley, Mary Gail Engle, Kathryn M. Albers, Brian M. Davis, Depts. of Anatomy & Neurobiology and Pathology, University of Kentucky College of Medicine, Lexington, KY.

Electron microscopy was used to compare the fiber composition in the deep vibrissal nerves (DVN) innervating the posterorbital sinus hair (POSH) of control mice and transgenic mice (NGF-mice) that overexpress nerve growth factor (NGF) in the epidermis. The POSH is an isolated vibrissa located approximately midway between the eye and the ear. The DVN, which is composed of myelinated and unmyelinated axons, pierces the outer capsule of the POSH, and then courses superficially, running parallel to the vibrissa. Cross sections were taken of the POSH where the DVN enters the outer capsule. Counts of axonal profiles revealed that NGF-mice had significantly more unmyelinated axons within the DVN than control mice, whereas there was no difference in the number of myelinated axons. In addition, large fascicles of unmyelinated axons were found outside the DVN of NGF-mice, in the dermal capsule surrounding the root sheath. These fascicles, which contained unmyelinated axons of a wide range of sizes, were not observed in control mice. The origin of the fascicles is not yet known. We are currently performing immunocytochemistry to further characterize these axons. These results indicate that increased levels of NGF selectively increase the number of unmyelinated axons in the vibrissae of mice. Funded by NS 31825 (BMD).

606.12

DIFFERENT EFFECTS OF FETAL NGF INJECTIONS AND TRANSGENIC NGF ENHANCEMENT ON THE INNERVATION OF THE WHISKER PAD IN NEONATAL RATS AND MICE.
F.L.Rice, T.A.Henderson, K.M.Albers, B.M.Davis, J.N.Carlson*, M.F.Jacquin. Dept. of Pharmacology and Neuroscience, Albany Medical College, Albany, NY 12208, Dept. of Neurology, Washington Univ., St. Louis, MO 63110, Dept. of Pathology and Dept. of Anatomy and Neurobiology, Univ. of Kentucky, Lexington, KY 40536.

Substantial neuron death occurs in the trigeminal ganglion (Vg) of rats and mice during the last week of gestation following a precipitous decline in the production of NGF in the mystacial pad. Previously we have found as much as a 36% increase in Vg neurons in neonatal rats after systemic injections of NGF on embryonic days 15 and 18 but whisker related projection patterns, normally present in the brainstem nuclei, were lost. In contrast, virtually no neuron loss occurred and whisker patterns were present in mice that had continuously enhanced NGF levels provided by an epidermal specific transgene construct. In this study, the innervation of the whisker pad was examined by immunofluorescence in such neonatal rats and mice. After NGF injections, the innervation of the skin between the whiskers as revealed by anti-PGP 9.5 was selectively increased and relatively more mature compared to controls. Also a far greater proportion of the innervation labeled with anti-CGRP. After transgenic enhancements, the interwhisker skin had even greater and more mature innervation but a relatively normal proportion labeled with anti-CGRP. Moreover, a component of whisker follicle innervation that is normally sparse and labels with anti-CGRP was enormously increased only in the transgenic paradigm. These results suggest that the NGF injections had a more selective effect among NGF responsive populations perhaps enhancing only a CGRP component of epidermal innervation. The relative imbalance between the impact on the interwhisker and whisker innervation after NGF injections might be related to the loss of the whisker related projection patterns in the brainstem. NIH DE 07734.

606.13

NERVE GROWTH FACTOR AND HYPERINNERVATION IN NEONATAL SKIN WOUNDS. M.L. Reynolds and M. Fitzgerald (SPON: Brain Research Association). Dept. Anatomy & Developmental Biology, University College London, U.K.

Skin wounds made during the newborn period result in sensory hyperinnervation of the wounded area which lasts for over 12 weeks (Reynolds & Fitzgerald, J. Comp. Neurol., in press). NGF levels (measured by ELISA) increased from 30-149 pg mg^{-1} tissue in surrounding skin during the 3 days following wounding, returning to control levels by P10 (Constantinou et al., NeuroReport 5: 2281, 1994). We have used a tissue culture model to examine the relationship between NGF upregulation, developmental stage and the production of hyperinnervation during wound healing in perinatal skin. Pieces of skin taken from around the scar area of wounds made 3 days before on the dorsal surface of the hindpaw of neonatal rat pups were cocultured in collagen gels with dorsal root ganglia (DRG) from E7 chick. The area surrounding the DRG explant was divided into 4 equal quadrants and the number of PGP9.5 immunostained neurites growing in each counted. With skin wounded 3 days previously the number of neurites in the quadrant adjacent to the skin was 15.1 ± 0.28 and on the segment away from the skin 4.9 ± 0.13. In age matched normal skin the comparable figures were 2.4 ± 0.18 and 3.2 ± 0.18. The addition of anti-NGF antiserum used in the ELISA estimations and shown to reduce neurite outgrowth in control ganglia by 75-94% resulted in an insignificant change in neurite outgrowth in these cultures; 13.7 ± 0.34 and 3.9 ± 0.12 neurites in adjacent and opposite quadrants respectively. Conditioned medium made by culturing 3 day post wound skin in serum free medium was shown by ELISA to have 3-5 times the NGF concentration of age matched P3 skin cultured in the same way. The results suggest that although NGF levels rise dramatically in neonatal skin after wounding other cytokines or growth factors may be involved in establishing the resulting hyperinnervation.

Sponsored by the MRC.

606.14

INFLUENCE OF STIMULATION AND NGF ON GENE EXPRESSION IN COLLATERALLY SPROUTING DRG NEURONS. K.M. Mearow*, Div. Basic Medical Sciences, Memorial University of Newfoundland, St. John's, NFLD, Canada, A1B 3V6.

Collateral sprouting of mature cutaneous nociceptive fibres is regulated by the availability of NGF. Furthermore, the onset of this sprouting is accelerated by electrical stimulation of the intact nerve. To investigate this influence of stimulation on the sprouting of DRG neurons the following experimental paradigms were used: adult rats were administered NGF (60 ug/100 g, ip) daily for 1-12 days; cytochrome c was injected in control animals. The dorsal cutaneous nerves were exposed, and those on the left side were electrically stimulated, while the ones on the right were not. At 12 days post-stimulation, the extent of nociceptive fibre sprouting was examined by an established behavioural mapping technique, and found to have occurred only in the NGF-treated animals. DRGs were removed at 6 hs, 1, 2, 6 and 12 days post-stimulation, cryosectioned and processed for in situ hybridization to examine mRNA expression of the NGF receptors (p75 and trkA), GAP-43 and α-tubulin; as well, the neurotrophins NGF, BDNF and NT-3 were examined. Expression of p75, trkA, α-tubulin and GAP-43 mRNAs was upregulated in the NGF-treated (2d and longer), but not in the control DRGs. Stimulation resulted in these increases being observed 1-3 days in advance of that in the absence of stimulation; nerve stimulation alone had no effect on expression of these mRNAs. Examination of neurotrophin mRNAs confirmed the absence of neuronal NGF and NT-3 expression, and the presence of BDNF expression. However, preliminary results suggest that stimulation significantly increased BDNF expression in these DRG neurons. These results demonstrate that stimulation influences the expression of mRNAs involved in the sprouting response, and suggest the possibility of the involvement of BDNF in the stimulation-induced acceleration of the sprouting response. Supported by NSERC.

606.15

NERVE GROWTH FACTOR ADMINISTRATION REVERSES DEFICITS IN SUBSTANCE P GENE EXPRESSION, BUT NOT TRKA AND NEUROFILAMENT L, IN SENSORY NEURONS OF DIABETIC RATS. L.T. Diemel*, P. Fernyhough and D.R. Tomlinson. Dept. of Pharmacology, Basic Medical Sciences, Queen Mary and Westfield College, University of London, U.K..

Effects of nerve growth factor (NGF) treatment on sensory neuron gene expression for substance P, neurofilament L (NFL) and trkA were studied in control and diabetic rats. Control rats were treated s.c. 3x weekly for 4 weeks with 0.2 and 1.0mg/kg hrNGF. Diabetic rats (65mg/kg streptozotocin i.p.) were treated for 1, 2 and 4 weeks with low dose hrNGF (total duration of diabetes was 8 weeks). Total RNA was isolated from the L_4 and L_5 dorsal root ganglia (DRG) and subjected to Northern blotting. Sciatic nerve samples were analysed for substance P and NGF protein. In control rats NGF treatment, at low dose, elevated γ-PPT mRNA (substance P precursor) greater than 2-fold ($p<0.05$) and trkA mRNA 1.33-fold in DRG. High dose NGF treatment had no greater effect even though NGF protein levels in sciatic nerve were raised 2-fold above those measured at low dose NGF. The levels of mRNA for γ-PPT, NFL and trkA were reduced by 25-40% in DRG of diabetic rats. NGF treatment for 4 weeks only reversed the deficit in γ-PPT mRNA. NGF treatment also reversed substance P protein levels, with an effect by 2 weeks of treatment of diabetic rats. At all time periods the levels of NGF protein in sciatic nerve of diabetic rats were significantly lower than control ($p<0.05$). The results show that diabetic sensory neurons have a reduced capacity to bind and/or retrogradely transport NGF. Furthermore, sensory neurons of diabetic rats mimic regenerating neurons in the ability of NGF treatment to reverse substance P gene expression but not that of NFL and trkA.

606.16

THE EFFECT OF NERVE GROWTH FACTOR ON AXOTOMIZED DORSAL ROOT GANGLION CELLS AND THEIR CENTRAL TERMINALS.
N.P. Eriksson, C. Rivero-Melián* and H. Aldskogius
Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden

Nerve Growth Factor (NGF) is known to have a function as a neuron-survival factor in dorsal root ganglion- (DRG) neurons as well as a differentiating factor for neuropeptides in their central terminals. However, NGF is unlikely to be of importance for the majority of the carbonic anhydrase- (CA) expressing DRG neurons, which lack the high-affinity NGF binding sites. This is also the case for the fluoride resistant acidic phosphatase- (FRAP) neurons in the spinal cord dorsal horn. It is not clear, however, whether the neuropeptides are really needed for neuronal survival. In our experiments we therefore wanted to study the effects of NGF on the differentiation of the neuropeptides, CA and FRAP as well as the neuron survival. The sciatic nerve was either transected or its transected proximal nerve stump was put in a capsule containing NGF or control substance. The animals were allowed to survive for two or four weeks whereafter they were sacrificed and the DRG:s L4-L6 as well as the spinal cord were dissected out and processed for SP, CGRP, CA and FRAP using either immunohistochemical-, radioimmunoassay- or ELISA-techniques. DRG:s were also cresyl stained for quantification. Ratios operated/unoperated sides were used as an index of depletion. Altogether, the neuropeptide-RIA results from DRG and spinal cord show no significant difference between the NGF- and the control substance groups. However, regardless treatment protocol, there is a larger side-to-side difference in the spinal cord than in the DRG:s. Further, following nerve transection only there is no significant decrease in the amount of CGRP in the DRG. On the other hand, the immunohistochemical material reveal a difference between the NGF-treated and untreated material, which is also the case for the DRG-counts. The CA- and the FRAP-results reveal no significant difference between the NGF- and the control substance groups. Thus, despite rescuing the neurons, NGF in the concentration and durations used does not completely reverse the axotomy induced depletion of neuropeptides in the dorsal horn.

606.17

CEREBROVASCULAR AXONS OF THE AGED RAT RESPOND TO INTRACEREBROVENTRICULAR INFUSION OF NERVE GROWTH FACTOR. K.L. Schwenk[1], G.N. Greenlees[1], R.G. Sherman[1]*, K.A. Crutcher[2], and L.G. Isaacson[1]. [1]Center for Neurosci Res, Dept Zoology, Miami Univ, Oxford, OH 45056; [2]Dept Neurosurg, Univ of Cinci Coll of Med, Cinci, OH 45267.

Although there is evidence for loss of neuronal plasticity with age, very few models permit assessment of the extent to which this loss of plasticity is due to changes in the neuronal environment or to a loss of growth potential of the aged neurons. A 2 week intracranial infusion of nerve growth factor (15 μg; NGF) into young rats (10 wks) results in sprouting of perivascular axons associated with the intradural internal carotid artery (ICA). This sprouting response results in a 2-fold increase in the total number of perivascular axons, the majority of which are sympathetic in origin. The robust response by young, intact sympathetic neurons provides the opportunity to determine whether aging affects such neuronal plasticity. Aged (24 mo.) Fischer female rats received a 2 week intracranial infusion of NGF (15μg; n=4) or VEH (n=4) and were perfused for electron microscopy. The mean number of axons associated with the ICA was increased by 120% following NGF infusion when compared with VEH controls (383 vs. 841 axons). These results suggest that aged cerebrovascular neurons retain their responsivity to exogenous trophic factors. The data are also consistent with previous findings suggesting that the decline in plasticity in the aged sympathetic nervous system is not due to the loss of growth potential of the aged neurons. (Supported by NS17131 and NS32876)

606.18

IN VIVO RESPONSIVENESS OF SENSORY AND SYMPATHETIC NERVES TO NGF IN MATURITY AND OLD AGE. I. Gavazzi*, K.L. Railton, T. Cowen, Dept. of Anatomy and Developmental Biology, Royal Free Hospital School of Medicine, London NW3 2PF, UK

Reduced responsiveness of ageing neurons to trophic factors could lead to decreased neuronal plasticity in old age. We have investigated this issue in the peripheral nervous system, evaluating the responsiveness to NGF of mature and aged sympathetic and sensory neurons supplying the innervation to the rat iris. Sprague-Dawley rats, aged 6 weeks or 24 months, received 4 weekly transcleral injections into the anterior eye chamber of 5μl (young rats) or 10μl (old rats) of: a) 100μg/ml Cytochrome C (CytC); b) 25μg/ml NGF; c) 100μg/ml NGF, or d) 200μg/ml NGF. CytC and NGF were dissolved in 1mg/ml rat albumin in PBS. One week after the final injection the irides were dissected and processed as whole mounts by conventional immunofluorescence techniques, using antibodies against TH (tyrosine hydroxylase, a sympathetic marker) or CGRP (calcitonin gene-related peptide, a marker for a subpopulation of sensory neurons). The density of innervation on the irides was measured by computerized image analysis. The density of CGRP-immunoreactive nerves was decreased on control irides from old animals as compared to young ($p<0.05$), whilst TH-positive nerve density was unaltered. CytC treatment had no effect on nerve density. The density of TH- and CGRP-positive nerves was increased by NGF treatment in both young and old rats. There appeared to be no loss of responsiveness in old age. However, CGRP-positive nerves were more sensitive to NGF treatment, showing significant growth responses to 25μg/ml NGF ($p<0.05$). In contrast TH-positive nerves responded only to NGF concentrations of 200μg/ml. We conclude that aged sympathetic and sensory nerves retain unaltered ability to respond to NGF as measured by changes in density of TH and CGRP immunoreactivity. Both in young and in old animals, sensory nerves are more sensitive than sympathetic nerves to variations in NGF availability.

606.19

INCREASED VULNERABILITY OF SYMPATHETIC, BUT NOT SENSORY, NEURONS TO NGF DEPRIVATION IN AGEING RATS: THE ROLE OF LOW-AFFINITY NGF RECEPTORS. T.Cowen*, R.E.M.Canavan and I.Gavazzi, Dept.of Anatomy and Developmental Biology, Royal Free Hospital School of Medicine, London NW3 2PF, UK.

In order to study the causes of selective neuronal vulnerability during normal ageing, sympathetic and sensory nerves to the irises of young (6wk) and old (24m) rats were treated daily for 7 days with anti-NGF antibody (αNGF; 0.5mg/ml) raised in sheep (Cedar Lane Ltd, Canada) or sheep IgG alone by transcleral injection into the anterior eye chamber under halothane anaesthesia. 0.5μl were injected into young rats and 10μl into old using sterile Hamilton syringes. Irises from untreated young and old rats were also studied. Animals were killed under terminal anaesthesia, irises were dissected and processed as whole mounts for CGRP immunohistochemistry to demonstrate sensory nerves or treated with glyoxylic acid for the demonstration of noradrenergic nerve fibres. Some untreated irises were double-immunolabelled for tyrosine hydroxylase (TH) and low-affinity NGF receptor (p75NGFr) or CGRP and p75NGFr. Nerve densities and co-localized double labels were assessed using computerised image analysis. Sympathetic nerve fibre density was reduced by about 40% ($p<0.05$*) in old rat irises following treatment with αNGF whilst in young rats little change was observed. In contrast, sensory nerve fibres to the iris were relatively unresponsive to αNGF treatment showing few changes in either young or old rats. p75NGFr showed substantial reductions in sympathetic nerve fibres from untreated old rat irises compared to young, whilst p75NGFr on sensory nerves showed less obvious changes with age. We conclude that sympathetic nerves become more vulnerable to deprivation of target-derived NGF with age and are more vulnerable than sensory nerves innervating the same target. We hypothesise that reduced p75NGFr expression contributes to the increased vulnerability of ageing sympathetic nerves.

606.20

AXONAL REGENERATION OF SENSORY NERVES IS REDUCED BY CONTINUOUS INTRATHECAL INFUSION OF NERVE GROWTH FACTOR (NGF). B.G. Gold*†^ M. Lagrange† and M. Zelany-Pooly. †Center for Research on Occupational & Environmental Toxicology (CROET) and ^Department of Cell Biology & Anatomy, Oregon Health Sciences University, Portland, OR 97201.

While it is well established that NGF is growth promoting for sensory neurons in culture, it is unclear whether it serves such a function *in vivo*. In fact, recent evidence indicates that NGF does not support regeneration of adult rat peripheral nerve (Diamond et al. *J Neurosci* 12:1467, 1992). Furthermore, the ability of NGF to prevent the induction of many axonal injury-induced changes in the neuronal cell body, such as increased *c-jun* expression (Gold et al., *Neurosci Letters* 154:129, 1993), suggests that NGF may actually impair axonal regeneration by reducing the cell body response to injury. To test this hypothesis, rats given a bilateral sciatic nerve crush received NGF (125 ng/hr) as a continuous infusion into the subarachnoid space of the lumbar spinal cord via an osmotic minipump (Alzet); controls received cytochrome C. At 7 or 10 days, the pump was removed and L4 or L5 dorsal root ganglion (DRG) exposed and injected with 50 μCi of [^{3}H]-leucine. Animals were killed 24 hours later, the sciatic nerves removed, cut into 3-mm segments and the radioactivity in each segment determined by liquid scintillation spectrophotometry. Maximal regeneration distances were determined from the front of the resultant transport curves. At 8 days, the apparent rate of regeneration (distance divided by the number of days following nerve crush) was significantly ($p < 0.001$) reduced (by 19%) in nerves from NGF-infused rats to 3.4 ± 0.04 mm/day (n=6) (mean ± SEM) from 4.2 ± 0.11 mm/day (n=7) in cytochrome C-infused rats. However, actual regeneration rates (between 8 and 11 days) were unaltered (regeneration distances were similarly reduced by NGF at 8 and 11 days) suggesting that NGF delays the onset of regeneration (*ca.* 3 days). The data support a role for two signals in the regenerative response: an early, induction signal (*e.g.* loss of NGF; present study) and a later, maintenance signal (see Gold et al., *Neurosci Letters* 176:123, 1994). *Supported by the Paralyzed Veterans of America Spinal Cord Research Foundation and NS19611.*

606.21

AUTOCRINE OR PARACRINE ROLE OF NGF IN DORSAL ROOT GANGLIA DURING DEVELOPMENT. J. S. Gill* and A. J. Windebank. Molecular Neuroscience Program, Mayo Foundation, Rochester, MN 55905.

NGF is a target derived growth factor. In the peripheral nervous system, it is produced by tissues innervated by the sympathetic nervous system and small sensory neurons. In the present study, we tested the hypothesis that an alternate source of NGF must be available to support dorsal root ganglion (DRG) neurons before they make connection with the target. Expression of NGF in rat DRG during development (E12 to adult) was examined. Using reverse transcriptase-polymerase chain reaction (RT-PCR), we were able to demonstrate NGF mRNA from E12-E17 (maximal at E13), while transcripts were not detected in adult DRG. Parallel studies using RT-PCR revealed mRNA for the high affinity NGF receptor/TrkA at all time periods studied. Functional assays were carried out to determine the level of receptor activation during development. Western blot analysis of DRG cell lysates (E12 to adult) revealed tyrosine phosphorylation of TrkA from E13-E17 only. Digoxigenin labelled riboprobes were constructed and used for *in situ* hybridization studies over the same time period to localize the site of NGF mRNA synthesis at the cellular level.

Conclusion: NGF mRNA is expressed in DRG after neuronal birth and before axons make contact with their targets. This demonstrates a potential paracrine or autocrine role for NGF during development of primary sensory neurons (supported by NS 29769).

606.22

AN EFFICIENT, UNBIASED METHOD TO ESTIMATE CELL NUMBER AND VOLUME IN MULTIPLE DORSAL ROOT GANGLIA. C. Avendaño* and A. Lagares. Dept. Morphology, Autónoma Univ., Sch. of Medicine, 28029 Madrid, Spain.

Few neuronal ensembles would seem better suited for being counted and measured than the primary sensory neurons in the dorsal root ganglia (DRG). Cell counts are useful to investigate developmental or degenerative processes in DRG, and cell body size is used, among other purposes, to recognize anatomical and functional subpopulations of neurons. However, despite a wealth of quantitative studies on DRG, progress is still hampered by conflicting results largely arising from methodological disagreements on sampling, counting or measuring procedures. Recent stereological developments have introduced unbiased methods which allow to efficiently obtain precise estimates of numbers and sizes of cell populations. We have applied the *optical disector, rotator* and *fractionator* principles (Gundersen et al., APMIS 96:857, 1988; Jensen and Gundersen, J. Microsc. 170:35, 1993) to estimate the total number of neurons, as well as the cell body size in groups of DRG. All left DRG of a rat, and sets of C_4 through T_2 DRG from both sides of 5 macaques (*M. nemestrina*) were orderly arranged and embedded in single blocks of celloidin. Sections cut at 50 μm were Nissl-stained. Cell counts and volume measurements were performed using an interactive computer system running the GRID® Stereological Software Package (Olympus Denmark). In the rat the total number of left DRG neurons was 155000. In monkeys, cell counts averaged 252000 per side (range: 203000 to 277000), with 45% belonging to the A type. Coefficients of error for these estimates were below 8%. Mean somatic volume (uncorrected for shrinkage) was 32400 μm^3 for A-cells and 4960 μm^3 for B-cells. Soma size distribution was skewed to the right in all cases and there was moderate overlap between the distributions of the two cell types. Cell counts performed on individual ganglia showed significant side differences in C7 and C8. It is expected that methodological developments like this one will encourage the use of quantitative approaches to the study of developmental, pathological and plasticity processes in the sensory systems.

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NEUROTROPHIC FACTORS: BIOLOGIC EFFECTS XII

607.1

DISTINCT CELLULAR TARGETS OF THE BONE MORPHOGENETIC PROTEINS IN NEURAL DEVELOPMENT. P. C. Mabie, M. Mehler, A. Papavasiliou, R. Marmur, S. Qingbin, K. Shen*, J. Kessler. Depts. of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Bone morphogenetic protein (BMP) members of the transforming growth factor beta superfamily have complex effects on developing neural cells in vitro, including region-specific induction of apoptosis, enhanced neuronal outgrowth and survival, promotion of astrocyte differentiation and proliferation, and modulation of neuropeptide gene expression. Regulated BMP transcript expression during neural development in vivo suggests that the BMPs may function as inductive signals for multiple lineages at different developmental stages. To define the direct target(s) of the BMPs and to elucidate their mechanism(s) of action, purified cultures of murine neurons, epidermal growth factor-generated multipotent progenitors, astroblasts, oligodendroblasts, and microglia were analyzed for transcript expression of activin receptor-like kinases (ALK) 3 and 6, putative BMP type I receptors, and for effects of BMP treatment. Neurons, multipotent progenitors, and protoplasmic astroblasts express both ALK 3 and 6, whereas oligodendroblasts and microglia express only ALK3. BMP treatment of primary embryonic serum-free cultures results in increased neuronal outgrowth and survival, as well as astroblast differentiation and proliferation. BMP treatment of multipotent progenitors favors differentiation and proliferation of astrocytes, while also enhancing outgrowth in surviving neurons. BMP-treated astroblasts and microglia do not significantly differentiate or proliferate. Preliminary experiments utilizing BMP-conditioned media from purified cell preparations suggest that the BMPs exert their trophic effects in part by actions on distinct cellular targets. These results suggest that the BMPs have distinct effects on neural cells of different lineages and stages of maturation and participate in complex signaling events between early diverging neural lineages.

607.2

OSTEOGENIC PROTEIN-1 (OP-1) INDUCES DENDRITIC GROWTH AND BRANCHING IN CULTURED HIPPOCAMPAL NEURONS. G. Withers[1], D. Higgins[2], D. Rueger[3], and G. Banker[1]*. [1]Dept. of Neurosci., Univ. Virginia Sch. of Medicine, Charlottesville, VA 22908, [2]Dept. of Pharmacology, State Univ. of New York, Buffalo, NY 14214, [3]Creative Biomolecules, Hopkinton, MA 01748

Osteogenic Protein-1 (OP-1), a member of the TGFβ superfamily, was originally isolated from bone but is expressed in both developing and mature brain. Previous studies have demonstrated that OP-1 stimulates dendritic growth in cultured sympathetic neurons. We examined the effects of OP-1 on process development in cultured hippocampal neurons.

Under our standard culture conditions (co-culture with astroglial in serum-free medium), dendritic development begins after several days in culture and by two weeks in culture, the cells have highly elaborate dendritic arbors. In cultures maintained in serum-free medium (without glia), treatment with OP-1 markedly enhanced the rate and extent of dendritic developmen'. By 1 day in vitro (DIV) most neurons had generated tapered and highly branched processes, several days before dendrites develop in our standard cultures. Dendritic length and branching was quantified after 3DIV using the Sholl concentric ring analysis. Neurons exposed to OP-1 were significantly increased on most branching and length measures compared with neurons from our standard coculture, e.g. number of Sholl ring intersections (39.6 vs. 16.02), number of terminal branches (13.05 vs. 6.64). There were no significant differences in the number of primary dendrites.

These observations provide further support for the view that neuronal morphology can be regulated by polypeptide growth factors. Supported by NS23094(GB) and NS09652(GW).

607.3

SOURCES OF TRANSFORMING GROWTH FACTORS AND THEIR ACTIONS ON DOPAMINERGIC NEURONS IN CULTURE. **J.W. Commissiong,* P. Tsoulfas, T. Takeshima,[1] and R.D. McKay.** LMB-NINDS-NIH, Bldg.36/3D02, Bethesda, MD 20892. [1]Div. Neurol., Tottori Univ. Sch. Med., 86 Nishimachi, Yonago 683, Japan.

The transforming growth factors (TGF) are a family of multifunctional compounds that promote the growth, differentiation and morphogenesis of cells. Glial cell line-derived neurotrophic factor (GDNF), (Lin et al., 1993), and TGF-β1, TGF-β2 and TGF-β3 (Krieglstein and Unsicker, 1994; Poulsen et al., 1994) were reported to enhance the survival of dopaminergic (TH-pos) neurons in culture. We tested each compound at 0.1, 1.0 and 10 ng/ml, under optimal conditions of culture of cells from the ventral mesencephalon of the E14 rat, and stained on the seventh day to identify TH-pos and total (MAP2-pos) neurons. GDNF, but not TGF-β1, caused an increase in TH-pos neurons per field (320 μm²), without a corresponding increase in MAP2-pos cells. TGF-β2 and TGF-β3 at 1.0 and 10 ng/ml, caused a decrease in TH-pos and MAP2-pos cells. Type-1 astrocytes (T1-As) and oligodendrocyte, type-2 astrocyte progenitor (O-2A) cells, expanded from the striatum of the E16 rat, and the B49 glial cell line, produce factors that promote the survival of TH-pos neurons in culture. We used the RT-PCR method to test for the expression of mRNA for the above mentioned compounds in all three glia. mRNA for TGF-β1, TGF-β2, TGF-β3 and GDNF was identified in T1-As and B49 cells. mRNA for TGF-β1, and TGF-β3 was identified in O-2A progenitor cells. Experiments are in progress to determine the corresponding degree of protein synthesis. The results indicate that GDNF is unlikely to be the dopaminergic neurotrophic factor that is produced by O-2A progenitor cells (Takeshima et al., 1994), and that the effects exerted by the TFG family of compounds on neurons may be critically dependent on the conditions of the assay. The results also indicate that caution must be exercised in any suggested use of these factors to treat neurodegenerative diseases.

Krieglstein. K. And Unsicker, K. (1994) Neuroscience 63:1189-1196
Lin, L.-F. H., et al. (1993) Science 260:1130-1132.
Poulsen, K, T, et al. (1994) Neuron 13:1245-1252.
Takeshima, T. et al., (1994) Neurosci. Letts. 166: 178-182.

607.4

6-OHDA LESION AND TGFα INFUSION-INDUCED PLASTICITY IN THE NIGROSTRIATAL DOPAMINE SYSTEM -- TRACING CHANGES WITH MARKERS FOR NEURODEGENERATION. S. Reid*, S. Patel, S. Mungania, S.E. Loughlin, R. Giolli and J.H. Fallon. Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

The nigrostriatal dopamine system displays considerable plasticity in response to injury or administration of exogenous dopaminotrophic factors. In a previous series of experiments, we examined the changes in nigrostriatal expression of transforming growth factor alpha precursor (TGFα-P) and epidermal growth factor receptor (EGFr) mRNAs and peptides in lesioned and TGFα-infused adult rats (Loughlin et al., 1992, 1994; Fallon et al., 1994). In related studies, we determined that TGFα infusions can reduce apomorphine-induced rotation behavior in unilaterally 6-OHDA lesioned rats (Loughlin et al., 1992) and may spare tyrosine hydroxylase-immunoreactive (TH-IR) afferents to the striatum (Loughlin et al., 1993). In the present study, we traced the 6-OHDA-induced degeneration of both striatal afferent fibers and mesencephalic dopaminergic cell bodies with markers selective for degeneration of each cellular structure and assessed the time course of protective effects of infused TGFα. Lesioned animals received either artificial cerebrospinal fluid (aCSF) alone or TGFα in aCSF (50 μg/ml, 0.5 μl/hr). A modified Nauta silver impregnation technique was used to label degenerating axonal processes at selected timepoints after lesion and infusion. The fluorescent stain, Fluoro-Ruby (courtesy L. Schmued), was used to identify degenerating cell bodies within the substantia nigra. The combined degenerative profiles from these stains are presented and compared with data from TH immunostaining during the progressive loss of nigrostriatal dopaminergic innervation. Supported by NS26761, NS15321, NIH Predoctoral Traineeship NS07351-5 (to S.R.), the American Foundation for Aging Research Graduate Fellowship (to S.R.), the UC Irvine Committee of 1000 Graduate Fellowship (to S.R.), the American Parkinson's Disease Association, the Parkinson's Disease Foundation, and the World Bank Training Grant, U. Nairobi.

607.5

BONE MORPHOGENETIC PROTEINS BMP-2 AND -4 STIMULATE THE EARLY DEVELOPMENT OF AVIAN SYMPATHOADRENERGIC CELLS.

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Adrenergic differentiation of sympathoadrenergic precursor cells is elicited early during development in the vicinity of notochord and dorsal aorta by interactions with the environment. Quail neural crest cell cultures were used to identify signals that affect development of sympathoadenergic cells. BMP-2 and -4 strongly increase the number of tyrosine hydroxylase-immunoreactive cells. When other members of the TGF-ß superfamily were tested, BMP-7 was found to be active at high concentrations, whereas TGF-ß1, TGF-ß3 and GNDF displayed no effect. BMPs, as well as TGF-ß1 and TGF-ß3 reduce the number of melanocytes developing in these cultures. Preliminary in situ hybridization experiments indicate expression of BMP-4 in dorsal aorta and the developing heart. We propose that BMP-2 and/or BMP-4 may play an important role for the induction of adrenergic differentiation of sympathetic neurons.

607.6

THE EFFECTS OF BMP-2, -4 AND -6 ON THE DEVELOPMENT OF THE ADRENERGIC PHENOTYPE IN QUAIL TRUNK NEURAL CREST CULTURES. J.E. Varley and G.D. Maxwell*, Dept. of Anatomy, Univ. of Connecticut Health Center, Farmington, CT 06030-3405

Members of the TGF-β superfamily of growth factors have a wide range of actions in developing and mature systems. Within this superfamily are the dpp and 60A subfamilies which contain several of the bone morphogenetic proteins (BMPs). The BMPs were originally identified based on their ability to stimulate new bone formation, but more recent investigations have revealed that they can act in nervous tissue as well. We have investigated the effects of BMP-2 and BMP-4, members of the dpp subfamily, and BMP-6, a member of the 60A subfamily, on the development of quail trunk neural crest cells in tissue culture. Cultures were grown in medium containing 15% horse serum and 10% chick embryo extract. When human recombinant BMP-2 or BMP-4 was present in the medium at 10 ng/ml the number of tyrosine hydroxylase (TH)-positive cells which developed after 7 days in vitro was increased 100-fold relative to control values. Total cell number was not altered by BMP-2, while melanocyte cell number was reduced to about two-thirds of the control value. In contrast to BMP-2 and BMP-4, BMP-6 at 10 ng/ml resulted in a less than two-fold increase in TH-positive cell number and 100 ng/ml of BMP-6 resulted in a six-fold increase. These results show that BMP-2 and -4 are potent stimulators of adrenergic development in avian trunk neural crest cultures, while BMP-6 is much less effective at promoting adrenergic development.

This work was supported by grant NS16115 from the NIH. Human recombinant BMPs were the generous gift of the Genetics Institute, Inc.

607.7

EXPRESSION OF ACTIVIN A AND FOLLISTATIN mRNA IN TARGETS OF THE CILIARY GANGLION. D.C. Darland* and R. Nishi. Dept. of Cell Biology and Anatomy, Oregon Health Sciences University, Portland, OR 97201

We are interested in examining target-derived factors that affect the neurotransmitter phenotype of neurons in the avian ciliary ganglion (CG). The CG consists of two populations of neurons: the ciliary neurons which innervate the ciliary body and iris, and the choroid neurons which innervate the arterial smooth muscle of the choroid layer. Both neuron types are cholinergic, but only the choroid neurons express the neuropeptide transmitter, somatostatin. Previous studies implicated a role in somatostatin induction for activin A, a member of the TGFβ superfamily of molecules. Activin A and follistatin, a protein which binds activin A and inhibits its action, have been proposed to act in concert to affect a number of critical processes in development. To assess whether both factors play a role in somatostatin induction in CG neurons, we established a multi-probe ribonuclease protection assay in which we could examine both activin A and follistatin mRNA in the same sample. Levels for mRNA were normalized to an internal loading control, chick ribosomal protein S17. Choroid cells in culture express high levels of activin mRNA and low levels of follistatin mRNA. In the choroid tissue in vivo from E9-E16, activin A levels remain fairly stable and follistatin is low. Iris cells in culture express similar levels of activin and follistatin mRNA. This is also seen in vivo, in iris tissue from E9-E16. These data lead to the hypothesis that differential expression of somatostatin in the CG neurons is due to the expression of follistatin in the iris, which may prohibit the ability of activin A to signal the somatostatin induction in the ciliary neurons. Further examination of these factors which affect neurotransmitter phenotype should help to clarify the role of neuron/target interactions during development of the CG. *Supported by NS25767 (RN) and NIH5T32EY07123 (DD).*

607.8

CHARACTERIZATION OF FOLLISTATIN PROTEIN IN TARGETS OF THE CILIARY GANGLION. B.A. Link* and R. Nishi. Dept. of Cell Biology and Anatomy, Oregon Health Sciences University, Portland, OR 97201.

Our goal is to understand the regulation of somatostatin expression in avian ciliary ganglion (CG) neurons. The CG is composed of ciliary neurons which innervate the ciliary body and iris muscles and the choroid neurons which innervate the choroid vasculature. Both ciliary and choroid neurons are embryonically derived from the mesencephalic neural crest, receive pre-synaptic input from cholinergic neurons of the accessory ocular nucleus, and are themselves cholinergic. However, the two populations of neurons differ not only by the targets that they innervate, but also by the selective expression of the neuropeptide somatostatin in choroid neurons. Results from our lab suggest a model where activin A, which induces somatostatin in choroid neurons, is expressed in all targets of CG neurons; however, activin A is prevented from acting on ciliary neurons by follistatin, an inhibitor of activin, which is expressed in the iris and ciliary body (see Darland and Nishi, Soc. Neurosci Abstr., 1995). In support of this model, we show that iris conditioned medium can inhibit the ability of activin A to induce somatostatin immunoreactivity in CG neuron cultures. In the same experiment iris conditioned medium did not affect neuronal survival or choline acetyltransferase activity, a marker of the cholinergic phenotype. In vivo, follistatin immunoreactivity can be detected in and around the muscle cells of the iris and ciliary body, but not in cells of the choroid coat. We are currently examining the developmental expression of follistatin in the eye.
Supported by NIH5T32EY07123 (BAL) and NS2567 (RN).

607.9

TGF-ß1 EFFECTS ON GLIAL CELLS: A DIFFERENTIAL DISPLAY STUDY.

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Transforming Growth Factor-ß1 (TGF-ß1) has been recently established as a potent factor during glial reactions after brain lesion and as a modulator of cytoskeletal protein expression in neurons and glia. This contribution reports ongoing studies that address TGF-ß1 effects on gene expression in glial cells. C6-glioma cells were treated with 0.2 to 5ng/ml TGF-ß1. Total RNA was extracted and reverse transcribed using 4 different $d(T)_{12}VN$ primers. In a Differential Display PCR (DDRT-PCR) approach cDNA from each experimental group was amplified using the 4 reverse transcription primers in combination with 9 different arbitrary primers. Nine fragments which suggest a differential expression were reamplified and cloned. For each fragment, 5 clones were sequenced. So far, 2 fragments were confirmed to be differentially expressed using northern blot hybridization. TGF-ß1 reduces the mRNA level corresponding to one cDNA identified as glycerol-3-phosphate dehydrogenase by 85%. Another fragment which does not show any homology to known sequences showed an 3-fold induction. This study further demonstrates that TGF-ß1 affects glial gene expression and that DDRT-PCR is useful in identifying novel changes in gene expression. (Supported by AG-07909 to CEF and a grant by DAAD to KK)

607.10

TGF-B1 PROTECTS SKELETAL MUSCLE FROM APOPTOTIC AND NECROTIC INJURY. S. Abu-Shakra*, M.S. Alhalabi, Dept. of Neurology, Wayne State Univ., Sch. of Med., Detroit, MI 48201.

Skeletal muscle is normally capable of mounting successful regenerative responses to injury. However, in certain muscle diseases, this regenerative capacity fails to compensate for cell death. Our aim is to enhance muscle recovery after an injury. We have established two models of acute muscle injury in the C2 differentiating skeletal muscle line. In one model, anabolic steroids (stanozolol) induce apoptosis of muscle cells (LD_{50} 100µM, at 3 hrs). In a second model, the calcium ionophore A23187, induces necrosis (LD_{50}: 1µM, at 3 hrs.). We asked whether the growth factors bFGF and PDGF-BB, and cytokines LIF and TGF-B1 protect skeletal muscle cells from these two acute injuries. Mature skeletal muscle expresses receptors to these factors. As an index of injury, cellular creatine kinase (CK) levels were assayed. Cultures were treated with growth factor/cytokine for 5 hrs. followed by injury (stanozolol at 100µM or A23187 at 1µM). Cellular CK levels were measured 3 hrs. later. We found that TGF-B1, at 0.5 ng/cc, protected skeletal muscle cells from necrosis (42%), and apoptosis (40%). In contrast, bFGF, PDGF-BB, and LIF afforded muscle < 10% protection each. We conclude that TGF-B1 rescues skeletal muscle from both apoptosis and necrosis, and may be of use in treating certain myopathies.

607.11

THE SWEAT GLAND-DERIVED CHOLINERGIC DIFFERENTIATION FACTOR IS DISTINCT FROM LIF, CNTF AND CT-1. N. Francis, B.Habecker, D.Pennica# & S. Landis* Dept. of Neurosci. Case Western Res. Univ., Cleveland, OH 44106 & Genentech, San Francisco, CA 94080#.

During development, rodent sweat glands release differentiation factors which induce their noradrenergic sympathetic innervation to decrease catecholamines and instead produce acetylcholine and vasoactive intestinal peptide (VIP). Two cytokines, leukemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF), promote similar changes in cultured sympathetic neurons. There is biochemical and immunological evidence for a CNTF-like activity in footpad extracts. While neither LIF nor CNTF have been localized in sweat glands, LIF mRNA is detected in footpads and CNTF is present in footpad extracts. Sweat gland neurons, however, in LIF-deficient mice change their phenotype appropriately. To determine whether CNTF or CNTF and LIF, alternatively or together, direct the phenotypic switch in sweat gland neurons, we analyzed the function of sweat glands and the phenotype of sweat gland innervation in mice in which the genes for CNTF or both CNTF and LIF were disrupted. Since development of secretory responsiveness requires cholinergic innervation, we assayed agonist-induced sweating and found that mutant and control mice showed equally robust sweat secretion. The activity of choline acetyltransferase (ChAT), which catalyzes acetylcholine production, was also similar in footpads from mutant and control mice. Further, the sweat gland innervation in mutant and control animals lacked catecholamines, but contained VIP and acetylcholinesterase. Taken together, these data indicate that CNTF and LIF are not required for acquisition of the mature phenotype of sweat gland neurons. The newly identified cytokine, Cardiotrophin-1 (CT-1), shares about 20% homology with LIF and CNTF and induces ChAT in cultured sympathetic neurons. However, ChAT induction by footpad extracts or co-culture with sweat gland cells is not altered by function-blocking antiserum to CT-1. In addition, CT-1 protein was not detected in footpad extracts by Western blot. Thus, the factor released by sweat glands that prompts developmentof cholinergic and peptidergic properties in sweat gland neurons is not LIF, CNTF, or CT-1. This work was supported by NS23678 and the McKnight Foundation.

607.12

CARDIOTROPHIN-1 INDUCES THE SAME NEUROPEPTIDES IN SYMPATHETIC NEURONS AS THE NEUROPOIETIC CYTOKINES. Jr-Gang Cheng*, Diane Pennica† and Paul H. Patterson, Division of Biology, California Institute of Technology, Pasadena CA 91125 and †Department of Molecular Biology, Genentech, South San Francisco, CA 94080.

Cardiotrophin-1 (CT-1) was cloned from a mouse embryoid body cDNA library by its ability to induce hypertrophic growth of neonatal rat cardiac myocytes (*PNAS* 92: 1142, '94). Predictions of its secondary structure suggest that CT-1 belongs to a family of cytokines with four amphipathic helices. Sequence comparison revealed a weak homology to leukemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF)(24% and 19%, respectively). Using a RT-PCR assay system with rat sympathetic neuron cultures, we find that CT-1 induces and suppresses the expression of the same set of neuropeptides and neurotransmitter synthesizing enzymes as do LIF and CNTF. This functional data confirms that CT-1 is a member of the neuropoietic cytokine family, and suggests that the CT-1 receptor complex contains the gp130 signal transducing component.

607.13

ASTROCYTES AND PARALBUMIN-POSITIVE NEURONS IN MICE LACKING THE GENE FOR LEUKEMIA INHIBITORY FACTOR S.S. Cheema[1]*, E.C. Lopes[1], F. Koentgen[2], M.Murphy[2] and P. F. Bartlett[2]. [1]Anatomy Dept., Monash University, Clayton and [2]The Walter & Eliza Hall Institute of Medical Research, Parkville, Victoria Australia.

Our previous studies have shown that leukemia inhibitory factor (LIF): i) promotes the in vitro survival and differentiation of sensory dorsal root ganglion neurons and fetal spinal cord cells, ii) is expressed in the target tissues of sensory neurones during neurogenesis, iii) can be retrogradely transported in situ, and iv) prevents the in vivo loss of axotomised sensory neurons in the dorsal root ganglia and axotomised motor neurons in the spinal cord of neonatal rats. We have now extended our studies to mice where the LIF gene is deleted by homologous recombination.

Adult mice were anesthetised with chloral hydrate (400 mg/kg, ip) and killed by intracardiac perfusion with 4% buffered paraformadlehyde. Brains were dissected out, frozen on a CO_2 freezing microtome and serial parasagittal sections cut at 40 µm. Sections were immunostained using polyclonal antibodies against GFAP and paralbumin. The number of astrocytes within a reference grid in CA3 of the wild-tupe (WT) and null-mutant (NM) was 54±2 and 40±3 (mean ± sem) respectively which represents a significant reduction ($p<0.001$). The number of astrocytes within a reference area in the dentate gyrus in the WT was 30±3 and in the NM 21±2 which also represented a significant ($p<0.001$) reduction. The numbers of paralbumin-positive neurones within reference areas of the hippocampus (WT 76±7 versus NM 67±6) and visual cortex (WT 31±3 versus NM 35±2) were not significantly reduced.

These studies indicate that LIF plays an important role in the development of astrocytes during the maturation of the hippocampus and dentate gyrus.

607.14

LEUKEMIA INHIBITORY FACTOR ENHANCES THE REINNERVATION OF MUSCLE. D.I. Finkelstein*, M.K. Horne, P.F. Bartlett[(1)], S.S. Cheema. Department of Anatomy, Monash University, Australia, 3168. Walter & Eliza Hall Institute for Medical Research Institute[(1)], Vic, 3052, Australia.

Transection of a peripheral nerve in the adult, results in atrophy of muscle. Subsequent regeneration of axons, results in regrowth of axons of reduced diameter. Leukemia Inhibitory Factor (LIF), is released by damaged muscle and has been shown to be important for motor and sensory neurones. The aim of these experiments was to determine the effect of intramuscular injections of LIF into denervated muscles and reinnervating muscles. Under sodium pentobarbitone anaesthesia, the nerve to medial gastrocnemius (MG) muscle was cut in both legs and sutured into the popliteal fat pad to prevent reinnervation, (Denervation Group n=12). In a second group the nerve to (MG) was crushed 10 mm from the muscle (Reinnervation Group n=7). LIF was injected into the left MG and vehicle on the right. After 6-8 weeks, LIF was found to provide a limited protection against denervation-induced changes and improves the reinnervation of muscle following a nerve crush and reinnervation. Muscle cross-sectional area was greater in LIF treated denervated (46 %) and reinnervating groups (60 %) compared to those of the control. Nerve fibre diameters below the crush were larger in LIF treated reinnervating group. The LIF treated nerves had 24% of the axons larger than 6 µm in diameter compared to 4% in the control.

607.15

LEUKEMIA INHIBITORY FACTOR-MEDIATED ACTIVATION OF STAT PROTEINS AFTER NEURONAL INJURY *IN VIVO*. P. Rajan*, C. L. Stewart† and J. S. Fink. Molecular Neurobiology Laboratory, Massachusetts General Hospital and Department of Neurology, Harvard Medical School, Boston, MA 02114, USA.†Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Nutley, NJ 07110, USA.

Leukemia inhibitory factor (LIF) is a member of a family of neuropoietic cytokines which influence neuronal survival, differentiation and response to injury. LIF synthesis is increased by neuronal injury, but the intracellular mechanisms of action of LIF in neurons have not been characterized *in vivo*. In cell lines LIF activates the Jak/Tyk tyrosine kinases and the STAT family of transcription factors. In this report we have investigated the intracellular signaling pathways activated by LIF after neuronal injury *in vivo*. Axotomy of postganglionic sympathetic nerves resulted in sustained activation of members of the STAT transcription factor family. This activation is dependent on LIF as axotomy failed to activate STAT proteins in LIF-deficient mice. These data indicate that LIF-dependent activation of STAT proteins is an essential molecular mechanism underlying the response of sympathetic neurons to injury. A similar activation of STAT proteins is seen in organ cultures of the superior cervical ganglion.

607.16

CDF/LIF mRNA INCREASES FOLLOWING ADJUVANT-INDUCED INFLAMMATION. L. R. Banner*†, C. J. Woolf‡ and P.H. Patterson†. †Biology Division, California Institute of Technology, Pasadena, CA 91125 and ‡Department of Anatomy, University College London, London WC1E 6BT.

Intraplantar injection of complete Freund's adjuvant (CFA) produces an inflammatory response characterized by local swelling, sensory hypersensitivity and a rapid up-regulation IL-1β and NGF. In addition, there is an increase in the neuropeptides substance P and CGRP in the neurons of the dorsal root ganglia. The neuropoietic cytokine, cholinergic differentiation factor/leukemia inhibitory factor (CDF/LIF), plays a role in the response of adult sympathetic neurons to injury, mediating neuropeptide induction. To test whether CDF/LIF regulates a neurogenic response to inflammation, CDF/LIF mRNA levels were determined following CFA injection. We find that LIF mRNA increases in both plantar skin and sciatic nerve following inflammation. This response occurs within 6 hrs on the ipsilateral side and also occurs to a lesser extent on the contralateral side. Although intraplantar injections of 50 or 100 ng of CDF/LIF produced no direct change in mechanical or thermal behavioral sensitivity, or in IL-1 or NGF levels in skin, increased levels in inflamed tissue may modify neuropeptide expression in primary sensory and sympathetic neurons.

607.17

CILIARY NEUROTROPHIC FACTOR (CNTF) AND NEUROTROPHIN-3 (NT-3) POTENTIATE ONE ANOTHER IN PROMOTING ENTERIC NEURONAL DEVELOPMENT. A. Chalazonitis*, T.P. Rothman and M.D. Gershon. Dept. Anat. & Cell Biol., Columbia Univ. P&S. New York, NY 10032

Pluripotent neural crest-derived precursors interact with microenvironmental signals in forming the enteric nervous system (ENS). To identify signaling molecules, we have studied the development of neurons *in vitro* from crest-derived cells isolated from the fetal rat gut by immunoselection. Immunoselected crest-derived cells express TRKC and NT-3 specifically promotes the development of enteric neurons. Both CNTF and the α subunit of the CNTF receptor are expressed in the fetal mouse gut. The present study thus determined the effect of CNTF, alone, and in combination with NT-3 on the *in vitro* development of enteric neurons. Crest-derived cells, which virtually all express $p75^{LNGFR}$, were immunoselected with IgG192. The isolated crest-derived cells were grown for 8 days in defined medium; neural markers included process formation and neurofilament or peripherin immunoreactivities. By itself, CNTF (0.01-100 ng/ml) promoted neuronal development. This effect was concentration-dependent and maximal at 0.1 ng/ml (178 ± 15 % of control). CNTF (0.01-100 ng/ml) was then applied together with NT-3 (40 ng/ml), which, by itself, promoted neuronal development to 207% of control. The effect of this combination of factors was greater than that of either factor alone; the number of neurons was 390% of control at 1 ng/ml CNTF and 529% of control at 100 ng/ml. At least some neurons became dependent on NT-3; thus, survival of neurons was reduced by 57 ± 6.1% when NT-3 was withdrawn for the final 3 of 8 days of incubation. When cells were co-treated with NT-3 and CNTF, survival was reduced from 222.7 ± 26.5% to 151 ± 20.5% if NT-3 was withdrawn for the final 3 of 8 days of incubation; thus CNTF may not fully compensate for the withdrawal of NT-3. These data are compatible with the idea that cytokines that react with CNTF receptors may combine with NT-3 in promoting the development of enteric neurons. Supported by NIH grants NS15547 and HD20470

607.18

MULTIPLE APPROACHES TO ALTERING LEVELS OF CILIARY NEUROTROPHIC ACTIVITY IN OVO. T.P. Finn and R. Nishi*. Dept. of Cell Biology and Anatomy, Oregon Health Sci. Univ., Portland, Oregon 97201.

Growth promoting activity (GPA), is a potent neurotrophic factor for chicken ciliary ganglion neurons *in vitro* which can be purified from both embryonic eye and adult sciatic nerve. We have previously shown by immunocytochemistry the presence of GPA within both targets of CG neurons- the choroid layer and iris of the eye. GPA message can first be detected at E6 and increases significantly through the critical time window of development when the CG neuronal population is being restricted. As part of an effort to determine whether or not GPA meets the requirements of a true target-derived trophic factor *in vivo*, we are attempting to alter the level of CG neuronal survival by adjusting the amount of GPA made available to the neurons. Four approaches have been taken: 1) direct injection of either recombinant GPA or blocking antibodies to GPA; 2) Immunization of adult hens with GPA in order to produce blocking GPA antibodies in the fertile eggs; 3) infection of chick embryos with replication competent retrovirus (RCAS) that either over-expresses GPA or produces GPA antisense; and 4) injection of chick embryos with antisense oligonucleotides against GPA. In order to facilitate injections *in ovo*, we have optimized methods for maintenance of chick embryos in shelless cultures from E2 through E18. Embryos injected with either GPA or GPA blocking antibodies survive and have a normal appearance. One adult hen injected with GPA antigen produced a high titer to GPA and the antibodies have partial blocking ability *in vitro*. We have expressed a marker gene within structures in the eye using the RCAS system and have GPA sense and antisense constructs prepared. Results from GPA over-expression and blocking experiments will be presented at the meeting. Funded by NS25767 to R.N.

607.19

CILIARY NEUROTROPHIC FACTOR REGULATES INJURY-INDUCED GROWTH FACTORS. T.L. Wood*, S. O'Donnell and S.W. Levison, Dept. Neuroscience and Anatomy, PSU College of Medicine, Hershey, PA 17033.

Growth factor induction is a major component of the response to central nervous system trauma. The insulin-like growth factors (IGFs) and IGF binding proteins (IGFBPs) are among the molecules induced by injury that have demonstrated neuroprotective actions. Ciliary neurotrophic factor (CNTF) recently has been described as a major injury signal and can induce several aspects of reactive gliosis. To establish whether this cytokine is responsible for inducing specific growth factors following CNS injury, we injected CNTF intracerebrally into the neocortex of adult rats and measured changes in mRNA expression for bFGF, IGF-I, IGF-II, IGFBPs, and the type I IGF receptor. By *in situ* hybridization analysis, bFGF mRNA increased by 10 hours following CNTF injection when compared to a control hemisphere injected with heat-inactivated CNTF. IGFBP-2 mRNA showed a dramatic increase following CNTF injection, peaking between 24-48 hours and then rapidly declining. The majority of the cells in which BP2 mRNA was induced also were immunopositive for GFAP, indicating that astrocytes are the source of the increased BP2. The type I IGF receptor mRNA also was up-regulated by CNTF. Interestingly, IGF-I and IGF-II were unchanged up to 72 hours following CNTF injection. Similarly, CNTF had no effect on either BP3 or BP5. These results suggest that CNTF is a major regulator of specific growth factor systems following CNS injury. Supported in part by NSF/IBN94-08860 to T.L. Wood and by NMSS #PP0407 to S. Levison.

607.20

VASOACTIVE INTESTINAL PEPTIDE STIMULATION OF EMBRYONIC GROWTH IN CULTURED E9 MICE IS PARTIALLY MEDIATED THROUGH ACTIVITY-DEPENDENT NEUROTROPHIC FACTOR. G. W. Glazner*, J. Y. Wu, P. Gressens, I. Gozes, D. E. Brenneman and J. M. Hill Lab. Dev. Neurobiol., NICHD, NIH, Bethesda, MD, 20892; Univ. of Louvain Med. Sch., Brussels, Belgium; Dept. of Clin. Biochem., Tel Aviv Univ., Tel Aviv, Israel.

Vasoactive intestinal peptide (VIP) increases growth in mouse embryos *in vitro*. VIP (10^{-7} M) increased somite number, DNA and protein content, embryo size, and BRDU incorporation. Recent work has shown that VIP is a secretagogue for a novel neurotrophic factor in cultured astrocytes: activity-dependent neurotrophic factor (ADNF). We tested the hypothesis that stimulation of embryonic growth by VIP was mediated through ADNF. Embryonic day 9.5 embryos (15 to 18 somites) were cultured in serum alone or with 10^{-7} M VIP, 10^{-15} M ADNF, anti-ADNF antiserum (AAA), or a combination of AAA and either VIP or ADNF. After four hours, embryos were measured for somite growth, protein and DNA content, and BRDU incorporation. VIP-stimulated growth was prevented by AAA. Both VIP and ADNF equally increased somite number relative to control animals (4.6 v. 2.3). ADNF treatment also significantly increased protein (15%) and DNA (22%) content, and number of BRDU-positive cells relative to control animals, indicating increased mitotic activity. As with VIP, ADNF-stimulated growth was blocked by AAA. Embryos treated with AAA alone had significantly decreased somite growth (0.8 v. 2.3) and reduced BRDU incorporation compared with controls. In an additional test of mediators of VIP action as a mitotic stimulator, cyclin A gene expression was measured in embryos grown for two hours in the presence or absence of VIP. Using both semi-quantitative RT- PCR and competitive PCR, cyclin A mRNA levels were significantly greater (2-3 fold) in VIP-treated embryos compared to control animals. These data indicate that VIP-stimulated growth in the embryo is mediated in part by ADNF, and is correlated with increased cyclin A mRNA.

607.21

VASOACTIVE INTESTINAL PEPTIDE-INDUCED SHORTENING OF S PHASE IN WHOLE CULTURED MOUSE EMBRYOS.

P. Gressens*, B. Paindaveine, J.M. Hill, P. Evrard & D.E. Brenneman. Lab. Dev. Neurol., Univ. of Louvain Medical Sch., Brussels, Belgium; Lab. Exp. Neuropathol. & Lab. Dev. Neurobiol., NIH, Bethesda, MD.

Vasoactive intestinal peptide (VIP), a neurotrophic and mitogenic factor, has been shown to stimulate embryonic and neural growth in a model of whole embryo culture (Nature 362: 155, 1993). *In vivo* inhibition of this growth function between embryonic day (E) 9 and E11 by the administration of a specific VIP antagonist induces severe microcephaly in mouse embryos (J. Clin. Invest. 94: 2020, 1994). In these models, VIP regulated cell multiplication (or survival) in the germinative neuroepithelium. To address the potential effects of VIP on the different phases of the cell cycle of neural cells, we used whole E9 embryo cultures combined with cumulative labelling of S phase cells by incorporation of BRDU. When embryos were cultured in the presence of 10^{-7}M VIP, S phase and G1 phase of neuroepithelial cells were shortened by 46% (295 to 160 minutes) and 57% (110 to 47 minutes), respectively. Consequently, the length of the mitotic cycle was reduced by 40% (500 to 302 minutes). G2 and M phases were not affected in this model. By *ex vivo* autoradiography, VIP binding sites were detected in the germinative neuroepithelium between E9 and E11 but not beyond E12, during neuronal migration. Together, these data support the hypothesis that VIP shortened S and G1 phases during premigratory stages of *in vivo* brain development. A critical component of VIP-induced embryonic growth probably resides in this reduction in S phase kinetics, the first demonstrated in response to a physiological peptide.

NEUROTROPHIC FACTORS: BIOLOGIC EFFECTS XIII

608.1

FIBROBLAST GROWTH FACTORS REGULATE CGRP mRNA EXPRESSION IN RAT MOTONEURONS AFTER LESION AND IN CULTURE.

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Adult motoneurons respond to axotomy with a rapid up-regulation of calcitonin gene-related peptide (CGRP) mRNA expression. This may reflect a direct response to injury or may be a consequence of a loss of target-derived factors. In this study, we have analyzed one *in vivo* and one *in vitro* system in an attempt to identify target-derived factors with a potential effect on CGRP levels in motoneurons. In *in vivo* experiments, young adult Sprague-Dawley rats were subjected to a unilateral transection of the sciatic nerve. The proximal nerve stump was inserted into a capsule filled with either brain derived neurotrophic factor (BDNF; 15 ug), basic fibroblast growth factor (bFGF; 0.2 or 1 ug) or saline. After 3 days survival, the animals were sacrificed and sciatic motoneurons were studied with *in situ* hybridization histochemistry for detection of alpha- and beta-CGRP mRNAs. In cell cultures, embryonic rat motoneurons were treated with BDNF (10 ng/ml) or fibroblast growth factor-5 (FGF-5; 50 ng/ml). After 24h incubation, RT-PCR was used to estimate the levels of alpha- and beta-CGRP mRNAs, with GAPDH mRNA as an external control. *In vivo*, the local administration of bFGF at a dose of 1 ug abolished the upregulation of alpha-CGRP following axotomy, while BDNF did not significantly alter the expression of CGRP mRNAs. The motoneuron cultures treated with FGF-5 had a lower ratio of alpha- and beta-CGRP mRNAs to GAPDH mRNA, than did control or BDNF treated cultures. The results suggest that members of the FGF family of growth factors influence the expression of CGRP in rat motoneurons, and that the increase of this neuropeptide induced by axotomy may, at least in part, be due to deprivation of these target-derived factors.

608.2

TISSUE SPECIFIC EXPRESSION OF bFGF BY A TYROSINE HYDROXYLASE PROMOTER IN TRANSGENIC MICE.

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Development, survival and plasticity of catecholaminergic (CA) systems may be controlled by number of sequentially expressed growth factors. One such protein is bFGF expressed in developing and adult NS in areas containing CA cells. In vitro bFGF stimulates mitosis, growth, synaptogenesis, and supports survival of CA cells and neurons. The role of bFGF in vivo is suggested by the observations that in Parkinson's Disease degeneration of dopaminergic neurons is preceded by depletion of bFGF, and that bFGF stimulates recovery of function in mice rendered Parkinsonian by MPTP. To investigate the roles of bFGF in development and plasticity of CA systems we have generated transgenic mice that carry human bFGF gene under the control of the rat tyrosine hydroxylase (TH) gene promoter. The construct contained a 940 bp human bFGF cDNA coding for all the known bFGF isoforms, the SV_{40} small T antigen splice and poly adenylation sites, and the 4.8 kb of the rat TH promoter fragment (previously shown to restrict expression of a reporter gene to CA cells-containing tissues; Banerjee et al., 1992). FVB embryos were injected with linearized DNA fragment purified on a sucrose gradient. Out of 107 live-born offspring, PCR screening of the tail DNA identified 8 potential founders. Offspring from one of these founders (#73) was also examined by Western blot analysis for tissue specific expression of human bFGF. A marked increase in bFGF expression was observed in adrenals, striatum, and olfactory bulbs in agreement with high expression of the TH gene in those tissues. Tissues devoid of CA cells (cerebellum, hippocampus, heart, lungs, liver or kidneys) expressed basal levels of bFGF in the transgenic progeny. (APDA and NPF).

608.3

IMMUNOHISTOCHEMICAL LOCALIZATION OF ALL 4 HIGH AFFINITY FGF-RECEPTORS IN ADULT RAT SENSORY GANGLIA, PERIPHERAL NERVES AND ADRENAL GLAND.

R. Westermann* & P. Hartmann, Dept. Anat. & Cell Biol. Philipps-Univ., D-35033 Marburg, Germany

FGF-2 is known to be expressed by and to act on cells of the peripheral nervous and endocrine systems. Action occurs via 4 families of high affinity receptors [FGFR-1 to 4]. We have used antibodies against the different receptors to identify possible target cells for FGF-2 in sensory ganglia, peripheral nerves and the adrenal gland. Results, summarized in the table below, show that i) a given cell may express simultaneously more than one receptor type, ii) nearly all cells analyzed express FGF-receptors, iii) nearly all cells may therefore be addressed by FGFs and iv) FGF seems to serve multiple auto- and paracrine functions in the peripheral nervous system and the adrenal gland.

	FGFR-1	FGFR-2	FGFR-3	FGFR-4
DRG neurons	+/f	+/A	+++/A	+/f
satellite cells	-	-	+++/A	-
nerve fibers/Schwann cells	+++/A	+/S	+/S	+/s
adrenal capsule	+/S	+++/A	+/S	-
Z. glomerulosa	++/S	++/S	+++/A	+/s
Z. fasciculata	+/s	++/S	++/S	+/s
Z. retucularis	-	+/s	+/s	-
adrenergic CCs	+++/A	-	-	-
noradrenergic CCs	-	++/A	++/A	++/A
symph. neurons	-	-	++/A	+/A
adrenergic vessels	-	+++/s	-	+++/s

+++/++/+/- = strong/medium/weak/no immunoreactivity; A = all cells; s/S = small/large subpopulation; f = only few cells; CCs = chromaffin cells

608.4

THE IN VIVO EFFECTS OF BASIC FIBROBLAST GROWTH FACTOR ON ASTROCYTES.

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Basic Fibroblast Growth Factor (bFGF) is a polypeptide with in vitro mitogenic effects on a variety of cells. The aim of this study is to investigate the in vivo effects of bFGF on astrocyte proliferation. Adult rats underwent intraventricular or intraparenchymal stereotactic implant of gelfoam (3mm^3) with 4μg (N=10) or 60μg (N=10) of recombinant human bFGF (Synergen, Boulder,CO) or saline (controls). To assess acute and delayed effects of bFGF, rats were sacrificed by intracardiac perfusion with carbon black and paraformaldehyde 4 (N=20) or 30 (N=20) days after the implant. Histological evaluation was done on paraffin embedded 10-μm-thick coronal sections stained with Nissl stain and immunohistochemically for glial fibrillary acidic protein (GFAP). Four days after bFGF treatment there was a mild increase in GFAP expression in 4μg-bFGF-rats and a moderate increase in 60μg -bFGF-rats around the site of implant. Thirty days after the implant 60μg-bFGF-rats had a robust increase in GFAP expression. These changes were not seen in controls. These data corroborate that bFGF plays a significant role on astrocytic proliferation.

608.5

ADULT HYPOTHALAMIC CELLS PROLIFERATE IN LONG-TERM CULTURE WITH FGF-2. P.W. Coates*, J. Miller†, H. Parikh†, L-X Liu†, L. A. Chiodo† and S. Thode Depts. Cell Bio/Bioch. & Pharm.†, TTUHSC, Lubbock, TX 79430, and Dept. Neurosci., UCSD, La Jolla, CA 92093.

A culture system patterned after that used to promote proliferation of adult hippocampal, nigral, septal and cortical neurons (Gage et al., SN Abs. 20: 670, 1994; Raymon et al., ibid.; Palmer et al., ibid.) has been developed for adult hypothalamus. Cells from normal rat hypothalami were grown in defined medium supplemented with N2 and recombinant human basic fibroblast growth factor (FGF-2, 20 ng/ml, courtesy Synergen) without antibiotics. Proliferation was assessed by quantitative analysis of cell numbers over time, incorporation of BrdU and microscopy. Phenotypic identification of cells was determined by immunostaining with neuronal or glial markers. Ultrastructure was evaluated with scanning and transmission E.M. Cultures have been maintained for over a year, can be passaged, cryopreserved and reestablished. Cell proliferation, organizational complexity and expression of immature and more differentiated features developed over time in vitro. Subsets of cells displaying morphological properties of neurons were positive for neuron-specific enolase or neurofilament protein. Large, flat stellate cells stained for glial fibrillary acidic protein. Results suggest that neural precursor or even stem cells may be present in normal adult hypothalamus and can be cultured long-term. Cells appear to progress along neuronal or glial lineage pathways, remain uncommitted or die. We are continuing to characterize the cultures with other markers. Preliminary RT-PCR data indicate a weak signal corresponding to tyrosine hydroxylase mRNA. We are also beginning to pharmacologically characterize the cells by calcium imaging and patch clamp recording. Supported by TTUHSC-RSG & MH-41557.

608.6

ACIDIC FIBROBLAST GROWTH FACTOR AND/OR ITS FRAGMENTS AMELIORATE LEARNING AND IMMUNOLOGICAL DYSFUNCTIONS IN SENESCENCE ACCELERATED MICE. K. Sasaki[1], Y. Oomura[2], A. Li[3], N. Hori[3], H. Yoshii[2], Y. Fukata[2], H. Yago[2], H. Kimura[4], I. Tooyama[4], K. Hanai[4], and N. Yanaihara[5] [1]Fac. Eng., Toyama Univ., Toyama 930, [2]Inst. Bio-Active Sci., Nippon Zoki Pharm. Co., Hyogo 673-14, [3]Fac. Med., Kyushu Univ., Fukuoka 812, [4]Inst. Molec. Neurobiol., Shiga Univ. Med. Sci., Shiga 520-01, [5]Fac. of Pharm. Sci., Univ. Shizuoka, Shizuoka 422, Japan

Subcutaneous (SC) injection of acidic fibroblast growth factor (aFGF) or its fragments such as [Ala[16]]-aFGF(1-29) into senescence accelerated mice (SAM-P/8) at one per week was continued for 7-9 months. Learning dysfunction appeared in controls was ameliorated in animals treated by fragments as well as aFGF, when tested in some learning paradigms. After 9 months, an induction of long-term potentiation was examined in hippocampal slices of those animals. As stimulation was given to the stratum radiatum, the magnitude of population spikes recorded in CA1 pyramidal cell layer increased in aFGF- and [Ala[16]]aFGF(1-29)-treated groups. During the period of SC injection, a delayed type hypersensitivity reaction (DTHR) in footpad caused by trinitrophenyl was also measured. The DTHR in controls became lower, but that in the aFGF-treated group did not. Results suggest aFGF and/or its fragments improve learning and immunological dysfunctions which are accompanied with senescence.

608.7

BASIC FIBROBLAST GROWTH FACTOR SELECTIVELY INCREASES DESENSITIZATION OF NMDA RECEPTOR CURRENTS IN CULTURED RAT HIPPOCAMPAL NEURONS A. L. Boxer* and E. B. Ziff Howard Hughes Medical Institute and Department of Biochemistry, New York University Medical Center, New York, NY 10016. Basic Fibroblast Growth Factor (FGF-2) has been shown to ameliorate excitotoxicty *in vitro*, possibly by inhibiting calcium entry through the NMDA receptor. Previously, we reported that when 40 µM glutamate was applied alone, then in the presence of CNQX (without washout of the first test pulse), to embryonic hippocampal neurons cultured in the absence or presence of FGF-2 (10 ng/ml, ≥5 days), NMDA receptor peak currents were 18.1% of controls ($p<.005$, n=13) in the FGF-2 treated cells. To further characterize this effect, E17/18 hippocampal neurons were grown in the presence of FGF-2 (10 ng/ml) for 24 or 120 hours, and peak current densities after superfusion of 500 msec. test pulses of 100 µM kainate, 1, 5, 10, 50, or 100 µM NMDA were assayed by whole cell patch clamp techniques after 7-10 days in culture. Cells exposed to FGF-2 for 24 hours had significantly smaller peak voltage-activated sodium channel and 100 µM kainate-evoked current densities than controls, which returned to control levels after 120 hours of treatment. Surprisingly, initial NMDA-evoked current densities were significantly larger in response to 10 and 50 µM NMDA in cells treated with FGF-2 for 120 hours than in control cells (cells treated for 24 hours had intermediate values between the two). To test whether increased desensitization of NMDA receptor currents could explain our original observation, we applied 10 µM glutamate (in 1 mM external Ca^{+2}) for 3 sec. out of every 30 sec. for 25 min. and then measured kainate- or NMDA-evoked currents. Under these conditions, NMDA receptor currents do not appreciably desensitize, however cells treated with FGF-2 for 24 or 120 hours both displayed significantly ($p<.05$ for both, pooled student's t test) increased desensitization (mean ±SEM ratio of currents after/before desensitization for 5, 10, 50, and 100 µM NMDA is 1.17 ± 0.23, n=29 in controls; 0.70 ± 0.08, n=11 after 24 hours; 0.70 ± 0.11, n=18 after 120 hours). There was no difference in kainate-evoked current desensitization. Analysis of kainate-induced current rectification suggested that a change in cultures' cellular composition did not explain the effect.

608.8

BASIC FIBROBLAST GROWTH FACTOR INDUCES THE PRODUCTION OF A GILA-DERIVED NEURON SURVIVAL FACTOR(S) FOR BASAL FOREBRAIN NEURONS. L.D. Cain* and L. A. Perkins, Dept. of Anatomy & Neurosciences, UTMB, Galveston TX 77555-1043.

Previous work in our laboratory has shown that in culture in the presence of basic fibroblast growth factor (bFGF), the number of embryonic and postnatal cholinergic basal forebrain neurons surviving after five days increases significantly over control cultures receiving no bFGF. These results occurred only in the presence of serum and glia. To determine the importance of serum in neuron survival, serum-containing cultures were treated with bFGF in the presence of Ara-C. We observed that serum and bFGF alone could not elicit survival. We now report that conditioned media (CM) removed from cultures stimulated with bFGF increases cholinergic neuron survival significantly over control cultures receiving no CM. This effect is seen in the absence of glia, in serum-free cultures containing Ara-C and anti-bFGF. Our data further indicates that the CM is not a mitogen for type 1 astrocytes as defined by glial fibrillary acidic protein staining. These results indicate that bFGF induces the survival of basal forebrain neurons by stimulating glia to produce a neuron-survival factor(s). The observation that this CM is not a mitogen for glia is significant for possible therapeutic purposes. (Supported UTMB Small Grant Program)

608.9

TEMPORAL ROLES OF BASIC FIBROBLAST GROWTH FACTOR (FGF-2) AND BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) IN THE DEVELOPMENT OF CHICKEN COCHLEO-VESTIBULAR GANGLION (CVG) IN VITRO. W.Amin Hossain*, A.Rutledge and D.Kent Morest , Dept. Anat., Univ. CT Health Ctr., Farmington, CT 06030.

Previous studies showed that FGF-2 can stimulate migration and differentiation of CVG cells by activating their receptors in vitro (Hossain et al., Assoc. Res. Otolaryngol. Abstr. 18: 109, '95). Adding FGF-2 increased CVG growth for the first 2 days in vitro, but after 5 days led to degeneration, implicating other factors in its later development. To see if BDNF could be such a factor otocysts were explanted from white leghorn embryos at Hamburger-Hamilton Stages 14-16, when CVG precursors normally start to migrate from the otic epithelium. Careful dissection and treatment with cold collagenase were effective in removing the adjacent mesenchyme and neural crest precursors of Schwann cells. Measurements of neuroblast migration and neurite outgrowth were made by time-lapse imaging techniques in living cultures. Cultures were grown in a defined medium [Ham's F12 base, L-glutamine, and other defined additives (ITS, Collaborative)] with or without human recombinant FGF-2 (UBI), 1ng/ml daily for 2 days. On day 3, FGF -2 was replaced completely either with BDNF (2ng/ml in defined medium) or with defined medium. With BDNF, cell migration and neurite outgrowth from the explant increased and continued for more than 3 weeks, but cultures receiving defined medium from day 3 gradually died after 10-12 days. However, control cultures (receiving only defined medium from day 1) did not survive more than 3-4 days. These findings suggest a temporal sequence in which FGF-2 acts early in development, while BDNF affects a later stage. Supported by NIH grant NS29613.

608.10

TROPHIC EFFECTS OF FGF-2 ON DEVELOPMENT OF COCHLEO-VESTIBULAR NEURONS IN VITRO DO NOT DEPEND ON MITOGENIC ACTIVITY IN THE CHICK EMBRYO. A. Hossain, W. Amin Hossain, A. Rutledge, and D. Kent Morest.* Dept. of Anatomy, Univ. CT Health Ctr., Farmington, CT 06030.

FGF-2 is well known as a mitogen for many cell types. It also has strong trophic effects on the early development of neurons of the cochleo-vestibular ganglion (CVG) and of the acoustico-vestibular nuclei (AVN) in vitro (Hossain et al.,Molec Biol Cell Suppl 4: 18a, '93; Morest et al., Soc Neurosci Abstr, 19, 1101, '93). Do these effects depend on cell proliferation in vitro? The otocyst, anlage of the CVG, was explanted from white leghorn embryos at Hamburger-Hamilton Stages 14-16, when CVG precursors normally start to migrate from the otic epithelium and continue to proliferate in vivo. The part of the rhombic lip that forms the AVN was explanted at Stage 28 (E5.5), when many precursors are still dividing in the matrix zone but the first postmitotic neuroblasts normally are starting to migrate in vivo. In vitro some neuroblasts continue to proliferate, while others migrate onto a substrate of purified collagen and differentiate. In both types of cultures, half were supplemented with fetal bovine serum, and half with human recombinant FGF-2 (UBI). The optimum FGF concentration for each type of explant was determined by dose response curves. FGF-2 increased migration and neurite outgrowth of CVG neuroblasts and early differentiation of AVN processes during the first 3 days in vitro. Cell proliferation in these cultures was measured on days 2 and 3 by using 5-bromo-2'-deoxyuridine (BrDU) pulselabeling for 4hrs or 24hrs and fluorescence immunoogytochemistry. In each condition about 20% of the neuroblasts in both control and treated groups in CVG and AVN cultures were immunolabeled. Hence there were no significant differences in the numbers of cells dividing in control and FGF-treated cultures. Thus the trophic effects of FGF-2 on the migration and early differentiation of cochleo-vestibular neurons do not depend on a mitogenic effect . Supported by NIH grant NS29613.

608.11

LONG TERM SURVIVAL OF NEURONS ISOLATED FROM THE ADULT GUINEA PIG CNS

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Cultures of central nervous system (CNS) neurons isolated from embryos and from young postnatal animals are a popular tool in neurobiology research. However, since several neuronal properties are differentially expressed during development, the use of mature neurons is preferable to that of embryonic or neonatal cultures, unless specific developmental studies are undertaken. Unfortunately, *in vitro* survival of mature CNS neurons is poor under culture conditions. The second postnatal week is the usual age limit to establish viable cultures of neocortical, hippocampal and diencephalic neurons.

Here we describe a method for long-term culturing of neurons isolated from adult (60 to 90-day-old) guinea pig CNS. Piriform cortex slices (500-µm thick) were incubated with papain (Worthington, type 3126, 14 U/ml) or tripsin (Sigma, type XI, 1 mg/ml) for 40-60 minutes at 37°C under continuous oxygenation and stirring. After a light mechanical trituration, cells were collected by centrifugation and were plated onto matrigel-coated glass coverslips or plastic Petri dishes (15,000/cm^2) in a chemically-defined DMEM/F12 medium containing 2 mM glutamine, 6 mg/ml glucose, 9.6 µg/ml putrescine, 6.3 mg/ml progesterone, 5.2 ng/ml sodium selenite, 25 µg/ml insuline, 0.1 mg/ml transferrine, 20 ng/ml basic fibroblast growth factor, 2 µg/ml heparin. After 5-6 days in culture cytosine arabinoside (1 µM final concentration) was added to prevent overgrowth of glial cells. At this time different neuron-like cell types were found, with multipolar, pyramidal or small globular shape, which gave off dendrite- or axon-like processes. Inthese culture conditions cell viability was maintained for up to 1 month. Indirect immunocytochemistry showed that the neuron-like cells expressed the neuronal antigens MAP-2 and Tau1. Electrophysiological current-clamp recordings, performed by means of the patch-clamp technique, demonstrated that these cells retain the membrane properties necessary to generate action-potential firing.

608.12

EPIDERMAL GROWTH FACTOR PREVENTS OXIDATIVE STRESS-INDUCED NEURONAL APOPTOSIS IN CULTURED CEREBRAL CORTICAL NEURONS.

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Epidermal growth factor (EGF) is a polypeptide growth factor which promotes cell proliferation of various types of cells including epithelial and fibroblast cells. In the central nervous system, EGF acts as a neurotrophic factor on cultured cerebral cortical, subneocortical telencephalic, and cerebellar neurons, enhancing neurite outgrowth and cell survival. In the present communication, we reported that EGF prevented the death of rat cerebral cortical neurons cultured in a 50% oxygen atmosphere. EGF protected the oxygen-induced neuronal cell death in a concentration-dependent manner. The maximal effect was exerted at an EGF concentration of 10 ng/ml, and the half maximal effect occurred at 0.3 ng/ml. Basic fibroblast growth factor (bFGF) also prevented this cell death, although there was no apparent additive effect of EGF and bFGF. This high oxygen-induced death showed a nuclear condensation and was blocked by RNA or protein synthesis inhibitor and antioxidants, vitamin E and N-acetylcystein. These results indicated that EGF prevented the oxidative stress-induced apoptosis of the cortical neurons. We confirmed that the cultured cortical neurons possessed the EGF receptor and expressed c-Fos protein in response to EGF. The viable astroglial cells were below 0.5% of that of the corresponding MAP2-positive neurons under the culture condition using a serum-free medium with cytosine arabinoside. Therefore, the effect of EGF on the cultured cortical neurons is thought to be due to a direct action. In addition, we examined EGF-induced signaling in the cultured cortical neurons. We found that EGF induced the sustained tyrosine phosphorylation of the EGF receptor and sustained the activation of mitogen-activated protein kinase in the cultured cortical neurons. We suggest that the sustained activation of the EGF signal is the mechanism by which it acts as a neurotrophic factor, preventing neuronal apoptosis.

608.13

DECREASED SKIN INNERVATION IN TRANSGENIC MICE OVEREXPRESSING INSULIN-LIKE GROWTH FACTOR II (IGFII) IN THE EPIDERMIS.

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There is increasing evidence that insulin-like growth factors have important stimulatory effects in the developing nervous system. We have examined the effects of IGFII on developing cutaneous sensory neurons in transgenic mice carrying multiple copies of the *Igf-2* gene driven by the epidermal keratin promoter. These animals have wrinkled skin due to hyperplasia of the epidermis corresponding to the expression of transgene transcripts in the skin from embryonic day 16 (E16) (Ward et al., 1994, PNAS 91:10365). Innervation of hindlimb skin in P1 and P22 transgenic mouse pups and their normal littermates was examined using PGP9.5 immunostaining. At both ages innervation was markedly reduced.particularly in distal regions. The fine terminal plexus of the epidermis was affected rather than the coarser subepidermal axonal branches. Innervation pad was reduced by 44% at birth and 68% at P21, ankle hairy skin by 40% at birth and 55% at P21. Cell numbers of L4 dorsal root ganglion (DRG) at P21 were the same in transgenics (9494±112) and controls (9307±637). The effects of IGFII on neurite outgrowth was tested in embryonic mouse L4 DRG explants in culture. In the absence of NGF, IGFII (20-100ng.ml^{-1}) stimulates neurite outgrowth at E18 but does not at E14. However, in the presence of NGF, the same doses of IGFII did not have an additive effect on neurite outgrowth and under some conditions (E18, 5ng/ml NGF) actually inhibits it. Overexpression of IGFII in the epidermis reduces cutaneous innervation density, while leaving DRG cell number intact. Tissue culture studies support the conclusion that under some conditions IGFII can inhibit sensory terminal growth.

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608.14

INSULIN ATTENUATES THE SURVIVAL EFFECTS OF IGF-1 ON CHICK MOTONEURONS *IN VITRO*.

A.P.D'Costa,* S.Wang, D.M.Prevette and R.W. Oppenheim. Dept. of Neurobiology and Anatomy, Bowman Gray School of Medicine, Winston-Salem, NC 27157.

Insulin-like Growth Factors (IGFs) have been shown to be involved in the regeneration, sprouting and survival of motoneurons (MNs) both *in vivo* and *in vitro*. However, in previous experiments, the administration of IGF-1 to purified avian MN cultures resulted in only a slight rescue effect (15%). We hypothesized that this may be due to a confounding effect of insulin present in the media used to culture MNs. Accordingly, we examined the survival effects of various concentrations of IGF-1 (0.1 to 100ng/ml) on MN cultures from the lumbar spinal cords of E5 chicken embryos, with or without insulin in the media. In the presence of insulin (5ug/ml), MN survival was modest (maximal effect=13%) and did not exhibit dose dependence, whereas in the absence of insulin, survival was dose dependent and reached 40% following IGF-1 treatment. Additionally, when IGF-1 was co-administered with CNTF (ciliary neurotrophic factor) and chicken muscle extract the synergistic effects on MN survival were more pronounced when insulin was absent in the media. These data suggest that supraphysiological amounts of insulin present in the media act to inhibit IGF-1 action, probably, by displacing IGF-1 from its receptor.

608.15

INSULIN-LIKE GROWTH FACTOR II REGULATION OF CELL NUMBER.

Robin L. Mozell* and Lydia Villa-Komaroff. Dept. of Neurology, Children's Hospital, Harvard Medical School, Boston, MA 02115.

Growth factors are critical signals in the regulation of cell growth, proliferation and differentiation. We have used a mouse line bearing an inactivated IGF-II gene (IGF-II nulls; Gift from A Efstratiadis, Columbia Med. Sch.) to investigate the function of IGF-II during development of the CNS. At birth, these mice are 60% the size of their wild type (WT) littermates, and remain so throughout their lifespan. However, the sizes of neural and non-neural cells in the IGF-II null mice are no different than cells in WT controls. This suggests that IGF-II regulates the number of cells produced and not the size of individual cells. The interplay between cell cycle length and programmed cell death is likely to determine the ultimate number of cells. As a first step in elucidating the mechanisms underlying the decreased cell number, we compared the cell cycle lengths of IGF-II null mice to WT controls. By BrdU/IrdU incorporation and double immunohistochemistry, we show that cerebellar granule cells in the IGF-II null mice have an altered cell cycle in that the S-phase is substantially elongated.

608.16

RETINAL GANGLION CELL SURVIVAL AND AXON GROWTH INTO ACELLULAR PNS GRAFTS WITH GROWTH FACTOR REPLACEMENT.

J. J. Norden* and B. Wouters. Dept. of Cell Biology, Vanderbilt University Medical School, Nashville, TN 37232.

The survival and axon growth of retinal ganglion cells (RGCs)were assessed in adult male Sprague-Dawley rats with cellular or acellular grafts into the optic nerve. A peroneal nerve segment was excised and transplanted into the transected optic nerve within 3 mm of the eye. This procedure results in growth of RGC axons into the grafts and in the sustained upregulation of GAP-43 (Doster et al. Soc. Neurosci., 14: 802, 1988; Wouters and Norden, Soc. Neurosci., 16: 338, 1990). To examine the effects of specific growth factors on axon growth and RGC survival, some grafts were rendered acellular by repeated freeze-thawing (which kills Schwann cells but leaves basal lamina intact) and an Elvax implant inserted which released BSA, basic fibroblast growth factor/BSA, or insulin-like growth factor-I/BSA. Animals survived for 4 wks. Axon growth was present in all cellular grafts and in acellular grafts in which growth factors were released. There was no RGC axon growth past the site of anastomosis in acellular grafts, or in acellular grafts with implants releasing BSA alone. Cresyl-violet stained retinal whole mounts were used to estimate the number of surviving RGCs. The results from this quantitative analysis indicated that the release of IGF-I in acellular grafts rescued the greatest number of RGCs. Supported by NIH Grant EY01117.

608.17

DEVELOPMENTAL ROLE OF INSULIN-LIKE GROWTH FACTOR SYSTEM GENES DURING INTEGRATION OF TRANSPLANTED PURKINJE CELLS INTO THE CEREBELLUM OF GENETICALLY ATAXIC MICE. W. Zhang, W.-H. Lee* and L. C. Triarhou. Dept. of Anatomy, Pediatrics, Pathology and Laboratory Medicine, Indiana Univ. Sch. of Med. Indianapolis, IN 46202.

Insulin-like growth factor I (IGF-I) plays a functional role in postnatal cerebellar development, as suggested by its spatially-temporally coordinated expression with the type I IGF receptor (IGFR-I) and with IGF binding proteins (IGFBP) 2 and 5. To understand the interaction between the IGF system and the developing cerebellum, we analyzed the distribution of IGF-I, IGFR-I, IGFBP2 and IGFBP5 mRNAs in normal embryonic cerebellar cells transplanted into the cerebellum of adult "Purkinje cell degeneration" (*pcd*) mutant mice. The *pcd* mutation leads to a degeneration of mature Purkinje cells, the only neurons that express IGF-I in the cerebellar cortex. Cell suspension (80,000 viable cells/2 µl) derived from normal cerebellum at embryonic day (E) 11-13 was implanted in adult pcd cerebellum. At 21 and 31 days after transplantation, IGF system gene expression was examined in cerebellar grafts by *in situ* hybridization histochemistry and by immunocytochemistry. Purkinje cells from the grafts migrated to form a distinct layer replacing the missing host Purkinje cells. Compared with the normal developing cerebellum, IGF-I mRNA in the cerebellar grafts was expressed at a similar level and showed a similar pattern of cellular distribution, as were IGFR-I, IGFBP2 and IGFBP5 mRNAs. These observations suggest that normal IGF system gene expression may be indispensable for cerebellar development, and that IGF-I may be important in the maturation and integration of the implanted Purkinje cells into the host cerebellum.

608.18

IGF AND IGF BINDING PROTEIN EXPRESSION IN SENSORY NEURONS. **A. Randolph* and E.L. Feldman, Department of Neurology, University of Michigan, Ann Arbor, 48109.**

The insulin-like growth factors (IGF-I and IGF-II) are polypeptides with both growth-promoting and insulin-like metabolic activity whose actions are modulated, in part, by a family of six proteins, the insulin-like growth factor binding proteins (IGFBPs). Diabetic neuropathy is primarily a disturbance of sensory nerves. IGF-I has been proposed as a treatment for diabetic neuropathy, however its effects on sensory dorsal root ganglion neurons (DRG) have not been well-characterized. In the current study, we examined the role of exogenous IGFs on growth and expression of IGFs and IGFBPs in ND26 cells, a cloned line derived from neonatal DRG neurons fused with a mouse neuroblastoma. ND26 cells were plated in either serum-free media (SFM) or with the addition of increasing concentrations of serum ± IGF-I. After 1, 2 and 3 days cell growth was measured using a colorimetric assay that detects reduction of the tetrazolium salt MTT. Cells remained viable in 0.3% serum, and at serum concentrations ≥ 0.6%, cell number increased in a dose-dependent fashion. IGF-I alone in SFM did not stimulate cell growth, however IGF-I + 0.3% serum resulted in a dose-dependent increase in cell number. α-IR$_3$, an antibody that inhibits the type I IGF receptor, blocked IGF-I coupled growth. Northern analysis of ND26 cells revealed IGF-I receptor and IGF-II gene expression. Western ligand blotting demonstrated ND26 cells highly express IGFBP-4 with lesser amounts of IGFBP-3 and IGFBP-5. In summary, ND26 cells express IGF-II, functional IGF-I receptors and 3 of 6 IGFBPs, capable of sequestering and targeting bioavailable IGF-I. Coupled with IGF-I's growth promoting effects on ND26 cells, these data suggest IGF-I may have a therapeutic role in the treatment of disorders of sensory nerves, such as diabetic neuropathy. Sponsored by R29 NS32843 and a grant from the Juvenile Diabetes Foundation #194130.

608.19

REGULATION OF NEURITE OUTGROWTH AND CELL DIVISION IN GT1 GnRH CELL LINES BY GROWTH FACTORS. A. Ochoa, G. Nava, C. Domenzain, C. Clapp and G. Martínez de la Escalera*, Neurobiology Center, National University of México, México City 04510, México.

Studying the mechanisms responsible for the development and function of GnRH neuronal networks is fundamental to understand the regulation of reproduction. However, GnRH neurons are sparse and scattered, impeding their experimental access. The availability of homogeneous populations of GnRH neurons such as the GT1 cell lines, facilitates the analysis of factors that may affect aspects such as cell division and differentiation. We tested the effects of various growth factors, including NGF, EGF, TGF-α, IGF-1 and bFGF on neurite outgrowth and mitotic rate in GT1-1 cells. Preliminary findings suggested that the number of neurites per cell was stimulated by protein kinase C activation (TPA 2.5 nM), while the length of the neurites was stimulated by cAMP (forskolin 10 µM). In 24 h, both treatments decreased ^{3}H-thymidine incorporation over 50%. While bFGF (10 ng/ml) by itself stimulated 50% the number of neurites per cell, TGF-α (10 ng/ml) and EGF (10 ng/ml) decreased over 50% and IGF-1 stimulated 30% the number of neurites induced by TPA. bFGF also increased (35%) ^{3}H-thymidine incorporation, while TGF-α, EGF and IGF-1 decreased the mitotic rate between 30 and 50%. NGF showed no effect on any parameter. These findings revealed that GnRH neuronal differentiation and reproduction may be affected in parallel or in opposed fashion by a single influence, and highlights the existence of various putative factors involved in this regulation. (Work supported by grants from the National University of Mexico (IN200393) and the Mexican Council of Science and Technology (1775N).

608.20

EFFECTS OF IGF-1 INFUSION ON SERUM IGF BINDING PROTEINS AND FUNCTIONAL RECOVERY FOLLOWING SCIATIC NERVE CRUSH. T. M. Engber*, R. V. Bhat, S. A. Dennis, Y. Zhu, M. Miller and P. C. Contreras. Cephalon, Inc., West Chester, PA 19380.

Insulin-like growth factor 1 (IGF-1) promotes the survival of neurons in a number of in vivo models of nerve injury and toxic damage. However, it is not clear whether it is advantageous to deliver IGF-1 by repeated injections or by continuous infusion, in particular since the pharmacokinetics of IGF-1 administration are complicated by the presence of specific binding proteins (IGFBPs) which may transport IGF-1 to target tissues and modulate its effects. We have used the mouse sciatic nerve crush model to compare the effects of s.c. infusion of rhIGF-1 at a variety of doses (0.003-8 mg/kg/day via osmotic minipump) with those of the optimal dose by repeated s.c. injection (1 mg/kg/day) on functional recovery and serum levels of rhIGF-1, glucose and IGFBPs. Continuous infusion of rhIGF-1 improved functional recovery of male CD-1 mice following bilateral sciatic nerve crush injury in a manner similar to repeated rhIGF-1 injections, but only at the highest dose tested (8 mg/kg/day). Serum levels of rhIGF-1 achieved after infusion at this dose in sham-operated animals did not differ from peak levels seen after s.c. injection at 1 mg/kg, but were 34% lower in mice with sciatic nerve crushes. Blood glucose levels decreased by 40% following injection of rhIGF and only 20% following infusion at 8 mg/kg/day. Infusion of rhIGF-1 increased serum IGFBP2 in a dose-dependent manner, but had no effect on serum IGFBP4. Serum IGFBP3 was also elevated following infusion of rhIGF-1 in sciatic nerve-crushed mice, but showed a bell-shaped dose response, with the peak increase seen at a dose of 3 mg/kg/day and levels close to control values following infusion of 8 mg/kg/day. These results indicate that the efficacy of IGF-1 in the sciatic crush model is related to serum IGF-1 levels and that IGF-1 infusion does not offer any obvious therapeutic advantage over IGF-1 injections.

608.21

IDENTIFICATION OF A BIOCHEMICAL SURROGATE MARKER FOR THE IN VIVO EFFECTS OF INSULIN-LIKE GROWTH FACTOR-1. **R.V. Bhat*, Y. Zhu, M. Miller and P.C. Contreras. Dept. of Pharmacology, Cephalon Inc., West Chester, PA 19380.**

Insulin-like growth factor-1 (IGF-1) plays an essential role during development of a variety of tissues, including brain. Recently, daily s.c. injections of rhIGF-1 (1-3 mg/kg, 17 days) has been shown to be efficacious in enhancing the regeneration of the injured sciatic nerve in mice. In an attempt to identify a biochemical marker that is altered in association with the administration of an efficacious dose of IGF-1, we have determined the acute and chronic effects of rhIGF-1 on serum levels of glucose and IGF binding proteins (IGFBPs). Serum glucose levels decreased in a dose-related fashion, following either acute (single) or chronic injections (1 mg/kg, s.c., once daily for 17 days) of rhIGF-1. Neither tolerance nor potentiation was observed to the peak hypoglycemic effects of rhIGF-1. However, the rate of recovery of serum glucose to normal levels in chronic rhIGF-1 treated mice was prolonged compared to that after an acute injection. Acute injections of 0.1, 1 and 10 mg/kg of rhIGF-1 resulted in a dose-dependent increase in serum IGFBP2 while IGFBP3 levels increased only after administration of 10 mg/kg of rhIGF-1. Although chronic administration of rhIGF-1 also resulted in a similar effect , it was observed that IGFBP2, but not IGFBP3 levels were significantly higher in the chronic treatment group, compared to the acute injection group. There were no significant changes in IGFBP4 observed. Since IGFBP2 levels are increased at doses of rhIGF-1 that are efficacious at enhancing regeneration of injured sciatic nerve, IGFBP2 may be useful as a surrogate marker for the *in vivo* neuroprotective effects of rhIGF-1.

608.22

IMMUNOCYTOCHEMICAL LOCALIZATION OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-2 IN PITUICYTES DURING POSTNATAL DEVELOPMENT IN THE RAT. X. Zhou and C.M. Paden*. Dept. of Biology, Montana State Univ., Bozeman, MT 59717.

The insulin-like growth factors (IGF-I and IGF-II) are a family of peptides with both growth-promoting and insulin-like activity. The action of the IGFs are modulated by insulin-like growth factor binding proteins (IGFBPs), and IGFBP-2 mRNA is known to be expressed at high levels in fetal and adult posterior pituitary. We have performed an immunocytochemical study to characterize the cellular localization of IGFBP-2 in the neural lobe (NL) of the rat at postnatal days (PD) 1, 11, 24, 37 and 53 using a polyclonal rabbit anti-human IGFBP-2 antisera (UBI). Cells immunoreactive for IGFBP-2 were identified as pituicytes by immunofluorescent co-localization of S-100 protein. In contrast, microglia labeled with antibody OX-42 were not IGFBP-2 positive. While IGFBP-2 staining was present at all ages, the pattern of immunoreactivity changed during maturation. At PD1 and PD11, IGFBP-2 immunoreactivity was primarily localized in the perinuclear cytoplasm of pituicytes, while at later ages staining appeared to be concentrated near the pituicyte cell membrane. Scattered cells immunoreactive for IGFBP-2 were also present in the intermediate lobe at each age. These results are consistent with a potential role for IGFs in development and/or function of the magnocellular neurosecretory system, and suggest that pituicytes may modulate any such effects through expression of IGFBP-2. Supported by NINDS NS32507 to CMP.

609.1

*TRK*C EXPRESSION IN INTACT AND INJURED ADULT RAT RETINAS. P. Kittlerová, G. M. Bray and A. J. Aguayo*. Centre for Research in Neuroscience, McGill University and Montreal General Hospital Research Institute, 1650 Cedar Ave., Montreal, Quebec, H3G 1A4.

After axotomy, NT-3 transiently increases retinal ganglion cell (RGC) survival by about 20% and dramatically enhances GAP-43 expression in 50% of the axotomized RGCs 2 weeks after injury. In contrast, BDNF induces an early up-regulation of GAP-43 in most RGCs (Fournier et al., unpublished observations) and markedly increases the survival of these neurons (Mansour-Robaey et al., PNAS 91: 1632, 1994). These effects of BDNF are presumably mediated by TrkB, the high-affinity receptor expressed by most RGCs (Jelsma et al., J.Neurobiol. 24: 23, 1993). We have now investigated TrkC expression in adult rat retinas using immunocytochemistry and *in situ* hybridization. Antibodies (provided by D.Kaplan) and oligonucleotide probes show similar distributions of the *trk*C mRNA and protein in the retina. Using cell size criteria, populations of both RGCs and amacrine cells expressed TrkC. Approximately 30% of RGCs were immunostained with the TrkC antibody. Labelled amacrine cells were found in the inner nuclear layer and in the ganglion cell layer.

Two weeks after axotomy, more than one-half of the surviving RGCs were TrkC positive. However, quantitative *in situ* hybridization revealed a 50% decrease of *trk*C mRNA levels in the axotomized RGCs. In surviving RGCs, neither the incidence of the TrkC expression, nor their mRNA levels were affected by the intravitreal injection of a single dose of NT-3 (5μg).

These results indicate that a sizable number of RGCs and amacrine cells express TrkC. Because TrkB is found on most RGCs, the demonstration that approximately one-third of RGCs is TrkC possitive suggests that some of these neurons express both these receptors.

609.2

CHANGES IN VGF mRNA EXPRESSION IN THE RAT BRAIN AFTER KAINIC ACID INDUCED SEIZURE AND CORTICAL LESION. S. E. Snyder[1]*, H.-W. Cheng[2], K. D. Murray[3], P. J. Isackson[3], T. H. McNeill[2], and S. R. J. Salton[1]. [1]*Fishberg Research Center for Neurobiology, Mount Sinai School of Medicine, New York, NY 10029;* [2]*Division of Neurogerontology, Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089;* [3]*Department of Biochemistry and Molecular Biology, The Mayo Clinic Jacksonville, Jacksonville, FL 32224.*

VGF was cloned on the basis of its rapid, selective induction by NGF in PC12 cells. Its expression is restricted to neurons and neurosecretory cells, is regulated during development, and is found in many neurons in the adult. The VGF protein is secreted and is likely cleaved into peptides.

In this study, recurrent seizures were induced in adult rats by i.p. injection of kainic acid. *In situ* hybridization to sections of rat brain perfused at various times after injection shows rapid induction of VGF mRNA in dentate granule cells, evident by 1 hr, which disappears by 48 hrs. VGF mRNA also increases in the hippocampal CA fields and the piriform cortex, following a more protracted time course.

In another paradigm, brains were taken at various intervals following unilateral cortical lesions. By 3 hrs, we observe a substantial induction of VGF mRNA throughout the ipsilateral cortex. By 6 hrs, we also see down-regulation of VGF in the ipsilateral striatum. By 3 days, cortical VGF levels have returned to normal, while the striatal levels remain depressed. Then, at 10 days, there is robust induction of VGF in a restricted area of the rostrodorsal ipsilateral striatum, coinciding closely in both timing and location with sprouting of contralateral cortical projections into this region.

Thus, kainate-induced seizure, either via depolarization or as a result of excito-toxicity, rapidly induces VGF mRNA in selected neurons. Also, direct lesion of the brain can both induce and repress VGF. Further studies will be needed to elucidate the cellular mechanisms by which such regulation occurs, and the function of VGF in response to such injury.

609.3

CONTRASTING CHANGES IN NEUROTROPHIN RECEPTOR AND TYROSINE HYDROXYLASE GENE EXPRESSION IN THE AGING SYMPATHETIC NERVOUS SYSTEM. **G. A. Kuchel* and C. Richard**, Geriatric Medicine, Montreal General Hospital Res. Inst., 1650 Cedar, Montreal, Quebec, Canada H3G 1A4

Quantitative *in-situ* hybridization with a riboprobe complementary to gp75 (low-affinity neurotrophin receptor) mRNA showed a 25% decrease in the mean neuronal *Labeling Index* (LI) in aged (24 m), as compared to mature (10 m) male rat SCG (superior cervical ganglia), while LI for cyclophilin mRNA did not change. A ribonuclease protection assay demonstrated a 25-30% decrease in levels of gp75 mRNA per cyclophilin mRNA. Although generalized, the decrease in the gp75 *Labeling Index* was greatest in <10% neurons (small & intermediate-sized), a population which resembles those neurons innervating the pineal and midline cerebral vessels. It is known that the innervation to these two SCG targets declines ~50% with aging, while that to other targets does not change.

Mean LI for *trkA* mRNA was decreased 13%, while the LI for tyrosine hydroxylase mRNA was 74% higher in aged SCG. The latter change was mostly due to very large increases in <10% neurons (small & intermediate-sized). Further studies will be needed to characterize the subset(s) of neurons in which these large changes take place and to determine whether they correspond to the neurons innervating the two targets whose innervation declines in old age. Our results demonstrate that when examining gene expression in neuronal aging, considerable specificity exists in terms of the genes and neuronal populations affected and that neuronal aging may also be associated with increased expression of some genes. Finally, decreased gp75 mRNA expression may be a marker of neuronal vulnerability and axonal degeneration in aging.

609.4

Tooth Injury Induces Expression of Nerve Growth Factor Receptors in the Trigeminal Ganglia.

E. F. Wheeler, Ph.D.* and M. Byers, Ph.D. The University of Texas at San Antonio , University of Washington, Seattle, WA

Six hours post injury, NGF mRNA and protein biosynthesis increase in the pulp of injured teeth to levels five to eight times those in normal teeth (Byers et al., Growth factors 6: 41-52, 1992). Profuse sprouting of sensory nerve fibers occurs in tooth pulp by 1 to 4 days following dentin injury. These events suggest that the trigeminal neurons that innervate the teeth may be responding to the increased levels of NGF post injury. To further characterize the effects of NGF on the trigeminal neurons after tooth injury, we tested the trigeminal ganglion for expression of the NGF receptors (trk A and p75) at at 4, 12, 28 and 52 hours after injury. For tooth injuries, the occlusal surface of rat molar cusps was drilled partway to the pulp and then etched, air dried and sealed with resin. At different times after injury, the trigeminal ganglia were harvested from perfusion-fixed rats and subjected to in situ hybridization analysis with riboprobes encoding sense and antisense sequences for trk A or p75. Trigeminal neurons that innervate the teeth showed increased levels of p75 and trk A 28 and 52 hours after injury that were 2-3 times higher than in uninjured controls. These results indicate that increased levels of NGF receptors in the trigeminal neurons are a part of the dental injury response. The timing of receptor upregulation suggests that retrograde transport of NGF may be necessary for the increases in the trkA and p75 receptors in the neurons that innervate injured teeth.

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609.5

MOLECULAR CHANGES ASSOCIATED WITH PERIPHERAL NERVE REGENERATION E.J. Livesey and S.P. Hunt (SPON: Brain Research Association), MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, U.K.

We have investigated the potential role in regeneration of candidate factors found in the peripheral nerve, in this case the neurotrophins, by studying the neuronal expression of receptors for those factors by *in situ* hybridisation under normal conditions, following injury and during regeneration. We found that the proportions of regenerating sensory neurons expressing the low-affinity neurotrophin receptor (LNGFR) and two of the high affinity receptors, *trkA* and *trkC*, decreased seven days after nerve crush to 89%, 68% and 81% respectively of control values. Similar changes were observed in a non-regenerating lesion of the nerve (LNGFR, 72%; *trkA*, 76%; *trkC*, 80%), while the number of neurons expressing *trkB* remained constant in both lesion models. Given the decrease in neuronal expression of neurotrophin receptors described here, we suggest that signalling through *trk* receptors may not be essential for neuronal regeneration.

Secondly, we are searching for genes which are differentially expressed in the peripheral nerve and in denervated peripheral targets during regeneration, assuming that some of the changes observed will be necessary for successful regeneration. We have used the mRNA differential display technique to compare more than 3,000 mRNAs from normal and injured sciatic nerve and innervated and denervated muscle and have found a small number of cDNAs which are reproducibly found in one tissue state but not the other. The true differential expression of each of these is presently being confirmed by northern blot analysis. The sequences of 10 of the muscle clones show no direct homologies to the non-redundant databases (GenBank BLAST search), although one of the clones contains the rat BC1 or ID motif, and some show partial homologies to human ESTs of unknown identity.

609.6

DIFFERENTIAL CHANGES IN TRK A AND TRK B NEUROTROPHIN RECEPTORS IN THE RAT HIPPOCAMPUS FOLLOWING TRIMETHYLTIN INTOXICATION. M. Skup, D. Koczyk, M. Zaremba and B. Oderfeld-Nowak. (SPON: European Brain and Behaviour Society). Department of Neurophysiology, Nencki Institute of Experimental Biology, 3 Pasteur Street, 02 - 093 Warsaw, Poland.

Trimethyltin (TMT) intoxication produces neuronal damage in the limbic system and in the hippocampal formation in particular. In the hippocampus TMT-evoked injury was reported to induce reactive sprouting of dendrites (D. Koczyk, Acta Neurobiol. Exp. 54, 1994) and nerve terminals. We have recently found that these responses are accompanied by an enhanced hippocampal neurotrophic activity. The aim of the present study was to examine the distribution of the high-affinity neurotrophin Trk A and Trk B receptors in the TMT-injured hippocampus to evaluate the presumably changed pattern of neurotrophin-responsive nerve terminals and to detect the putative targets of altered trophic activity. The immunocytochemical study with anti-Trk A and anti-Trk B (full-length) polyclonal antibodies (Santa-Cruz, CA) was carried out on the adult male Wistar rats at 4, 7, 14, and 21 days after TMT administration (8mg/kg, i.p.). Trk A labeling of punctate appearance suggesting nerve-terminal localization detected in the control PBS-treated rats, in the hilus, along the mossy fibres pathway, in CA3 and in CA2 pyramidal cell layers was not changed in TMT-treated animals. An intense Trk B immunoreactivity (IR) in neurons of CA1 and CA2/CA3 pyramidal cell layer with dense dendritic labeling in CA1 as well as Trk B-IR in hilar interneurons were found in control rats. A loss of Trk B-IR in TMT-treated rats occurred. A decrease of labeling was observed predominantly in CA1 damaged neurons. The pattern of loss of dendritic Trk B-IR detected in CA1 and CA2 layers paralleled that one of dendritic marker (MAP 2) found by us previously. No induction of Trk B-IR was found in non-neuronal elements. On the other hand multiple cells of astroglial appearance became Trk A-IR. Low number of Trk A-IR astrocytes detected at 4 and 7 days after TMT-intoxication increased significantly at 14 and 21 posttreatment days. Trk A expression in astrocytes may indicate responsiveness of reactive astrocytes to NGP and further supports the postulated (Oderfeld-Nowak et al., IX Int. Symp. on Cholinergic Mechanisms, Mainz, 1995) involvement of glial neurotrophic activity in neuronal survival and/or plastic postinjury rearrangements.

609.7

NEUROTROPHIN-4/5 UPREGULATES PROTEIN LEVELS OF NITRIC OXIDE SYNTHASE IN CULTURED STRIATAL NEURONS. H.R. Widmer*, H. Heider, H. Hoppeler, B. Knüsel and F. Hefti. Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089 and Institute of Anatomy, University of Bern, CH-3012 Bern, Switzerland.

We have recently shown that chronic treatment of the neurotrophin NT-4/5 promotes the survival and differentiation of striatal GABAergic neurons prepared from fetal rat brain. Furthermore, this treatment elevates levels of the calcium-binding protein calretinin, suggesting that NT-4/5 may exert neuroprotective actions in striatal cells. Nitric oxide (NO) mediates a variety of physiological functions in the central nervous system and has been proposed to protect neurons from cytotoxic events. We now found that chronic treatment of primary cultures from embryonic rat striatum with NT-4/5 increased the amount of nitric oxide synthase (NOS) as measured by Western blot analysis. The treatment elevated cell number and protein content of the cultures. No significant increase in thymidine incorporation was observed during any culture period investigated, indicating that NT-4/5 treatment reflects increased survival rather than cell proliferation. In support of this conclusion we found levels of cdc2 p34, a cyclin-dependent protein kinase involved in cell cycle regulation, were not altered after NT-4/5 treatment. Furthermore, no alterations were detected for c-jun, erk-1 and ras-GAP protein levels. The findings suggest that NT-4/5 improves survival of striatal neurons by a mechanism involving NOS.

609.8

PRODUCTION AND BIOLOGICAL ACTIVITY OF NGF:NT-4/5 HETERODIMERS. B. Knusel*, F. Hefti, L.E. Burton and J.J.S. Treanor. University of Southern California, Los Angeles, CA 90089 and Genentech, Inc., South San Francisco, CA 94080.

Neurotrophins (NGF, BDNF, NT-3, NT-4/5) exist in solution as non-covalently linked dimers. The interface residues of neurotrophins are highly conserved and heterodimeric neurotrophins can be formed. We generated recombinant human (rh) NGF:NT4/5 by acid-driven rearrangement and purification by HPIEC. rhNGF:NT4/5 was assayed for heterodimeric stability and analyzed by radioligand binding and ligand induced tyrosine phosphorylation in cell lines expressing individual neurotrophin receptors. Functional activity was tested in PC12 cells and primary cultures of rat brain cells. Compared with respective homodimers, NGF:NT4/5 was as potent at binding and signaling through rat trkA but 10-20 fold less potent through rat trkB receptors. In cultures containing cholinergic neurons of the basal forebrain, the order of potency for stimulating choline acetyltransferase activity was NGF > NGF:NT-4/5 > NT-4/5. The shape of the dose-response curve for NGF:NT-4/5 suggested involvement of more than one receptor. In cultures of the striatum, NT4/5 was approximately 3-10-fold more potent than the heterodimer in increasing GABA uptake activity. A similar difference was found in neurotrophin induced Trk tyrosine phosphorylation in cultured neurons. Our results indicate that NGF:NT-4/5 heterodimers are biologically active under physiological conditions. Such heterodimers might exist in-vivo and present a control mechanism of neurotrophin action. Heterodimers and other pan-neurotrophins should become useful in the treatment of neurodegenerative diseases with more than one afflicted cell population.

609.9

EXTRACELLULAR MUTANTS OF THE RAT TRKA RECEPTOR. S. O. Meakin*[1,2], H. Schneider[1] and J. I. S. MacDonald[1]. [1]Neurodegeneration Research Group, The John P. Robarts Research Institute, [2] Department of Biochemistry, University of Western Ontario, London, Ontario, N6A 5K8, Canada]

TrkA is a ligand-activated tyrosine kinase receptor which binds to the closely related neurotrophic molecules, nerve growth factor (NGF) and neurotrophin-3 (NT-3). The extracellular domains of rat and human trkA contain 3 highly conserved regions, namely, the second and third leucine rich motifs (96% and 91% identity respectively) and the second IgC_2 domain (91% identity). Despite the conservation of these sequences, the functional roles of these domains have not yet been determined. Using standard genetic approaches we have introduced uni-directional 5' and 3' deletions into the extracellular domain of rat trkA and assayed the effects of these mutations on ligand binding, activated tyrosine kinase activity and neurite outgrowth. All mutants were engineered to contain a wild type signal sequence to facilitate similar levels of cell surface receptor expression. A third trkA mutant was also generated which lacks 20 amino acids in the hinge region just 5' to the transmembrane domain. Interestingly, the leucine rich motifs were found to be dispensable for ligand binding, ligand-activated tyrosine kinase activity and ligand-induced neurite outgrowth. In contrast, both the hinge region and the IgC_2 domains were found to be indispensable for ligand binding. These data indicate that sequences in the IgC_2 domains form the NGF binding site(s) in trkA and suggest an alternative role of the leucine rich motifs in trk-mediated protein-protein interactions.

609.10

CHOLINERGIC NEURONS IN TRANSGENIC MICE WITH AN ALTERED LOW-AFFINITY NGFr GENE T.T. Yeo*, J.H. Kunimitsu, D.H. Bredesen#, and L.L. Butcher. Departments of Psychology and Neurology#, UCLA, Los Angeles, CA 90095-1563.

The functions of the low-affinity NGF receptor (p75NGFr) in the nervous system are poorly understood. Although *in vitro* cell culture studies have indicated that it may facilitate high-affinity binding of NGF to Trk and mediate apoptosis in NGF-dependent neurons upon trophic factor withdrawal, virtually nothing is known about its function *in vivo*. The recent production of a "p75-knockout" mouse (transgenic mouse with an altered p75NGFr gene; Lee et al., Cell, 1992) offers new opportunities in further elucidating the role(s) of the p75NGFr in neuron physiology and biochemistry. This mouse can also serve as an *in vivo* model for studying the role of the p75NGFr in mediating NGF actions in the development and survival of basal forebrain cholinergic neurons, which undergo degeneration in Alzheimer's disease.

We found that in the normal (wildtype) mouse, as in the rat, the p75NGFr is colocalized with cholinergic neurons in the basal forebrain (i.e. medial septal nucleus, diagnal band, magnocellular preoptic area, ventral pallidum, nucleus basalis, and nucleus of the ansa lenticularis) but not the local-circuit cholinergic neurons of the dorsal and ventral striata and mesopontine complex. In "p75-knockout" mice, basal forebrain cholinergic neurons are devoid of p75NGFr immunoreactivity, but the number and distribution of neurons displaying immunopositivity for choline acetyltransferase (ChAT) remain unchanged. Levels of ChAT-like immunoreactivity also appear normal. These data suggest that the p75NGFr may not be crucial for proper development of the cholinergic phenotype in the basal forebrain. [Support: Gustav Rose Fellowship, French Foundation for Alzheimer Research (T.T.Y); Research Grant from the Retirement Research Foundation, UCLA Center on Aging (L.L.B.)]

609.11

***trk*A-MEDIATED REGULATION OF ACETYLCHOLINESTERASE IN ADRENAL MEDULLA AND SPINAL CORD: EVIDENCE FROM THE *trk*A KNOCKOUT.** A. Schober,[1] L. Minichiello,[2] R. Klein,[2] M. Keller,[3] P.G. Layer,*[3] and K. Unsicker.[1] [1]Dept. Anatomy & Cell Biology III, Univ. Heidelberg, [2]EMBL, Heidelberg, [3]Dept. Zoology, Technical Univ. Darmstadt, Germany.

NGF induces neuritic growth of adrenal medullary chromaffin cells. To determine whether NGF has physiologically relevant functions for the adrenal medulla and its innervation, we have studied the distribution and activity of acetylcholinesterase (AChE) in adrenal medullae and spinal cords of *trk*A-/- mice. Histologically, adrenal medullae and spinal cords of mutants appear grossly normal. Shortly after birth (P0 and P3), patterns and intensities of staining for AChE enzyme activities do not differ between wildtypes and mutants. At P6/7 and P12, however, AChE staining is strongly decreased in spinal cords and in adrenal medullary nerve fibers of *trk*A-/- as compared to wildtype animals. Quantitative measurements of AChE activities reflect this decrease in staining intensities (adrenal: -64%; spinal cord: -25% relative to wildtypes). Interestingly, immunocytochemical staining with an antibody to AChE does not detect differences in the adrenal medullae of wildtype and mutant animals. Our results suggest a role of NGF in the regulation of AChE activity of nerves innervating chromaffin cells, possibly also chromaffin cells themselves. The molecular basis of this regulation will now be explored.
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609.12

SOME NOCICEPTIVE NEURONS INNERVATING THE CORNEA SURVIVE IN *trkA* KNOCKOUT MICE. M. López de Armentia[1], F. de Castro[1], I. Silos-Santiago[2], M.A. Garber[2] M. Barbacid[2] and C. Belmonte[1]*. Instituto de Neurociencias Universidad de Alicante, Spain[1]; Dept. Molecular Biology, Bristol-Myers Squibb, Princeton NJ[2].

Mutant mice in which NGF/*trkA* signaling has been abolished by inactivation of the trkA gene, have depleted populations of nociceptive neurons in sensory ganglia and do not respond to painful stimuli. Whether or not all subtypes of nociceptive neurons are absent is unknown. The cornea is innervated by trigeminal nociceptive neurons, that respond to different noxious stimuli. Only sensation of pain can be elicited by corneal stimulation. We have compared corneal innervation and responsiveness to noxious stimulation in trkA knockout (-/-), wild type (+/+) and heterozygous (+/-) mice. Corneal nerves were stained using gold chloride impregnation. Blinking movements evoked by von Frey hairs (7-144 mN/mm^2) and topical application of 0.1-10 mM acetic acid, saline at 0-90°C and 33 mM capsaicin were counted. A dramatic reduction of nerve trunks in the corneal stroma and of thin nerve terminals was observed in (-/-) animals. Blinking in response to various modalities of noxious stimuli was either absent or significantly reduced. We concluded that most corneal trigeminal nociceptive neurons depend on NGF for survival. However a small population of corneal sensory neurons appears to be NGF-independent and may belong to all functional types. (CITYT SAF93-0267, Spain; F.C.& M.L.A.:are MEC fellows).

609.13

p75 INHIBITION AND KNOCK-OUT EFFECTS ON ADULT CHOLINERGIC FOREBRAIN NEURONS C.E.E.M. Van der Zee*, G.M. Ross#, R.J. Riopelle#, T. Hagg Dept. Anatomy & Neurobiology, Dalhousie Univ., Halifax, B3H 4H7, Canada ; # Dept. Medicine, Queen's University, Kingston, K7L 2V7 Canada.

NGF (nerve growth factor) can activate its transducing TrkA receptor and can bind to the low-affinity NGF p75 receptor. The role of p75 is unclear but it can enhance the transduction efficiency of TrkA. p75 also may have a TrkA-independent signaling capacity by activation of the sphingomyelin pathway and has been implicated in developmental apoptosis. We have used a conformationally constrained synthetic peptide designed from putative p75-interacting NGF residues 28-36. This cyclic peptide (c28-36) blocked neurite outgrowth in cultured chick DRG neurons and inhibited crosslinking of ^{125}I-NGF to p75 receptor but not to TrkA. We are investigating potential p75-specific signaling functions in the basal forebrain cholinergic system. After a 14 day infusion of c28-36 (6 nmol/d; ~7 µg/d) into the lateral septum or hippocampal formation of normal adult female Sprague-Dawley rats, the diameter of medial septum cholinergic (ChAT-positive) neurons was reduced by 35%, similar to the atrophy seen after axotomy or in aging. In animals with an entorhinal lesion, non-lesioned septal cholinergic axons undergo collateral sprouting in the outer molecular layer of the dentate gyrus. An 8-day infusion of c28-36 into the hippocampal formation caused neuronal atrophy but did not diminish the extent of sprouting. Transgenic mice (4-6 weeks old) with a p75 null mutation had almost twice as many medial septum ChAT-positive neurons compared to F2 control mice and these neurons were 30% larger in diameter. Thus, in the cholinergic basal forebrain, p75 receptor appears to be involved in regulation of developmental neuronal death and cell body size.

Supported by Network of Centres of Excellence, Canada.

609.14

EXPRESSION OF p75 IN THE TRIGEMINAL GANGLION OF TRANSGENIC MICE THAT OVEREXPRESS NGF IN THE SKIN. P.H. Kitzman*, K.M. Albers and B.M. Davis. Depts. of Pathology and Anatomy and Neurobiology. University of Kentucky Lexington, KY

During development a subset of neural crest -derived sensory neurons and sympathetic neurons are dependent on NGF for survival and differentiation. NGF has been shown to bind to both the trk A tyrosine kinase receptor and the low affinity NGF receptor (or p75). However, at present, whether binding to both the trk A and p75 receptors is required for NGF-induced activity remains controversial. Recently it has been shown that exogenously increased target-derived NGF increases p75 expression (Miller et. al., 1994). Our lab has previously described a transgenic mouse line that overexpresses NGF in the skin (Albers et. al., 1994). These mice demonstrated a two-fold increase in the number of neuronal cell bodies in the trigeminal ganglia as compared with control mice. In addition, the number of trk A expressing neurons increased 5-fold and the number of trk C expressing neurons increased 2-fold. To determine whether expression of p75 is similarly effected, *in situ* hybridization using a riboprobe for the p75 receptor was performed. Results of this study suggest a trend for an increase in the number of neurons expressing p75 in trigeminal ganglia of the NGF transgenic mice. RT-PCR was performed to determine the change in p75 mRNA expression in the trigeminal ganglion as well as in other tissues. Preliminary results indicate an increase in p75 mRNA expression in the skin and tongue of the NGF transgenic mice as compared with control mice. Finally, immunohistochemistry utilizing an antibody to the p75 receptor was performed to determine whether there is a change in the number of neurons expressing the p75 protein

609.15

TrkB/TrkC DOUBLE KNOCKOUT MICE COMPLETELY LACK SEVERAL PERIPHERAL SENSORY GANGLIA BUT EXHIBIT ONLY MINOR ABNORMALITIES IN THE CENTRAL NERVOUS SYSTEM.
I. Silos-Santiago*[1], A.M. Fagan[1], D.R. Foschini[1], S. Ozaki[2], W.D. Snider[2] and M. Barbacid[1]. [1]Dept. of Molecular Oncology, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543; [2]CSNSI, Washington University, St. Louis, MO 63110.

The paucity of deficits found in the CNS of "knockout" mice lacking either TrkB or TrkC tyrosine kinase receptors suggests that the survival of most CNS neurons depends on multiple growth factors. Indeed, many CNS neurons, such as hippocampal and motor neurons, express both TrkB and TrkC receptors *in vivo* and respond to their cognate neurotrophins (BDNF/NT-4 and NT-3, respectively) *in vitro*. Therefore, the absence of dramatic CNS phenotypes in single *trk*(s) knockout mice may reflect compensatory survival-promoting effects of the unaffected neurotrophin signalling pathway. To address this possibility, we have generated double knockout mice in which both *trk*B and *trk*C genes have been inactivated. These *trk*B(-/-)/*trk*C(-/-) mice develop until birth, the latest time point evaluated. Gross histological evaluation indicates that CNS structures, including the hippocampal formation and motor pools, appear to be formed normally in these double *trk*B/*trk*C mutants. Preliminary quantitation analysis demonstrates only a slight decrease in motor neuron number as compared to wildtype controls. Expression of parvalbumin, calbindin, and tyrosine hydroxylase appears to be normal in CNS regions. In contrast, several peripheral sensory ganglia, including the vestibular, cochlear and geniculate ganglia, are completely missing in the double *trk*B/*trk*C *null* animals. Other sensory ganglia, although present, have more neuronal cell loss than in single *trk*B or *trk*C knockout mice. These results indicate that neurotrophin signalling may not be required for survival of CNS neurons, at least during embryonic development. However, in the PNS, neurotrophin signalling is absolutely required and may cooperate in promoting neuronal survival.

609.16

OVEREXPRESSION OF Bcl-2 DOES NOT PREVENT THE DEATH OF NEUROTROPHIN-DEPENDENT PNS NEURONS IN THE TrkA KNOCKOUT MOUSE. A.M. Fagan*[1], M.A. Garber[1], O. Bernard[2], M. Barbacid[1], and I. Silos-Santiago[1]. [1]Dept. of Molecular Oncology, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543; [2]The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria, Australia.

Neurotrophin signalling is absolutely required for the survival of specific populations of developing neurons in the PNS. We have recently shown that ablation of *trk*A, the gene encoding the NGF signalling receptor, results in the developmental death of sympathetic and nociceptive sensory neurons. However, the intracellular mechanisms involved in neurotrophin-dependent cell survival are unknown. Bcl-2 is a candidate molecule for mediating the survival effects of neurotrophic factors since it has been shown to enhance the survival of PNS neurons following neurotrophin withdrawal *in vitro*. *In vivo*, Bcl-2 has been reported to protect PNS neurons from programmed cell death during development, as well as prevent the axotomy-induced death of neonatal motor neurons. In an effort to explore the role of Bcl-2 in neurotrophin-dependent cell survival, we have generated *trk*A(-/-) mice which constituitively overexpress *bcl*-2 under the direction of the neuron-specific enolase promoter. Developmental expression of the Bcl-2 protein in these animals was confirmed by Western blot analysis. However, Bcl-2 expression was unable to rescue sympathetic or sensory neurons from cell death in the neonatal *trk*A(-/-) mouse. These data indicate that whereas Bcl-2 may confer a survival advantage for neurons at earlier stages of neurotrophin-dependence, its overexpression is not sufficient to compensate for the absence of NGF signalling in these *trk*A *null* animals.

609.17

NEURONAL DEFICITS IN THE TRIGEMINAL SYSTEM OF KNOCK OUT MICE LACKING THE TrkA NGF RECEPTOR
D.R. Foschini*[1], D.P. Crockett[2], M.D. Egger[2], M. Barbacid[1], and I. Silos-Santiago[1]. [1]Dept. of Molecular Oncology, Bristol-Myers Squibb, Princeton, NJ 08543 and [2]Dept. Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ 08854.

Mice carrying germ line mutations in the genes encoding the TrkA, TrkB, and TrkC neurotrophin receptors display an array of distinct neurological deficits, mainly limited to the PNS. Whether or not these neurons die before or after reaching their peripheral and central targets remains to be determined. In the trigeminal (TRG) ganglia, the period of naturally occuring programmed cell death is simultaneous with TRG neuron target innervation and precedes the emergence of the vibrissae-related cytochrome oxidase (CO) patterns in the trigeminal brainstem complex. We have studied the development of this system in the absence of neurotrophin signalling. We have observed that in the *trkA null* mice TRG sensory neurons are born. Some neurons are likely to be NGF-dependent since transcripts for the extracellular domain of the trkA receptor have been detected in these mutant mice by *in situ* hybridization at early embryonic stages. However, by E11.5 there is an increase in the rate of cell death in these mutant mice, and by E13.5, 50% of the TRG neurons are lost and levels of trkA transcripts are lower than in wild-type mice. By E15.5 the TRG ganglion of the *trkA null* mice only had 16% of the neurons found in their wild-type littermates. At this time expression of trkA mRNA is completely absent. In addition, the whisker-related CO pattern appears to be less prominent in the *trkA null* mice. These results suggest that the death of NGF-dependent trigeminal neurons may occur before they reach their targets and have an effect on central pattern formation.

610.1

AGE-RELATED DNA REPAIR ACTIVITY IN NEURONAL CORTICAL CULTURES. P. Corsi*, G. Brescia and G. Assennato. *Istituto di Fisiologia Umana, Istituto di Medicina del Lavoro, Facoltà di Medicina e Chirurgia, Bari, Italy.

Possible age-related deterioration in the efficacy of DNA repair was investigated in neuronal cortical cultures as a function of degree of cellular differentiation and ageing. DNA repair capacity was measured as spontaneous and UV induced unscheduled DNA synthesis (UDS). While not necessarily related to the induction of accelerated ageing in neurons, ultraviolet (254 nm) light (UV) may represent an excellent model agent for studying the role of DNA damage in ageing. At 7, 14, 21 days in vitro, the neuronal cultures were incubated with hydroxyurea (HU), a DNA replicative synthesis inhibitor and irradiated with a UV light at 24 J/m^2. The DNA content was determined and UDS measured by the DNA incorporation of [^{3}H]thimidine and expressed in cpm/μg of DNA; the amount of DNA damage has been evaluated as sensitivity to S1 nuclease representing the proportion of single stranded DNA. In UV irradiated cultures, parameters reffering to total cell survival, neuronal cytotoxicity and morphology were also considered. Our experimental evidence suggest that a lowered performance of age-related repair activity has been observed indicating that a DNA damage potentially accelerates the ageing processes alterating the primary information of the DNA macromolecule.

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610.2

CROSSLINKING OF MEMBRANE GLYCOPROTEINS BY CONCANAVALIN A INDUCES APOPTOSIS IN CORTICAL NEURONS. V.M. Kreng, D.H. Cribbs, A.J. Anderson, A. Russo-Neustadt* and C.W. Cotman. Irvine Research Unit in Brain Aging, University of California, Irvine, CA 92717.

Recently apoptosis has been implicated in the loss of neurons during normal aging and in neurodegenerative diseases. This has led to an increased interest in the type of stimuli that initiate neuronal apoptosis *in vivo*. Unfortunately, many agents capable of activating apoptosis do not mimic *in vivo* events and also hit multiple targets (i.e., radiation, hydrogen peroxide, and staurosporine). However, activation-induced cell death (AICD), which occurs when the oligomerization of membrane receptors by multivalent ligands leads to signal transduction that triggers apoptosis is one well defined activating event. We have used the lectin concanavalin A (Con A), to study whether crosslinking of membrane glycoproteins on neurons will activate apoptosis. Cortical neurons treated with Con A exhibit the morphological and biochemical characteristics of apoptosis, including membrane blebbing, condensation of nuclear chromatin and internucleosomal DNA fragmentation. In addition, Con A produced a rapid, robust and prolonged increase in the immediate early gene c-jun which precedes the onset of other markers for apoptosis in neurons. Interestingly, succinyl Con A, the dimeric form of the lectin which is much less effective at crosslinking, was not neurotoxic although it did bind to the neurons and Con A specific monosaccharides were able to block Con A-induced apoptosis. Thus, Con A provides a clearly defined activating signal to study apoptosis in cultured neurons.

610.3

ANTI-OXIDANTS AND NMDA RECEPTOR ANTAGONISTS PROTECT AGAINST AGE-INDUCED APOPTOSIS IN CEREBELLAR GRANULE CELLS. D.-M. Chuang[1], P. Leeds[1], K.-G. Zhang[1], X.-M. Gao*[1] and R. Ishitani[2]. [1]Section on Molecular Neurobiology, Biological Psychiatry Branch, NIMH, NIH, Bethesda, MD 20892 and [2]Group on Cellular Neurobiology, Josai Univ., Saitama 350-02, Japan.

Cerebellar granule cells (CGC) prepared from neonatal rats undergo apoptosis within 3 weeks in culture under typical growth conditions, i.e. in the presence of 25 mM KCl without medium change and glucose supplement. This age-induced apoptosis involves over-expression of glyceraldehyde-3-phosphate dehydrogenase (Ishitani et al., this volume). Here, we found that NMDA antagonists, MK-801 (0.1-10 μM) and AP-V (1-100 μM), dose- and time-dependently protected against age-induced death of CGC as evidenced by microscopic inspection and MTT assays after 20 days in vitro (DIV). The anti-oxidants, vitamin E and melatonin, also dose-dependently protected against age-induced apoptosis with an EC_{50} of about 3 μM and 30 μM respectively. Maximal protection occurred when vitamin E (10^{-5} M) or melatonin (10^{-4} M) was added after 10 DIV. Confirming previous observations, periodic supplements with glucose markedly prolonged the life span of CGC. These results suggest that age-induced death of CGC involves over-stimulation of NMDA receptors by the release of endogenous glutamate. This NMDA receptor activation may trigger the production of reactive oxygen intermediates which contribute to age-induced CGC apoptosis.

610.4

COMPARISON OF CARBAMAZEPINE, PHENYTOIN, AND AGE-INDUCED CELL DEATH IN CEREBELLAR GRANULE CELLS P.A. Saunders*, R. Ishitani@, P.Leeds, and D.-M. Chuang Sect. Molecular Neurobiol., Biol. Psychiatry Branch, NIMH, NIH, Bethesda, MD 20892, @ Josai U., Sakado, Saitama 350-02, Japan.

We have used primary cerebellar granule cells to study the mechanisms involved in neuronal apoptosis. The properties of the apoptosis caused by the anticonvulsants carbamazepine (CBZ) and phenytoin (PHE) are different from that found with aging. Age-induced apoptisis was inhibited by the anti-oxidants, vitamin E and melatonin (Chuang et al., this volume). In addition, chronic exposure to the glutamate receptor agonst, NMDA, hastened the onset of apoptosis. In contrast to aging, short pretreatment of the cells with NMDA protected the cells against CBZ- and PHE-induced apoptosis and the antioxidants were not protective. Increased levels of a 38 kDa protein identified as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and its mRNA were seen during age-induced apoptosis. An antisense oligonucleotide against its mRNA delayed age-induced apoptosis (Ishitani et al, this volume), suggesting the protein plays a role in programmed cell death. In contrast to aging, CBZ and PHE caused a rapid and persistent decrease in GAPDH mRNA levels. Pretreatment with NMDA caused an increase of GAPDH mRNA and prevented the CBZ- and PHE-induced GAPDH mRNA loss. These observations suggest that age-induced apoptosis which is due, at least in part due,to oxidative or free radical damage to the cell, while the anticonvulsant induced apoptosis is due to another mechanism which may not require induction of GAPDH.

610.5

APOPTOSIS INDUCTION IN A CNS NERVE CELL LINE BY MICROTUBULE DEPOLYMERIZATION: SELECTIVE CHANGES IN PROTEIN PHOSPHORYLATION. M.P. Lambert*, B. Chromy, G. Stevens, and W.L. Klein. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL , 60208.

The B103 cell line is a spontaneously neuritogenic CNS nerve cell model recently used in studies of cell death evoked by the Aβ peptide (Zhang et al., 1994 J.Biol.Chem.269:25247). It also can be used, as shown here, for studies of an apoptotic cascade evoked by colchicine. Colchicine is a microtubule depolymerizing agent that recently has been reported to cause programmed cell death in several non-neuronal cell lines. Viewed by VEC-DIC microscopy, B103 cells exposed to chronic colchicine round up, undergo neuritic atrophy and retraction, begin extensive membrane blebbing and lose adhesion to the culture substratum -- morphological changes similar to those seen in apoptosis and in mitosis. During the response to colchicine, significant decreases occur in levels of phosphorylated nuclear tau (detected by PHF-1 immunofluorescence; Lambert et al., Neuro.Aging, in press). Cytoplasmic PHF-1 immunofluorescence increases, most likely due to the concomitant increase of a novel PHF-1 immunoreactive protein seen in immunoblots at 120 kDa. Nocodazole also induces the 120 kDa band, as well as several PHF-1 immunoreactive bands not seen with colchicine. Cells that have detached from the culture substratum comprise two populations. Some remain viable, shown by replating sans colchicine. The others have apoptosed, as evidence by the diagnostic 180-200 kb DNA ladder. The disadherent cells show a striking increase in a tyrosine phosphorylated protein at ~90 kDa. We hypothesize that kinase activities associated with mitotic control of adhesion and the cytoskeleton lead to apoptosis when aberrantly activated.

610.6

GLIAL AND OXIDATIVE STRESS MARKERS IN TWO MODELS OF GLIAL ACTIVATION IN RATS. K. Sugaya*, S.J. Xu, T. Pauly and M. McKinney. Dept. of Neuropharmacology, Mayo Clinic Jacksonville, Jacksonville, FL 32224.

Nitric oxide synthase (NOS) containing cells are relatively preserved in certain neurodegenerative diseases and in some lesion animal models. NOS-positive cells may have more robust protection systems for oxidative stress that may be generated by nitric oxide (NO). The inducible type of NOS (i-NOS) is one source of NO. We have found indications of gliosis in aged memory-impaired rats; such activated glia might express i-NOS. In the present study of glial activation, we injected ibotenic acid into the nucleus basalis magnocellularis (NBM), or endotoxin (LPS) into the lateral ventricle. Glial fibrillary acidic protein (GFA), amyloid beta precursor protein (APP), i-NOS and Mn-superoxide dismutase (Mn-SOD) mRNAs were assessed with *in situ* hybridization histochemistry (ISHH) combined with GFA or OX-42 immunohistochemistry. The production of NO (as nitrate) after LPS injection was measured by *in vivo* microdialysis and ion exchange chromatography.

By 6 hr post-treatment, GFA mRNA had increased in the LPS-injected rat, but delayed to a later period in the NBM-lesioned rat. In the NBM lesion model, APP mRNA was increased early and was followed by an increase in i-NOS mRNA. In the LPS-injected rat, APP mRNA was elevated within the same time frame as GFA mRNA. LPS also elicited an increase in i-NOS mRNA at the injection site by 6 hr after the injection, and this was accompanied by an increase in nitrate levels in the ventricle. Mn-SOD mRNA was elevated in parallel with i-NOS mRNA in both animal models. The number of GFAP and OX-42 immunostained cells were increased and their processes were heavily stained in both models, following peak of GFA and i-NOS mRNA elevation.

These results indicate that the involvement of astrocyte and microglial activation differs in these two animal models and that APP may involved in the glial activation cascade. The increase in levels of Mn-SOD mRNA that paralleled the increase in i-NOS mRNA may indicate the induction of cellular oxidative stress protection systems.

610.7

DOUBLE *IN SITU* HYBRIDIZATION FOR NITRIC OXIDE SYNTHASE AND SUPEROXIDE DISMUTASE mRNA COMBINED WITH ChAT IMMUNOCYTOCHEMISTRY IN THE RAT BRAIN. S-J. Xu*, K. Sugaya, C.Kent and M. Mckinney. Dept. of Neuropharmacology, Mayo Clinic Jacksonville, Jacksonville, FL 32224.

Neurons expressing the constitutive nitric oxide synthase (c-NOS) are relatively resistant to degeneration in several diseases including Alzheimer's disease (AD). This may indicate the existence of neuroprotective mechanisms against diffusible free radicals like nitric oxide in these cells. One candidate for this protection mechanism is superoxide dismutase (SOD), since Cu/Zn-SOD is elevated in AD hippocampus and SOD protects cells from free radicals. Differential expression of these protection mechanisms could explain the differential vulnerability in neuronal systems in AD.

In this study, we focused mainly on cholinergic systems and we examined whether SOD mRNA was co-localized with c-NOS mRNA in ChAT-positive cells. For this purpose, we developed a method of "double" *in situ* hybridization histochemistry (ISHH), in which one probe is labeled by [^{35}S] and another probe is labeled by digoxigenin (DIG), and ISHH is combined with ChAT immunocytochemistry.

SD rats were perfused with 4% buffered paraformaldehyde and brain sections were immunostained for ChAT. The sections were hybridized with a [^{35}S]-labeled Cu/Zn-SOD or Mn-SOD riboprobe and a DIG-labeled c-NOS riboprobe. After hybridization, sections were incubated with alkaline phosphatase (AP) conjugated anti-DIG-IgG and c-NOS positive cells were visualized with a substrate for AP. The sections were then emulsion-coated and developed to visualize the radioactive probe. Brown ChAT-immunostained cells expressing c-NOS had a purple deposit. These cells were overlaid with silver grains if Cu/Zn- or Mn- SOD mRNA was also present. The c-NOS message level varied in ChAT-positive neurons in different division of the basal forebrain system, while all the upper brainstem cholinergic cells contained c-NOS mRNA. High level of mRNA for the SODs were not frequently found in cNOS-positive cells These data suggest that c-NOS has a variable relationship to the cholinergic phenotype, perhaps in a way related to differential vulnerability in disease, and that SOD may not the protection mechanism against NO in these c-NOS positive cells.

610.8

EVIDENCE THAT GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE MEDIATES AGE-INDUCED APOPTOSIS IN MATURE CEREBELLAR NEURONS IN CULTURE. R. Ishitani[1], K. Sunaga[1], A. Hirano[2], N. Katsube*[2], P. Saunders[3] and D.-M. Chuang[3]. 1)Group on Cellular Neurobiology, Josai Univ., Saitama 350-02, Japan. 2)Minase Research Institute, Ono Pharmaceutical Co., Ltd., Osaka 618, Japan. 3)Section on Molecular Neurobiology, BPB, NIMH, MD 20892, U.S.A.

Under typical culture conditions, cerebellar granule cells die abruptly after 17 days *in vitro*. This burst of neuronal death involves ultrastructural changes and internucleosomal DNA fragmentations characteristic of apoptosis and is effectively arrested by pretreatment with actinomycin-D, cycloheximide, or aurintricarboxylic acid. The level of a 38-kDa protein in the particulate fraction is markedly increased during age-induced cell death. This increment is suppressed by all above-mentioned agents. N-terminal microsequencing of the 38-kDa protein revealed sequence identity with glyceraldehyde-3-phosphate dehydrogenase (GAPDH). A GAPDH antisense oligodeoxyribonucleotide blocks age-induced expression of this 38-kDa protein and effectively prevents neuronal apoptosis, while the corresponding sense oligonucleotide was completely ineffective. Thus, over-expression of GAPDH in the particulate fraction has a direct role in age-induced apoptosis of cerebellar neurons.

610.9

GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GAPDH) ANTISENSE OLIGODEOXYNUCLEOTIDE PROTECTS AGAINST LOW K^+-INDUCED APOPTOSIS OF CULTURED CEREBELLAR NEURONS. K. Sunaga[1], M. Tanaka[2], H. Aishita[2], D.-M. Chuang*[3] and R. Ishitani[1]. 1)Group on Cellular Neurobiology, Josai Univ., Saitama 350-02, Japan. 2)Fukui Research Institute, Ono Pharmaceutical Co., Ltd., Fukui 913, Japan. 3)Section on Molecular Neurobiology, BPB, NIMH, Bethesda, MD 20892, U.S.A.

D'Mello et al. have reported the induction of apoptosis in cerebellar granule cells (CGC) by lowering K^+ to 5 mM in culture medium. We have shown that GAPDH is involved in age-induced apoptosis of CGC. Therefore, we examined the role of GAPDH in low K^+-induced apoptosis of CGC. Within 24 hrs after exposure of mature CGC to low K^+, a fraction of neurons undergo apoptosis, as revealed by ultrastructural changes and DNA fragmentation. Preceding cell death, the levels of mRNA and protein of GAPDH are markedly increased. Antisense, but not sense, oligodeoxyribonucleotide to GAPDH effectively protects against low K^+-induced apoptosis. Cycloheximide and actinomycin-D block the low K^+-induced increase of GAPDH mRNA and cell death. These results suggest that over-expression of GAPDH mRNA and protein is intimately coupled to low K^+-induced apoptosis in CGC.

610.10

ANTISENSE OLIGODEOXYNUCLEOTIDE TO GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GAPDH) BLOCKS AGE-INDUCED APOPTOSIS IN CEREBRO-CORTICAL NEURONS IN CULTURE. M. Kimura[1], D.-M. Chuang[2] and R. Ishitani*[1]. 1)Group on Cellular Neurobiology, Josai Univ., Sakado, Saitama 350-02, Japan. 2)Section on Molecular Neurobiology, BPB, NIMH, Bethesda, MD 20892, U.S.A.

We have shown that GAPDH is involved in age and low K^+-induced apoptosis of cerebellar granule cells. To further examine the role of GAPDH in the apoptosis of other cell types, we have extended the study using cerebro-cortical neurons. Similar to cerebellar granule cells, cortical neurons undergo age-induced apoptosis after 15 days in culture which is characterized by DNA fragmentation and chromatin condensation. Cycloheximide, actinomycin-D and aurintricarboxylic acid markedly protect against this apoptosis of cortical neurons. Preceding the apoptosis, we found a marked increase in the GAPDH mRNA level. A GAPDH antisense oligodeoxyribonucleotide markedly delays this cell death by 3 to 4 days, an effect which is as robust as that of cycloheximide. In contrast, the corresponding sense oligonucleotide is totally ineffective in the protection. Theses results suggest that GAPDH is involved in the apoptosis of not only granule cells but also cortical neurons.

610.11

TETRAHYDROAMINOACRIDINE AND ONO-1603, A POTENTIAL ANTIDEMENTIA DRUG, ATTENUATE AGE-INDUCED APOPTOSIS OF CULTURED CEREBELLAR NEURONS. N. Katsube[1,2], K. Sunaga[1], M. Kimura[1], M. Yamamoto*[2], H. Aishita[2] and R. Ishitani[1]. 1)Group on cellular Neurobiology, Josai Univ., Sakado, Saitama 350-02, Japan. 2)Minase Research Institute, Ono Pharmaceutical Co., Ltd., Mishima-Gun, Osaka 618, Japan.

Tetrahydroaminoacridine (THA) is effective in the treatment of some Alzheimer's patients. It seems unlikely that THA's therapeutic effect is simply due to its anticholinesterase activity, since other cholinesterase inhibitors show little clinical efficacy. Our present report demonstrates that a lower dose of THA (3-10 μM) added to mature cerebellar granule cells (CGC) significantly attenuates apoptotic cell death and this neuroprotection is correlated with the ability of THA to suppress the expression of the particulate 38-kDa protein, i.e., glyceraldehyde-3-phosphate dehydrogenase. These novel effects of THA provide a new avenue to investigate the mechanisms of action of this drug. This paradigm may also be used to screen for more effective drugs in the treatment of Alzheimer's disease. ONO-1603, a novel prolyl endopeptidase inhibitor and possessing an anti-amnesic effect, significantly delays the age-induced apoptosis of CGC at a dose of 0.03-0.1 μM, as similar to THA.

610.12

EXPRESSION OF p53 IN THE PONTINE PRINCIPAL SENSORY NUCLEUS (PSN) FOLLOWING TRANSECTION OF THE INFRAORBITAL NERVE. P.E. Kuhn* and M.W. Miller. Prog. Cell & Devel. Biol., Rutgers Univ., Piscataway NJ 08854, Depts. Psychiatry & Pharmacology, Univ. Iowa Coll. Med., Iowa City IA 52242 and Res. Serv., V.A.M.C., Iowa City IA 52246.

We tested the hypothesis that increased expression of p53 heralds neuronal death by examining the expression of p53 in the PSN (second order neurons) after transecting a primary afferent, the infraorbital nerve (IO) on the right side. In addtion, we examined the temporal expression of ALZ-50 immunoreactivity, for it has been shown that the ALZ-50-positive 56 kDa antigen is a marker for dying neurons. Fresh tissue was harvested 1 hr, 2 d, 12 d, and 21 d post-lesion for immunoblots and fixed tissue was prepared immunohistochemically. The analysis of the blots showed that p53 was significantly higher in the right pons (i.e., the side ipsilateral to the lesion) using the left side as a control. This increase was lost 2 d post-lesion. The expression of p53-immunoreactivity was increased in the ventral, ipsilateral PSN (the target of the transected IO) and the adjacent trigeminal sensory tract of the neonate. In contrast, an increase in ALZ-50 immunoreactivity (in blots and sections) achieved significance only 2 d post-lesion. Thus, it appears that as neurons degenerate, p53 is upregulated early, even before the ALZ-50-positive antigen. Funded by the Department of Veterans Affairs and NIH grants DE 07734, AA 06916, and AA 07568.

610.13

RADIATION-INDUCED APOPTOSIS IN RAT DRG NEURONS. J.X. Tong, R. Drzymala, J.R. Simpson, V.S. Tantuwaya, and K. M. Rich* Department of Neurological Surgery, Washington University School of Medicine, St. Louis, MO 63110.

Embryonic dorsal root ganglion (DRG) neurons are susceptible to trophic factor deprivation-induced apoptotic cell death *in vitro*. Radiation is known to induce apoptosis in several cell lines. We examined radiation-induced cell death in DRG neurons *in vitro*.

Primary dissociated culture of DRG neurons were prepared from embryonic-day-15 (E-15) Sprague-Dawley rats. Cells cultured for 6, 11, and 21 days were exposed to varying doses of radiation, and death was assessed by cell counts after crystal violet staining. Maximal cell death occurred in 6 and 11 day old neurons at 24 Gy radiation (24% death for 6-day-old neurons, 18% death for 11-day-old neurons). Twenty one-day-old neurons underwent significant cell death only at higher doses (10% death at 32 Gy; 13% death at 40 Gy).

Five, 10, and 21-day-old DRG neurons were stained with the fluorescent DNA-binding dye Hoechst 33258 at various time points after 24 Gy irradiation. The percentage of cells displaying DNA condensation were compared with non-radiated controls. DNA condensation increased 4.5% in 5-day-old DRG neurons 12 hours after irradiation. In 10-day-old DRG neurons, the percentage of cells displaying DNA condensation increased 2.5% at 12 hours after irradiation. Twenty one-day-old DRG neurons demonstrated no significant increase in DNA condensation after 24 Gy radiation at any of the time points tested.

DRG neurons are sensitive to radiation-induced death. This death is dose-dependent and is less effective in older neurons. The DNA condensation seen after radiation is consistent with apoptotic cell death. These changes are age dependent, similar to trophic factor deprivation-induced apoptosis in DRG neurons.

610.14

CORTICAL EFFECTS OF SELECTIVE IMMUNOLESIONS IN THE BASAL FOREBRAIN CHOLINERGIC SYSTEM. S. de Lacalle* and R.G. Wiley. Dept. of Neurology, Beth Israel Hospital/Harvard Medical School, Boston, MA 02115 and Neurology Service, VAMC, Nashville, TN 37212.

Excitotoxic lesions of the basal forebrain cholinergic neurons induce a decrease in ChAT activity in the neocortex, which recovers over time. The compensatory mechanisms responsible for this restoration are not known, although some authors have postulated a compensatory increase of the functional activity of intrinsic cholinergic neurons in the cortex. We set out to investigate the effect that lesions of the basal forebrain cholinergic system exert on cholinergic cortical interneurons. Unilateral infusion of 20 ng of 192 IgG-saporin in the nucleus of the horizontal diagonal band (HDB) induced a significant 30% decrease in the number of ChAT immunoreactive (-ir) neurons in the lesioned side, compared with the contralateral side. Qualitative and quantitative estimation of the effects of these lesions in the entorhinal cortex showed a moderate loss of ChAT-ir fibers and a significant 11% decrease in the number of ChAT-ir interneurons ipsilateral to the lesion. In order to assess whether this effect was indeed specific to the target area for HDB neurons, similar counts were performed in the anterior cingulate cortex, where no significant difference was found in the number of ChAT-ir neurons between the two sides. In contrast to what has been previously reported, we conclude that the loss of cholinergic neurons in the HDB induces transsynaptic changes in the population of ChAT interneurons in the cortex. It remains to be seen whether these changes are reversible, and the implications in the understanding of cholinergic deficits in the cortex.

610.15

NEURONAL DEATH DOES NOT PLAY SIGNIFICANT ROLE IN THE RODENT SUBPLATE. L. López-Mascaraque*, J.A. De Carlos, M. Santacana and F. Valverde. Laboratorio de Neuroanatomía Comparada. Instituto Cajal (CSIC), Madrid, Spain.

In the rat, the lower neocortical layer forms a conspicuous cell band known as subplate (layer VIb). Subplate cells are among the first to differentiate during cortical development. Cell death in this layer has been shown in carnivora and primates and it is considered as universal feature for subplate cells. In order to assess the validity of this assert, we examined the sequence of generation and the extent of cell death in the rodent subplate layer. We used injections of ^{3}H-thymidine and two methods for direct visualization of apoptotic figures (Nissl staining and in situ apoptosis detection kit). Single injections of ^{3}H-thymidine were performed between E12 and E15 (E0 is the day of insemination) and the brains were examined at different postnatal ages between P1 and P63. The number of heavily labeled cells were counted through the subplate layer in six standard equally spaced coronal sections for each brain. Single injections at E12 labels about 3% of the entire population of layer VIb cells, 17% at E13, 30% at E14 and <1% at E15. Our results indicate that the absolute number of heavily labeled cells in subplate layer remains constant. The analysis of variance (one-way ANOVA) showed that the difference among the group means was not significant from P1 to P63 after injections at either age of E12, E13 or E14. In order to confirm these results we evaluated the distribution of pyknotic (apoptotic) cell bodies in neocortex. The visualization of apoptotic cells was performed using Nissl stained preparations and by immuno staining using an in situ apoptosis detection kit (Apoptag, Oncor). The analysis was done in rats from E18 to P15. Both methods gave comparable results. We found that the amount of cell death in subplate layer is neither particularly prominent nor significantly different than that occurring in the remaining neocortical layers, except in layer II and in the white matter of the corpus callosum. We conclude that neuronal death does not play any significant role in the rodent subplate. (Supported by MEC Research Project PB 91-0066).

610.16

INCREASED APOPTOSIS IN THE ZEBRAFISH OPTIC TECTUM FOLLOWING ENUCLEATION. M.E. Murray* and L.S. Ross. Neurobiology Program, Dept. of Biological Sciences and College of Osteopathic Medicine, Ohio University, Athens, OH 45701.

Programmed cell death is a common process during development of the nervous system. Apoptosis is genetically programmed cell death, mediated by an endonuclease's fragmentation of genomic DNA. During neural development, ingrowing afferents to a brain region may secrete a trophic factor that maintains the target cells. In the absence of this innervation and the associated trophic factor, the target cells may die by apoptosis. We investigated the hypothesis that removing the eye may increase apoptosis in the eye's target, the developing optic tectum. Using the ApopTag™ kit (Oncor, Gaithersburg, MD), we examined the distribution of labeled apoptotic nuclei in the optic tectum following unilateral enucleation. Since the zebrafish retina innervates only the contralateral tectum, the ipsilateral tectum serves as a convenient control. Enucleation increases apoptotic nuclei numbers in the two day old experimental tectum. Labeled apoptotic nuclei are located dorsally throughout the rostrocaudal extent of the experimental tectum. After two days, apoptotic nuclei numbers decreased dramatically, and by three days, there was no difference in the number of apoptotic nuclei on the experimental and control sides. These results suggest that retinal innervation has a trophic effect on optic tectal cells by maintaining the target cells and thereby preventing apoptosis.

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610.17

TRK ONCOGENE EXPRESSION PROTECTS AXOTOMY-INDUCED APOPTOSIS IN ADULT RAT RETINAL GANGLION CELLS. S.C. Sharma*, E. Garcia-Valenzuela. Dept. of Cell Biology, New York Medical College, Valhalla, NY 10595.

Reporter plasmid application to terminal areas of retinal ganglion cell (RGC) axons results in retrograde transport to their somas and consequently to its specific expression. Here we utilize similar gene transfer approach to study transfection of RGCs with TRK-T1 oncogene under the promoter control of CMV. TRK-T1 contains the cytoplasmic domain of the high affinity NGF receptor and has been shown to initiate signal transduction common to all memmbers of the TRK family.

pCMV/TRK-T1 was injected in rat superior colliculi. Controls were rats with either uninjected tectum or injected with pRSV-lacZ. Three days following plasmid injections, optic nerves were transected and fast blue (FB) applied to the cut ends. Rats were sacrificed 7-30 days post optic nerve section. Retrograde transport of the plasmids and their expression were confirmed through biotinylation of plasmid DNA and immunocytochemical staining. Counts of FB-labeled RGCs and terminal transferase labeled apoptotic profiles were made on flatmounted retinae. Expression of TRK-T1 correlated with survival of RGCs, and was over 50% higher than control animals. In addition, there were less apoptotic profiles. These in-vivo results support the dependence of RGCs on neurotrophins for survival. The present approach provides an avenue for rescuing and promoting regeneration of retinal ganglion cells.

610.18

VISUAL RESPONSES OF AXOTOMIZED RETINAL GANGLION CELLS ARE PRESERVED IN TRANSGENIC MICE OVEREXPRESSING BCL-2. Porciatti V.†, Pizzorusso T.†‡, Martinou J.§, Maffei L.†‡* ‡ Scuola Normale Superiore, Pisa, Italy; † Institute of Neurophysiology, CNR, Pisa Italy; § Glaxo Institute for Molecular Biology, Geneve Switzerland.

Bcl-2 is a membrane protein which it has been shown to protect neurones from apoptosis. Recently, transgenic mice have been generated which contain the gene coding the human Bcl-2 protein. In these mice (bcl-2 mice), neurones have a larger than normal amount of Bcl-2, and most of them are preserved from natural and pathological cell death. We asked the question of whether the function of axotomized retinal ganglion cells is preserved in mice overexpressing bcl-2.

The ganglion cell visually driven activity has been recorded by means of the electroretinogram in response to alternating gratings (P-ERG). It is known that the P-ERG depends on ganglion cell activity, since it disappears progressively after optic nerve cut, with a time course comparable to that of ganglion cell degeneration. The experiments have been performed on bcl-2 (n=5) and wild type mice (n=2) before and at different times after unilateral optic nerve cut.

In wild type mice, the P-ERG is abolished already one month after axotomy, when ganglion cells have undergone massive degeneration. In bcl-2 mice, by contrast, the P-ERG is in the normal range three and half months after axotomy.

Results indicate that overexpression of bcl-2 in transgenic mice allows the survival of axotomized retinal ganglion cell and preserves their function after optic nerve cut. Experiments are in progress to establish whether bcl-2 overexpression preserves indefinitely ganglion cells from death or simply delays their death destiny.

610.19

LONG-TERM SURVIVAL OF RETINAL GANGLION CELLS AFTER OPTIC NERVE TRANSECTION IN bcl-2 TRANSGENIC MICE: ANATOMY.

M.C. Cenni[1], E. Strettoi[1]*, L. Bonfanti[1], G.M. Ratto[1], S. Rabacchi [2], J-C. Martinou[3] and L. Maffei[1,2]. [1]Istituto di Neurofisiologia CNR, Pisa, [2]Scuola Normale Superiore, Pisa Italy, and [3]Glaxo Institute for Molecular Biology, Geneva, Switzerland.

Bcl-2 is one member of a family of related genes encoding proteins that regulate apoptotic cell death. Recent studies on transgenic mice overexpressing the human bcl-2 gene in neurons show that the BCL-2 protein protects adult nerve cells from death following ischemia (Martinou *et al.*, Neuron 1994). Transection of the optic nerve in adult mice induces massive degeneration in retinal ganglion cells (RGCs). To investigate whether in the adult bcl-2 transgenic mice RGCs are protected from axotomy, we performed intracranial optic nerve transection on both wild type and bcl-2 adult mice. We tested survival of RGCs 3.5 months following axotomy. We counted the cell bodies of all the nerve cells in retinal ganglion cell layer of whole-mounted retinas stained with cresyl violet. In addition, we examined the morphology and fine structure of the proximal stump of the sectioned optic nerves at the light and electron microscope. It has been reported that, in mice, two months after axotomy 50/60 % of all the cells in the retinal ganglion cell layer have degenerated (Grafstein and Ingoglia, Exp. Neurol., 1982). We confirmed this report in wild type animals at 3.5 months. In bcl-2 transgenic mice, however, up to 75% of all neurons in retinal ganglion cell layer survived. The difference between wild type and bcl-2 was even more evident comparing the proximal stumps of sectioned optic nerves. These showed a very large number of surviving axons with normal ultrastructure in bcl-2 transgenic animals, but only few axons within a general degenerating picture in wild type animals. We conclude that the overexpression of BCL-2 protein in nerve cells is a very effective strategy to ensure long-term survival in axotomized neurons.

610.20

Survival Of Retinal Ganglion Cells Over-Expressing *Bcl-2* Protein In Neonatal Transgenic Mice Following Optic Nerve Section.

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bcl-2 protein overexpressed in neurons of transgenic mice reduces natural cell death (Martinou et al, 1994, Neuron). We have previously found that optic nerve section induces massive apoptosis in retinal ganglion cells of neonatal rats, with a peak of degeneration at 24 hours post-lesion (Rabacchi et al, 1994, J. Neurosci.).

Here, we compare the effects of section to the optic nerve between neonatal *bcl-2* transgenic mice and their wild-type littermates. Whole-mounted retinae were stained with cresyl violet 24 h post-lesion and the apoptotic and surviving cells were counted. While optic nerve transection in neonatal wild-type mice causes a great increase in apoptotic ganglion cells (61,387 $\pm$ 9,793 SE n=5), the number of degenerating cells in *bcl-2* transgenic mice was found to be remarkably reduced (7,761 $\pm$ 1,198 SE n=5, $p<0.05$).

We have also found that in the *bcl-2* transgenic animals the number of surviving cells (138,332 $\pm$ 9,990 SE n=3) was much greater with respect to that found in the axotomized retinae of wild-type mice (65,514 $\pm$ 11,839 SE n=3, $p<0.05$).

These results demonstrate that the *bcl-2* protein protects ganglion cells from axotomy and that most of the cellular degeneration induced by axotomy follows a *bcl-2* dependent pathway.

RETINAL DEVELOPMENT

611.1

INVOLVEMENT OF E-BOX ELEMENTS IN THE DEVELOPMENTAL REGULATION OF THE PHOTORECEPTOR-SPECIFIC GENES. I. Ahmad*. Dept. of Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, NE 68198.

The homologues of *Drosophila achaete-scute* complex genes play an important role in vertebrate neurogenesis. These genes encode bHLH transcription factors that are thought to regulate downstream genes by interacting with the cis-acting element, E-box.We have shown that Mash-1, a mammalian achaete-scute homologue, may regulate opsin gene expression by binding to the E-box element, E_{opsin} during photoreceptor differentiation (ARVO Abst. 1995).To evaluate whether Mash-1 participates in the developmental regulation of other photoreceptor-specific genes we have begun identifying and analyzing E-box elements in their promoters. We have identified an E-box element, $E_{\alpha\text{-}transducin}$ in mouse α-transducin gene which is situated upstream of Tα-1, a RET-1/PCE1 cis-acting element that binds a developmentally regulated retina-specific factor (Ahmad et al. J. Neurochem, 1994 62:396-399). DNase I footprint and mobility shift assay showed that $E_{\alpha\text{-}transducin}$ binds factors present in the retinal nuclear extract. We have also identified an E-box element, $E_{\beta\text{-}PDE}$ in the human β-PDE gene which, like E_{opsin} and $E_{\alpha\text{-}transducin}$ is situated upstream of RET-1/PCE1 like element and forms complexes with retinal nuclear extracts. Experiments are underway to determine whether the complex formation at $E_{\alpha\text{-}transducin}$ and $E_{\beta\text{-}PDE}$ is due to Mash-1 or other bHLH transcription factors. The location of the E-box and RET1/PCE1 elements suggests the involvement of bHLH transcription factors in the multifactorial regulation of the photoreceptor specific genes. Supported by NIH EY10313-03.

611.2

EFFECTS OF ANTI-β_1 INTEGRIN ANTIBODIES ON THE HISTOGENESIS AND DIFFERENTIATION OF THE *XENOPUS* RETINA *IN VIVO*. J. Leonard and D.S. Sakaguchi*. the Department of Zoology and Genetics, the Signal Transduction Training Group and the Neuroscience Program, Iowa State University, Ames, IA 50011

In the present study we have examined the role of β_1 integrins during the normal histogenesis and differentiation of the *Xenopus* retina. Immunohistochemical analysis has revealed that β_1 integrins are expressed in the developing neural and pigmented retina, as well as in the neural tube. *In vitro* assays have demonstrated that function blocking antibodies directed against the β_1 integrin subunit inhibit axon and glial cell outgrowth from embryonic retina growing on extracellular matrix substrates in a dose-dependent fashion. To examine the possible role of β_1 integrins during *in vivo* retinal differentiation the function blocking antibodies were injected into the optic vesicle of stage 23 embryos. After injections, embryos were allowed to develop for 36 hours. The retinas were then examined immunohistologically using antibodies directed against neurons (XAN-5) or photoreceptors (XAP-1 and 2). In control eyes XAN-5 immunoreactivity (IR) displayed a laminar pattern with the outer nuclear layer and the outer and inner plexiform layers exhibiting the strongest IR. XAP-1 and 2 IR was restricted to the outer neural retina, labeling the photoreceptor outer segments (XAP-1: rods and cones or XAP-2: rods). Compared to controls, those eyes injected with the anti-β_1 antibodies often displayed abnormal lamination patterns. In some cases XAP-1/2 IR was observed in ectopic locations within the inner retina. Taken together, these results provide evidence that β_1 integrins play an important role during the normal development of the vertebrate retina. Supported by grants from the NSF, the ISU Biotechnology Council and the Carver Trust.

611.3

IN VITRO DEVELOPMENT AND DIFFERENTIATION OF THE POSTNATAL RETINA OF THE BRAZILIAN OPOSSUM, *MONODELPHIS DOMESTICA*. M.H.West*[1,2,3], C.D. Jacobson[1,2,4] and D.S. Sakaguchi[1,2,3] [1]Department of Zoology and Genetics, [2]Neuroscience Program, [3]Signal Transduction Training Group, and [4]Department of Veterinary Anatomy, Iowa State University, Ames, Iowa 50011.

In the present analysis we have used an *in vitro* system to examine the postnatal differentiation of the retina of the Brazilian opossum, *Monodelphis domestica*. *Monodelphis* is a small, pouchless marsupial whose young have a protracted period of postnatal neurogenesis and differentiation. In this study, retinas from postnatal day (PN) 1,2,5 and 12 opossum pups were cultured on laminin containing substrates. Within three days, most explants have attached to the substrate and elaborated an extensive carpet of non-neuronal cells. These cellular carpets provided a substrate that supports extensive neurite outgrowth. Many of the neurites growing on the cellular carpets were immunoreactive for neurofilament protein, providing evidence that they may be axons of retinal ganglion cells (RGCs).

Antibodies directed against presynaptic terminal-associated proteins were used to examine retinal differentiation after seven days *in vitro*. *In vivo*, immunoreactivity (IR) for Synaptosomal Associated Protein-25 (SNAP-25), which is not present at birth, appears by PN5 in RGCs, their axons, and in the outer portion of the retina. PN1 retinas after 7 days in culture, exhibit IR for SNAP-25 in occasional cell bodies and processes. In PN5 retinas Rab3A IR is observed along the inner half of the retina. However, by PN10 the retina exhibits more lamination with Rab3A IR in the RGC axons and inner plexiform layer. PN5 retinas after 7 days in culture, also exhibited a laminar pattern of Rab3A reactivity. Taken together these results show that development and differentiation of the *Monodelphis* retina continues *in vitro* and may parallel *in vivo* development. Supported by grants from the ISU Biotechnology Council, the Carver Trust and the NSF.

611.4

EMBRYONIC RETINAL ANATOMY AND PAX2 IMMUNOSTAINING IN THE NORMAL AND *Krd* MUTANT MOUSE. **P.F. Hitchcock*, J.T. VanDeRyt*, J.M. Jones^ and M.H. Meisler^. Depts. of *Ophthalmology, *Anatomy and Cell Biology and ^Human Genetics. The University of Michigan, Ann Arbor.**

***Krd* mice contain a transgene-induced mutation of chromosome 19 that includes the developmental regulatory gene, *Pax2*. *Krd*/+ adults have anatomical defects in both kidney and retina that range from mild to severe (Keller et al., (1994) Genomics, 23:309; see also Sanyanusin et al., (1995) Nature Gen., 9:358). Defects in the retinas of severely affected mice include spherical aggregates of photoreceptors and laminar malformations in central retina and hypocellularity of all nuclear layers. We have now examined heterozygous embryos (E15.5-E18.5) and newborns, and our observations indicate that the defects seen in the adult are of embryonic origin. In the central retina of transgenic embryos, ectopic cells and folds in the outer neuroblastic layer are present, and with the formation of the IPL (E18.5 to P1), in some retinas there are markedly fewer cells in the ganglion cell layer. Further, in affected retinas the anatomical organization of the optic disc, the site of *pax2* expression (Nornes et al., (1990) Development, 109:797), is abnormal. Based upon this latter observation, we also examined the expression of *Pax2* in the retinas of normal and *Krd* embryos (E14.5-E17.5) using polyclonal antibodies against a recombinant Pax2 protein (a gift from G. Dressler). This immunostaining confirmed the original observations (see above), and revealed an additional population of Pax2-containing cells that reside within the neural retina and encircle the optic nerve. Together our observations suggest that the genetic defect in the *Krd* mouse affects the cellular organization of the neuroepithelium, cell proliferation andthe normal formation of the optic disc.**

EY07060 and EY07003 (PFH); GM24872 (MHM).

611.5

INSULIN-RELATED FACTORS STIMULATE *IN VITRO* PROLIFERATION OF PLURIPOTENT NEUROEPITHELIAL CELLS BUT NOT ROD PRECURSORS OR INJURY-INDUCED NEURAL PROGENITORS IN ADULT GOLDFISH RETINA. S. E. M. Boucher* and P. F. Hitchcock. The University of Michigan, Depts. of Ophthalmology and Anatomy and Cell Biology and The Neuroscience Program, Ann Arbor, MI.

The retina of the adult goldfish continually generates new neurons and regenerates when injured. Two populations of progenitors are normally present in the goldfish retina: pluripotent neuroepithelial cells at the margin and rod precursors in the outer nuclear layer. Rod precursors are presumed to be the origin of injury-induced neural progenitors. Little is known about molecules that modulate the normal or injury-induced neurogenesis. We assayed the ability of individual peptide growth factors to modulate proliferation of neural progenitors using an organ culture system. Retinas were surgically injured, and eyecups (1 week post-lesion) were incubated in defined media for three days with or without a growth factor. On day 2, the eyecups were pulsed overnight with bromodeoxyuridine (BrdU) to label dividing cells. The eyecups were fixed, frozen, sectioned and processed for BrdU immunocytochemistry. BrdU-labeled cells in retinal sections were counted and averaged. Confirming earlier results (S.E.M. Boucher and P. Hitchcock, (1994) Soc. Neurosci. Abstr. 20: 1322), treatment with 25 nM insulin, insulin-like growth factor-I, or insulin-like growth factor-II increased the number of BrdU-labeled cells in the margin by 461, 845, and 702 %, respectively, over media alone. In contrast, these insulin-like factors did not significantly increase the number of BrdU-labeled rod precursors or injury-induced neural progenitors. These data suggest that one of these insulin-related factors may regulate normal proliferation of pluripotent neuroepithelial cells, but may not regulate the proliferation of rod precursors and injury-induced neural progenitors. Supported by NIH (NEI) grants EY07060 and EY07003 (CORE).

611.6

THE VERVET *(CERCOPITHECUS AETHIOPS SABEUS)* RETINA. [1,2,3]M. Ptito*, [1,2,3]M. Herbin. [1,3]D. Boire,[1]S. Montfort [1]Neuropsychol. Unit and [2]CRSN, Université de Montréal and [3]McGill University, Montreal, CAN.

This study is to establish normative data on the organization of the retina of the Vervet monkey, a species increasingly used in visual neuroscience. The axial length of the eyeball is 21.3 mm. Ganglion cells (RGC) distribution and morphometry were studied in Nissl-stained whole-mounts retinae using a computer controlled image analysis system developed in our laboratory. RGC isodensity maps showed that the fovea is located temporally and at about 3.4 mm from the optic disk. This region has the highest density (ca 38 000) of RGC that are densely packed forming four to five layers of cells. From the fovea, cell density falls rapidly until 3-4 mm (d= 5000) and the decrease is slower thereafter (d=<1000). The RGC counts were confirmed by the electron microscopy of the optic nerves thus eliminating the possible overestimation of RGC due to displaced amacrine cells. The soma size of RGC range from 5.73 µm to 22.87 µm and appear to form three distinct groups: small (x= 7.84 um), medium (x= 12.32 µm) and large (x= 16.50 µm). The foveal region comprises exclusively small RGC; in the periphery medium and large cells are added and the frequency distribution of cell size appears to become trimodal. These results are comparable to those reported for several species of Old World monkeys such as *Macaca mulatta* and *Macaca fascicularis* and indicate that the Vervet monkey has similar visual capabilities.

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611.7

ORGANIZATION OF THE RETINA FOLLOWING CEREBRAL HEMISPHERECTOMY IN THE VERVET MONKEY. [1,2]M. Herbin*, [1,3]D. Boire, [1,2,3]M. Ptito, and [3]A. Ptito. [1]Neuropsychol. Unit and [2]CRSN, Université de Montréal and [3]McGill University, Montreal, CAN.

Vervet monkeys underwent the surgical removal of a whole cerebral hemisphere at various post-natal ages. After 48 months of survival, their retinae were removed, flat mounted and Nissl stained. Retinal ganglion cell (RGC) distribution and morphometry were studied using a computer controlled image analysis system. Our results on RGC counts were verified by studying Nissl-stained horizontal sections of the retinae and with electron microscopy of the optic nerves, thus eliminating the possible overestimation of RGC due to displaced amacrine cells. Moreover, the results obtained on the young-lesioned monkeys (YLM) were compared to adult-lesioned (ALM) ones and to normal controls (NC). The axial length of the eyeball was identical for all groups of animals (ca 21.3mm). RGC were dispersed throughout the retina in YLM whereas in ALM they were mainly restricted to the intact hemiretinae. The highest density of RGC was found in the fovea of all groups but was significantly lower in ALM and YLM compared to NC. In the "blind" hemiretinae of ALM there was very few RGC; for YLM, the "blind" hemiretinae comprised numerous RGC whose distribution in regards to eccentricity followed the one observed in NC. The only difference between YLM and NC concerned the relative density of RGC. As far as soma size is concerned, we found in the "blind" hemiretinae mainly small (W-like) and large (Y-like) cells. Results are discussed in terms of plasticity of the retinofugal pathway.

* Supported by grants from NSERC and FCAR to M.P. and a Cordeau post-doctoral fellowship to M.H.

611.8

TIME BETWEEN CELL BIRTH AND OPSIN EXPRESSION IS GREATER FOR GOLDFISH RETINAL CONES THAN FOR RODS. D.L. Stenkamp* & P.A. Raymond. Dept. Anat. & Cell Biology, Univ. of Mich., Ann Arbor, MI 48109.

Cone photoreceptors in teleost retinas are arranged in a precise mosaic in which the 4 spectral subtypes (red, green, blue, ultraviolet [uv]) have consistent relative positions. By using *in situ* hybridization with opsin cRNA probes to identify specific photoreceptor types, we showed that rod opsin was the first retinal opsin to be expressed in goldfish embryos (*Invest Ophthal Vis Sci* 36:S1055, 1995). Red cone opsin expression began 10 hours later, followed by green, then blue, then uv; this temporal order predicts the spatial positions of cone types in the mosaic. We proposed a model in which precocious rods establish the spacing of the mosaic unit, and a cascade of lateral signals from sequentially differentiating photoreceptor subtypes induces neighboring presumptive photoreceptors to become committed to specific spectral subtypes.

Since birthdating studies have shown that teleost cones are born before rods, one prediction of the model is that onset of opsin expression is delayed in cones relative to rods. To test this prediction, we injected juvenile goldfish eyes with BrdU to label proliferating cells in the marginal germinal zone and later examined the newly generated cells with *in situ* hybridization and BrdU immunocytochemistry. By 3 days, photoreceptor cells were double-labeled for BrdU and rod opsin mRNA; however, cells double-labeled for cone opsins were not found until 9 days. At 9 days, the number of double-labeled cells was greatest when retinas were hybridized with the red cone opsin probe, followed in descending order by green, then blue, then uv, suggesting that the order of opsin expression in adults may mimic the order observed in embryos. These results verify the prediction that opsin expression in cones is de-layed as compared to rods, and are consistent with the hypothesis that rods may play a role in commitment of cones to specific phenotypes. NIH EY06612 & NSF IBN9222046.

611.9

EXPRESSION OF CONE OPSIN mRNA IN THE ADULT AND FETAL PRIMATE RETINA. K.M. Bumsted*, C.L. Jasoni and A.E. Hendrickson. Departments of Biological Structure and Ophthalmology, University of Washington, Seattle, WA 98195.

Adult Macaca monkey retina contains an average of 3.1 million cones which are divided into long (L), medium (M), and short (S) wavelength-specific subtypes. During development, primate cones are generated in a foveal to peripheral sequence between Fetal day (Fd) 38 and Fd100. The aim of this study was to determine a) the distribution of opsin mRNA in adult cones and b) the temporal and spatial pattern of M/L and S opsin expression during retinal development. We used in situ hybridization on retinal whole mounts and sections with digoxigenin-labeled RNA probes to human M/L and S opsin.

The majority of message was localized to the inner segment below the ellipsoid in adult and fetal cones. In adult cones, 8-12% expressed S and 88-90% expressed M/L mRNA. At Fd88 in the nasal retina no cones contained M/L mRNA. At the most central point, 7% expressed S mRNA, but none were present in the periphery. By Fd97, cones expressing either S or M/L mRNA were present centrally with 85-90% containing M/L mRNA. This percentage of M/L mRNA expressing cones decreased to 10-20% at 2mm and to 0% at 3mm eccentricity. In a matched retinal sample, 10-13% of central and 4-8% of cones at 2.7mm eccentricity expressed S mRNA. At 6mm eccentricity, the most peripheral extent of S mRNA expression, 1% of cones are recognized.

These results confirm the adult percentages of S and M/L cones determined with opsin antibodies. In fetal retina, our results indicate that S mRNA expression precedes that of M/L mRNA at any given eccentricity. These data support our previously-identified sequence of S before M/L opsin expression which was based on immunocytochemical labeling in the developing primate retina. Supported by EY01208, EY04536, EY07031

611.10

RETINOIC ACID INDUCES THE EXPANSION OF ROD PHOTORECEPTORS IN THE DEVELOPING ZEBRAFISH RETINA. G.A. Hyatt, E. A. Schmitt, and J. E. Dowling* Dept. Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138.

Retinoic acid (RA) alters the expression of cellular and molecular markers of dorso-ventral polarity in the early zebrafish retina (Schmitt et al., this meeting) These observations suggest that RA is instrumental in establishing ventral retinal characteristics. To determine if RA is involved in cellular patterning at latter stages of retinal development, zebrafish embryos were exposed to RA or to inhibitors of endogenous RA synthesis during the onset of photoreceptor histogenesis (approx. 2 days pf). An increase in the number of rod photoreceptors was observed at 3 days pf within the ventral retina, 24 hours following the application of RA. The continued exposure of embryos to RA increased the number of rods within both the ventral and dorsal segments of the eye by 4 days of development. By treating zebrafish embryos with citral, a competitive inhibitor of a dehydrogenase that converts endogenous retinaldehyde to retinoic acid within the ventral segment of the retina, a dose dependent decrease of rod photoreceptor differentiation was observed. These treatments generated only slight effects upon the histogenesis of the uv-light sensitive photoreceptor cells.

611.11

POSTNATAL EXPRESSION OF DEVELOPMENTALLY REGULATED MOLECULES IN CULTURED MOUSE RETINA. Zhi-Ping Mi*, and C. Lagenaur Dept. Neurobio., Univ. Pittsburgh, Pittsburgh, PA 15261.

Our earlier studies identified retinal cell surface molecules that defined important developmental events in vivo. To determine if these molecules are similarly expressed in vitro, we stained postnatal retina cultures with M6, L1, M4(Thy-1) and NGP-1 antibodies. In culture, over 90% of the cells were M6 positive. One population of M6 positive cells had relatively flat cell bodies with a dandelion-like morphology and may be glial cells or progenitors. The second group of M6 positive cells were cluster of small spherical cells with few processes. Occasionally, M6 immunoreactivity was also seen on isolated round cells without processes. In contrast, L1 staining was found on relatively few cells. These L1- positive cells had two or three long processes. In addition, both L1 and M4 were found on large dying cells with the immunoreactive remnants surrounding the cell bodies, indicating that the cells had been undergoing degeneration. NGP-1 is a newly identified antibody that recognizes a subset of neural glycoproteins and stains apical region of rod photoreceptors in vivo. It stained a type of small cells in vitro with one or rarely two processes. Most of these cells had an apical cilium known to be one of the characteristics of cultured photoreceptor cells. To further define the staining by NGP-1, we used Ret P1, a marker for photoreceptors to compare the staining pattern to that of NGP-1. We found that almost all the NGP-1 positive cells were Ret P1 positive, although about two-thirds of the Ret P1 positive cells were NGP-1 negative. NGP-1 may recognize subpopulation or maturational state of photorecceptors.

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611.12

NUMBERS AND DISTRIBUTIONS OF LARGE RETINAL GANGLION CELLS IN THE FROG: CORRELATION WITH MYELINATION OF OPTIC AXONS: [1]S.A. Dunlop* and [2]D.E. Playford [1]Dept. Zoology, University of Western. Australia and [2] Current address: Dept. Neuroscience, McGill University, Canada.

In the optic nerve of the adult frog *Litoria moorei*, 3% of axons are myelinated. These axons acquire mycin in a biphasic pattern (1). The first phase is complete at metamorphic climax and the second in fully mature adults. Both phases result in optic axons being myelinated along their length, but for most of tadpolc and post-metamorphic life, many axons are myelinated only partway. Cresyl-violet stained retinal whole-mounts were used to determinc whether, as in cat (2), numbers of large retinal ganglion cells correlated with myelinated axon counts. We studied 2 and 6 month tadpoles, animals at metamorphic climax, 5 and 7cm (body length) frogs and 9cm adults, thus encompassing both phases of myelination. Tadpoles were anaesthetised in MS222 (immersion, 2%) and post-metamorpluc animals with Saffan (0.4-0.6 ml; 1.8mg/g Alphaxalone, 0.6mg/g Alphadolone acctatc, ip). At all stages, there was a distinct population of pale staining large cells (range x± sd: 11.6± 2.1-12.5± 1.7 μm); the remaining cells were darker and smaller (range x+sd :5.7± 1.2-7.9± 1.5 μm). The correlation (r^2 = 0.99) of large cell totals with peak values of myelinated axons (1) suggests that large cells give rise to myelinated axons and that these cells assume a large size even if their axons are only myelinated partway along their length. Analysis of cell densities revealed a regular mosaic of large cells at all stages but a changing distribution of the total cell population as the area centralis and visual streak emerged. The pan-retinal increase in densities of large cells at metamorphic climax indicated the late maturation of a large cell class with mycinated axons, a phenomenon which also occurs in *Rana pipiens* (3).

1. Playford, D.E. and Dunlop, S.A. (1993) J. Comp. Neurol. 333, 83-93.
2. Fitzgibbon, T. et al., (1991) Vis. Neurosci. 6, 159-174.
3. Frank, B.D. & Hollyfield, J.G. (1987) J. Comp. Neurol. 266, 435-444.

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611.13

EXPRESSION OF GAP-43 IN THE RETINA OF RATS FOLLOWING PROTRACTED ILLUMINATION. **J. J. López Costa*, J. Goldstein and J. Pecci Saavedra.** **Instituto de Biología Celular y Neurociencias "Prof. E. De Robertis". Fac. de Med., Univ. de Buenos Aires, Argentina.**

Neuronal expression of the Growth Associated Protein-43 (GAP-43) has been reported in developmental and regenerative stages of the CNS, including the normal retina. Continuous illumination produces degeneration of photoreceptors, which is reversible after an adequate period of darkness.

Distribution of GAP-43 in the retinas of rats during the regenerative period after continous illumination (12000 LUX during 8 days) was studied in 30/35-days Wistar rats, followed by different darkness periods. Retinas were fixed by perfusion with a 4% paraformaldehyde solution, in phosphate buffer, immediately after the period of illumination or after 2, 10 or 20 days of total darkness. Criostat sections were immunocytochemically stained with antibodies to GAP-43 (generously provided by G. Wilkin) (dil. 1:5000), sheep anti-rabbit IgG (Sigma) and rabbit peroxidase anti-peroxidase IgG complex (Sigma).

GAP-43 Immunoreactivity was detected in the outer segment of photoreceptor membranes and in the plexiform layers in all observed retinas. It was maximum in the outer segments of rats kept in total darkness for 10 days and decreased thereafter, while in the plexiform layers a delicate plexus of fibers with a minimum of immunolabelling was found at 10 days and recovered the original parameters at 20 days. In both cases staining stabilized at 20 days.

We conclude that the described changes in GAP-43 expression could indicate the participation of the protein in plastic changes ocurring in photoreceptor outer segments and across plexiform layers.

611.14

Development of Tyrosine Hydroxylase Immunoreactive (TH-I) Amacrine Cells in Primate Retina. AE Hendrickson*[1], A Erickson[1] and JM Provis[1,2],Dept Biological Structure[1],Univ.Washington, Seattle, WA 98195 and Depts Anatomy and Histology and Clinical Ophthalmlogy[2], Univ.Sydney, N.S.W. 2006, Australia.

The appearance of TH-I was correlated with birthdates of amacrine cells in primate retina. Pregnant *M. fascicularis* monkeys were injected twice with 5mCi/kg tritiated thymidine 1hour apart on embryonic day (E)42, E43, E47, E55, E57 or E72. Fetuses or infants were sacrificed between E112 and P11mo. Frozen sections including the entire horizontal meridian were stained for TH-I which was visualized with diaminobenzidine and then coated for autoradiography. Frozen sections from twenty-four other fetal monkey retinas between E45 and P6weeks were also stained for TH-I.

Large TH-I+ somas first appeared at E60 in the inner nuclear layer (INL) of the developing fovea. By E70 numerous intensely TH-I somas were present in foveal retina and pale TH-I+ somas extended to the optic disk with a TH-I plexus in s1 of the central IPL at E74. By E97 heavily TH-I large cells and the s1 plexus extended throughout much of peripheral retina with smaller pale TH-I cells in far periphery. At E115 both cells and plexus reached the retinal edge. TH-I double-labeled cells born at E57 are located in mid-peripheral retina at E105. The preliminary data suggest that TH-I amacrine cells are some of the first amacrines generated, but TH-I does not appear until 2-3weeks later. Supported by EY04536 and Research to Prevent Blindness, Inc.

611.15

BDNF ENHANCES DIFFERENTIATION OF NEURONS OF THE ROD PATHWAY IN ORGANOTYPIC CULTURES OF NEONATAL RAT RETINA. Dennis W. Rickman*. Department of Ophthalmology, Anheuser-Busch Eye Institute, St. Louis University, St. Louis, MO 63104.

We have previously shown that the high-affinity neurotrophin receptors, trk_A and trk_B, are expressed in ganglion cells and in some cells of the inner nuclear layer (INL) of the developing rat retina. We have also shown that intraocular treatment with an antisense oligonucleotide targeted to trk_B mRNA in the developing retina alters the expression of parvalbumin (PV)-immunoreactivity (IR), a morphological marker for the narrow-field, AII, amacrine cell in the rod pathway. The present studies examined the effects of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), the primary ligands for trk_A and trk_B, respectively, on neuronal differentiation in explant cultures of neonatal rat retina. Neonatal retinas were explanted onto laminin-coated inserts and cultured in media supplemented with NGF (Boehringer Mannheim) or BDNF (Regeneron, Inc.). After 15 days in culture (a time that represents eye opening *in vivo*), cultures were fixed and either embedded in Epon or cryosectioned and processed for immunohistochemistry using antibodies to parvalbumin (PV) and protein kinase C (PKC), a marker for the rod bipolar cell. Analyses showed that explants treated with either NGF or BDNF displayed well-defined nuclear and plexiform layers, as well as inner and outer limiting membranes. Ganglion cells, however, were sparse. PV-IR in NGF-treated cultures revealed the presence of well-stained horizontal cells and occasional lightly-stained cells in the proximal INL; weak PKC-IR was present in a few cells in the outer INL. In BDNF-treated cultures, PV-IR showed well-stained horizontal cells and also numerous robustly-labeled cells in the proximal INL and their processes in the inner plexiform layer (presumed AII amacrine cells). Furthermore, PKC-IR was strong, labeling numerous bipolar cell somata in the outer INL, as well as their radially-oriented processes. These results suggest that BDNF enhances the expression of specific neurochemical phenotypes in a well-defined retinal circuit during the postnatal period.

611.16

SUPPRESSION OF trk_B EXPRESSION BY ANTISENSE OLIGONUCLEOTIDES ALTERS NEUROCHEMICAL PHENOTYPES IN THE DEVELOPING RAT RETINA. C. Bowes Rickman* and D.W. Rickman. Department of Ophthalmology and Anheuser-Busch Eye Institute, St. Louis University, St. Louis, MO 63104.

Brain-derived neurotrophic factor (BDNF) has a purported role in retinal neuronal development. Since considerable neuronal differentiation in the rodent retina occurs postnatally, transgenic mice in which BDNF or its high-affinity receptor, trk_B, have been "knocked out" are of limited use because they die shortly after birth. Therefore we have designed a strategy to selectively block expression of trk_B in the postnatal retina in order to assess the role of BDNF in its neurochemical differentiation. Phosphorothioated oligonucleotides were generated in sense and antisense orientations and injected intraocularly into neonatal Sprague-Dawley rats following a defined regimen. On postnatal days 10-15, animals were sacrificed and retinas were processed for immunohistochemistry using antibodies to trk_A and trk_B. Alternatively, retinal RNA was extracted for Northern blot analysis using ^{32}P-labeled probes for trk_B. Retinas were also analyzed for expression of specific neurochemical phenotypes using antibodies to parvalbumin (PV) and protein kinase C (PKC), markers for the narrow-field, AII, amacrine and the rod bipolar cell, respectively. In retinas treated with sense oligonucleotides, both trk_A- and trk_B-immunoreactivities (IR) were present in numerous cells in the ganglion cell and inner nuclear layers (INL) and in fibers in the inner plexiform layer (IPL) and nerve fiber layer. In trk_B antisense-treated retinas, trk_A-IR was unchanged, while trk_B-IR was greatly reduced. Northern blot analysis showed that trk_B mRNA transcription was not affected in antisense-treated retinas. In controls, PV-IR labeled numerous cells in the proximal INL and their fibers in the IPL (presumed AII amacrine cells); PKC-IR labeled numerous cells in the distal INL and their radially-oriented fibers (rod bipolars). In trk_B antisense-treated retinas, PV-IR and PKC-IR were decreased in both the numbers of cells stained and in staining intensities. These data suggest that the ligands for trk_B influence the neurochemical differentiation of specific neurons of the rod pathway.

611.17

REORGANIZATION OF RETINAL AXONS IN THE OPTIC FISSURE OF THE CHICK. U. Rager and G. Rager*. Institute of Anatomy, University of Fribourg, CH-1700 Fribourg, Switzerland.

From the data of our earlier tracer study (Rager et al., Anat. Embryol. 179, 135-148, 1988 we postulated that the organization of retinal fibers is transformed in the optic fissure. In order to analyze the rules according to which the reorganization of fibers takes place in the optic fissure, we injected DiI and DiA into various sites of the retina in chick embryos and in adult chicks. The position of the injection sites was determined by measuring their polar coordinates with respect to the coordinate system defined by the longitudinal axis of the optic fissure and a line which was set perpendicular to this axis at the apex of the optic fissure.

The results show that axons originating in an early formed ganglion cell field (Rager et al., J. Comp. Neurol. 334, 529-544, 1993) enter the optic fissure near its apex, axons originating from later formed ganglion cell fields enter it further ventrally. Thus, the time of origin of retinal axons is represented along a dorsoventral gradient in the optic fissure, i.e. axons are organized chronotopically in the optic nerve head. They form bands which are slightly curved ventrally and resemble crescents as we have observed them earlier in the embryonic period. Axons originating in the peripheral temporal retina stay on the same side in the nerve. Axons originating in the peripheral nasal retina cross to the other side. They are represented in the temporal periphery of the nerve and overlap with peripheral temporal axons. Although axons are organized chronotopically in the optic fissure, an additional process may be required to sort out temporal and nasal fibers. Supported by Swiss N.S.F. grant 31-36468.92.

611.18

FORM DEPRIVATION MYOPIA (FDM) IN THE CHICK RETINA - A PHARMACOLOGICAL INVESTIGATION OF THE OPIATE AND NMDA SYSTEMS R.L.P. Seltner and W.K. Stell*, *Lions Sight Centre/Dept. of Anatomy, Faculty of Medicine, University of Calgary, Calgary, Alberta, CANADA T2N 4N1*

Form-deprivation myopia (FDM) is characterized by increased axial and equatorial eye length, increased eye weight and large negative refractive error. It can be induced in hatchling chicks by monocular application of a goggle which blurs the image to the retina. Induction of FDM leads to changes in the enkephalin containing retinal amacrine cells. Pharmacological studies were therefore undertaken to investigate the role of the opiate system in the genesis of FDM. The opiate antagonist naloxone blocked development of FDM in a dose-dependent manner when injected daily into the vitreous chamber, while the opiate agonist morphine had no effect on FDM at any dose tested. Opiate antagonists specific for μ and δ receptors had no effect on development of FDM. A κ specific antagonist reduced development of FDM by only a maximum of 50 %, while κ specific agonists completely eliminated development of FDM in a dose dependent manner. Naloxone was developed as a non-specific opiate antagonist. However, it has also been shown to be an ion channel blocker at the NMDA receptor (NMDA-R). The inconsistency between actions of naloxone and those of other opiate antagonists and agonists suggests that the naloxone induced prevention of FDM may occur as a result of antagonism at the NMDA receptor rather than opiate receptors. Two NMDA specific receptor antagonists were therefore tested:MK-801 and AP5 which act at the NMDA-R channel and at the glutamate site respectively. Both NMDA-receptor antagonists completely blocked development of FDM. **Conclusions:**The results of these studies suggest independent roles for both opiate and NMDA systems in the genesis of FDM. The opiate receptor agonists appear to prevent FDM via κ receptors. NMDA receptor antagonists also prevent FDM, suggesting that glutamatergic retinal pathways utilizing NMDA-receptors are instrumental in controlling ocular growth.

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611.19

DEVELOPMENTAL CHANGES IN THE PATTERN OF CORRELATED SPONTANEOUS BURSTING ACTIVITY IN THE FERRET RETINA. K.R. Fischer, D.M. Oakley and R.O.L. Wong*. Dept. of Anatomy and Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

Ganglion cells (GCs) of the neonatal ferret retina undergo periodic spontaneous increases in intracellular calcium levels prior to eye-opening (postnatal day, P30). Calcium waves, composed of correlated bursts of neighboring cells, propagate across the retina. With increasing age, cells participate in the waves to varying degrees. We are examining whether the degree of participation of GCs in the waves reflect their class or subclass ('on' or 'off'), and which physiological changes account for these variations. Using calcium imaging techniques, the activity of GCs was recorded between P1 and P24, and bursting cells were classified after intracellular dye filling.

Between P1 and P24, every wave involved α, β, and γ classes of GCs, but the bursting patterns of each cell class changed with age. Neighboring presumed 'on' and 'off' β GCs, classified by their dendritic stratification, participated in every wave between P9 and P16. However, presumed 'on' and 'off' β GCs differed in the degree to which they participated in the waves at P21 and P24. The age-related changes among cell types was also reflected in the effects of bicuculline, a $GABA_A$ receptor antagonist, and adenosine on the waves. Between P1 and P14, bicuculline (100 μM) blocked the bursting of most GCs, while it was less effective at P21. Likewise, most cells at P1-P14 responded to adenosine (50-100 μM) with a 2-5 fold decrease in their interburst intervals, but only a subpopulation of cells responded to adenosine in this manner at P21.

Maturation of the physiological properties and connectivity of retinal cells is likely to underlie the age-related changes in the waves.

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611.20

SELECTIVE LOSS OF ALPHA GANGLION CELLS IN THE POSTNATAL CAT RETINA CONTRIBUTES TO THE FORMATION OF MATURE DISTRIBUTION PATTERNS. G. Jeyarasasingam, G. M. Ratto* and L. M. Chalupa. Section of Neurobiology, Physiology & Behavior, UC Davis, CA 95616.

We assessed the possibility that retinal ganglion cell loss during postnatal development contributes to the formation of orderly distribution patterns characterizing these neurons at maturity. The distribution and density of retrogradely labeled alpha cells in central regions of postnatal retinas (beginning at P12) were compared with those found in equivalent regions of mature retinas. This showed that the density of alpha cells was significantly greater in neonatal retinas (at P12: 27-34%) than at maturity. This density decrease reflects cell loss since the central region of the postnatal retina does not undergo significant expansion (Mastronarde et al., 1984). Nearest neighbor analyses revealed that alpha cells in the neonatal retina are distributed in a significantly different pattern than at maturity. Subtraction of adult nearest neighbor distributions from neonatal patterns indicated that the "excess" cells were differentially distributed within bins corresponding to the shortest distances from their neighbors. These observations indicate that selective alpha cell loss contributes to the formation of mature distribution patterns. (Supported by NSF IBN 94-12593)

AXONAL REGENERATION II

612.1

DISRUPTION OF THE MYELIN-ASSOCIATED GLYCOPROTEIN (MAG) GENE IMPROVES AXONAL REGENERATION IN C57BL/WLD^S MICE. M. Schäfer*, M. Fruttiger, D. Montag, M. Schachner, and R. Martini. Department of Neurobiology, Swiss Federal Institute of Technology, Hönggerberg, 8093 Zürich, Switzerland.

It has recently been shown that the cell recognition molecule MAG is inhibitory for neurite outgrowth in vitro. These findings have been used to explain in part the poor regenerative capacity of injured myelinated tracts in CNS. Moreover, the unusually poor axonal regrowth into lesioned peripheral nerves of mouse mutants with abnormally slow Wallerian degeneration (C57BL/Wld^S mice) has been attributed to the prolonged presence of MAG-positive structures in denervated nerve stumps. To test this hypothesis, we generated MAG-deficient C57BL/Wld^S mice by cross-breeding C57BL/Wld^S mice with mice deficient in the MAG gene. Axonal regeneration in crushed femoral nerves was assessed at the light- and electron microscopic levels by counting anterogradely labeled axons having regenerated into the first 2 mm of the distal nerve stumps at postlesion day 7. The number of regenerating axons in association with persisting, apparently intact myelin sheaths was increased in MAG-deficient C57BL/Wld^S mice versus C57BL/Wld^S mice expressing MAG. Thus, MAG could contribute to the poor substrate properties of intact myelin in peripheral nerves. Alternatively, N-CAM which is upregulated in MAG-deficient mice and/or other disregulated molecules could influence the axonal regeneration along myelinated fibers.

612.2

LACK OF EVIDENCE THAT THE MYELIN-ASSOCIATED GLYCOPROTEIN (MAG) IS A MAJOR INHIBITOR OF AXONAL REGENERATION. U. Bartsch, L. Schnell[1], S. Bartsch, D. Montag, M. Schwab[1] and M. Schachner* Dept. of Neurobiology, Federal Institute of Technology, Zürich, Switzerland and [1]Brain Research Institute, Univ. of Zürich, Switzerland

It has recently been suggested that the myelin-associated glycoprotein (MAG) inhibits axonal regeneration *in vivo*, since MAG interferes with neurite elongation from distinct nerve cell types at certain developmental ages (McKerracher et al., 1994; Mukhopadhyay et al., 1994). To test this hypothesis, we have analyzed regeneration in lesioned optic nerve and corticospinal tract of adult wild-type and MAG-deficient (Montag et al., 1994) mice. Length of regrowing axons in both fiber tracts distal to the lesion site was not significantly different between wild-type and mutant mice. In both fiber tracts, however, length of regrowing axons increased considerably after application of IN-1 antibodies (Schwab et al., 1993). Again, length of regrown axons in each fiber tract was similar for both genotypes. Strongest inhibitory activity of MAG *in vitro* has been demonstrated for small cerebellar neurons. Axons of these nerve cells are normally not myelinated. Therefore, small cerebellar neurons were cultured on optic nerve cryosections from adult wild-type and MAG-deficient mice. On both substrates, cerebellar neurons extended neurites of similar length. Together, our observations do not support the view that MAG is a significant inhibitor of axonal regeneration.

612.3

MAG INTERACTS WITH A NEURONAL SIALO-GLYCOPROTEIN TO AFFECT NEURITE OUTGROWTH. M.T. Filbin*, M.E.de Bellard and S. Tang, Biology Dept., Hunter College, CUNY, New York, NY 10021

Depending on the age and the type of neuron, myelin-associated glycoprotein (MAG) can either inhibit or promote axonal regeneration. Neurite outgrowth from all post-natal cerebellar and from adult dorsal root ganglion (DRG) neurons is inhibited by 40-70% on MAG-expressing transfected Chinese hamster ovary (CHO) cells, while neurites from newborn DRG neurons are twice as long on MAG-expressing cells compared to on control cells. Furthermore, MAG binds specifically to a sialic acid residue attached to a neuronal glycoprotein in a 2,3 O-linkage, regardless of whether neurite outgrowth is promoted or inhibited. To determine if, like the binding, the effect of MAG on neurite outgrowth is dependent on its interaction with a sialo-glycoprotein on the neuronal plasma membrane, first, neurons were desialyated prior to comparing the neurite outgrowth on MAG-expressing and control cells, and, second, small sialic acid sugars were included in the co-cultures. The removal of sialic acid from the neuronal cell surface abolished completely the ability of MAG to promote neurite outgrowth from newborn DRG neurons. Similarly, the ability of MAG to inhibit neurite outgrowth from cerebellar neurons was reversed by about 50% if the neurons were desialyated prior to the neurite outgrowth assay. Two sialo-sugars were tested, 2,3 dehydro-2-deoxy-N-acetylneuraminic acid (DD NANA) and sialo-lactose. At 20 mM DD NANA completely blocked the promotion of neurite outgrowth from newborn DRG neurons by MAG and reversed the inhibition of neurite outgrowth from cerebellar neurons by 65%. Sialo-lactose, at 9 mM, blocked promotion by 80% and reversed inhibition by 40%. Lactose, as a control, had no effect. These results suggest that the affect of MAG on both promotion and inhibition of neurite outgrowth from neurons is mediated through a neuronal sialo-glycoprotein and that the specific binding we measure is, most likely, to the physiological receptor for MAG. Supported by the RCMI and NS 26242.

612.4

DISCOVERY OF A BRAIN-DERIVED PEPTIDE, WHICH PROVIDES A BIOCHEMICAL BASIS FOR IMMUNE PRIVILEGE AND THE EVOLUTIONARY LOSS OF CNS REGENERATION. P. Beserman, D.L. Hirschberg and M. Schwartz*. Dept. of Neurobiology, Weizmann Institute of Science, Rehovot, Israel.

The evolutionary basis for the loss of the ability of mammalian CNS to regenerate is not known. One of the features of regeneration failure is an inadequate posttraumatic macrophage response. We have recently shown that this impairment of macrophage response is due, at least in part, to a CNS-resident inhibitor of macrophage migration. We here identify the inhibitor as a CNS endogenous peptide and demonstrate its presence in the CNS, but not in the PNS of mammals. It is possible that this peptide provides the mammalian CNS with an immune brain barrier (IBB), in addition to the evolutionarily early barrier, the blood brain barrier (BBB). The presence of this inhibitor in the CNS may underlie the poor ability of macrophages to invade the CNS, thus providing a molecular link between the well-known phenomenon of immune privilege and poor regeneration in the CNS. Since communication of an injured nerve with the immune system is probably an early event, possibly governing all subsequent steps in regeneration, it may be possible to enhance the regenerative capacity of the mammalian CNS by creating a temporary breach in this immune barrier.

612.5

NEURITE GROWTH INHIBITORY SCARS ARE INDUCED IN NEWBORN SPINAL CORD BY LESION BUT NOT BY CYTOKINES AND GROWTH FACTORS.
M.E. Schwab*, R. Schneider, L. Schnell. Brain Research Institute, University of Zurich, CH-8029 Zurich, Switzerland.

After partial transsection of spinal cords of adult rats followed by injections of NT-3 or E14 spinal cord transplants, many regrowing corticospinal tract (CST) fibers end at glial scars (Schnell and Schwab 1993, Schnell et al. 1994). When we lesioned the growing CST in the thoracic spinal cord (postnatal day, P5) or the future CST area before the tract has reached the lesion site (P2) most of the CST fibers were again arrested at the small scar areas. In spite of an intact ventral gray and white matter, most of the growing or regenerating CST fibers were unable to circumvent the lesion. Microglia and astrocytes were activated at these lesion sites. In order to induce the expression of putative growth inhibitory scar constituents, various factors involved in inflammation and scaring reactions were injected into 2-day old rat spinal cords. Although they often produced microglia activation, neither LPS, Il-1, IFNγ, TNFα and MIP1α, nor CNTF, LIF, PDGF or FGF alone or in combinations arrested the growing CST fibers.

612.6

INDUCTION OF MITOSIS BY TRANSECTION INJURY MAKES SCHWANN CELLS SUSCEPTIBLE TO CHEMOTHERAPEUTIC CYTOTOXICITY. D. A. Wittrock, X. Sun,* and A. J. Windebank, Molecular Neuroscience Program, Mayo Foundation, Rochester, MN.

Chemotherapeutic neurotoxins produce axonal injury and do not damage myelin nor stable adult Schwann cells (SC). Axonal injury results in Wallerian degeneration which induces a wave of Schwann cell division. The mitotic Schwann cell is then potentially susceptible to cytotoxic effects. SC may be a source of trophic or guidance factors for regenerating axons. Loss of SC may retard recovery from neurotoxic injury. To test this hypothesis, we treated 250 g rats with fluorodeoxyuridine (FUDR, 1 mg/kg daily i.p.) or saline control for one week and then crushed the left sciatic nerve at the notch. Treatment was continued for 4 weeks. Two weeks and 4 weeks later, near nerve conduction velocities and amplitudes were measured. After 4 weeks, animals were euthanized, nerves removed, fixed, and embedded. 0.75 μm sections prepared for morphometric analysis.

Nerve action potential amplitudes and conduction velocities (8.2 ± 3.2 vs. 0.01 ± 0.01 $msec^{-1}$; p=.013) were significantly reduced in FUDR treated animals at six weeks. Morphometric analysis of peroneal nerve at the knee showed a small reduction of mean of myelinated fiber (MF) number per nerve (controls, 3789 ± 125 vs FUDR, 3509 ± 267 p=.23). Means of median MF fiber diameter (3.89 ± .24 vs 3.37 ± .15 μm; p=.037) mean of median axon diameter (2.26 ± .09 vs 2.01 ± .07 μm; p=.027) and mean of median myelin thickness (.176 ± .07 vs .63 ± .04 μm; p=.049) were all significantly reduced in FUDR treated animals. There was no significant difference between the corresponding uncrushed nerves. FUDR (10^{-6} to 10^{-5} M) has no effect on axonal growth rate from DRG neurons *in vitro*.

Conclusions. FUDR is not toxic to the normal PNS and has no direct effect on axonal growth. Regeneration after crush injury is significantly retarded by FUDR treatment because of loss of trophic support from SC (supported by NS14304).

612.7

PURIFICATION OF A NEURITE GROWTH INHIBITOR FROM BOVINE CNS MYELIN. A.A. Spillmann, C.E. Bandtlow, F. Keller*✉ and M.E. Schwab. Brain Research Institute, University of Zurich, August-Forel-Strasse 1, CH-8029 Zurich, Switzerland.

Inhibitors of neurite growth play a substantial role in the development and regeneration of the nervous system. Two of them, NI-35 and NI-250, cause collapse of neuronal growth cones and strongly inhibit neurite growth *in vitro*. These low abundant proteins are associated with oligodendrocyte membranes and are present in the myelin sheath of the CNS in higher vertebrates. Previous studies have demonstrated both *in vivo* and *in vitro* that the monoclonal antibody (IN-1), which has been raised against NI-250, neutralizes the neurite growth inhibiting properties of differentiated oligodendrocytes and of CNS myelin, and allows regeneration of lesioned nerve fibers in the adult rat spinal cord and brain.

We report here the protein purification of an inhibitory protein of 250 kd starting with crude bovine spinal cord myelin and using various chromatography techniques in combination with three bioassays. The first two bioassays are screening assays which test the effect of different column fractions on the spreading of 3T3 fibroblasts and on neurite outgrowth of PC12 cells. The third bioassay tests for the collapsing activity on growth cones of primary rat dorsal root ganglion neurons. Using a five step purification protocol, which includes discontinuous flotation sucrose density centrifugation, strong anion exchange chromatography and size exclusion chromatography, a highly active protein fraction was obtained. The biological activity of this fraction is enriched 15000 fold, is fully neutralized by the monoclonal antibody IN-1 and shows one detectable band at 250 kd in the SDS-PAGE (silver stain). Microsequencing the gel eluted protein band reveals an amino acid sequence which could not be found in SwissProt and GenEMBL data bases.

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612.8

REGULATION OF ANDROGEN RECEPTOR mRNA EXPRESSION IN ADULT HAMSTER FACIAL MOTOR NEURONS (FMN). S.M. Drengler*, R.J. Handa, K.J. Jones, Dept. of Cell Biology, Neurobiology and Anatomy, Loyola University Chicago, Maywood, IL 60153.

We have previously shown that exogenous testosterone propionate (TP) differentially regulates both the rate of facial nerve regeneration after injury and androgen receptor (AR) mRNA levels in FMN of both intact and gdx male/female hamsters. Since TP can be converted to estrogen by endogenous aromatization, it is possible that a component of the steroidal regulation of AR mRNA in FMN could be due to estrogen. In this study, we examined regulation of AR mRNA in FMN by the non-aromatizable androgen, dihydrotestosterone (DHT). Four groups of hamsters, intact and gonadectomized (gdx), males/females, were implanted with DHT capsules for 0,1,2, or 7 d. *In situ* hybridization was performed using an ^{35}S-labeled antisense AR cRNA probe (encoding 350 NTs of the ligand binding domain). Unlike our previous studies using TP, DHT was effecive in rapidly upregulating AR mRNA only in intact female rats. These data demonstrate a sex difference in the regulation of AR mRNA which is dependent on the presence of the gonad, and support the hypothesis that there is a synergism between androgen and estrogen in regulating AR mRNA in FMN. Studies are in progress to evaluate the interactive effects of hormonal/injury states on AR mRNA expression in HFMN. Supported by NIH grant NS28238.

612.9

REGENERATION OF SEPTO-HIPPOCAMPAL CHOLINERGIC FIBERS AND EXPRESSION OF CELL ADHESION MOLECULES IN THE ADULT RAT BRAIN. I. Aubert*, J.-L. Ridet and F.H. Gage. Salk Institute, Laboratory of Genetics, La Jolla, California 92037-1099.

Cell adhesion molecules (CAMs), such as polysialylated NCAM (PSA-NCAM) and nerve growth factor inducible large external glycoprotein (NILE), are thought to play an important role in cell migration, axonal growth and guidance, as well as maintenance of stable contact between cells. In the present study, we investigated the immunoreactivity of PSA-NCAM and NILE in relation to choline acetyltransferase (ChAT) and glial fibrillary acidic protein (GFAP) in adult rats which received unilateral aspirative lesions of the fimbria-fornix and implantation of bridging grafts containing either β-galactosidase (β-gal) or nerve growth factor (NGF)-producing fibroblasts. The relationship between CAMs and neuronal or glial cells is investigated by confocal and electron microscopy. After two weeks survival, PSA-NCAM, NILE and GFAP immunoreactivities are observed in the host tissue surrounding the β-gal or NGF grafts. One month post-operation, PSA-NCAM, NILE and GFAP positive fibers start to invade the NGF, but not the β-gal, producing grafts. After two months survival, most of the surface of NGF grafts is filled with PSA-NCAM and ChAT positive fibers. The β-gal grafts do not contained significant amount of PSA-NCAM and ChAT immunoreactivity. In contrast to PSA-NCAM and ChAT, NILE and GFAP positive fibers are limited to the periphery of the NGF grafts. Simultaneous labeling of PSA-NCAM, NILE, ChAT and GFAP suggest that in the NGF grafts, at all time points, PSA-NCAM, NILE, ChAT, and GFAP positive fibers are in close association in the host tissue and in the periphery of the graft. However, at two months post-operation, only PSA-NCAM and ChAT positive fibers are present in significant amounts in the center of NGF grafts. The results suggest that PSA-NCAM and NILE, along with reactive astrocytes, are permissive substrates for regeneration of cholinergic fibers in presence of NGF. Supported by the MRC, Canada (I.A.), AFRT (J.-L. R.).

612.10

SPATIAL CONTROL OF NEURONAL CELL ATTACHMENT AND NEURITE OUTGROWTH BY LIGHT-INDUCED IMMOBILIZATION OF A LAMININ-DERIVED PEPTIDE. J.-F. Clémence[1], J.P. Ranieri[2], P. Aebischer[2], H.R. Lüscher[3]* and H. Sigrist[1]. [1]Institute of Biochemistry, University of Berne, Berne, Switzerland. [2]Division of Surgical Research, Lausanne University Medical School, Lausanne, Switzerland. [3] Institute of Physiology, University of Berne, Berne, Switzerland.

Tissue engineering in the nervous system requires topical organization of cells in three dimensions (3-D). Photolabel-derivatized, bioactive laminin fragments were synthesized, characterized, and photoimmobilized onto biocompatible materials. Laminin-derived CDPGYIGSR was thermochemically coupled to either diazirine or benzophenone photo crosslinker. Covalent high-resolution (20 and 300 μm) patterning of CDPGYIGSR to hydroxylated fluorinated ethylene propylene (FEP-OH), poly(vinyl alcohol), and glycophase glass was achieved. Radiolabeled, photolabel-derivatized peptide patterns were visualized by autoradiography. The cellular interaction of photoimmobilized CDPGYIGSR was demonstrated by the selective, receptor-mediated attachment of NG 108-15 neuroblastoma×glioma cells. On peptide-patterned FEP-OH membranes, NG 108-15 cells differentiated within one day. Specific cell attachment to immobilized CDPGYIGSR was assessed through competitive binding studies. Further, an extracellular matrix analogue was developed on agarose as 3-D scaffold. Agarose provided the chemical basis for functionalization, and the physical support for neurite extension from primary cells. Topically selective peptide immobilization of agarose gels in 3-D was explored with benzophenone-modified CDPGYIGSR using an argon laser light source. The 3-D functionalization of agarose hydrogels sets the basis for spatially adressable directed neurite outgrowth *in vitro* and *in vivo*.

612.11

NERVE DERIVED SCHWANN CELLS PRODUCE ACETYLCHOLINESTERASE. B. Haesaert, S. Rotshenker, R. Cohen and L. Anglister*. Dept. of Anatomy, The Hebrew University Medical School, Jerusalem, Israel.

Acetylcholinesterase (AChE) is highly concentrated at the neuromuscular junctions (NMJs) and appears in several molecular forms: A12, G1, G2 and G4. It is not clearly understood which cell type(s) (nerve, muscle or Schwann) produce and deposit AChE at NMJs. All forms of AChE can be extracted from intact peripheral nerves that innervate muscle. The A12 isoform is carried by fast anterograde axonal transport, thus suggesting its neural origin. The major AChE isoform detected in peripheral nerves undergoing Wallerian degeneration (WD), thus lacking axons, is G4. The G1 and G2 are detected to a much lesser extent and A12 is nondetectable. To examine whether the Schwann cells that reside in the WD nerves produce G4 we studied AChE production in nerve-derived Schwann cell cultures. Fourteen days WD-frog sciatic nerves were dissociated and highly enriched Schwann cell cultures (>95%) obtained (modified after Reichert et al., J. Neurosci. 14:3231-3245, 1994). Schwann cell cultures contained AChE activity, extracted primarily as globular forms G4, and to a lesser extent as G1 and G2. These AChE isoforms could have been synthesized by Schwann cells or acquired by them by phagocytosis of degenerating axons. To determine which is the case, Schwann cells were first exposed to DFP to inhibit irreversibly all AChE present in them. Then, after 4-10 days in culture Schwann cells were analyzed for AChE activity and immunostaining. Newly synthesized AChE, mainly the G4 species, was extracted. Thus, nerve derived Schwann cells produce G4 isoforms. (Supported by Israel Acad. Sci.- Revson Found. 675/94).

TRANSPLANTATION: GROWTH FACTORS

613.1

MODULATION OF O-2A PROGENITOR CELLS IN THE RAT CERVICAL SPINAL CORD BY EX VIVO GENE DELIVERY OF PLATELET DERIVED GROWTH FACTOR. F. Noel*, A. Ichiji, S. Sakuma, M. Weil and P.J. Tofilon. Dept. of Experimental Radiotherapy, MD Anderson Cancer Center, Houston, TX 77030.

A target cell population for radiation-induced spinal cord myelopathy is considered to be the oligodendrocyte-type 2 astrocyte (O-2A) progenitor. The loss of the reproductive potential of these cells after irradiation leads to a depletion of functional oligodendrocytes and ultimately demyelination. Platelet derived growth factor (PDGF) enhances the proliferation and migration of O-2A cells. To increase or replenish the O-2A cells in the cervical spinal cord, we have begun to develop an ex vivo gene therapy approach for delivering PDGF. Initial studies using recombinant Rat-1 fibroblasts expressing β-galactosidase indicated that cells injected into the cisterna magna of adult rats localized in the subarachnoid space of the brain stem and cervical spinal cord. The effects of cisterna magna injection of recombinant fibroblasts expressing PDGF A chain on O-2A progenitors were evaluated using an excision/clonogenic assay. In this procedure the cervical spinal cord is disaggregated, seeded onto a monolayer of astrocytes and the number of O-2A colonies (A2B5+) determined 3 weeks later. The number of O-2A colonies was increased by 4 days after injection of the PDGF expressing cells reaching a maximum (approximately 2-fold over sham injected rats) by 8 days. These data indicate that the implantation of PDGF expressing cells into the cisterna magna can increase the number of O-2A progenitors in the rat cervical spinal cord and suggest that the cell-mediated delivery of growth factors may be used in the treatment of demyelinating disorders.

613.2

AN ALTERNATIVE TO THE REPLACEMENT THERAPY IN THE TREATMENT OF THE EFFECTS OF BRAIN NEURONS DEGENERATION. A. Ennaceur*, S.M. Ennaceur, P. Monmaur, Univ. Durham, Dept Psychol., South Road, England & Univ. Paris 7, Lab. Psychopharmacol. & Cognit. Process, Place Jussieu, Paris 5, France.

It has been reported that lesions of the basal forebrain cholinergic neurons prevent the hippocampus and the frontal cortex from their main input of Acetylcholine (Ach) and produce memory dysfunction. Neuropharmacological studies of memory have generally focused on the properties of the damaged neurons and tried to replace them either by increasing the release of Ach from the remaining neurons or by implanting a graft that provide new inputs to the target of the lesioned neurons. Nobody have paid attention to the fact that there are many adaptive changes that follow a brain damage, among which the target of the degenerated neurons may increase in number, size or change their affinity. Furthermore, there are other neurons that were acting presynaptically and deprived from their main target by the lesion. It is not clear how to increase the release of Ach from mostly lacking pre synaptic neurons and stimulate their post synaptic target. Furthermore, the acetyl cholinesterase inhibitors and the muscarinic agonists are often applied too late after the damage and for a short lasting duration. The projections sent by a graft also reach their targets too late. We suggest here a new alternative to the replacement strategy: the post synaptic targets of the lesioned neurons should be maintained "normal" by an immediate and continuous stimulation (osmotic minipump) that prevent their adaptation to the new environment induced by the lesions. Therefore, if a graft is implanted and succeed in sending new projections, these will find a "normal" target to bind to.

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613.3

THE CHRONICALLY INJURED SPINAL CORD EXHIBITS RESPONSIVENESS TO NGF DELIVERED LOCALLY BY GENE THERAPY.

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Previously we reported that grafts of NGF-secreting genetically modified fibroblasts into the acutely injured spinal cord elicited outgrowth from local spinal sensory and motor neurites. In the present experiment, we determined whether these neuritic populations retained NGF responsiveness after chronic injury.

Primary Fischer 344 rat fibroblasts were transduced with MLV retroviral vectors to produce human ß-NGF. *In vitro*, transduced cells secreted 2 ng NGF/hr/10^6cells.

Adult Fischer 344 rats underwent spinal dorsal hemisection lesions at the T7 level. 6 weeks later, the spinal injury site was re-exposed and NGF-secreting fibroblasts (n=7) or control, uninfected fibroblasts (n=3) were grafted in collagen gels into the lesion cavity. 4 weeks later, NGF-secreting grafts were extensively penetrated by neurofilament-, p75-, and CGRP-immunolabeled fibers. Control grafts contained siginificantly fewer neurites. ChAT-labeled fibers did not penetrate either NGF- or control grafts. Thus, sensory neurites continue to exhibit NGF responsiveness after chronic injury, whereas local motor neurites do not. Temporal changes in neurotrophin receptor expression after injury in relation to neuritic responses to NGF are being examined. Some neuritic populations maintain neurotrophin responsiveness in the chronically injured spinal cord, providing a potential means of promoting axon regeneration.

613.4

TRANSPLANTATION OF POLYMER-ENCAPSULATED HUMAN NGF-SECRETING CELLS PROMOTES CHROMAFFIN CELL SURVIVAL AND BEHAVIORAL RECOVERY IN HEMIPARKINSONIAN RATS.

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Encapsulated cell grafting is one approach for the delivery of neurotransmitters and/or neurotrophic factors into the brain. Baby hamster kidney (BHK) cells were genetically modified to secrete high levels of human nerve growth factor (BHK-hNGF). Following polymer encapsulation, the NGF-secreting devices were implanted into the left lateral ventricle or into the left striatum 1.5 mm (in both cases) from cografted rat adrenal medullary chromaffin cells in hemiparkinsonian rats. Although the animals receiving chromaffin cells alone or chromaffin cells with intraventricular hNGF-secreting devices did not show recovery from apomorphine-induced rotational behavior, the animals receiving chromaffin cells with intrastriatal hNGF-secreting cell implants showed a significant reduction in rotational behavior 2 and 4 weeks after transplantation. Histological analysis revealed that in animals receiving chromaffin cells and an intraventricular hNGF-secreting device, there was an approximately 5-6 fold increase in chromaffin cell survival (TH-IR) when compared to animals receiving chromaffin cells alone. In contrast, in those animals receiving chromaffin cell grafts together with intrastriatal hNGF-secreting devices, the number of surviving chromaffin cells that were TH-IR was more than 20 times higher than in animals receiving adrenal medullary cells alone. Analysis of retrieved capsules revealed that hNGF continued to be released by encapsulated BHK-hNGF cells after 4 weeks *in vivo*. These results indicate the potential use of intrastriatal implantation of encapsulated hNGF-secreting cells for augmenting the survival of cografted chromaffin cells.

613.5

SPECIFIC HEPARAN SULFATES CAN POTENTIATE THE EFFECTS OF FGF-1 ON NEURONAL DEATH AFTER EMBRYONIC TRANSPLANTION

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Embryonic transplants have been extensively used over the last few years in order to ameliorate the death of neurons in many neurological disease models. Generally these models have indicated that very early embryonic cells transplant much better than neurons of differentiated phenotype. Recently it was found that fetal dopaminergic neurons grafted in conjunction with cells engineered to express high levels of FGF-2 survived in up to 10 times greater numbers than dopaminergic neurons alone, indicating that FGF-2 may be an important co-factor for the successful integration of transplanted cells [1]. We have successfully isolated FGF-specific heparan sulfates [2] from neural precursor cells and shown them to be an absolute requirement for the binding of FGFs to their high affinity receptors. The aim of this work was to transplant an FGF-1- producing cell line, created by inserting full length FGF-1 coding region cDNA into the murine neuroepithelial cell line 2.3D [2], into the ventricular space within the optic tectum of the embryonic chick, with or without increasing concentrations of specific heparan sulfate, and monitor the effects on developing neuronal populations. Much greater levels of FGF-1 were able to reach susceptible neuronal compartments when presented to the embryo in conjunction with heparan sulfate, confirming a protective role for the sugar. Significantly greater numbers of motor neurons within the lumbar spinal cord of the embryo were able to survive the period of normal histogenetic death when the FGF-1 was presented to the neurons in conjunction with their appropriate heparan sulfate co-factor. The heparan sulfates may therefore have important uses in controlling the survival of neuronal cells responsive to FGF-1 in a transplantation paradigm.

1. Takayama et al., *Nature Medicine* 1:53-58 (1995).
2. Nurcombe et al., *Science* 260:103-106 (1993).

613.6

OPTIMAL EFFECTS OF EXOGENOUS BDNF ON GRAFTS OF FETAL DOPAMINE NEURONS COINCIDES WITH THE ONTOGENIC PERIOD WHEN DOPAMINE CONTENT AND BDNF EXPRESSION INCREASE WITHIN THE STRIATUM.

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The expression of brain-derived neurotrophic factor (BDNF) within the developing striatum was measured using Northern blot analysis of BDNF mRNA and an enzyme-linked immunoassay (EIA) for BDNF protein. BDNF mRNA within the striatum is not detectable before P7, increases transiently between P10-P28, and then decreases in adult. Similarly, BDNF protein levels in the striatum show a sharp increase between P10-P30, which is closely paralleled by increases in striatal dopamine content. These data suggest that BDNF may act as a target-derived trophic factor for dopaminergic neurons destined to innervate the striatum.

The hypothesis that developing dopamine neurons might be most responsive to BDNF during this discrete developmental period was tested in grafts of fetal dopamine neurons implanted into the denervated striatum. Rats received unilateral 6-OHDA lesions and four weeks later were transplanted with E14 ventral mesencephalic tissue, then infused with BDNF at different time points. Animals receiving grafts and immediately infused with BDNF for two weeks showed slight enhancement in rotational behavior compared to that observed in grafted animals receiving PBS infusions. Grafts infused with BDNF 3-4 weeks after transplant surgery, which is equivalent to neuronal age P7-P21, showed a profound affect on rotational behavior and extensive TH+ outgrowth when compared to grafts infused with PBS or BDNF 1-2 weeks after surgery. These data suggest that transplanted immature dopamine neurons may be more responsive to the neurotrophic action of BDNF during a critical period of neuronal development which ordinarily occurs postnatally.

613.7

EFFECTS OF BDNF INFUSION ON LOCOMOTOR BEHAVIOR AND DOPAMINE NEURITE OUTGROWTH FROM FETAL MESENCEPHALIC TRANSPLANTS.

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Infusion of BDNF adjacent to grafts of fetal ventral mesencephalon (VM) enhances dopamine neurite outgrowth and augments the effects of the transplants on amphetamine-induced turning in hemiparkinsonian rats (Yurek et al, Exp. Neurol. 129:14, 1994). To determine if similar results obtain when BDNF is infused at a distance from the transplant, fetal VM (E14) were grafted into the medial or rostral striatum and BDNF (36 µg/d) or PBS was infused into the striatum 1-2 mm from the transplant site. Pumps were removed 4 weeks post-transplantation, and the animals allowed to survive for an additional 4-8 weeks. Grafted animals that received BDNF showed a complete amelioration of amphetamine-induced ipsiversive rotation, whereas grafted animals infused with PBS showed a modest improvement. Non-grafted animals that received BDNF or PBS showed no improvement, or a worsening of behavior. Grafts contained numerous tyrosine hydroxylase-positive (TH+) neurons in both PBS and BDNF-infused animals, and a plexus of TH+ fibers was apparent within the host striatum in the immediate vicinity of the transplants. When the BDNF infusion cannula was located within ~1 mm of the transplanted VM, a markedly enhanced outgrowth of TH+ neurites was seen, directed towards the cannula. No selective outgrowth of fibers in the direction of the cannula was noted in PBS-infused animals, or in BDNF-infused rats when the cannula was located >1.5 mm from the graft. Thus, transient infusion of BDNF produces a long-lasting change in the functional integration of fetal VM grafts, and can exert a tropic effect on dopamine neurite outgrowth.

613.8

Effects of NGF-Secreting Genetically Modified Cell Grafts on Cholinergic Neuronal Morphology and Cognition in Aged Primates.

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Basal forebrain cholinergic neuronal atrophy occurs in Alzheimer's disease and in aged rats and monkeys. Age-related deficits on selected cognitive tasks also occur in rats and primates. Nerve growth factor (NGF) can ameliorate age-related cognitive dysfunction in rats and can reduce injury-induced cholinergic neuronal degeneration in primates. In the present study, we examined the effects of genetically modified NGF-secreting fibroblast grafts in aged rhesus monkeys.

18 aged subjects were pre-operatively assessed on a delayed response (DR) task. 9 of these subjects showed DR deficits relative to young control animals, whereas 9 subjects showed no impairment.

The 9 unimpaired subjects then received grafts of either NGF-secreting fibroblasts (n=6; NGF production *in vitro* 15 ng/10^6cells/h) or control, unmodified fibroblasts (n=3) into 10 intraparenchymal sites spanning the Nucleus Basalis of Meynert (NBM) bilaterally. On post-operative testing, NGF-graft recipients showed a decline in performance on the DR task compared to control subjects, indicating that modulation of cholinergic systems by NGF influences cognition in primates.

The 9 aged-impaired subjects have also received grafts of NGF-secreting cells (n=5) or control cells (n=4) into the NBM. Effects of NGF on age-related declines in cholinergic neuronal morphology and function will be presented.

613.9

ASTROCYTE-INDUCED NEUROTOXICITY OF DOPAMINERGIC GRAFTS IN A RAT MODEL OF PARKINSON'S DISEASE: EFFECTS OF ASTROCYTE AGE AND EXPRESSION OF RECOMBINANT BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF). I. Lopez-Colberg, K.A. Krobert, P.A. Castillo, G. Laks and L.A. Cunningham*. Dept. Pharmacology, Univ. New Mexico School of Medicine, Albuquerque, NM 87131.

These studies were performed to determine whether primary astrocytes genetically engineered to express recombinant BDNF could enhance the survival or function of co-grafted embryonic dopaminergic (DA) neurons in the unilateral 6-OHDA-lesioned rat model of Parkinson's disease. Using a MoMLV-based retroviral vector, primary type I astrocytes derived from newborn (P0) rat striatum and maintained in culture for at least two weeks were genetically transduced in vitro to express recombinant BDNF and tested for their survival effects on embryonic day 15 midbrain DA neurons in culture and following intracerebral transplantation. In culture, both control non-transduced astrocytes and BDNF-producing astrocytes supported the survival of DA neurons, however, only BDNF-producing astrocytes were neuroprotective for DA neurons following a 24 hour exposure to 100 μM 6-OHDA. Following intrastriatal transplantation, control astrocytes reduced the survival of DA neurons within embryonic ventral midbrain (VM) grafts by ~2-fold compared to grafts of VM alone. This astrocyte-induced toxicity was attenuated when VM was co-grafted with BDNF-producing astrocytes. In addition, the neurotoxic effect of the astrocytes was age-dependent; astrocytes derived from embryonic day 18 striatum and maintained in short-term culture were not neurotoxic for dopaminergic neurons. These studies suggest that a) astrocyte age and maturation are important considerations when using astrocytes as gene delivery vehicles and b) co-delivery of recombinant BDNF may provide protective effects to DA neurons following intrastriatal transplantation. Supported by NIH-NINDS R29-NS32562 and The National Parkinson Foundation.

613.10

A GENE THERAPY APPROACH FOR THE TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS. P. Aebischer[1]*, N. Déglon[1], B. Heyd[1], A.D. Zurn[1], A.C. Kato[2], J.P. Hammang[3], E.E. Baetge[3]. [1]Gene Therapy Center and Surgical Res. Div., Lausanne University Medical School, Switzerland; [2]Div. of Clinical Neurophysiology, Geneva University Medical School; [3]CytoTherapeutics Inc., Providence, RI.

Amyotrophic lateral sclerosis (ALS) is a disease characterized by the progressive degeneration of motoneurons leading to paralysis and death in 2-3 years. Even though the cause of the disease remains unknown, gene therapy strategies using neurotrophic factors have been developed, since recent experiments demonstrated increased survival of motoneurons *in vitro* and in animal models of neurodegeneration. Exposure to ciliary neurotrophic factor (CNTF), for instance, has been shown to reduce motoneuron cell death associated with peripheral nerve axotomy. The present study used an encapsulation technology to deliver CNTF directly into the CNS. Baby hamster kidney cells (BHK) were transfected with an expression vector containing the human CNTF gene. About $1x10^4$ parent BHK cells or hCNTF-producing cells were loaded in polyethersulfone fibers. BHK-hCNTF cells were shown to release approximately 5 $\mu g/10^6$ cells/day as measured by Elisa assay. The *in vivo* bioactivity of the BHK-hCNTF cells was tested using the pmn/pmn mutant mouse model of motoneuron degeneration. Animals implanted with BHK-hCNTF capsules showed a significantly longer survival time and improved motor functional activity as compared to control mice. In order to proceed with a clinical trial, tumorigenicity and toxicity studies were performed. Unencapsulated BHK-hCNTF cells were injected into the lateral ventricle of rats ($2x10^6$ cells), sheep and primates ($1x10^7$ cells). Histological analysis showed no tumor formation in any of the animals during the 3 month trial. No side effects, such as fever or weight loss, were noticed in rats implanted with capsules containing up to 100x the human dosage. Finally, the BHK-hCNTF cells, which express the suicide gene thymidine kinase (HSV-tk), were sensitive to ganciclovir treatment *in vitro* and *in vivo*. A clinical program aimed at the treatment of ALS through the transplantation of encapsulated cells genetically engineered to release hCNTF has just been initiated.

AGING PROCESSES: ANATOMY

614.1

AGE-RELATED REGRESSIVE CHANGES IN MOTONEURON ULTRASTRUCTURE IN AN ANDROGEN-SENSITIVE RAT SPINAL NUCLEUS. M.C. Clark-Phelps* and D.R. Sengelaub. Program in Neural Science, Indiana University, Bloomington, IN 47405.

Motoneurons in the spinal nucleus of the bulbocavernosus (SNB) in rats are sensitive to androgens during development, at adulthood, and in old age. Castration of young adults significantly reduces SNB soma size and dendritic length (Kurz, *et al.*, 1986). Ultrastructurally, synapse number and size are also reduced following castration (Matsumoto, *et al.*, 1988). Androgen titers decline with normal aging in male rats, and we have previously reported androgen-dependent declines in SNB target muscle weight, soma size, dendritic length, and steroid receptor density through 22 months of age. In this study, we have extended our analysis of age-related changes in the SNB to the ultrastructural level, examining whether synapse number and size also decline with advancing age.

SNB synapse number was assessed in male rats at 4 and 22 months of age (4-5 motoneurons per animal, n=6). SNB motoneurons were retrogradely labeled with cholera toxin-HRP, reacted with TMB/DAB, and processed for electron microscopy. Somatic and proximal dendritic membranes of HRP-labeled SNB motoneurons were examined for synaptic contacts. Preliminary results reveal a significant decrease (33%) in the number of synapses per micron of somatic membrane in aged rats. Like the other morphological regressive changes we have observed, it is likely that the age-related decline in SNB synapse number results from the normal decline in circulating androgen and can be reversed with androgen treatment. (Supported by NIH AG09309)

614.2

QUANTITATIVE ANALYSIS OF THE CEREBELLAR PURKINJE CELLS DURING AGING OF THE HETEROZYGOUS STAGGERER MOUSE. N. Hadj Saharaoui*, H. Zanjani*, F. Frédéric*, N. Delhaye-Bouchaud*, K. Herrup and J. Mariani*'* Université P.& M. Curie, CNRS, URA 1488, 75005 Paris. * Alzheimer Research Laboratory, Cleveland, OH 44106.

The staggerer (sg) mutation causes a severe ataxia correlated with digenesis of the cerebellar cortex in the sg/sg mouse. The Purkinje cells (PC) are reduced in number by about 75 % and the remaining ones are ectopic and reduced in size with a profound dendritic atrophy. The mutation is described as recessive because the heterozygote, +/sg, is behaviorally normal. However when the +/sg mouse advances in age, 25-30 % of the PC are gone as early as 12 months postnatal. In the present study we aimed to see whether morphological abnormalities accompany the PC loss. We used a semiquantitative method to characterize several morphological parameters of a large number of Golgi-impregnated PC in 4, 12 and 22 month old +/sg and +/+ mice. In 4-month old +/sg mice, most cells are indistinguishable from+/+. The dendrites are profusely branched and extend to the pial surface but they frequently deviate from the ideal, perpendicular orientation. By 12 months, +/sg PCs are slightly but significantly atrophic compared to +/+. In 22-month-old the PC branching complexity is reduced in both genotypes: many cells appear atrophic and dendritic trees often fail to reach the pial surface but the +/sg cells deteriorate far more and completely than +/+ ones. From these results it appears that the heterozygous staggerer is a model of early aging of the cerebellum.

614.3

PURKINJE CELL DENDRITIC SPINE ALTERATIONS IN AGING. S. Chen*, R. Bing, R. Llinas and D.E. Hillman. Depts. of Otolaryngol. and Phys./Neurosci., NYU Medical Center, New York, NY 10016.

The inability to demonstrate major decreases in neuronal number during normal aging has directed our attention to dendritic synapses. Spines of Purkinje cells are major indicators for synaptic inputs from granule cells. Using immunoreactions for the cytosolic protein-calbindin, computer assisted light and electron microscopy were used to analyze the shape and number of synapses between young (3 month) and aged (24 month) Fisher 344 rats. The animals were perfused, under pentobarbital anesthesia, through the aorta with 4% paraformaldehyde, 0.5% glutaraldehyde and 1% sodium bisulfate for 10 minutes. The cerebellar vermis was sectioned sagittally with a vibratome into 6x50μm and 300μm thick serial sections. Immunohistochemical reactions for calbindin were processed by either HRP-DAB or postembedding nanogold reactions on one micron plastic sections. Calbindin clearly labeled somata, dendrites and spines of Purkinje cells in both methods. The thin plastic sections provided excellent resolution for accurate sampling of spine density. The spine density in aged animals was substantial less than in young. Osmicated thin sections examined in electron microscopy revealed changes in spine and synaptic sizes.

The reduced number of synapse on Purkinje cells in the absence of cerebellar cell losses raises the question of selective dendritic spine death or pruning granule cell axons. These findings suggest that aged rats have some compensatory capability by increasing the size of synapses with synapse loss. Grant NIA AG-09480

614.4

AGING OF THE CEREBELLAR HEMISPHERES AND VERMIS OBSERVED *IN VIVO*. J.H. Dupuis*, C.A. McGavran, N. Raz, S.D. Briggs, and J.D. Acker*. Department of Psychology, The University of Memphis, and *Baptist Memorial Hospital, Memphis, TN 38152.

We investigated patterns of cerebellar aging using MRI based morphometry in a sample of 93 healthy volunteers (19 to 77 years old). Cerebellar volume was computed for each hemisphere from series of consecutive 1.5 mm coronal sections. On midsaggital sections, we measured the area of five vermal regions: (1) lingula and centralis, (2) culmen, (3) declive, folium and tuber, (4) pyramis, and (5) uvula and nodulus. We also measured the cross sectional area of the ventral pons. The volume of the cerebellar hemispheres decreased significantly with age. The rate of decline was consistent for both hemispheres, and for men and women. The vermis as a whole was also affected by age, but not as strongly as the hemispheres. The degree of shrinkage associated with age varied among the vermal regions. Only the posterior vermis showed an age related shrinkage. More specifically, the cross sectional area of the declive, folium, and tuber was most strongly affected. In contrast, the anterior vermis and the ventral pons appear resistant to the effects of age. The pattern of age effects found here with the cerebellar hemispheres, the vermis, and the pons is consistent with the notion that phylogenetically older structures tend to be more resistant to the effects of age, whereas relatively newer structures tend to significantly decrease in size over the life span. Supported by the NIA grant AG11230 to N.R.

614.5

AGE, GENDER, AND HEMISPHERIC DIFFERENCES IN THE HUMAN STRIATUM. F. Gunning*, D. Head, N. Raz, J. McQuain, & J.D. Acker^. Department of Psychology, The University of Memphis, and ^Baptist Memorial Hospital, Memphis, TN 38152.

Age and gender differences as well as lateral asymmetries have been noted in motor behavior. These differences may be related to the morphology of the basal ganglia. In this study we attempted to replicate and extend previously reported findings of the variability in the basal ganglia volume by gender, hemisphere, and age. We examined the neostriatum of 66 healthy right-handed adults (19 - 77 years old). MRI-based morphometry was used to quantify the volume of the head of the caudate nucleus (Cd) and the putamen (Pt). Our analysis revealed bilateral age-related shrinkage in both the Cd ($r=-.34$, $p<.006$) and the Pt ($r=-.41$, $p<.001$). In this sample age-related differences in neostriatal nuclei were associated neither with gender nor with hemisphere. However, both Cd and Pt were larger in males than in females, even after adjustment for body size (height) differences. There was a signficant rightward asymmetry in the Pt, whereas no hemispheric differences were observed in the Cd. Supported by the NIA grant AG11230 to N.R.

614.6

AGE-RELATED CHANGES IN THE RHESUS MONKEY: MEMORY, EXECUTIVE FUNCTION AND "IQ" IN A NON-HUMAN PRIMATE MODEL OF NORMAL HUMAN AGING. RJ Killiany, MB Moss, DL Rosene, J Herndon* & ZC Lai, Dept. of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA 02118 and Yerkes Regional Primate Research Center, Emory University, Atlanta, GA. 30322.

As part of an ongoing project aimed at identifying the neurobiological bases of cognitive decline with normal aging, a group of 8 young (5 - 14) and 16 elderly (20 - 32 yrs of age) rhesus monkeys were behaviorally tested on tasks designed to assess specific cognitive functions that have been previously shown to undergo age-related declines in human subjects. The monkeys were administered two tasks of recognition memory; the Delayed Non-Matching to Sample and Delayed Recognition Span Task as well as two tasks of executive function; Spatial & Object Reversal Learning. A standardized composite score, weighted equally on measures of initial learning, recognition memory, and reversal performance, was derived for each monkey. The composite score served as a global index of each monkey's level of cognitive ability. Overall, the findings of this study revealed that monkeys, like healthy adult humans, evidence significant age-related declines in both memory and executive system function. The onset of the memory impairments preceded those of executive function. Secondly, aged monkeys, like healthy aged humans, evidence marked individual differences in the extent of memory impairment. Thirdly, the data suggest that spatial abilities may be more vulnerable to age than non-spatial abilities. Finally, our data suggest that in monkeys, as in healthy adult humans, select individuals age more "successfully" than others. (Supported by a grant from the Dana foundation and by NIH grants PO1 AG00001 and RR 00165)

614.7

AGE-RELATED CHANGES IN THE RHESUS MONKEY: MRI CHANGES IN FOREBRAIN WHITE MATTER BUT NOT GRAY MATTER. ZC Lai*, DL Rosene, RJ Killiany, D Pugliese, MS Albert and MB Moss, Dept. of Anatomy & Neurobiology, Boston University School of Medicine, Boston MA 02118, Dept of Psychiatry & Neurolology, Massachusetts General Hospital, Boston MA 012114 and Yerkes Regional Primate Research Center, Emory University, Atlanta, GA 30322.

To study the neurobiological bases of age-related decline in cognitive function in vivo, 8 young (5 - 14) and 8 elderly (20 - 32 years of age) monkeys that were behaviorally tested were given MRI scans in a GE Signa scanner (3D SPGR T1-weighted, TR56, TE12, 1.3 mm thickness, 512 x 512). The total forebrain volume was measured using in-house software on a Sun Sparcstation. To determine relative volumes of forebrain gray matter (cortex, basal ganglia and diencephalon), white matter (subcortical white and cerebral peduncles) and ventricles, the scans were analyzed by point counting using either a plastic overlay on the Sun or a grid macro for NIH Image on a Macintosh Quadra. There was a significant ($r=.55$, $p<.05$) age-related decline in the overall forebrain volume (gray and white matter and ventricles combined) but no significant change in the gray matter volume. Additionally, there was a significant decline in the white matter volume ($r=.63$, $p<.05$) while ventricular volume appeared to decrease ($r=.36$, $p<.10$). Comparison of these results with our studies of age-related cognitive dysfunction revealed a significant correlation between the white matter loss and performance on the acquisition component of the delayed non-matching to sample test ($r=.52$, $p<.05$). This suggests that there may be an impairment in connectivity resulting from white matter loss that underlies age-related cognitive changes. (Supported by a grant from the Dana foundation and by NIH grants AG00001 and RR00165)

614.8

AGE-RELATED CHANGES IN THE RHESUS MONKEY: PET IMAGING AND REDUCTIONS IN CYTOCHROME OXIDASE MARKERS. DL Rosene, ZC Lai, AA Bonab, N Alpert, R Rizkalla, KJ Page, BT Hyman, R Killiany*, MS Albert & MB Moss, Dept. of Anatomy and Neurobiology, Boston University School of Medicine, Boston MA 02118 and Depts. of Psychiatry and Neurology and of Radiology, Massachusetts General Hospital, Harvard Medical School, Boston MA 02114 and the Yerkes Regional Primate Research Center, Emory University, Atlanta, GA 30322.

To identify the neurobiological bases of observed age-related declines in cognitive function, young (5 - 14) and elderly (20 - 32) behaviorally tested monkeys were examined with a GE4096 PET camera using 15-O-water to assess cerebral blood flow and cortical metabolism. Results demonstrated an apparent decline in global cerebral blood flow with age. Regional analyses revealed that the greatest reductions occurred in posterior slices of the brain. To further examine the basis of this apparent decline in metabolism, fresh frozen cryostat sections through the forebrain were processed with oligonucleotide probes to the RNA message for nuclear subunit IV and mitochondrial subunit II of cytochrome oxidase (COX). Adjacent sections were processed with COX histochemistry to assess functional activity. All three measures demonstrated a significant decline in COX with age. Since other studies from this laboratory indicate that there is an age-related loss of white matter without a significant loss of gray, these results suggest that age-related declines in cognition may result from an anatomical and/or functional deafferentation of the cortex that is reflected in reduced cerebral blood flow, cytochrome oxidase activity and overall cortical metabolism. (Supported by a grant from the Dana foundation and by NIH grants AG00001 and RR00165)

614.9

AGE-RELATED CHANGES IN THE RHESUS MONKEY: CHOLINERGIC RECEPTOR BINDING IN THE HIPPOCAMPUS AND TEMPORAL LOBE. TJ Nicholson, EC Johnson, A Doolittle, MB Moss, and DL Rosene*, Dept of Anatomy and Neurobiology, Boston University School of Medicine, Boston MA 02118 and Yerkes Regional Primate Research Center, Emory University, Atlanta, GA 30322.

Previous research has demonstrated that the cholinergic neurotransmitter system may contribute to changes in memory that occur in normal aging. To characterize the neurobiological changes underlying these cognitive deficits, three cholinergic receptors (M1, M2, and the high affinity choline uptake site [HACU]) were examined using in vitro, on the slide, autoradiography of 15 micron thick coronal sections from the brains of old (>20) and young (<14) monkeys. The binding of tritiated ligands (5nM H^3-hemicholinium to HACU, 10nM H^3-pirenzipine to M1, InM H^3-oxotremorine to M2) in different regions of the hippocampus and temporal lobe was measured. A significant overall decrease in binding was found for the M1 ($p<.0001$) and M2 ($p<.0001$) receptors in all areas. In contrast the HACU receptors showed a significant ($p<.0094$) increase in binding with age but only within the hippocampus and not in the remainder of the temporal lobe. Until saturation binding experiments can be completed it is not clear if these changes are due to a change in affinity or the number of receptors but the increase in HACU sites suggests a compensatory response to cholinergic deafferentation. These results compared with our studies of age-related cognitive dysfunction revealed a significant correlation ($p<.05$) between the decreased M2 binding and impairments of memory on the delayed non-matching to sample basic task. (Supported by NIH grants PO1 AG00001 and RR 00165)

614.10

AGE-RELATED CHANGES IN THE RHESUS MONKEY: EXCITATORY AND INHIBITORY AMINO ACID RECEPTOR BINDING IN THE HIPPOCAMPUS AND TEMPORAL LOBE. MB Moss*, TJ Nicholson, R Rizkalla, C Fitzgerald, and DL Rosene, Dept of Anatomy and Neurobiology, Boston University School of Medicine, Boston MA 02118 and the Yerkes Regional Primate Research Center, Emory University, Atlanta, GA 30322.

Previous research has demonstrated that the excitatory amino acid and inhibitory GABAergic systems are potential contributors to changes in memory that occur in normal aging. To characterize the changes in these two systems, the N-methyl-D-aspartate (NMDA), kainate (KAI), gama-aminobutyric acid type a ($GABA_A$), and benzodiazepine (BZD) receptors were examined using in vitro, on the slide, autoradiography of 15 micron thick coronal sections from the brains of old (>20) and young (<10) monkeys. The binding of tritiated ligands (7nM H^3-MK-801 to NMDA, 15nM H^3-KAI to KAI, 5nM H^3-muscimol to $GABA_A$, 2nM H^3-flunitrazepam to BZD) in the temporal lobe was determined. The NMDA ($p<.0001$), KAI ($p<.0001$), and $GABA_A$ ($p<.0001$) receptors all decreased binding significantly whereas the BZD ($p<.0001$) receptors increased binding with age. Saturation binding experiments must be completed to determine whether changes in binding are due to a change in affinity or the number of receptors. The decrease in NMDA and KAI binding may reflect degenerative changes in dendrites. The increase in BZD binding suggests alteration in the subunit composition of the $GABA_A$ receptor. These results compared with our studies of age-related cognitive dysfunction revealed a significant correlation ($p<.05$) between the increase in BZD binding and impairments of spatial short term memory . (Supported by NIH grants PO1 AG00001 and RR 00165)

614.11

AGE RELATED CHANGES IN THE RHESUS MONKEY: NEURONAL LOSS IN SUBSTANTIA NIGRA-PARS COMPACTA AND VENTRAL TEGMENTAL AREA. Z. A. Siddiqi, T.L. Kemper, D. L. Rosene and M. Feldman*, Dept. of Anat. and Neurobiology, Boston Univ. Sch. of Med., Boston, MA & The Yerkes Regional Primate Center, Amory Univ., GA

Advancing age in rhesus monkeys is associated with a reduction in the dopamine concentration in the prefrontal association cortex (Goldman-Rakic and Brown, Neurosci. 6:177-187,1981). The cells of origin of this prefrontal dopaminergic projections are mainly in the anterior part of ventral tegmental area (VTA) and medial one third of the substantia nigra, pars compacta (PC). We report initial morphometric findings in PC and the cortically projecting subnuclei of VTA, Pars nigralis (PN) and Pars brachialis pigmentosus (PBP) in rhesus monkeys ages 5, 15, 22 and 32 years. The analysis was made using every 10th section in a series of 30μ thick Nissl stained frozen sections. The extent of these dopaminergic nuclei was determined with an immunohistochemical stain for tyrosine hydroxylase. Location of soma, somal size, cell packing density and melanin were used in defining PN and PBP. We used the three dimensional cell counting system of Williams & Rakic (J. Comp. Neur. 278:344-352) and systematic sampling was done with an X-Y stage encoder. All three regions showed a progressive decrease in neuronal packing density with age. This ranged up to 25%, 24% and 12% in the PC, PBP and PN respectively in the oldest monkey as compared to the youngest monkey. The most severe reduction was observed in the monkey aged 22 years, 37%, 36% and 35% in PC, PBP and PN respectively. This animal had shown severe age-related behavior deficits on a variety of cognitive tests. These preliminary results support the earlier neurochemical findings that the dopaminergic system is effected by age and may play a pivotal role in age- related cognitive decline. (Supported by NIH Grants P01 AG00001 & RR 00165).

614.12

THE HUMAN NUCLEUS COERULEUS IN AGING - A STEREOLOGICAL ANALYSIS OF FEMALES AND MALES

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The few recent studies on the effect of aging on the total cell number of noradrenergic (i.e. neuromelanized) neurons of the nucleus coeruleus are detracted either from the lack of detailed neuropathological characterization of the used cases or because no unbiased modern stereological tools were applied. Moreover, no gender-related analysis was performed so far. In the present approach we used a newly developed unbiased sampling scheme and examined the total number of neuromelanized neurons in 31 individuals between 49 and 98 years of age. All were clinically without signs for neurological or psychiatric disorders, however, at histopathological inspection four meet the criteria for stage II (Braak and Braak, 1991) of Alzheimer's disease-related neurofibrillary changes, and 12 for stage III. We did not uncover a significant decrease in total neuron numbers, neither when analyzing females (n=14) and males (n=17) together or separately: There was no correlation between the stage and the total cell numbers. In line with previous findings, we did not detect side differences. However, a gender difference in total cell numbers is suggested. Females showed a mean (± SD) of 18913 (±3023) with a range from 13501-26031 whereas males showed 14844 (± 2494) with a range of 11737-20707. Taken together our data show (i) no age-related cell loss between 50 and 100 years of age, (i) a sexual dimorphism with males having 20% fewer neuromelanin-containing neurons, and (III) Alzheimer's disease-related cell loss in the nucleus coeruleus is not an early event.
Supported by the DFG (Oh48/1-2)

PHARMACOLOGY OF SYNAPTIC TRANSMISSION II

615.1

A NOVEL SUBTYPE OF THE CNS PROSTACYCLIN RECEPTOR: ITS INVOLVEMENT IN SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS H. Takechi*, K. Matsumura, Yu. Watanabe, and Y. Watanabe, Subfemtomole Biorecognition Project, Research Development Corporation of Japan, and Department of Neuroscience, Osaka Bioscience Institute, Osaka 565, Japan.

We found a new presumable subtype of the prostacyclin receptor abundantly expressed in the central nervous system. Several prostacyclin analogues showed the different binding properties of the subtype compared with the known prostacyclin receptor, which was tested by *in vitro* autoradiography in male adult rats. Isocarbacyclin (TEI7165), which is a potent agonist for the known prostacyclin receptor, had high affinity for this novel subtype (the dissociation constant, *Kd*, was 7.8 nM). However, iloprost, which had been usually used as a stable prostacyclin agonist and had a high affinity site in the mastocytoma cell line or CHO cells transfected with a cloned prostacyclin receptor cDNA, showed a low affinity binding (the *Kd* was 159.3 nM) for this subtype. Other prostaglandins had no or little affinity for the subtype. The density of the subtype was high in the thalamus, lateral septal nuclei, hippocampus, cerebral cortex, striatum and dorsal cochlear nucleus. Although the nucleus of the solitary tract (NTS) and spinal trigeminal nucleus showed a high density binding of [^{3}H] isocarbacyclin, [^{3}H] iloprost had also high affinity in these regions and the binding specificity was similar to that found in the peripheral organ. Hemilesion studies of striatal neurons or afferents revealed that the binding sites of the novel subtype existed on neuronal cells in the striatum, not on presynaptic terminal of dopaminergic afferents nor glial cells. Since the expression of the receptor in the hippocampus was observed along with the pyramidal cell layer, we tested whether prostacyclin analogues had some effects in synaptic transmission in the CA1 region using rat hippocampal slices. We found that prostacyclin analogues caused a transient increase of field epsp. Surgical removal of the CA3 region did not affect the enhancement. These results suggest that prostacyclin had some physiological roles in synaptic transmittion.

615.2

THE MEMBRANE PERMEABLE CALCIUM CHELATOR, BAPTA-AM, ATTENUATES SYNAPTIC TRANSMISSION *IN VITRO* IN THE HIPPOCAMPUS CA1 REGION. A. Ouanounou, L. Zhang, M. Tymianski, M.P. Charlton, C.M. Wallace and P.L. Carlen*, Playfair Neuroscience Unit, Toronto Hospital Research Institute, Depts of Medicine (Neurology), Physiology and Surgery (Neurosurgery), Bloorview Epilepsy program, University of Toronto, Toronto, Ont, Canada.

To assess a possible neuroprotective mechanism of the membrane-permeable calcium chelator, BAPTA-AM, we examined its effects on synaptically evoked field potentials in the stratum radiatum of the hippocampal CA1 region in rat brain slices. Perfusing with 50-0.5 μM BAPTA-AM caused a reversible, concentration-dependent reduction of the synaptically evoked field potential by 45-23%, without affecting the presynaptic volley or antidromically evoked responses. Similar application of 5'5'-dinitro BAPTA-AM, which is a low-affinity BAPTA analogue, did not decrease synaptic field potentials. Repetitive stimulation (1 Hz) in the presence of BAPTA-AM caused an increase in the orthodromically evoked field potentials. These results demonstrate that extracelluar BAPTA-AM, at a concentration as low as 0.5 μM, can depress synaptic transmission in mammalian CNS, likely by decreasing evoked neurotransmitter release.

615.3

PHARMACOLOGICAL CHANGES IN PAIRED PULSE DEPRESSION OF DENTATE GRANULE CELL FIELD POTENTIALS. LJ Burdette*, SM Lasley & ME Gilbert, Dept Neurology, Graduate Hospital, Philadelphia, PA, 19146, Dept Basic Sciences, U. of Illinois, Peoria, IL, 61656, and Curriculum in Toxicology, U. of North Carolina, Chapel Hill, NC 27599

We previously have shown that moderate to high stimulation reduces late paired pulse depression and the 1 Hz depression of the population spike (PS) in field potential recordings. Antagonists to $GABA_B$ (CGP-35348, 50 mg/kg ip) receptors and NMDA (MK-801, 0.25 mg/kg ip) or Ca^{2+} channels (flunerazine, 80 mg/kg po) were given to evaluate cellular processes that modulate paired pulse depression of dentate granule cell field potentials in the unanesthetized rat. Following drug or vehicle administration, paired pulses (0.1 ms, 20 or 200 ms interpulse interval) were delivered to the perforant path at 0.05 Hz and 1 Hz (30 sec) for a range of stimulus intensities. MK-801 increased the strength of late paired pulse depression and the 1 Hz depression of the PS at all but the highest stimulus intensity. CGP-35348 had no effect on any measure of paired pulse depression. Flunerazine exerted a marginal decrease in both early and late paired pulse depression that was not significant. The results suggest that NMDA-mediated currents reduce the strength of late paired pulse depression at all stimulus intensities but do not account for the loss of late paired pulse depression with maximal stimulation. Consistent patterns exhibited by late paired pulse depression and the 1 Hz depression of the PS provide confirmation that the same currents participate in both events. As a loss of late paired pulse depression precedes a failure of early paired pulse depression and seizure initiation in naive rats, additional study is needed to identify mechanisms responsible for the disinhibition observed with moderate to high stimulation. (Supported by MH45961(LJB), ES06253(SML,MEG) & U.S. EPA (MEG)).

615.4

THE QUANTITATIVE SENSITIVITY OF SYMPATHETIC GANGLION TRANSMISSION TO VOLATILE ANESTHETICS. J.B. McCallum, Q. Hogan, J.L. Seagard*, and Z.J. Bosnjak, Dept. of Anesthesia, Med. College of Wisconsin, Milwaukee, WI 53226.

Inhalational anesthetics depress peripheral ganglionic transmission, but the quantification of this effect remains unknown. The purpose of this study was to compare the effects of halothane (H), isoflurane (I) and enflurane (E) on ganglionic transmission at equimolar anesthetic concentrations using isolated *in vitro* canine stellate ganglia. Eleven ganglia were isolated from adult mongrel dogs after halothane anesthesia, desheathed, and superfused with Krebs' solution equilibrated with a mixture of 97% O_2 and 3% CO_2 maintained in a specially designed tissue chamber at a pH of 7.4±0.5 and a temperature of 37±0.5^0 C. Compound action potentials (CAP) were generated by supramaximal stimulation of the preganglionic T3-ramus at a frequency of 0.4 Hz. The percent CAP depression from control was measured 10 min. after exposure to increasing anesthetic concentrations delivered to the ganglia through a vaporizer attached to the gas flow. The following superfusate concentrations (in mM) were measured in the superfusate by gas chromatography. H: 0.099, 0.16, 0.3, and 0.72. I: 0.09, 0.17, 0.32; and 0.72. E: 0.1, 0.19, 0.31, and 0.74. The EC_{50} value (with its 95% confidence limits) for each anesthetic are as follows: H: 0.3(0.27-0.33). I: 0.32(0.29-0.35). E: 0.34(0.31-0.37). Equivalent MAC values for these concentrations are 1.25, 1.03 and 0.5, respectively. While the percent suppression achieved at equimolar anesthetic concentrations was essentially the same, inhibition of ganglionic transmission was significantly different in terms of equivalent MAC values. These results indicate that the mechanisms underlying anesthesia in the peripheral ganglia are more sensitive to plasma concentrations than partial pressures.

615.5

A SLOW EXCITATORY SYNAPTIC CURRENT RECORDED FROM DOPAMINERGIC NEURONS IN RAT MIDBRAIN SLICE.
K.-Z. Shen* and S. W. Johnson. Department of Pharmacology, Oregon Health Sciences University, Portland, OR 97201.

Synaptic currents were evoked with bipolar electrodes while recording whole-cell currents with patch pipettes from ventral tegmental neurons in rat midbrain slices. A late-onset slow inward current (50 - 300 pA at -70 mV) was evoked by a train of stimuli in most of dopaminergic neurons superfused with solution containing APV, CNQX, bicuculline and CGP 35348. These slow EPSC was voltage-dependent, and was abolished in low calcium/high magnesium solution. Antagonists of muscarinic, serotonergic (5-HT2), and noradrenergic receptors failed to block the slow EPSC, as well as by antagonists of neurotensin and NK1 receptors. Senktide (0.1 - 5 μM), an agonist of NK3 receptors, and substance P (3 - 5 μM) evoked inward current and occluded the slow EPSC in some neurons. In contrast, NMDA (10 μM) failed to occlude the slow EPSC although it also evoked an inward current. In neurons recorded with cesium gluconate in the pipette, baclofen (3 - 10 μM) reduced the amplitude of the slow EPSC by 80%, this effect was blocked by CGP 35348 (300 μM). These results suggest that the slow EPSC is mediated by substance P acting at NK3 receptors. Furthermore, GABA acting at $GABA_B$ receptors mediate presynaptic inhibition of the slow EPSC.

615.6

GLUTAMATE AND GABA IN ANESTHETIC-INDUCED NEOCORTICAL BURST SUPPRESSION ACTIVITY *IN VITRO*.
H. S. Lukatch* and M. B. MacIver. Dept. of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305.

The present study used an *in vitro* neocortical EEG brain slice preparation to determine the neuronal loci for propofol, thiopental and isoflurane-induced burst suppression activity. Control EEG responses within the theta frequency range (4-8 Hz) were electrically evoked (0.5 ms, 5 V, 0.033 Hz) in the presence of carbachol (100 μM) and bicuculline (10 μM) in layers 2/3 of Oc2MM neocortex. Clinically relevant concentrations of propofol (10 μM, n=3), thiopental (50 μM, n=12) and isoflurane (0.9 vol %; ~ 140 μM, n=4) produced burst suppression EEG patterns in neocortical brain slices. Bursts ranged in amplitude from 150-500 μV, and occurred with a frequency of 0.3 - 2.0 Hz similar to anesthetic-induced burst activity seen *in vivo*. The $GABA_A$ agonist muscimol (1 mM, n=5) produced burst suppression activity in the absence of anesthetics. Glutamate receptor antagonists CNQX (8 μM, 5 of 5 slices) or APV (42 μM, 3 of 3 slices) forced anesthetic-induced burst suppression activity to isoelectric activity. These results indicate that enhanced tonic GABAergic transmission and intact glutamatergic transmission are required for burst suppression activity.

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615.7

RECURRENT EXCITATION IN THE DENTATE GYRUS REVEALED BY OPTICAL AND MICROELECTRODE RECORDING.
Meyer B. Jackson* and Helen E. Scharfman#, *Dept. of Physiology, University of Wisconsin, Madison, WI 53706. #NRC, Helen Hayes Hospital, NY State Dept of Health, W. Haverstraw NY 10993 and Depts. of Pharmacology and Neurology Columbia University, NY, NY 10032.

A voltage-sensitive dye (RH414) was used to image electrical activity in the dentate gyrus in rat hippocampal slices. Electrical stimulation of the molecular layer (ML) evoked large responses throughout the ML and hilus. Bath application of the excitatory amino acid receptor antagonists DNQX and APV blocked ML responses distal to the stimulating electrode, indicating a dependence on excitatory synaptic transmission. In slices with a lesion through the outer two thirds of the ML, made by cutting radially through the crest, ML stimulation on one side of the lesion evoked nearly equal responses on both sides. EPSPs that triggered action potentials could be recorded in granule cells on the side of the lesion opposite from the stimulating electrode located in the ML. When field potentials were recorded on the side of the lesion opposite from the stimulating electrode, the largest negative potentials were seen in the inner ML. Focal application of excitatory amino acids to the hilus evoked negative field potentials in the ML. In slices with a lesion in the hilus, parallel and subjacent to the granule cell layer, responses to distal ML stimulation were much reduced in the ML above the lesion. Intracellular recording from granule cells above the lesion demonstrated that they had normal membrane properties (i.e. they were not damaged by the lesion). These results confirm that hilar neurons form excitatory synapses on granule cell dendrites, and indicate that the strength of this pathway has been underestimated by previous studies. Supported by NINDS grants NS30016 to MBJ and NS30831 to HES.

615.8

FUNCTIONAL CHARACTERIZATION AND PLASTICITY OF FEEDBACK INHIBITORY CIRCUITS IN AREA CA3 OF HIPPOCAMPAL SLICE CULTURES.
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Brain Research Institute, University of Zurich, CH-8029 Zurich, Switzerland

Pyramidal neurons of the hippocampus, expecially in the CA3 region, are interconnected by recurrent excitatory axon collaterals. The spread of excitation is controlled by feedback and feedforward inhibitory circuits. Intracellular recordings in current clamp mode were used to study feedback inhibition. Single action potentials (APs) were evoked by brief depolarizing current pulses in CA3 pyramidal cells. In 40% of pyramidal cells, the AP was followed by an inhibitory postsynaptic potential (IPSP) within 3-5 ms, which was blocked by bicuculline, hence mediated by $GABA_A$ receptors. The probability of activation of the inhibitory cell was decreased by reducing the probability of its excitation with adenosine (0.1-0.2 μM). Adenosine increased the number of failures of transmission from <10% to >50% of APs, indicating that the EPSPs in the interneurons were indeed reduced, and decreased the amplitude of non-failure IPSPs. The comparison of the IPSP amplitude distribution before and after adenosine application suggests that the simultaneous activation of multiple interneurons was decreased by adenosine. In some experiments, adenosine increased the failure rate, but did not change the IPSP amplitude, suggesting that feedback inhibition was mediated by only one interneuron. When the frequency of APs was increased from 1/6 to 2 Hz for 20-40 seconds, the IPSP amplitude decreased by ~35%. CGP 35-348 (0.5-1 mM), a $GABA_B$ receptor antagonist, did not affect the amount of depression. We conclude that more than one inhibitory interneuron is usually involved in feedback inhibition in area CA3 of hippocampal cultures. When the interneurons fire at a frequency of 2 Hz, IPSPs are depressed in a $GABA_B$ autoreceptor-independent manner.

615.9

SYNAPTIC INTERACTIONS IN COCULTURES OF POSTNATAL RAT SENSORY AND SPINAL CORD NEURONS.
E. Sermasi, P. Feltz* and R. Schlichter. Lab. Physiologie générale, Univ. Louis Pasteur, URA 1446 / CNRS, 21 r. Descartes, 67084 Strasbourg, France.

The aim of this study was to characterize the synaptic interactions between postnatal(1-3 days old) rat dorsal root ganglion (DRG) and spinal cord neurons in a coculture preparation. It was possible to distinguish between both cell populations on the basis of morphological and electrophysiological criteria. DRG neurons have large (25 to 40 μm diameter), spheric, phase-bright cell bodies with a prominent nucleolus and one or two neurites whereas spinal cord neurons are small in size (5 to 15 μm diameter), irregularly shaped phase-dark cells with multiple processes. Whole-cell patch-clamp recordings were made from visually identified spinal neurons from 1 to 3 weeks old cocultures. Care was taken to record only from neurons having a projecting DRG neuron nearby. At a holding potential of -60 mV, spontaneously occuring synaptic events were characterized as transient inward currents of variable amplitude (10-500 pA) and short duration (10-50 ms). Occasionally, it was also possible to observe synaptic events having a slower time course (>50 ms). The majority of the synaptic activity is reversibly abolished by application of CNQX (5 μM) but not by a similar concentration of APV (5 μM) or bicuculline (5μM). However a small subpopulation of fast (< 15 ms) and small-sized (<25 pA) synaptic events was resistant to the blocking action of CNQX. Application of capsaicin (0.5 μM) appeared to enhance and/or to evoke these fast synaptic currents. Our results suggest that at least part of the synaptic activity in spinal cord neurons is due to an input from Aδ and C fibers acting on non NMDA glutamate receptors. Thus our model is useful to study in detail the synaptic interactions of Aδ and C nociceptive neurons with postnatal spinal cord neurons under experimental conditions of ideal electrophysiological and pharmacological access.
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615.10

ESTABLISHMENT OF LONG TERM PRIMARY NEOSTRIATAL NEURONAL MICROCULTURES OF POSTNATAL RAT.
P. B. Senatus*. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

The rat neostriatum is known to consist of various morphological and cytochemical neuronal types including GABAergic medium spiny projection neurons (~95%) [Wilson and Groves (1980) J Comp. Neurol. 194: 599-615] and several classes of nonspiny interneurons (~5%) [Kawaguchi (1993) J Neuroscience 13: 4908-23; Kubota (1993) Neuro-Report 5: 205-8)]. A major concern of this study is the investigation of synaptic interactions among rat neostriatal neurons.

Neuronal microcultures and mass cultures from the postnatal rat neostriatum were prepared by methods similar to those described by Segal and Furshpan (1990) [J Neurophysiology 64: 1390-99] and Furshpan and Potter (1989) [Neuron 3: 199-207], and could be maintained up to four weeks in culture. The microculture format was chosen in order to reduce the number of interacting neurons and to permit electrophysiological, cytochemical, and electron microscopic examination of the same neuron. By two days in culture, the neurons increased in size and extended neuritic processes. After one week, dendritic arbors became elaborate and axonal varicosities, including terminal boutons, could be visualized by light microscopy. On the basis of prevalence and somatic diameter, distinct sets of neurons could be identified. After 2 weeks in culture, about 90% of the neurons were GABA-immunoreactive, and just under 2% of the neurons expressed NADPH diaphorase activity. These results are consistent with proportions observed *in vivo* in the adult rat neostriatum. Supported by 5 F31 GM 14736 to P. B. Senatus and NS02253 to D. D. Potter, Ph. D..

615.11

EFFECTS OF MELATONIN ON SYNAPTIC TRANSMISSION AND LONG-TERM POTENTIATION IN THE DENTATE GYRUS OF THE HIPPOCAMPUS. R.G. Gonzales* and D.L. Armstrong. Div. of Life Sciences, University of Texas at San Antonio, San Antonio, TX 78249.

The indoleamine, melatonin, was investigated as a possible modulator of synaptic transmission in the dentate gyrus of rat hippocampal slices. Reports demonstrating specific binding of melatonin to both calmodulin and CNS receptors coupled to regulatory G-proteins suggest that this hormone could mediate profound effects on neuronal function, including the induction and maintenance of long-term potentiation (LTP). Mature male rats housed under a 12:12 LD cycle were used, with hippocampal slices prepared midway through the dark period. Evoked field excitatory postsynaptic potentials (fEPSP) were recorded in the dentate molecular layer following perforant path stimulation at 0.1 Hz. Bath application of 0.1 to 0.5 mM melatonin for thirty min. reduced fEPSPs as compared to basal levels. The average percent decrease in the slope and amplitude was 9% and 13%, respectively. To test the effect of melatonin on LTP induction, the hormone was present during the application of three 100 Hz trains with an intertrain interval of 10 sec, and then washout was begun. Increases of greater than 20% in fEPSP slope and greater than 14% in amplitude were observed 30 min. after tetanization. This potentiation was within the range of that observed under control conditions. By contrast, in slices where melatonin was applied prior to, during and following tetanization (n=3), no LTP was induced in two cases, but rather a slow decrease in fEPSP response was produced. These findings suggest that melatonin not only depresses normal synaptic transmission, but might also modulate the processes involved in the induction and maintenance of LTP.

615.12

NITRIC OXIDE INCREASES CALCIUM/CALMODULIN-DEPENDENT PHOSPHORYLATION OF PROTEINS IN POSTSYNAPTIC DENSITY FRACTIONS FROM ADULT RAT CEREBRAL CORTEX. K. Wu*, H.T.J. Mount, J.L. Xu and I.B. Black. Dept. Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Nitric oxide (NO) plays important roles in diverse processes, including neurotransmission within the peripheral and central nervous systems. Nitric oxide synthase (NOS), the enzyme that catalyzes formation of NO from L-arginine, is an intrinsic component of the postsynaptic density (PSD), an electron-dense proteinaceous specialization of the postsynaptic membrane. This raises the possibility that NO may have actions on enzyme functions within this structure.

To begin defining local actions of NO, we examined effects of NO on calcium/calmodulin-dependent phosphorylation (C/C-DP) of proteins in cortical PSD from adult rat brain. Treatment of PSD with sodium nitroprusside (1 mM, 5 min), a NO donor, caused a dramatic 10-fold increase in C/C-DP, relative to C/C treatment alone. S,S'-Dinitrosodithiol (0.1 mM), another NO donor, elicited a 2-fold increase in C/C-DP. Treating PSD fractions with L-arginine (1 mM, 10 min), to produce endogenous NO, also doubled C/C-DP activity. The effect of L-arginine was blocked by a competitive NOS inhibitor, N^G-Nitro-L-arginine methyl ester (N-LAME). Pretreatment of cortical tissue with N-LAME (0.5 mg/ml, 5 min), before isolation of PSD fractions, decreased basal C/C-DP of PSD proteins by 50%. The inhibitor had no effect on cAMP-dependent phosphorylation of the proteins, suggesting specificity of NO action on C/C-DP.

Our observations indicate that NO enhances C/C-DP of PSD proteins. It has been shown previously that C/C-DP inactivates NOS. These findings raise the possibility that NO effects on C/C-DP may constitute a mechanism for feedback regulation of NOS activity. (Supp: NICHD 23315 and MRCC.)

615.13

CHRONIC CYCLOSPORIN A TREATMENT DECREASES ELECTRICALLY STIMULATED RELEASE OF [3H]-D-ASPARTATE FROM RAT HIPPOCAMPAL SLICES. S.A. Queen*, J. Stepanek, and W.C. Buss. Dept of Pharmacology, Univ. New Mexico Sch. Med., Albuquerque, NM 87131.

The immunosuppressant cyclosporin A (CsA) forms a binary complex with cyclophilin, interrupting the immune response by inhibiting protein phosphatase 2B (PP2B) in T-cells. Although highly effective as an immunosuppresssant, CsA is neurotoxic in some patients. We hypothesized that some of the neurotoxic symptoms of CsA, such as cortical epileptiform activity and seizure, result from altered release of excitatory amino acid neurotransmitter in the hippocampal formation.

Sprague-Dawley rats were injected daily with control vehicle or 20 mg/kg CsA for 14 days. This dose of CsA has been shown to generate cortical epilieptiform activity (Famiglio et al., 1989). Hippocampal tissue slices (350 m) were prepared and preloaded with 300 nM [3H]-D-aspartate (D-Asp). Following a 1 hour equilibration in a superfusion apparatus at a flow rate of 0.4 mL/min, tissue slices were superfused at 0.8 mL/min and 3 minute aliquots were collected for 1 hour. Tissue slices were equilibrated and superfused in continuously oxygenated Krebs-Hensleit buffer, pH 7.4. L-Cysteine sulfinic acid (500 mM) was included during the last 20 minutes of equilibration and throughout superfusion to inhibit neurotransmitter reuptake. Release of D-Asp was stimulated electrically by two 5 Hz, 3 minute stimulations, separated by a 21 min interval. Release of D-Asp from CsA exposed tissue was equivalent to controls in response to the first electrical stimulus, but significantly decreased by 27% in response to the second stimulus. We propose that the reduced release of [3H]-D-aspartate is due to changes in phosphorylation of presynaptic proteins resulting from inhibition of PP2B by a CsA-cyclophilin complex. (Supported by NIH R01-AI25555)

615.14

INHIBITION OF ELECTRICALLY-STIMULATED [^{3}H]GLUTAMATE RELEASE BY THE SUBSTITUTED BENZIMIDAZOLONE NS-004. H.L. Wiener* and J.R. Torrente, CNS Drug Discovery, Bristol-Myers Squibb Co., Wallingford, CT 06492.

During ischemic brain damage, excess release of the excitatory amino acid glutamate may damage brain tissue by a multi-faceted process. Since stroke-related damage can potentially be reduced by limiting glutamate release, we developed an assay that measures the amount of preloaded [^{3}H]glutamate released from rat hippocampal slices in response to electrical stimulation. NS-004 (5-trifluromethyl-1-(5-chloro-2-hydroxyphenyl)-1,3-dihydro-2*H*-benzimidazole-2-one) opens neuronal and vascular smooth muscle large-conductance calcium-activated potassium (maxiK) channels. In addition, NS-004 blocks delayed rectifier and ATP-sensitive potassium channels in smooth muscle and is both cardio- and neuroprotective. The present studies investigated the ability of NS-004 to modulate electrically-stimulated [^{3}H]glutamate release in rat hippocampal slices *in vitro*. NS-004 inhibited [^{3}H]glutamate release in a concentration-dependent and saturable manner. To test the hypothesis that maxiK channel opening by NS-004 contributed to its ability to attenuate [^{3}H]glutamate release, NS-004 was assayed in the presence of iberiotoxin (IbTX), a highly specific blocker of the maxiK channel. Although 100 nM IbTX did not itself affect [^{3}H]glutamate release, it prevented NS-004-induced inhibition of [^{3}H]glutamate release. That the response to NS-004 was IbTX-sensitive suggests that NS-004 inhibits electrically-stimulated [^{3}H]glutamate release in hippocampal slices *in vitro* by opening the maxiK channel and that this may contribute to its neuroprotective action.

615.15

FUNCTIONAL UNMASKING OF L-TYPE VOLTAGE-SENSITIVE CALCIUM CHANNEL INVOLVEMENT IN K$^+$-EVOKED [^{3}H]-NORADRENALINE RELEASE FROM RAT AND HUMAN NEOCORTICAL SLICES. D.J. Dooley*[3], T. Ginap[1], R. Scheremet[2], and T.J. Feuerstein[1]. [1]Klinische Neuropharmakologie, Neurozentrum, 79106 Freiburg, Germany; [2]Neurochirurgische Universitätsklinik, 79106 Freiburg, Germany; and [3]Dept. of Neuroscience, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Co., Ann Arbor, MI 48106.

Central noradrenaline (NA) release is predominantly subserved by N- and P/Q-type voltage-sensitive calcium channels (VSCCs). The role of L-VSCCs in this release has been demonstrated to be minimal or nonexistent. There are, however, two conditions known which permit an increase in *in vitro* neurotransmitter release by the L-VSCC activators Bay K 8644 and FPL 64176: a reduction of the K$^+$ stimulus concentration and the block of N-VSCCs by ω-conotoxin GVIA. In the present study, L-VSCC-mediated [^{3}H]-NA release from rat or human neocortical slices, induced by Bay K 8644 (1 μM) or the endogenous indirect L-VSCC activator endothelin-3[3] (10 nM), was assessed as a function of K$^+$ stimulus concentration, N-VSCC antagonism (ω-conotoxin GVIA (1 μM)), and P/Q-VSCC antagonism (ω-agatoxin IVA (1 μM)). Bay K 8644 or endothelin-3 markedly increased [^{3}H]-NA release in the presence of 25 mM K$^+$ and N- and/or P/Q-VSCC blockade, or 15 mM K$^+$. These results suggest that a decrease of NA release effected by several mechanisms, including N- and P/Q-VSCC antagonism or a lower stimulus intensity, permits functional consequences of either direct or indirect L-VSCC activation.

[1]Eur. J. Pharmacol. 198 (1991) 37. [2]Neurosci. Lett. 156 (1993) 35. [3]Biochem. Biophys. Res. Commun. 157 (1988) 977.

615.16

ARG-VASOPRESSIN REDUCES FAST EXCITATORY SYNAPTIC TRANSMISSION IN THE SUPRAOPTIC NUCLEUS *IN VITRO*. Samuel B. Kombian*, Jeffrey A. Zidichouski & Quentin J. Pittman. Neuroscience Research Group, University of Calgary, Calgary, AB Canada T2N 4N1.

The supraoptic nucleus (SON) is a hypothalamic structure that contains vasopressin and oxytocin releasing cells. There is evidence that these peptides are released locally and may influence SON cells. These cells receive glutamatergic and GABAergic afferents which may regulate them. We examined the action of vasopressin on glutamate-mediated fast synaptic transmission in an *in vitro* slice preparation of the SON using the whole cell nystatin patch recording technique. 400 μm thick coronal hypothalamic slices containing the SON were made from 25-40 days old Sprague-Dawley rats. Nystatin patch recordings were made with K-acetate filled electrodes (5-10 MΩ). Access (resistance 20-30 MΩ) was attained in 1-30 min using an Axopatch 1D amplifier. Synaptic responses were evoked (5-50V, 100-500 μs) through a bipolar electrode placed dorso-medial to the SON. Stable recordings (lasting 45-150 min) were made from SON cells with RMP of -84.6 ± 2.1 mV, SEM, n=35 cells. All cells were voltage clamped at -80 mV (V_h) and intensity dependent inward currents (I_{in}) could be evoked in >80% of cells at this V_h. The evoked I_{in} were mediated by both non-NMDA and $GABA_A$ receptors as CNQX (10 μM) decreased the response by 46.7 ± 14.2% ($p<0.05$, n=3) while picrotoxin (25 μM) reduced it by 25.4 ± 7.3% ($p<0.05$, n=3). A combination of these antagonists reduced the I_{in} by 94.1 ± 3% ($p<0.001$, n=3). To examine the pure glutamate-mediated I_{in}, picrotoxin (25 μM) was present throughout the remaining experiments. Arginine-vasopressin (AVP; 1 or 3 μM) caused no change in holding current (I_h, 6/8 cells). However the synaptically evoked I_{in} was reduced by 33.7 ± 5.8%, $p<0.005$, n=6 in these cells. AMPA-induced inward currents were not altered 4 of 6 of these SON cells (0.1 ± 1.2%, $p>0.5$, n=4). AVP therefore reduces fast excitatory synaptic transmission in the SON mainly by a presynaptic mechanism. Supported by MRC; SBK is supported by H&SF, AHFMR and MRC.

615.17

cAMP-BINDING PROTEINS AFTER PAROXETINE AND FLUVOXAMINE ADMINISTRATION S. Mori[1], M. Caivano[1], S. Garbini[1], A. Dorigo[1], M. Motta*[3], G. Racagni[1], J. Perez[1-2]. 1- Center of Neuropharmacology, Inst. Pharmacological Sciences. 2- Department of Neuropsychiatry HSR, University of Milan. 3- Inst. of Endocrinology, University of Milan, Via Balzaretti 9, 20133 Milan, Italy.

cAMP-dependent protein kinase (PKA) is a serine-threonine kinase, which plays a crucial role in the cAMP signaling. The activation of this enzyme proceeds by a reaction where cAMP binds to the regulatory (R) subunits enabling free catalytic (C) moieties to phosphorylate specific substrate proteins, which in turn are involved in a wide variety of cellular functions. Recent data obtained in our and other laboratories have suggested that the cAMP binding to R subunit, as well as the activity of the enzyme, can be markedly modified following administration of different antidepressants. We have now extended these studies to determine whether cAMP binding activity could be affected in cerebrocortical soluble (S1 or S2) and microtubule fractions after short and long term administration of paroxetine (5 mg/kg) and fluvoxamine (15 mg/kg), two selective serotonin reuptake inhibitors (SSRIs). The results show that either paroxetine and fluvoxamine after 12 days, but not after 5 days of treatment, significantly enhanced the covalent binding to 52 KDa R subunit in the S1 as well as in the microtubule fractions. Following 21 days of treatment these compounds markedly increased the cAMP binding to R subunits only in the S2 fraction, which is devoid of cytosolic microtubules, suggesting that long-term administration of SSRIs could induce a translocation of cAMP receptors from the cytoskeletric to the cytosolic compartment of the cell. In conclusion, our results seem to demonstrate that cAMP binding proteins could be involved in the biochemical action of SSRIs.

PHARMACOLOGY OF SYNAPTIC TRANSMISSION III

616.1

VERY SLOW CURRENTS RECORDED FROM DEEP DORSAL HORN NEURONS IN ADULT MOUSE SPINAL CORD *IN VITRO*. B. A. Miller and C. J. Woolf. (SPON: Brain Research Association) Dept. of Anatomy & Developmental Biology, University College London, Gower Street, WC1E 6BT.

Slow excitatory synaptic events, lasting up to 60 seconds, have been recorded from neonatal rat spinal cord neurons *in vitro* in response to stimulation of high-threshold afferents (Aδ- and C-fibers). However, it is uncertain whether such slow events reflect the immaturity of the preparation or whether they also occur in the adult. To investigate this, we recorded postsynaptic currents from deep dorsal horn neurons in adult mouse spinal cord *in vitro*.

Thick (700 μm) horizontal spinal cord slices with intact dorsal roots were prepared from adult male mice (MF1 strain, 21-27 g). Neurons at the lamina IV/V border were whole-cell voltage-clamped at -70 mV. Currents were evoked by stimulation (500 μA/500·μs) of high-threshold afferents in dorsal roots L4 or L5, via a suction electrode.

Single-shock stimulation evoked **very slow** inward currents (peak amplitude, 612.0 ± 63.3 pA; duration, 4.1 ± 0.8 mins; mean ± S.E.M., n = 10), such currents were not observed with stimulation of the low-threshold Aβ-fibers. The currents were voltage-dependent, becoming larger at depolarized potentials and reversing around O mV.

Stimulation of primary sensory afferents, therefore, evokes very slow postsynaptic currents in the deep dorsal horn of the adult mouse spinal cord. These currents are considerably longer than any recorded from neonatal *in vitro* preparations. Understanding the transmitter and cellular mechanisms involved may contribute to an understanding of activity-dependent synaptic plasticity in the spinal cord.

This work was supported by the Wellcome Trust.

616.2

MODULATION OF EXCITATORY SYNAPTIC TRANSMISSION IN THE NUCLEUS ACCUMBENS BY DOPAMINE D_1 RECEPTORS. J. Harvey & M.G. Lacey (SPON: Brain Research Association), Dept. of Pharmacology, The Medical School, University of Birmingham, Birmingham, B15 2TT, UK.

In the nucleus accumbens (NAc), dopamine (DA) is known to be involved in locomotion and behavioural reward, but the cellular mechanisms underlying these actions of DA remain unclear. In this study, whole-cell patch clamp recording has been used to investigate the actions of DA and psychostimulants on excitatory synaptic transmission in the NAc. Experiments were performed on 350 μm horizontal rat forebrain slices, maintained at 32-33 °C and perfused with a standard superfusate. Fast glutamate receptor-mediated synaptic currents (EPSCs) were evoked by cortical stimulation, in the presence of picrotoxin (50 μM). In 9 of 11 cells, DA (10-60 μM) produced a reversible and dose-dependent depression of the peak EPSC amplitude, in the absence of any discernable postsynaptic effect. DA (30 μM) produced a depression of 56 ± 11% (mean ± SEM; n=6), which was completely reversed by the selective D_1 receptor antagonist, SCH23390 (1 μM) (n=3). The depressant actions of DA were mimicked by the D_1 receptor agonists, SKF 38393 (10 μM; 42 ± 6%; n=4) and SKF 81297A (1 μM; 39 ± 4%; n=2). In contrast, the selective D_2 receptor agonist, quinpirole (10 μM), failed to affect the peak EPSC amplitude or the holding current (n=4). These data suggest that D_1 receptors, possibly located presynaptically, modulate excitatory synaptic transmission in the cortico-accumbens pathway. In a further 11 of 23 cells, cocaine (10 μM), the DA uptake inhibitor, depressed the peak amplitude (40 ± 6%) of EPSCs in a reversible manner. The D_1 receptor antagonist, SCH23390 (1 μM), completely reversed the depression evoked by 10 μM cocaine (n=3). Amphetamine (10-30 μM), also induced a reversible depression of the peak EPSC amplitude (62 ± 9%; n=6/11), which was antagonised by 1 μM SCH23390 (n=2). Together these data suggest that endogenous DA depresses glutamate receptor mediated synaptic transmission in the NAc, via an action at D_1 receptors.

616.3

EFFECTS OF TRH ON GABAERGIC SYNAPTIC TRANSMISSION OF THE RAT HIPPOCAMPUS. M. Atzori, A. Nistri, M. Sciancalepore* and G.Stocca. Biophys.Lab.,Int. Sch. Adv. Studies (SISSA), 34013 Trieste, Italy.

The effects of Thyrotropin Releasing Hormone (TRH), a neuropeptide present in the mammalian hippocampus, on inhibitory synaptic transmission of the rat hippocampal slice were studied with intracellular or patch clamp recording. After block of glutamate receptors 10 μM TRH increased the frequency of spontaneous $GABA_A$ rerceptor mediated currents of CA1 pyramidal neurons (from 1.07±0.68 Hz to 3.16±0.73 Hz) and of Stratum Lacunosum Moleculare (SL-M) interneurons (from 1.6±0.5 to 3.4±0.9). In TTX solution TRH did not change miniature currents. Simultaneous patch recordings from one pyramidal cell and one SL-M interneuron displayed synchronous events suggesting that they shared a common input. Conversely, TRH depressed by 39±6 % average GABAergic responses evoked by stimulation of SL-M whereas it enhanced (by 43±13 %) those induced by Stratum Pyramidale (SP) stimulation. TRH decreased (by 35%) intracellularly recorded $GABA_B$ receptor mediated potentials evoked from SL-M in half of the cells tested, while leaving unchanged those evoked by SP stimulation. Responses to 10 μM isoguvacine or 10 μM baclofen remained the same in the presence of TRH (104±1% or 105±4%, respectively). The simplest interpretation of these data is that pyramidal neurons and SL-M interneurons received a common GABAergic inhibitory input by a distinct class of SP interneurons selectively modulated by TRH. In this way, spontaneous GABAergic activity and SP-evoked responses of CA1 cells were enhanced while SL-M induced inhibition was depressed by upregulated SP interneurons. The limited sensitivity of $GABA_B$ receptor mediated responses to TRH might reflect their relatively small contribution to the overall process of GABAergic synaptic inhibition. Supported by a HCMP grant f rom the EU.

616.4

LOW CONCENTRATIONS OF ADENOSINE AND THE A2 AGONIST CGS 21680 FAIL TO ELICIT EXCITATORY EFFECTS IN HIPPOCAMPAL BRAIN SLICES. L.H. Diao*, C.R. Lupica and T.V. Dunwiddie. University of Colorado Health Sci. Cntr., Dept of Pharmacology, and VA Medical Center, Denver, CO 80262.

The inhibitory effects of adenosine and related compounds on evoked field excitatory postsynaptic potentials (fEPSP) and population spikes (PS), have been consistently observed in a number of laboratories, and are likely due to both pre- and postsynaptic actions at adenosine A1 receptors. In contrast, the excitatory effects of adenosine, which have been hypothesized to reflect the activation of A2 receptors, have not been consistently observed. We have attempted to characterize the excitatory effects of adenosine using protocols shown to generate this phenomenon in other laboratories. Thus, low concentrations of adenosine and the selective A2a receptor agonist CGS 21680 were tested on fEPSPs and PSs in the CA1 area of hippocampal slices obtained from male Sprague-Dawley and Wistar rats, and Guinea pigs. Neither adenosine (10 nM-1 μM) nor CGS 21680 (1 nM-100 nM) consistently altered the amplitudes of these responses. Also, pretreatment with the selective A1 adenosine receptor antagonist 8-cyclopentyltheophylline (CPT, 0.1 μM) did not reveal excitatory effects of adenosine (30 μM-100 μM) on fEPSPs and PSs. Higher adenosine concentrations (250-500μM) significantly decreased both fEPSPs and PSs in the presence of CPT. The reason(s) why low concentrations of adenosine and CGS 21680 were unable to consistently produce excitatory effects are unclear at this time. One possibility is that A2a receptors exist only in specific strains of animals that were not tested in the present experiments. Alternatively, these receptors may be present, but are not coupled to the modulation of transmitter release under the present experimental conditions. However, these findings, together with the observation that antagonists such as theophylline, CPT and DPCPX produce only increases in synaptic responses, suggest that the predominant effect of both exogenous and endogenous adenosine in this preparation is inhibitory. Supported by NS 29173 and VA Medical Research Service.

616.5

DIRECT AND DISINHIBITORY EFFECTS OF NICOTINE IN THE HIPPOCAMPAL SLICE. C.J. Frazier*, G.M. Rose, and T.V. Dunwiddie. Neuroscience Training Program, UCHSC, and Medical Research, VAMC, Denver, CO 80220

We have used extracellular recording techniques, in conjunction with an antidromic-orthodromic paired pulse paradigm, to examine the effects of nicotine on GABAergic inhibition in area CA1 of rat hippocampal brain slices. In this system we are able to measure the effects of nicotine on the amplitude of control population spikes generated by direct orthodromic activation of Schaffer and commissural afferents, and on orthodromic population spikes that were inhibited by prior antidromic (alvear) activation of CA1 interneurons. Our results indicate that 300 μM nicotine exerts a small excitatory effect on the control population spike, which is likely to be mediated either by direct depolarization of pyramidal neurons, or by antagonism of feedforward inhibition. However, the most pronounced affect of nicotine is a dose dependent reduction of the inhibition produced by antidromic stimulation. This disinhibitory effect is likely due to nicotinic inhibition of interneuron function. Further efforts towards characterizing this system have indicated that acetylcholine (50-200 μM), and 1,1,-dimethyl-4-phenylpiperazinium iodide (DMPP; 200 μM) are also effective agonists, while cytisine (30-200 μM), methyl-carbachol (5-50 μM), and 2,4-dimethoxybenzylidene anabaseine (DMXB; 5-50 μM) are not. We have also demonstrated that hexamethonium (5 mM), and methyllycaconitine (1-5 μM) partially antagonize the effects of 300 μM nicotine, while α-bungarotoxin (300 nM - 1 μM), mecamylamine (100-500 μM), Dihydro-β-erythroidine (10-40 μM), and the serotonergic antagonist MDL 72222 (50 μM) do not. These results suggest the existence of an α7 mediated component in hippocampal nicotinic function, however they do not rule out the possibility of a significant α4 component. The emerging pharmacological profile, and the lack of complete antagonism, may indicate a more complex system, involving multiple receptor types.

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616.6

SEROTONERGIC MODULATION OF SYNAPTIC TRANSMISSION IN THE BASOLATERAL AMYGDALA. D.G. Rainnie*: Harvard Medical School & Brockton VAMC, Department of Psychiatry, Brockton, MA 02401, USA

Altered neuronal activity within the amygdala has been regarded as a substrate for several pathophysiological states such as epilepsy, depression and schizophrenia. Although the response of basolateral neurones to extrinsic fibre stimulation has been reported in several studies, little information is available concerning the regulation of synaptic transmission by catecholamines.

Whole cell patch-clamp records were obtained from neurones of the basolateral amygdala in an *in vitro* slice preparation using standard techniques. Patch electrodes (6-10 MΩ) were filled with recording solution containing, in mM: $KMeSO_4$, 140, $MgCl_2$, 3; EGTA, 3; HEPES, 4; $CaCl_2$, 1; MgATP, 2; NaGTP, 2; and biocytin 0.25%. Stable records were obtained from 64 neurones, approximately 6% of which were considered to be putative interneurones. Passive membrane properties reveal no significant difference in the resting membrane potential or input resistance of these neurones (-60 ± 6 mV compared to -62 ± 3 mV, and 195 ± 90 MΩ compared to 135 ± 58 MΩ respectively). Application of serotonin (5HT, 5-100 μM) had no effect on membrane properties of the majority of projection neurones examined (n=20). In contrast, 5HT caused a membrane depolarization (5.0 ± 1.8 mV, n=5) and c-oncomittant increase in the input resistance of putative interneurones. This action was not mimicked by 1-(*m*-chlorophenyl)-biguanide (1 μM) or 8-OH-DPAT (1 μM) suggesting that $5HT_3$ and $5HT_A$ receptors are not involved in this response. Serotonin application also caused a reduction in the amplitude of evoked synaptic potentials and had a biphasic effect on spontaneous potentials. In control ACSF, spontaneous IPSPs occur at ~ 0.7Hz in projection neurones. Superfusion of 5HT (30 μM) increased IPSC frequency to 1.4Hz but then caused a progressive decrease in IPSC amplitude until they were abolished. Experiments are in progress to determine the subtype/s of serotonin receptor mediating these responses.

616.7

CANNABINOID RECEPTOR AGONISTS INHIBIT GLUTAMATERGIC SYNAPTIC TRANSMISSION IN RAT HIPPOCAMPAL CULTURES. M. Shen, T. M. Piser and S. A. Thayer* Dept. of Pharmacology, Univ. of Minnesota, Minneapolis, MN 55455.

Reduced extracellular Mg^{2+} (0.1 mM) elicited repetitive intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) spikes in cultured rat hippocampal neurons as detected by indo-1-based microfluorimetry. Combined whole-cell current clamp and microfluorimetry showed that a burst of action potentials underlies each $[Ca^{2+}]_i$ spike. $[Ca^{2+}]_i$ spiking activity was inhibited by the NMDA receptor antagonist CGS19755 (10 μM) and abolished by either the non-NMDA receptor antagonist CNQX (10 μM), or TTX (1 μM), indicating that the spiking results from glutamatergic synaptic transmission. Anandamide, an endogenous cannabinoid receptor ligand, inhibited the frequency of $[Ca^{2+}]_i$ spiking with an EC_{50} of 71 nM. Inhibition of $[Ca^{2+}]_i$ spiking by cannabinoid receptor agonists was prevented by pretreatment of the cultures with pertussis toxin. Activation of cannabinoid receptors did not affect kainate or AMPA-induced $[Ca^{2+}]_i$ transients, but did inhibit CNQX-sensitive excitatory postsynaptic currents elicited by stimulation of the presynaptic neuron with an extracellular electrode. Thus, activation of cannabinoid receptors inhibits the presynaptic release of glutamate via an inhibitory G protein. Modulation of excitatory neurotransmission may account for many of the CNS effects of the cannabinoids and suggests that cannabinoid receptor agonists may reduce synaptically mediated excitotoxicity.

616.8

COCAINE, AMPHETAMINE, AND DOPAMINE DEPRESS SYNAPTIC TRANSMISSION IN THE NUCLEUS ACCUMBENS *VIA* A PRESYNAPTIC MECHANISM. S.M. Nicola*, S.B. Kombian, and R.C. Malenka. Dept. of Psychiatry and Program in Neuroscience, University of California, San Francisco, CA 94143.

We have continued to investigate the actions of psychostimulants on excitatory synaptic transmission in rat nucleus accumbens slices. Previously (Soc. Neurosci. Abstr. 20: 1517, 1994) we reported that cocaine and amphetamine depressed EPSP's at cortico-accumbens synapses, and that dopamine (DA) mimicked these effects. The DA-induced depression of synaptic transmission was accompanied by an increase in paired-pulse facilitation, suggesting a presynaptic site of action. We have now extended this analysis by examining miniature EPSC's. DA (100 μM, n=15) and amphetamine (10 μM, n=6) decreased the frequency of mEPSC's by 33% and 44%, respectively, while leaving their amplitude unchanged. These findings confirm that the mechanism of psychostimulant-induced depression is presynaptic at this synapse.

We found previously that the actions of cocaine, amphetamine, and DA were antagonized by the D1 receptor antagonist SCH23390 (2 to 10 μM), while the D2 antagonist sulpiride (10 μM) had no effect. However, upon re-examination, the effects of agonists are less clear. Neither the D2 agonist quinpirole (20 μM, n=6) nor the D1 agonists (+)-SKF38393 (10 to 30 μM, n=6) and (±)-SKF81927 (30 μM, n=11) appreciably depressed synaptic transmission. However, (±)-SKF38393 at 100 μM depressed EPSP's by 23% (n=4). To determine whether a different catecholamine or receptor contributed to the psychostimulant-induced synaptic depression, we applied 2 μM serotonin (n=7) which decreased the EPSP by 20%; however, this effect was not antagonized by SCH23390 (10 μM, n=9). Norepinephrine (100 μM, n=11) depressed synaptic transmission by 30%; this depression was blocked by the α-adrenergic antagonist phentolamine (10 μM, n=7) whereas 10 μM phentolamine had no effect on the depression induced by 10 μM amphetamine (n=6). These results are consistent with the hypothesis that psychostimulant-induced synaptic depression is mediated primarily by DA.

616.9

MU OPIOID AND METABOTROPIC GLUTAMATE RECEPTOR AGONISTS MODULATE NMDA SYNAPTIC POTENTIALS IN NUCLEUS ACCUMBENS. G. Martin, T. Krucker* and G.R. Siggins, Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Opiates decrease glutamatergic EPSPs in the nucleus accumbens (NAcc; Yuan et al., Neurosci. Lett. 134: 223, 1992). Therefore, we investigated the effects of a specific μ opioid receptor agonist DAMGO ([D-Ala^2-N-Me-Phe^4,Gly-ol^5]-enkephalin) and the metabotropic glutamate agonist trans-ACPD (trans-(±)-1-amino-1,3-cyclopentanedicarboxylic acid) on events mediated by N-Methyl-D-Aspartate (NMDA) receptor activation, using intracellular recording in rat NAcc slices. The mean resting membrane potential of NAcc core neurons was -85 mV; mean input resistance was 78 MΩ and spike size, 124 mV. Superfusion of DAMGO (1 μM) or trans-ACPD (10-50 μM) did not alter membrane potential or input resistance in any cell. In the presence of 10 μM CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) to block non-NMDA glutamate receptors and 15 μM bicuculline to block $GABA_A$ receptors, EPSPs evoked by local stimulation displayed the characteristics of NMDA components, including voltage-dependence and blockade by the NMDA receptor antagonist d-APV (60 μM). DAMGO (1 μM) significantly decreased NMDA-EPSP amplitudes by up to 50% of control, with reversal of this effect by naloxone and the μ-selective opiate antagonist CTOP (0.5 μM). To examine possible postsynaptic actions of DAMGO, we superfused slices with TTX (1 μM) under voltage-clamp and evoked inward currents by pressure application of NMDA from pipettes. Here, 1 μM DAMGO markedly enhanced (by 75%) NMDA currents (n = 8), with naloxone reversal. In 18 cells, trans-ACPD (10 to 50 μM), like DAMGO, decreased NMDA-EPSC amplitudes dose-dependently. Suprisingly, this effect was not mimicked by quisqalate (1 μM, n = 6). Neither L-AP3 (50 μM, n = 5), MCPG (0.5 mM, n = 3), nor naloxone (1 μM, n = 3) antagonized the trans-ACPD-induced decrease of NMDA-EPSCs. Unlike DAMGO, trans-ACPD (5 μM) markedly decreased the current evoked by pipette application of NMDA. These results suggest a complex modulation of the NMDA glutamatergic neurotransmission by μ opioid and metabotropic receptors: both agonists decrease the amplitude of NMDA-EPSCs but have opposite effects on currents evoked by exogenous NMDA.

Supported by NIH grants DA03665 and AA06420 and Swiss NSF.

616.10

METABOTROPIC GLUTAMATE RECEPTORS AT THE MOSSY FIBER SYNAPSES OF THE GUINEA PIG HIPPOCAMPUS. P.E. Castillo*, O.J. Manzoni and R. A. Nicoll Depts. Cellular and Molecular Pharmacol. and Physiol., UCSF, San Francisco, CA 94143-0450.

We have tested the ability of several specific agonists of metabotropic glutamate receptors (mGluRs) to depress synaptic transmission at mossy fiber (MF) synapses in the CA3 region of the guinea pig hippocampus. 1S,3R-1-amino-cyclopentyl-1,3-dicarboxylate (ACPD) reversibly inhibited monosynaptic MF field potentials, presumably by a presynaptic mechanism, with an EC_{50} of 2.0 ± 0.4 μM. This finding suggests that mGluRs of the group 1 or 2 category are on MF synaptic terminals. L-2-amino-4-phosphono butanoate (L-AP4) also inhibited responses with an EC_{50} of 1.1 ± 0.2 μM, suggesting that mGluRs of the group 3 (mGluR4, 6, 7 and 8) category of receptors are also present on MF terminals. Both (2S,1'S,2'S)-2-(2'-carboxycyclopropyl)glycine (L-CCG1) and (S)-4-carboxy-3-hydroxyphenyl-glycine (4C3HPG) were also efficacious at blocking MF transmission, with EC_{50}s of 1.1 ± 0.1 μM and 4.8 ± 0.6 μM, respectively. The latter finding suggests the involvement of mGluRs belonging to the group 2 (mGluR2,3) category of receptors. The effects of L-AP4 and L-CCG1 were both antagonized by (+)-α-methyl-4-carboxyphenylglycine [(+)MCPG]. MAP4, an antagonist of group 3 mGluRs in other systems, blocked the effect of L-AP4, but not the effect of L-CCG1. These pharmacological findings provide strong evidence for the coexistence of group 2 and 3 mGluRs on the terminals of MFs in the guinea pig.

616.11

PROPERTIES OF ACPD-INDUCED LTD OF SYNAPTIC TRANSMISSION IN IMMATURE CA1 L.S.Overstreet,* J.F.Pasternak, N.T.Slater, J.W. Cozzens, B.L. Trommer, Pediatric Neurology Research Laboratory, Northwestern Univ. Medical School, Evanston Hospital, Evanston, IL, 60201

Bath application of the metabotropic glutamate receptor (mGluR) agonist 1S,3R-ACPD (10μM, 20 min) induces an acute partially reversible decrease (~60% baseline) followed by long-term depression (LTD, ~75% baseline at 30 min) of the field EPSP slope at the Schaffer collateral-CA1 synapse of 8-12 day rats. We examined several pharmacologic properties of this phenomenon. ACPD-induced LTD was blocked by the NMDA receptor antagonists AP5 (20 μM, n=7) and MK-801 (20 μM, n=4), although the acute reversible depression was observed in 9/11 slices. The enzyme glutamic pyruvic transaminase (5u/ml) in pyruvic acid (2mM) also blocked ACPD-induced LTD (acute depression of EPSP slope to 79 ± 14%, recovery to 94±12% of baseline, n=4; interleaved controls=77±10% of baseline). The protein kinase inhibitor staurosporine (0.4μM) blocked ACPD-induced LTD (99±21% baseline; control=62±6% baseline, n=5). ACPD-induced LTD was not blocked by the L-type Ca^{2+} channel blocker nitrendipine (5 μM, 77±26% baseline, n=6) or the A-1 adenosine receptor blocker CPT (5 μM, 68±10% baseline, n=5). These data suggest that 1) ACPD-induced LTD is independent of both L-type voltage-gated Ca^{2+} channels and A-1 adenosine receptors; 2) ACPD-induced LTD is NMDA receptor dependent and the requisite NMDA-mediated current results from background activity generated by extracellular glutamate rather than glutamate released by axon terminals; and 3) ACPD-induced LTD depends on the activation of protein kinase C.

616.12

PHARMACOLOGICAL CHARACTERISATION AND NOVEL ANTAGONISM OF METABOTROPIC GLUTAMATE RECEPTORS IN THE LATERAL PERFORANT PATH OF THE NEONATAL RAT HIPPOCAMPUS. T.J. Bushell[1], D.E. Jane[2], J.C. Watkins[2], E.J. Coan*[1] and G.L. Collingridge[1].
Depts. of Anatomy[1] & Pharmacology[2], University of Bristol, Bristol, U.K. BS8 1TD.

Standard extracellular recording techniques were used to investigate the actions of various mGluR agonists and antagonists on synaptic transmission in the lateral perforant path of hippocampal slices prepared from neonatal (12-16 day old) rats.

The lateral perforant path is highly sensitive to low concentrations of the group III mGluR agonist, L-AP4 (i.c. > 50% depression using 10 μM L-AP4). In this study we confirmed this observation and also showed that this pathway is depressed by group II mGluR agonists, (1S,3S)-ACPD (46 ± 4 %; n=12;10 μM) and DCG-IV (63 ± 3 %; n=3; 500 nM). In contrast, the group I selective mGluR agonist DHPG (50-100 μM; n=5) had no effect. These data suggest that group II and group III mGluRs mediate the depressions of synaptic transmission in the lateral perforant path of rat hippocampus.

Novel mGluR antagonists, (R,S)-α-methyl-4-phosphonophenylglycine (MPPG), (R,S)-α-methyl-4-sulphonophenylglycine (MSPG) and (R,S)-α-methyl-4-tetrazolylphenylglycine (MTPG) have been shown to antagonise L-AP4 and (1S,3S)-ACPD-induced depressions in the neonatal rat spinal cord (Jane et al. Neuropharmacology, in press). The actions of these new antagonists were tested against the synaptic depressions induced by L-AP4 and (1S,3S)-ACPD in the lateral perforant path. MPPG most potently antagonised L-AP4-induced depressions with a K_B of 8 μM. The potency for the antagonism of (1S,3S)-ACPD-induced depressions was MTPG > MSPG = MPPG > MCPG. The advent of these new mGluR antagonists may help determine the physiological roles of class II and III mGluRs.

Supported by the BBSRC.

616.13

ACTIVATION OF GROUP I MGLURS REVERSIBLY DEPRESSES GABAERGIC INHIBITION IN THE HIPPOCAMPUS. V.R.J. Clarke[1], C. H. Davies[3], G. Henderson*[2] & G.L. Collingridge[1]. *Depts. of Anatomy[1] & Pharmacology[2], University of Bristol, Bristol, U.K. BS8 1TD & Dept. of Pharmacology[3], University of Edinburgh, Edinburgh, U.K. EH8 9JZ.*

Activation of metabotropic glutamate receptors (mGluRs) by ACPD has been shown to depress GABAergic inhibition in CA1 hippocampal neurones (Desai, M.A. & Conn, P.J., 1991, J. Neurophysiol, **66**, 40-52; Liu, Y-B. *et al*, 1993, J. Neurophysiol., **69**, 1000-1004). We have examined the effects of (1S,3R)-ACPD and the more selective agonists DHPG (group I), DCG-IV (group II), and L-AP4 (group III) on monosynaptically-evoked inhibitory postsynaptic potentials (IPSPs) and currents (IPSCs).

Standard intracellular recordings were obtained from 400 μm thick transverse rat hippocampal slices. Monosynaptic inhibitory responses were evoked by low frequency stimulation (0.033Hz) of the Schaffer collateral-commissural pathway close to the recording site in the presence of 20 μM CNQX and 50 μM D-AP5. CA1 neurones were manually current clamped at -62 ± 1 mV (mean ± S.E.M.; n=19) or voltage clamped at -60 ± 3 mV (n=3). (1S,3R)-ACPD (50 μM; 10 mins) induced an inward current, increased input resistance (by 11 ± 1%), blocked action potential accommodation and depressed the peak amplitude of the IPSP (by 17 ± 3%) or IPSC (by 24 ± 5%). On washout, all the effects were fully reversible. The IPSPs and IPSCs recovered to 97 ± 2% (n=19) and 97 ± 5% (n=3) of control, respectively. Similar results were obtained under current clamp conditions for DHPG (50-100 μM; n=8). In contrast, no effect of DCG-IV (5 μM; n=3) or L-AP4 (50-500 μM; n=5) was observed. These data suggest that activation of a group I mGluR produces a reversible depression of inhibitory synaptic transmission.

Supported by the Wellcome Trust.

616.14

ELECTROPHYSIOLOGICAL EFFECTS OF KAINATE RECEPTOR ACTIVATION IN THE HIPPOCAMPUS M. Vignes, Z. I. Bashir* and G. L. Collingridge. Dept. of Anatomy, University of Bristol, BS8 1TD. UK.

By the use of the selective AMPA receptor antagonist GYKI 52466 and whole-cell patch-clamp technique we have investigated the effects of the activation of kainate (KA) receptors in CA1 hippocampal neurones. Experiments were performed on hippocampal slices, obtained from 12-16 day old rats, perfused with a standard Mg^{2+}-containing medium at room temperature. NMDA receptor-mediated synaptic currents, isolated by using GYKI 52466 (100 μM) picrotoxin (50 μM) and intracellular Cs^{+}, were evoked by stimulation of Schaffer collateral-commissural pathway. Neurons were voltage-clamped at -45 mV.

Three effects were observed. KA (1 μM; 5 min) evoked, firstly, an increase in the NMDA receptor-mediated synaptic noise, secondly, an NMDA receptor-independent inward current and, thirdly, a reversible depression of the NMDA receptor-mediated synaptic current (by on average 60%). The depression of the synaptically-evoked current outlasted the inward current. All three effects of KA were mimicked by domoate (100 nM) but not by AMPA (10 μM). The effects of kainate were antagonised reversibly by CNQX (10-20 μM).

It is suggested that activation of kainate receptors can have both postsynaptic excitatory and presynaptic inhibitory effects within the hippocampus.

Supported by the European Community

616.15

PHARMACOLOGY OF METABOTROPIC GLUTAMATE RECEPTORS INHIBITING HIPPOCAMPAL CA3-CA1 SYNAPSES.
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CNRS-UPR 9023, Rue la Cardonille 34094 Montpellier Cedex 5 France

In the CA1 region of rat hippocampal slices, we have studied the ability of several specific agonists of metabotropic glutamate receptors (mGluRs) to depress transmission at the CA3-CA1 pyramidal cells synapses. We found that the broad-spectrum agonist (1S,3R)-ACPD and the group 1 specific agonist (R,S)-dihydroxyphenyl-glycine both reversibly inhibited evoked field excitatory post-synaptic potentials (with EC50s of 13 ± 1 μM (n=6) and 18 ± 3 μM (n=5) respectively), indicating the presence of group 1 mGluRs. (R,S)-dihydroxyphenyl-glycine inhibited transmission via a presynaptic mechanism, as whole-cell voltage-clamp recordings revealed that inhibition of the synaptic transmission was always accompanied with an increase of paired-pulse facilitation (176 ± 30 % of control, n = 5). Treatment with a specific blocker of mGluR1 receptors, (+)-4-carboxyphenylglycine, was without effect on the (1S,3R)-ACPD-induced depression of the field excitatory post synaptic potentials, strongly suggesting that mGluR5 receptors are responsible for the (1S,3R)-ACPD effect. Two selective agonists of group 2 mGluRs, L-CCG1 and (S)-4C3HPG, were totally inefficacious at blocking CA3-CA1 evoked synaptic transmission excluding the involvement of mGluR2/3 subtypes. Finally, we confirmed that the specific group 3 agonist L-AP4 was also capable of reducing transmission with an EC50 of 112 ± 22 μM (n=4) and we propose that mGluR7 mediate this inhibition. We are currently investigating the transduction mechanisms responsible for these effects.

616.16

METABOTROPIC GLUTAMATE RECEPTOR TYPE 1/5 MEDIATES BOTH DIRECT AND INDIRECT EXCITATION OF CA1 PYRAMIDAL CELLS. P.J. Conn* and R.W. Gereau IV Department of Pharmacology and Program in Neuroscience. Emory Univ. School of Medicine Atlanta, GA 30322.

Previous studies have shown that the excitatory effects of metabotropic glutamate receptor (mGluR) activation on CA1 pyramidal cells are mediated by direct excitatory effects and a reduction of synaptic inhibition in area CA1. Studies that have examined the physiological roles of mGluRs have generally used agonists that do not differentiate between the various subtypes. We have performed a detailed pharmacological analysis, including examination of the effects of agonists which are selective for Group I (DHPG), Group II (DCG-IV), and Group III (L-AP4) mGluRs as well as rank orders of agonist potencies, to determine the specific mGluR subtypes involved in the direct and indirect excitatory effects of mGluR activation in area CA1. All direct excitatory effects on CA1 pyramidal cells tested including depolarization, increased input resistance, blockade of the slow afterhyperpolarization (AHP), and spike frequency adaptation have pharmacological profiles that are consistent with mediation by a Group I mGluR (mGluR1/5), but not consistent with mediation by Group II (mGluR2/3) or III (mGluR4/6/7/8) mGluRs. The mGluR that reduces transmission at inhibitory synapses in area CA1 also has a Group I-like pharmacological profile. Analysis of miniature IPSCs suggests that this mGluR is localized presynaptically on GABAergic nerve terminals in area CA1. *Supported by grants from the NIH (PJC) and a predoctoral fellowship from the Howard Hughes Medical Institute (RWG).*

616.17

MULTIPLE METABOTROPIC GLUTAMATE RECEPTORS REDUCE EXCITATORY SYNAPTIC TRANSMISSION IN AREA CA1 BY DISTINCT PRESYNAPTIC MECHANISMS. R.W. Gereau IV* and P.J. Conn. Department of Pharmacology and Program in Neuroscience. Emory Univ. School of Medicine Atlanta, GA 30322.

Many studies have shown that metabotropic glutamate receptors (mGluRs) reduce excitatory synaptic transmission in the hippocampus. The conventional view is that this effect is mediated by an L-AP4-sensitive mGluR (mGluR4, 6, 7, or 8). However, some studies suggest that other mGluR subtypes may also be involved. We have found that two pharmacologically distinct presynaptic receptors are involved in the depression of excitatory postsynaptic currents (EPSCs) at the Schaffer collateral-CA1 synapse. Consistent with previous studies, one receptor subtype is an L-AP4-sensitive receptor that is pharmacologically similar to mGluR4 or mGluR7. However, we have found that a second mGluR subtype, which is pharmacologically similar to mGluR1 or mGluR5, can also reduce EPSCs in area CA1. While the mGluR1/5-like receptor is sensitive to inhibition by the mGluR 1/2 antagonist, MCPG, the mGluR4/7-like receptor is insensitive to MCPG. Analysis of miniature EPSCs suggests that both receptors are localized presynaptically, but act to reduce glutamate release via different mechanisms. *Supported by grants from the NIH (PJC) and a predoctoral fellowship from the Howard Hughes Medical Institute (RWG).*

616.18

METABOTROPIC GLUTAMATE RECEPTOR AGONISTS MODULATE PAIRED-PULSE DEPRESSION IN THE DENTATE GYRUS OF THE RAT IN VITRO. R. E. Brown*, and K. G. Reymann. Dept. of Neurophysiology, Federal Inst. for Neurobiology, Brenneckestr. 6, POB 1860, D-39008, Magdeburg, Germany.

The medial perforant path input to the dentate gyrus exhibits paired-pulse depression (PPD), a presynaptically-mediated, short-term form of synaptic plasticity, whereby the synaptic response elicited by the second of a pair of pulses delivered with inter-pulse intervals (ISIs) of up to 2 seconds is depressed relative to the first. We have analyzed the effects of agonists acting at different classes of metabotropic glutamate receptors (mGluRs) on PPD to reveal which classes regulate this process and therefore, which are located presynaptically at this synapse. Hippocampal slices were maintained according to standard procedures; picrotoxin (50 μM) was present in all experiments. A monopolar stimulating electrode was placed in stratum moleculare of the dentate to stimulate the medial perforant path input and field excitatory postsynaptic potentials were recorded. Paired pulses were given at 3 minute intervals during drug application. Interpulse interval was 40 or 500ms.

None of the drugs tested affected PPD with an ISI of 500 ms. In contrast, both 1S, 3R-1-aminocyclopentane-1,3-dicarboxylic acid (100 μM, n = 5), which acts at class I (mGluR1, 5) and class II (mGluR2, 3) mGluRs and L-2-amino-4-phosphobutyric acid (100 μM, n = 4) which is specific for class III (mGluR 4, 6-8) mGluRs , strongly reduced PPD with an ISI of 40 ms ($p < 0.01$). The class I specific agonists trans-azetidine-2,4,dicarboxylic acid (100 μM, n=7) and 3,5,dihydroxyphenylglycine (100 μM, n = 4) did not affect PPD. The relatively specific class II agonists S-3-carboxy-4-hydroxyphenylglycine (n = 5) and 2S,3S,4S-α-carboxycyclopropyl-glycine (n = 4) did reduce PPD but only at very high concentrations (500 and 40 μM respectively). These results suggest that 2 types of mGluRs control PPD at this synapse - a class III mGluR and a class II-like mGluR which may not correspond to one of the currently cloned receptors.

CALCIUM CHANNEL STRUCTURE, FUNCTION AND EXPRESSION II

617.1

L-TYPE CALCIUM CHANNELS MODULATE EVOKED TRANSMITTER RELEASE AT NEWLY FORMED NEUROMUSCULAR JUNCTIONS. Y. Sugiura* and C.-P. Ko Dept. Biol. Sci., University of Southern California, Los Angeles, CA 90089.

Evoked-transmitter release at adult mammalian neuromuscular junctions (NMJs) is blocked by P/Q-type voltage-sensitive calcium channel (VSCC) blockers but not by N- or L-type VSCC blockers. To study the ontogenic change of VSCC subtypes, we examined the effects of several Ca^{2+} channel ligands on synaptic transmission at developing rat NMJs. Similar to adult NMJs, omega-conotoxin MVIIC blocked evoked transmitter release at developing NMJs. Nifedipine, a dihydropyridine (DHP) that blocks L-type VSCCs, *increased* evoked transmitter release at developing NMJs in dose- and age-dependent manners. At embryonic day 17, one, five, and 10 μM nifedipine increased endplate potentials (epps) amplitude 1.8-, 2.5- and 5.4-fold, respectively. This potentiation effect of nifedipine was dramatically weakened after birth. 10 μM nifedipine increased epp amplitude by only 30% at two weeks, and had little effect at one-month. Nimodipine, another L-type blocker, showed a similar effect. The potentiation effect of nifedipine was also observed during the initial stage of reinnervation at the adult mouse and frog NMJs. Nifedipine had little effect on the amplitude and frequency of miniature epps. Charybdotoxin (ChTX), a Ca^{2+}-activated K^+ channel (gK_{Ca}) blocker, increased epp amplitude at regenerating NMJs similar to normal NMJs. However, nifedipine following ChTX showed no additive effect on epp amplitude at regenerating NMJs. The results indicate that L-type VSCCs modulate evoked transmitter release at newly formed, but not mature, NMJs. Blocking L-type VSCCs may prevent the activation of nearby gK_{Ca} and result in a lengthening of presynaptic action potential duration and thus an increase in transmitter release. (Supported by NIH grant NS 30051).

617.2

A CALCIUM CHANNEL FROM THE PRESYNAPTIC NERVE TERMINAL OF THE *NARKE JAPONICA* ELECTRIC ORGAN CONTAINS AN L-TYPE $\alpha_2\delta$ SUBUNIT. Y. Kirino[a,b]*, H.Tokumaru[a,b], S. Shojakua[b], H. Takehara[b], N.Hirashima[a,b], T. Abe[c], H.Saisu[c]. [a]Faculty of Pharmaceutical Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113, JAPAN, [b]Faculty of Pharmaceutical Sciences., Kyushu University, Higashi-ku, Fukuoka 812, Japan, [c]Department of Neurochemistry, Brain Research Institute, Niigata University, Niigata 951, Japan

A monoclonal antibody (MCC-1) that recognizes the $\alpha_2\delta$ subunit complex of L-type Ca channels from rabbit skeletal muscle membranes partially inhibited the evoked release of acetylcholine from synaptosomes isolated from the electric organ of the marine electric ray, *Narke japonica*. A potent L-type Ca channel blocker, calciseptine, inhibited the evoked release of acetylcholine to the same extent. Digitonin extracts of synaptosomal plasma membranes were subjected to immunoaffinity column chromatography on MCC-1-Sepharose. The purified fraction contained a 170 kDa protein, which reacts with MCC-1 and dissociates into smaller polypeptides under reducing conditions. In addition, immunoblotting analysis revealed the existence of syntaxin in the purified fraction, suggesting that the Ca channel forms a complex with syntaxin. However, MCC-1 did not immunoprecipitate an ω-conotoxin GVIA-binding protein. These findings indicate that the 170 kDa protein may be the $\alpha_2\delta$ subunit of an L-type Ca channel that is distinct from the ω-conotoxin GVIA-sensitive N-type Ca channel and partially responsible for the Ca influx that triggers the evoked release of acetylcholine.

617.3

STRUCTURE OF CALCIUM CHANNEL α_1 SUBUNITS FROM CNIDARIANS AND PLATYHELMINTHS. R.M. Greenberg*, K.S. Clark, M.C. Jeziorski, G.B. White, and P.A.V. Anderson. Whitney Lab, University of Florida, St. Augustine, FL 32086.

The phylum Cnidaria is the lowest extant phylum to possess a recognizable nervous system. The platyhelminths, which include the flatworms and flukes, are the earliest animals with a distinct rostral brain and nervous system plasticity, and are thought to have emerged close to the divergence of the deuterostome and protostome branches. We are characterizing the molecular structure of components underlying nervous system function in these animals to provide clues to nervous system evolution as well as insights into structure/function relationships in these molecules. We have recently used degenerate primers and RT-PCR to clone cDNA fragments from neuron-enriched perirhopalial tissue from the jellyfish *Cyanea capillata*, and from the ectoparasitic flatworm *Bdelloura candida*, that are homologous to vertebrate Ca^{2+} channel α_1 subunits. In *Cyanea*, the single Ca^{2+} channel sequence we have obtained is encoded by a ≈8.5 kb mRNA and is most similar to vertebrate L-type α_1 subunits. In *Bdelloura, fragments of two separate* α_1 subunit cDNAs were cloned, each encoded by a distinct mRNA of either 7.2 kb or 7.6 kb. Preliminary evidence suggests that they are most similar to vertebrate L-, and N-type α_1 subunits, respectively. All three of the sequences show extensive similarity to the vertebrate Ca^{2+} channels, particularly in the transmembrane domains. For example, the *Cyanea* fragment shows as much as 72% amino acid sequence identity to the Ca^{2+} channel from rabbit cardiac muscle, suggesting that rather rigorous constraints are operating on the structure of these channels. (Supported by NSF grant #BIR9204017).

617.4

DISTRIBUTION OF ALPHA 1A, ALPHA 1B AND ALPHA 1E VOLTAGE-DEPENDENT CALCIUM CHANNEL SUBUNITS IN THE HUMAN MOTOR SYSTEM. N.C. Day[1]*, P.G. Ince[1], P.J. Shaw[2], S.G. Volsen[3], A.L. McCormack[3], P.J. Craig[3], W. Smith[3], A. Gillespie[4], S.B. Ellis[4] and M.M. Harpold[4]. MRC Neurochemical Pathology Unit[1] and Division of Clinical Neurosciences[2], University of Newcastle upon Tyne, England; Lilly Research Centre[3], Surrey, England; SIBIA Inc.[4], La Jolla, CA 92037, USA.

Voltage-dependent calcium channels (VDCCs) play an important role in neuronal excitability and neurotransmitter release. Six classes of VDCC, termed T, L, N, P, Q and R, have been identified. Expression data for cloned alpha 1 (α1) calcium channel subunits suggest that α1B is a major component of the N channel, while α1A and α1E may correspond to P/Q and R channels respectively. We have investigated the distribution of α1A, B and E in the normal human motor system by *in situ* hybridization and immunocytochemistry. The *in situ* hybridization studies were performed using riboprobes directed towards the intracellular loop between transmembrane domains IIS6 and IIIS1 of α1A, B and E. Fusion proteins of this region were used to generate subunit-specific rabbit polyclonal antisera which were used for immunocytochemistry.

In this study there was good correlation between the distribution of subunit mRNA and protein. α1A subunits were expressed in spinal cord, XII nucleus and III nucleus motor neurones, as well as in Betz cells in the motor cortex. Preliminary studies suggest that the α1B subunit exhibits a similar pattern of distribution to α1A, while the α1E subunit is only weakly expressed in motor system neurones. In general, α1A and α1E antisera stained somatodendritic compartments while α1B antisera labelled cell bodies but exhibited diffuse neuropil staining. Thus α1A, B and E exhibit a differential distribution pattern in the human motor system which may reflect their different functions.

617.5

LOCATION OF L-TYPE Ca^{2+} CHANNELS AT PERISYNAPTIC GLIAL CELLS OF THE FROG NEUROMUSCULAR JUNCTION. R. Robitaille*, M.J. Bourque and S. Vandaele. Départements de Physiologie et Pathologie and CRSN, Université de Montréal, Montréal, Canada, H3C 3J7.

Glial cells may modulate synaptic activity because of their close proximity of synapses. To understand the roles of glial cells at synapses, it is important to know the subcellular distribution of glial receptors and channels in relation with transmitter release sites. This work aims at characterizing the type and subcellular distribution of Ca^{2+} channels at perisynaptic Schwann cells (PSC) of the frog neuromuscular junction. The activity of Ca^{2+} channels was tested by monitoring changes in intracellular Ca^{2+}. Depolarisation of PSCs with KCl (25 mM) produced Ca^{2+} entry which was not blocked by ω-conotoxin GVIA (2 μM) but prevented by L-Type Ca^{2+}channel blockers ((+)R-BayK 8644, 2 μM; nimodipine, 5 μM). In addition, L-type Ca^{2+} channels are functionnaly modulated by an ATP receptor of the P_{2x} subtype. The distribution of L-type Ca^{2+} channels was studied using a monoclonal antibody directed against the α_2/δ subunit of these channels and observed using confocal microscopy. L-type Ca^{2+} channel immunoreactivity was distributed as irregular clusters along the frog endplate, mainly absent from the PSC cell bodies. A similar pattern was observed using a fluorescent phenylalkylamine. Clusters of L-type Ca^{2+} channels were preferentially located in between bands of cholinergic receptors stained with α-bungatotoxin-TRITC. Ca^{2+} channel labeling was absent after mechanical removal of nerve terminal and PSCs but remained after denervation. Cross sections of motor endplate showed that Ca^{2+} channels are strictly located at the processes of the PSCs. Thus, perisynaptic Schwann cells present a highly organized distribution of L-type Ca^{2+} channels around the presynaptic nerve terminal. (Supported by MRC, FRSQ , FCAR and the Sloan Foundation).

617.6

LOCALIZATION OF CALCIUM CHANNEL AND PLASMALEMMA CALCIUM PUMP PROTEINS ON COCHLEAR STEREOCILIA. D.E. Hillman*, S. Apicella, I, Arital, S. Chen, R. Bing, J.T.B. Penniston, R. Llinas. Depts. of Otolaryngol., Phys./Neurosci., NYU Medical Center, New York, NY 10016 and Biochem. Mayo Clinic & Foundation, Rochester, MN 55905

This study determined the location of specific protein epitopes belonging to calcium voltage activated channels and the plasmalemmal calcium pump. Cochlear hair cells transform mechanical energy into neurochemical signals via ion-fluxes with subsequent neurotransmitter release. Electrophysiological evidence supports the existence of voltage activated calcium and potassium channels in cochlear hair cells. Five types of calcium channels have been described but none explain the mechanism of mechanical transduction. We tested the location of proteins of voltage activated channels and the plasmalemmal calcium pump in the organ of Corti using immunohistochemical assays. The antibody reaction product to P-type voltage activated calcium channels was observed on the tips of stereocilia of both outer and inner hair cells.

The plasmalemmal calcium pump protein localized by the 5F10 antibody revealed reaction product all along the stereocilia. The presence of labeling for P-type voltage activated calcium channels on stereocilia may indicate association with mechanical activation or the usual voltage activation via membrane depolarization. Although preliminary analyses show promising evidence that voltage activated calcium channels and a related calcium pump protein are located on cochlear stereocilia, ultrastructural localization and functional testing are needed to clarify the precise location and role of these channel proteins. NIH Grant NS-13742

617.7

IMMUNOCHEMICAL IDENTIFICATION AND DIFFERENTIAL DISTRIBUTION OF ALTERNATIVELY SPLICED FORMS OF THE α_1 SUBUNIT OF NEURONAL CLASS A CALCIUM CHANNELS. T. Sakurai, R. E. Westenbroek, J. Rettig, and W. A. Catterall*. Dept. of Pharmacology, SJ-30, Univ. of Washington, Seattle, WA 98195.

Biochemical properties and subcellular distribution of the α_{1A} subunits were examined in rat brain using anti-peptide antibodies (anti-CNA3 and anti-NBI-2) against unique sequences in the rat rbA and rabbit BI α_{1A} cDNAs, respectively. In immunoblotting, anti-CNA3 recognized two α_{1A} polypeptides, a major 190 kDa form and a minor 220 kDa form. The 220 kDa and 190 kDa forms were also recognized by antibodies against the predicted NH_2- and COOH-termini of α_{1A}, indicating that these isoforms of α_{1A} arise from alternative RNA splicing rather than post-translational proteolytic processing. Anti-NBI-2 specifically recognized a 190 kDa form of α_{1A} in rat brain membrane. Both anti-CNA3 and anti-NBI-2 specifically immunoprecipitated high affinity receptor sites for ω-conotoxin MVIIC ($K_d \sim 100$ pM). Immunofluorescence studies reveal that calcium channels recognized by anti-NBI-2 are localized predominantly in dendrites and nerve terminals forming synapses on them while calcium channels recognized by anti-CNA3 are localized more prominently in neuronal cell bodies and to a lesser extent in punctate structures in dendritic fields. These results indicate that an α_{1A} subunit with a sequence related to the rabbit BI cDNA is present in the rat brain, and that the rbA and BI cDNAs encode α_{1A} subunits with distinct biochemical properties and subcellular distribution.

617.8

CLUSTERING OF Ca^{2+} CHANNELS IN RAT CEREBELLAR PURKINJE CELLS. J.R.B.Dupere and M.M.Usowicz*. Dept. Pharmacology, University of Bristol. UK

The spatial localisation of high voltage activated Ca^{2+} channels determines the electrical properties and influences local changes of $[Ca^{2+}]_i$ in cerebellar Purkinje neurons. However, the elucidation of the sub-cellular distribution of these channels is incomplete. Therefore, to examine this distribution, we made cell-attached patch clamp recordings from Purkinje cells in slices of mature rat (5-7 week) cerebellum. Currents were evoked in somatic patches by voltage ramps (160mV, 100ms) from a holding potential 30-40mV negative to the resting potential. Patch pipettes (5-12MΩ) contained $BaCl_2$ (5mM), TTX (1μM), TEA-Cl (134mM), HEPES (10mM), EGTA (0.1mM), pH 7.4 (TEA-OH).

Current amplitudes varied considerably between patches. Peak currents (at -10mV) ranged from -0.1 to -67.7pA, with a non-normal distribution and a marked skew towards more negative values. The average size was -7.9±10.4pA (±s.d., 93 patches). There was no correlation between peak current and pipette resistance.

The Ca^{2+} channels in cerebellar Purkinje cells were examined further pharmacologically. A blocker of N-type Ca^{2+} channels, ω-conotoxin-GVIA (3μM), reduced the mean current to -3.2±4.0pA (±s.d., 31 patches). The distribution of currents was less skewed in the presence than in the absence of ω-conotoxin-GVIA. The proportion of currents more negative than -10pA was 6% (2/31 patches) with ω-conotoxin-GVIA and 27% (25/93 patches) without. The effect of ω-conotoxin-GVIA is consistent with immunohistochemical studies which identified N-type Ca^{2+} channels in cerebellar Purkinje cells (Westenbroek et al., Neuron 9: 1992, 1099). In contrast, electrophysiological studies revealed few ω-conotoxin-GVIA-sensitive Ca^{2+} channels in these cells (Llinàs *et al.*, PNAS 86:1689, 1989; Mintz *et al.*, Neuron 9: 85, 1992).

In summary, cerebellar Purkinje somata possess N-type channels in addition to P-type Ca^{2+} channels. The relatively large size of ω-conotoxin-GVIA-sensitive currents indicates clustering of N-type channels.

617.9

LOW THRESHOLD (T TYPE) Ca^{2+} CHANNELS CONTROL MINIATURE EPSC FREQUENCY IN THE SPINAL SUBSTANTIA GELATINOSA. J. Bao, J. Li, B. Wall and E. R. Perl*. Dept. of Physiology, CB #7545, Univ. of North Carolina, Chapel Hill, NC 27599

The relationship of presynaptic Ca^{2+} influx to release of transmitter evoking miniature EPSCs (mEPSCs) is not clearly established. We explored the effects of Ca^{2+} channel manipulation on synaptic events in 500-700 μm transverse spinal cord slices prepared from deeply-anesthetized 45-65 gm Syrian Golden Hamsters. The slices were maintained in vitro by superfusion with oxygenated artificial cerebrospinal fluid (ACSF). Cs^+ internal solution electrodes were used to make tight-seal whole-cell recordings from neurons of the substantia gelatinosa (SG). Inward currents representing mEPSCs or EPSCs evoked by stimulation of dorsal rootlets (DR) or adjacent regions of superficial dorsal horn were used to evaluate synaptic events.

Substances modifying Ca^{2+} channel function proved to have differential effects on evoked EPSCs and mEPSCs. Cd^{2+} <50μM blocked evoked responses without appreciable effect upon mEPSC frequency. A putative selective T-type Ca^{2+} channel blocker, Ro 40-5967, decreased mEPSC frequency 45% (n=16), while reducing average DR-evoked EPSC amplitudes only 16% (n=12); in 6 neurons Ro 40-5967 reduced mEPSC frequency 43% without any significant effect on DR evoked EPSCs. La^{3+}, another reported T-type channel blocker, decreased mEPSC frequency 35% (n=10) while reducing DR-evoked EPSCs amplitude only 5.5% (n=10). Increasing $[K^+]_o$ from 2.5 to 5 or 10mM, causing relatively small membrane depolarizations, sharply increased (>70%) EPSC frequency (N=7). These results suggest that transmitter release contributing to mEPSCs is governed by Ca^{2+} channels of the low threshold (T) type, opening near background transmembrane potentials. Low threshold Ca^{2+} channel kinetics in the SG thereby may be a factor in modulating the background excitability of neurons concerned with pain and temperature functions. Supported by NINDS Research Grant NS-10321.

617.10

UP-REGULATION OF L-TYPE CALCIUM CHANNELS IN REACTIVE ASTROCYTES FOLLOWING BRAIN INJURY AND HYPOMYELINATION. R.E.Westenbroek*[1], S.B. Bausch[1], J. E. Franck [2], J.L. Noebels[3], and W.A. Catterall[1]. Depts of Pharmacology[1] and Neurosurgery[2], Univ. of Washington, Seattle, WA 98195 and Developmental Neurogenetics Lab, Dept. of Neurology[3], Baylor College of Medicine, Houston, Texas 77030.

Affinity-purified anti-peptide antibodies which distinguish the alpha 1 subunits of class C L-type (anti-CNC1, Hell et al., JCB 123: 949-962, 1993) and class B N-type calcium channels (anti-CNB2; Westenbroek et al., Neuron 9: 1099-1115, 1992) in rat brain along with a monoclonal antibody to the alpha 2 subunit of L-type calcium channels (MANC1; Ahlijanian et al., Neuron 4: 819-832, 1990) have been well characterized and shown to have distinct patterns of staining in the CNS. These antibodies have been used in combination with the indirect peroxidase-anti-peroxidase technique to investigate if the expression pattern of various voltage-gated calcium channels is affected by brain injury. Light microscopic studies reveal that reactive astrocytes within the CA3 region of the hippocampus following intra-ventricular injection of kainic acid (KA) become immunoreactive for anti-CNC1 and MANC1, but not for anti-CNB2. In KA animals the normal pattern of neuronal staining remains unaltered in regions of the hippocampus outside of the CA3 region. Normal patterns of staining were observed in sham operated control animals. In the *shiverer* mouse, a deletion at the *shiverer* locus (chr 18) results in the absence of myelin basic protein gene expression and development of hyperexcitability. In these animals reactive gliosis was observed in hypomyelinated fiber tracts including the corpus callosum, internal capsule, fimbria, fornix, and cerebellum, which was not observed in normal mice. The reactive astrocytes stained positive with the anti-CNC1 and MANC1, but not with the anti-CNB2 antibodies. These findings demonstrate that L- , but not N-type calcium channels are expressed in vivo in reactive astrocytes in response to brain injury and hypomyelination and suggest that L-type calcium channels may play an important role in the ability of reactive astrocytes to promote neuronal survival.

617.11

DOPAMINE D_2 RECEPTORS REGULATE MELANOTROPE P-TYPE VOLTAGE OPERATED CA^{2+} CHANNELS. D.M. Beatty, P. Sharma, K.E. Hagler, S.A. Sands, B.M. Chronwall, S.J. Morris*. School of Biological Sciences, University of Missouri-Kansas City, MO 64110.

Chronic dopamine D_2 receptor stimulation of primary intermediate lobe cultures depresses melanotrope P-type voltage operated Ca^{2+} channel activity as well as P-type α_1 subunit mRNA. This was measured by real time image analysis fluorescence microscopy and *in situ* hybridization histochemistry.

In untreated melanotropes, K^+ depolarization induced an increase in $[Ca^{2+}]_i$ which was partially sensitive to ω-agatoxin, a P-type channel blocker. The P-type Ca^{2+} channel activity increased during maturation of the rat. When melanotropes were treated for 4 days with bromocriptine, a dopamine agonist, the K^+ depolarization-induced Ca^{2+} influx *via* P-type Ca^{2+} channel was decreased.

Melanotropes chronically treated with bromocriptine showed a decrease in the relative levels of the α_1 subunit mRNA of the P-type channel, while haloperidol, a dopamine antagonist, increased the relative mRNA levels as compared to controls. These results confirm the presence of P-type Ca^{2+} channel in melanotropes and that channel activity and mRNA are regulated by dopamine receptor stimulation.

This work was supported by NIH Grant NS-28019, NSF Grant IBN-9211912, grants from the Kansas Affiliate of the American Heart Association, and the Loeb Charitable Foundation. P.S. is a Marion Merrell Dow-Scientific Education Partnership Fellow.

CALCIUM CHANNEL STRUCTURE, FUNCTION AND EXPRESSION III

618.1

A CELL LINE EXPRESSING ONLY A T-LIKE CALCIUM CHANNEL. W. GOTTSCHALK+, D.S. KIM#, H. CHIN# *, E.F. STANLEY+ SMS+, LNC#, NINDS, NIH, Bethesda, MD 20892.

We have examined calcium current components in a series of neuroblastoma cell lines (from Busis *et al.* Brain Res., 324: 201-210, 1984). One line, 140-3, is deficient in high-voltage activated calcium channels, and only expresses a T-like current. This current is recruited at about -40 mV from a holding potential of -90 mV and is inactivated at a holding potential of -50 mV. The calcium channel is insensitive to agents reported to block T-type current, including nickel, ethosuximide and RO 40-5967, but is blocked by 250 μM cadmium and 10 μM nifedipine. Both low- and high-voltage activated currents were recorded in a control cell line, NG108-15. We have examined the expression of the α_1 subunit transcripts by the RT-PCR method. The NG108-15 cells express α_1 subunits, but we have not as yet detected any α_1 subunits in the 140-3 cells.

618.2

STRUCTURAL IMPLICATION OF THE ABSENCE OF β SUBUNIT-INDUCED FACILITATION OF L-TYPE CA CHANNEL WITH β2. T. Cens, Mangoni, M., J. Nargeot and P. Charnet*. CRBM-CNRS UPR9008, Route de Mende, 34033 Montpellier France

We have recently characterized a facilitation of the class C L-type Ca channel expressed in Xenopus oocyte. This facilitation was recorded when positive predepolarisations were applied before the test potential and required the coexpression of the β1b subunit with the class C α1 subunit (Bourinet *et al.*, 1994, EMBO J., **13**: 5032-5039). It could also be recorded when β1a or β3 subunits were coexpressed with α1C in oocytes, but was specific of the class C channel.

Here we show, by using a similar approach, that this facilitation is also observed in oocytes injected with the β4, but not the β2 subunit, and the α1C Ca channel subunits. Specific structural determinants on the α1 and β subunits are thus necessary for the induction of facilitation. The four β subunit genes have a similar structure composed of two conserved regions flanked by variables, alternatively spliced, NH2 and COOH termini. A third variable region resides between the two conserved segments. These three variable regions are potential candidates to account for the modulation of the facilitation recorded when different β subunits are expressed. Deletions experiments and single point mutations on these segments are under way to identify the molecular determinants of the induction of facilitation.

618.3

Genomic Analysis of a Human N-Type Calcium Channel α1 Subunit. J Francis *, JH Eubanks, and OT Jones, Playfair Neuroscience Unit, The Toronto Hospital (Western Division), Toronto, Ontario, Canada.

We are interested in studying the targeting and maintenance mechanisms governing N-type voltage-dependent calcium channel (N-VDCC) distribution in neurons, where these multimeric channels display a marked degree of polarization. To this end, an understanding of the genomic regulation of these channels is necessary. Since little is presently known about the genomic characteristics of the N-VDCC subunits, we have attempted to identify the human gene for the N-VDCC pore-forming (α1) subunit.

A human pWE2 placental cosmid library was screened with a random-primed probe derived from a 1243 bp PCR fragment from the C-terminal of the rat rbB1 α1 subunit. A single, positively hybridizing colony was identified which was confirmed to be homogeneous on a secondary screen. The cosmid has an insert of approximately 38 kb. Rare-site mapping indicates the presence of two ClaI and five SacII sites in the insert. A 4.4 kb EcoRI fragment containing a dinucleotide repeat (CA_n), identified in the insert, has been subcloned and is being investigated for possible polymorphisms. Details of PCR, sequencing and genomic structural analysis will be presented and discussed. These studies attempt to provide a first look at the genomic organization of this important class of human calcium channel subunit gene.

618.4

STRUCTURE/FUNCTION STUDIES OF THE RAT N-TYPE CALCIUM CHANNEL EXPRESSED IN *XENOPUS* OOCYTES. D. Shuey, R. Numann, Rod Parsons and R. Franco* CNS Disorders, Wyeth-Ayerst Research, Princeton, NJ 08543-8000

Modulation of the N-type Ca^{++} channel appears to play an important role in both neurotransmission and ischemic cell death (Dunlap *et al.*, 1995). In this study, we analyze the Ca^{++} currents that result from the introduction of cloned rat calcium channel cDNA's into Xenopus oocytes. Control experiments demonstrate that the elicited Ca^{++} currents are almost entirely dependent on nuclear microinjection of the rat alpha-1B cDNA (α2, β3 mean = 0.128μA; α1, β3, α2 mean = 6.6μA).

We wished to study how cyclic AMP dependent protein phosphorylation (kinase A) might alter the function of this channel. Application of forskolin (50μM) produced an immediate decrease in N-type Ca^{++} current amplitude (41.5% n=2). This decrease continued for 10-15 min. at which time a steady-state level of 74% was reached. In parallel studies we activated protein kinase A by perfusing the selective lipid soluble SP isoform of cAMP (100mM). This produced a similar decrease in the inward current (41.2% at 3 min. and 60% at 15 min., mean of two). This down-regulation was reversible.

To further explore the effects of phosphorylation and allosteric modulation, we utilized site-specific mutagenesis to create three point mutations in the alpha-1B subunit. Consensus sequences for cAMP dependent protein kinase phosphorylation (aa 898 S-A) and nucleotide binding (aa 458 K-A) were altered. Preliminary studies of these mutants have failed to detect any measurable current. Injection of a mutated putative tyrosine kinase site (aa 789 Y-F) cDNA, however, expressed as robustly as wild type and showed a consistent 12.4mV negative shift in steady-state activation (n=3). Observed changes in the inactivation of this current are also being investigated.

K. Dunlap, J.I. Luebke, and T.J. Turner (1995). Trends in Neuroscience 18:89-98

618.5

PCR AMPLIFICATION AND CLONING OF HUMAN GENOMIC AND cDNA SEQUENCES SIMILAR TO A P-TYPE Ca^{2+} CHANNEL SEGMENT FROM SCLC CELL LINES. R.K. Hajela* and W.D. Atchison, Department of Pharmacology & Toxicology and Program in Neuroscience, Michigan State University, E. Lansing, MI 48824-1317.

We are interested in studying the biology of the Ca^{2+} channels responsible for transmitter release at the motor neuron terminals using molecular clones of genes coding these channels by expression in the *Xenopus* oocyte. This information may help us gain a better understanding of presynaptic neuromuscular disorders like Lambert-Eaton Myasthenic Syndrome (LEMS). Since the soma of the motor neurons resides in the spinal cord, we decided to isolate genes from a cDNA library of human spinal cord. P-type Ca^{2+} channels, believed to be the main types responsible for Ca^{2+} influx resulting in transmitter release and muscle contraction at the neuromuscular junctions, were targeted first for these studies. PCR primers were synthesized based on the published sequence of a P-type Ca^{2+} channel segment spanning regions IV5 and IV6 (Barry *et al., 1995, J. Neurosci. 15:274-283)*. Template DNA prepared from human spinal cord cDNA libraries or cultured HPBL cells was used to amplify a corresponding fragment using these primers. The amplified DNA was cloned in pAMP1 cloning vector (Gibco-BRL Life Technologies), sequenced to ascertain the identity of cloned inserts and used as probe to isolate cDNA clones from the human spinal cord CDNA libraries. Isolated clones are being sequenced and used to assemble the entire coding sequence for expression experiments. This work was supported by grant #1-R01 NS33006 from the NIH.

618.6

THE EXPRESSION OF THE α_{1E} VDCC SUBUNIT IN HUMAN CNS.
N. Moore*[1], S.Volsen[1], A. McCormack [1], P. Craig [1], W. Smith [1], N . Day [2], P. Ince [2], P. Shaw [3], N. Smith [4], S. Ellis[5], A.Gillespie [5] and M. Harpold [5]. Lilly Research Centre[1], Windlesham, Surrey, UK ; MRC Neurochemical Pathology Unit [2] and Division of Clinical Neuroscience[3], University of Newcastle Upon Tyne, UK; Department of Paediatric Pathology [4], Royal Hospital for Sick Children, Edinburgh, UK ; SIBIA Inc[5]. La Jolla, CA 92037.

Little is known about the regional distribution of the α_{1E} VDCC subunit in human CNS. We have studied the expression of α_{1E} by in situ mRNA hybridisation and immunohistochemistry in a range of neuroanatomical sites. Areas of particular interest, for example the cerebellum and hippocampus, were examined for age related changes in α_{1E} expression by studying tissues obtained from individuals 3 weeks to 74 years of age.

Both α_{1E} mRNA and subunit protein had discrete yet widespread localisation in the adult human brain. Expression was observed, though at different apparent levels, in areas including the hippocampus, para hippocampal gyrus, inferior temporal cortex , basal ganglia, midbrain and spinal cord. The most intense signal was however noted in the cerebellum.

Within the cerebellar cortex, α_{1E} transcripts were at highest density in the Purkinje cell layer and antibody staining showed that these cells labelled intensely both in their soma, dendritic tree and axons. There was however little evidence of staining in the neuropil of the dentate nucleus. In cerebellar sections from infant brains, α_{1E} expression was less extensive than that seen in the adult and immunohistochemical analysis revealed that only a population of Purkinje cells appeared to express α_{1E}. These data show that α_{1E} is differentially distributed in the adult CNS and that its pattern of expression alters during development.

618.7

FUNCTIONAL AND PHENOTYPIC ANALYSIS OF THE EXPRESSION OF α_{1A} AND α_{1E} VOLTAGE DEPENDENT CALCIUM CHANNEL (VDCC) SUBUNITS IN RAT CEREBELLAR PURKINJE CELLS . S. Volsen, P. Craig, A. McCormack, D. Bell, C. Bath, W. Smith, D. Tupper* and D. Bleakman. Lilly Research Centre, Erl Wood Manor, Windlesham, Surrey, GU20 6PH, UK.

We have examined the relationship between whole cell VDCC current characteristics and the expression of α_{1A} and α_{1E} subunits in cerebellar tissues derived from 6-9 day old rats. These analyses were performed on Purkinje cells in parallel patch clamp-single cell reverse transcription PCR, in situ mRNA hybridisation and immunohistochemical experiments.

Whole cell voltage clamp recordings were made in acutely isolated cerebellar Purkinje cell bodies and inward calcium currents evoked by depolarisation from a holding potential of -90mV to various test potentials. Analysis of current-voltage relationships showed no evidence for the presence of T-type VDCCs. The majority of the whole cell current was inhibited by 50nM ω-Aga-IVA (89 ± 2%, n=6) and the remainder blocked by. 1μM ω-CgTx GVIA (9 ± 2%, n=4) and 5μM nimodipine (2 ± 1%, n=4). Whole cell currents were also blocked by Cd^{2+} (IC_{50} value ~ 5μM) and Ni^{2+} (IC_{50} value > 300μM).

Single cell RTPCR experiments demonstrated the presence of α_{1A} and α_{1E} transcripts in Purkinje cells which was supported by the results from in situ mRNA hybridisation analysis of cerebellar sections using α_{1A} and α_{1E} specific riboprobes. The protein products of both α1 subunits were demonstrated in Purkinje cells by immunohistochemical analysis.

The combined results demonstrate that both α_{1A} and α_{1E} subunits are present in Purkinje neuron cell bodies. Since we have no evidence for the presence of either the T or R-type VDCC in Purkinje soma, the precise role of the α_{1E} subunit in these cells remains to be elucidated.

618.8

MOLECULAR CLONING OF A CLASS D L-TYPE CALCIUM CHANNEL WITH AN ELONGATED CARBOXYL TERMINUS. E. Mathews and T.P. Snutch*. Biotechnology Laboratory, University of British Columbia, Vancouver, B.C., Canada V6T 1Z3.

Molecular cloning and transient expression studies have identified five major types of calcium channel α_1 subunits expressed in the mammalian nervous system (α_{1A}, α_{1B}, α_{1C}, α_{1D} and α_{1E}). Both the α_{1C} and α_{1D} subunits encode L-type calcium channels and are colocalized to the cell bodies and proximal dendrites of many central neurons (Hell et al., J. Cell Biol. 123:949-962). In contrast to other classes of rat brain α_1 subunits, a previously reported α_{1D} subunit (RBα1; Hui et al., Neuron 7:35-44) terminated approx. 170 residues past domain IV S6 resulting in a 1634 amino acid protein with a predicted molecular mass of 187 kDa. Utilizing the screening of a rat brain cDNA library and the PCR amplification of rat cerebellar RNA we have isolated an α_{1D} isoform similar in size to other calcium channels. Compared to RBα1, the $\alpha_{1D\text{-}a}$ protein is extended by greater than 500 amino acids in the carboxyl terminus and has a predicted molecular mass of ~ 244 kDa. The extended carboxyl terminus of $\alpha_{1D\text{-}a}$ contains several consensus sites for phosphorylation by PKA and PKC and may be involved in modulation of α_{1D} channels. Northern blot analysis indicates that the $\alpha_{1D\text{-}a}$ isoform is encoded by an ~ 9.5 kb mRNA and is expressed throughout the rat CNS. Examination of several cDNAs indicates that additional α_{1D} isoforms are generated by alternative splicing of at least two regions: the domain I-II cytoplasmic linker and the domain IV S3 transmembrane segment. While the α_{1C} and $\alpha_{1D\text{-}a}$ calcium channels share ~ 75% amino acid identity in domains I - IV, they are significantly different in the carboxyl tail (<50% identity). Current experiments are aimed at comparing the biophysical and modulatory properties of the α_{1C} and α_{1D} L-type calcium channels.

618.9

SPLICE VARIANTS OF THE *DROSOPHILA* CALCIUM CHANNEL β SUBUNIT DIFFERENTIALLY STIMULATE α_1-MEDIATED CALCIUM CURRENTS IN XENOPUS OOCYTES. Maninder Chopra*, Dejian Ren and Linda M. Hall. Dept. of Biochemical Pharmacology, SUNY at Buffalo, Buffalo, NY 14260.

The mammalian calcium channel β subunit enhances expression and modifies the kinetics and voltage-dependence of activation of α_1 subunits expressed in Xenopus oocytes. It has two conserved domains: Domain I (toward the amino end) and Domain II (toward the carboxyl end). Structure-function analysis has shown that a conserved proximal region of Domain II is necessary for its interaction with α_1 subunits (De Waard et al., *Neuron* **13**:495, 1994). A recently cloned *Drosophila* calcium channel β subunit resembles mammalian β subunits, since it has both the conserved domains. Analysis of *Drosophila* cDNA clones shows that they fall into three categories. One class (β-DN1C1) encodes both domains. The second class (β-DN1ΔC, β-DN2ΔC) encodes only Domain I. The third class (β-ΔNC1) encodes only Domain II. In addition, alternative splicing at the N-terminus generates at least two different N-terminal regions preceding Domain I. The presence of two highly conserved domains in the β subunit in species ranging from *Drosophila* to human suggests that both domains must play important roles in the organism. Expression of spliced forms with Domain II (β-ΔNC1) with a cardiac α_1 subunit in Xenopus oocytes alone was sufficient to stimulate calcium channel currents, while coexpression with Domain I alone (β-DN1ΔC) was without an effect. *Drosophila* genetic tools will be useful to determine the functional significance of conserved Domain I.

618.10

PREPULSE FACILITATION OF CARDIAC ALPHA ONE AND ENDOGENOUS CALCIUM CHANNELS IN *Xenopus laevis* OOCYTES. M. Chahine[1], A. Figourov*[2] and A. Sculptoreanu[3]. Laval Hospital Research Center[1], Laval University, Ste-Foy, Qc, Canada, Roche Inst. for Mol. Biol[2]. Nutley, NJ, Lady Davis Institute of Jewish General Hospital,[3], McGill University, Montreal, Qc, Canada.

We reported previously that cardiac L-type calcium channels are facilitated by depolarizing prepulses and this type of facilitation requires phosphorylation by cAMP dependent protein kinase (PKA). *Xenopus laevis* oocytes can be selected to express relatively high levels of endogenous Ca-currents. These currents are facilitated by prepulses. Facilitated endogenous Ca-currents are unaffected by okadaic acid, Rp-cAMP or the dihydropyridine (DHP) antagonist (+)PN200-110. The endogenous current and facilitation of endogenous current are fully blocked by 1 mM Cd^{2+}. In contrast, oocytes injected with mRNA encoding for the rabbit cardiac α_1-subunit, express prepulse facilitated Ca-channel currents which are highly enhanced by phosphoprotein phosphatase inhibitor okadaic acid (3 fold), blocked by Rp-cAMP and the DHP antagonist (+)PN200-110. While okadaic acid selectively stimulates prepulse facilitated cardiac Ca-currents, L-type Ca-channel agonist (+)SDZ202-791 largely increases (5 fold) both the control (before prepulse) and prepulse facilitated currents. (+)SDZ 202-791 did not prevent the effect of Rp-cAMP or okadaic acid on facilitation of cardiac α_1 suggesting that stimulation by Ca-agonist is independent of phosphorylation leading to channel facilitation. In conclusion, oocytes express a Cd-sensitive-DHP-insensitive endogenous Ca-current which is facilitated by depolarizing prepulses. However, unlike facilitation of cardiac α_1 expressed in oocytes which is mediated by PKA phosphorylation, prepulse facilitation of endogenous Ca-channels may be mediated by a yet a unrecognized protein kinase. (Supported by grants from MRC to AS and MC and Heart Foundation to MC and AS. PN200-110 & SDZ 202-791 were gifts from Sandoz Canada.)

618.11

HUMAN CALCIUM CHANNEL α_{1C} SUBUNIT SPLICE VARIANTS SHOW DIFFERENT VOLTAGE-DEPENDENT INHIBITION BY DIHYDROPYRIDINES. Nikolai M. Soldatov, Alexandre Bouron, Harald Reuter and Erwin Sigel*. Dept. of Pharmacology, Univ. of Bern, Friedbuehlstrasse 49, CH-3010 Bern, Switzerland.

Voltage-dependent inhibition by 1,4-dihydropyridines is a characteristic property of L-type calcium channels. Eight out of 50 exons of the channel α_{1C} subunit gene are subjected to alternative splicing, thus generating channel isoform diversity. Using *Xenopus* oocytes as an expression system, we have found that transmembrane segments IIIS2 and IVS3 of human α_{1C} subunit, genetically regulated through alternative splicing of exons 21/22 and 31/32, respectively, are involved in the control of voltage-dependence of dihydropyridine action. When elicited from the holding potential V_h = -40 mV, Ba^{2+} current through both $\alpha_{1C,21,32}$ and $\alpha_{1C,22,32}$ channels was inhibited by (+)-isradipine with $IC_{50} \approx 7$ nM. However, at V_h = -90 mV the inhibitory potency of the drug towards $\alpha_{1C,21,32}$ was 8.6-fold lower than for $\alpha_{1C,22,32}$. Site-directed mutagenesis points to two amino acids (Gly/Phe-954 and Tyr/Ile-958), which determine the difference of the splice variants $\alpha_{1C,21,32}$ and $\alpha_{1C,22,32}$ in their sensitivities to dihydropyridines. This difference was sharply diminished by incorporation of exon 31 instead of exon 32. Our finding provides new insight into molecular mechanisms of calcium channel inhibition by this important class of drugs.

This work was supported by grants from the Swiss National Science Foundation (31-29862.90) and from the Sandoz Foundation.

CALCIUM CHANNELS: MODULATION BY PEPTIDES AND HORMONES

619.1

SELECTIVE VOLTAGE-DEPENDENT AND VOLTAGE-INDEPENDENT INHIBITION OF CALCIUM CHANNEL SUBTYPES BY OPIOIDS IN BOVINE CHROMAFFIN CELLS. A. Albillos, E. Carbone§, L. Gandia, A.G. García and A. Pollo*§. Dept. of Pharmacology, Univ. of Madrid, Spain. (§)Dept. of Anatomy and Human Physiology, Univ. of Turin, Italy.

The opioid agonists methionine-enkephalin and DAGO (0.1-10 μM), markedly and reversibly inhibited the high-voltage-activated Ba^{2+} currents of bovine chromaffin cells. Inhibition depended on the day of cell culture (50-60% in 1-2 days old cells, 20% in cells 7 days old) and caused a pronounced slow-down of current activation together with a marked amplitude depression. The slow phase of activation was relevant around 0 mV but decreased at more positive potentials (>+30 mV). Strong facilitating pre-pulses (50 ms, +90 mV) induced only a partial recovery of the inhibition (60%, voltage-dependent) suggesting the presence of a significant voltage-independent component (40%). In most cells, both inhibitions were effectively removed by intracellular perfusion with GDP-β-S (0.2-1 mM) or by cell preincubation with pertussis toxin (100 ng/ml, 24 h). The opioid-induced inhibition was not selective for any Ca^{2+} channel subtype, being not prevented after the addition of specific Ca^{2+} channel antagonists (nifedipine, ω-CTx-GVIA, ω-Aga-IVA, ω-CTx-MVIIC). However, when separately analyzing the contribution of each channel subtype to the voltage-dependent and voltage-independent modulation, a sharp distinction could be achieved. The voltage-dependent process was abolished by acute application of 3 μM ω-CTx-MVIIC and unaffected by 3 μM nifedipine, suggesting a selective action on non-L-type channels (N-, P-, and Q-types). On the contrary, the voltage-independent opioid inhibition was sensitive to all Ca^{2+} channel antagonists, particularly to nifedipine (3 μM), suggesting that the voltage-insensitive modulation is common to all Ca^{2+} channel subtypes but predominant on L-type channels. Our data suggest that Ca^{2+} channels which are crucial for the control of catecholamine and opioid secretion from chromaffin cells (L- and Q-type) may be differently modulated by the secreted material itself.

619.2

MODULATION OF CALCIUM CHANNELS BY OPIOIDS, BACLOFEN AND SOMATOSTATIN IN ACUTELY DISSOCIATED NEURONS FROM THE RAT NUCLEUS TRACTUS SOLITARIUS (NTS). Hyewhon Rhim*, Peter T. Toth and Richard J. Miller. Department of Pharmacological and Physiological Sciences, The University of Chicago, Chicago IL 60637.

We previously reported that activation of opioid receptors inhibit N- and P/Q-type Ca^{2+} channels but not L-channels in neurons acutely isolated from the rat NTS (J Neurosci, 14:7608, 1994). In the present studies, we further investigated the coupling mechanism between opioid receptors and Ca^{2+} channels and compared this with the effects of baclofen and somatostatin in these cells. 30 μM baclofen and 300 nM somatostatin suppressed 40% (n=9) and 28% (n=6) of peak Ca^{2+} whole cell currents respectively and targeted the same types of Ca^{2+} channels (N & P/Q) which were inhibited by opioids. The inhibition of Ca^{2+} currents by the μ-selective opioid agonist, DAMGO, baclofen and somatostatin was reduced by overnight treatment of 200 ng/ml PTX. It is known that G-protein-mediated inhibition of Ca^{2+} currents can be relieved by strong depolarization. We examined neurotransmitter-mediated inhibition of Ca^{2+} currents using 50 ms prepulse to +80 mV prior to the test pulse. The mean inhibition of peak current produced by DAMGO, baclofen and somatostatin was 42(n=4), 41(6), and 36(6)% for a constant voltage step (HP = -80 mV, TP = 0 mV). This inhibition was decreased to 23, 25, and 18% by a strongly depolarizing prepulse. In order to investigate which PTX-sensitive G-protein transduces the inhibitory signal to Ca^{2+} channels, we examined cells dialyzed with the specific G-protein antibody, GC/2, raised against the N-terminal of $G_{\alpha o}$. A 30 min perfusion of cells with non-immune serum did not alter the inhibitory response to the three neurotransmitters. Intracellular loading with GC/2 markedly attenuated the somatostatin-induced inhibition (n=11), but did not block the DAMGO- (n=5) and baclofen- (n=14) induced inhibition. These findings suggest at least two different PTX-sensitive G-protein-mediated pathways are involved in receptor-induced inhibition of Ca^{2+} currents in the NTS.

619.3

ENKEPHALINS MODULATE DEPOLARIZATION-ACTIVATED CALCIUM CONDUCTANCES IN RAT INTRACARDIAC NEURONS. D.J. Adams*, and C. Trequattrini. Dept. of Physiology & Pharmacology, Univ. of Queensland, Brisbane, QLD 4072, Australia and Dept. of Pharmacology Univ. of Miami, FL 33101, USA.

Modulation of depolarization-activated Ca^{2+} channels by enkephalins was investigated in isolated parasympathetic neurons of rat intracardiac ganglia using the amphotericin B perforated-patch technique. Focal application of met-enkephalin (10 μM) to the soma membrane did not alter the resting membrane potential but reduced the maximum amplitude and slowed the rate of rise and repolarization of the action potential reversibly. Under voltage-clamp, Met-enkephalin reversibly inhibited the peak amplitude of Ba^{2+} currents evoked at 0 mV by 53 % and increased 3-4-fold the time to peak. Met-enkephalin had no effect on either voltage-dependent Na^+ or K^+ currents in rat intracardiac neurons. Half-maximal inhibition of I_{Ba} was obtained with ~300 nM met-enkephalin or leu-enkephalin. The enkephalin derivatives, DAMGO (1 μM) and DADLE (5 μM) but not DPDE (10 μM) inhibited Ca^{2+} channel currents and were antagonized by the opioid receptor anatgonists, naloxone and naltrindole with IC_{50}'s of ~100 nM and 1 μM, respectively. Taken together, these data suggest that enkephalin-induced inhibition of Ca^{2+} channels in rat intracardiac neurones is mediated by the μ- and δ-opioid receptor types. Inhibition of I_{Ba} amplitude by enkephalins was abolished using the conventional (dialyzed) whole cell recording configuration but was observed when 100 μM GTP was added to the pipette internal solution. Internal perfusion of the cell with GDP-β -S or incubation of the neurons in *Pertussis* toxin (PTX) also abolished the modulation of I_{Ba} by enkephalins, suggesting the involvement of a PTX-sensitive G-protein in the signal transduction pathway. Given that enkephalin peptides have been shown to be synthesized and secreted from cardiac muscle (Springhorn & Claycomb, 1992, *Am. J. Physiol.*, 263, H1560-H1566), the activation of opioid receptors in postganglionic intracardiac neurons suggests that it may serve for n-egative feedback regulation of vagal transmission to the heart.

619.4

ACETYLCHOLINE AND ENKEPHALIN INHIBIT HIGH AND LOW VOLTAGE-ACTIVATED CALCIUM CHANNELS IN THALAMIC RELAY NEURONS. A. Formenti*, E. Arrigoni, A. Bejan, G. Avanzini#, M. Mancia. Inst. of Human Physiol. II, Univ. of Milan and #Dip. of Neurophysiol., Nat. Inst. of Neurol. C. Besta, Milan I-20133 Italy.

Thalamic relay neurons present two different functional states. During wakefulness they send sensory information to the cortex from the periphery. During synchronized sleep and epileptic seizures they show an oscillatory burst discharge. Ca^{2+} currents are crucial in determining the characteristic firing patterns. LVA Ca^{2+} channels can give rise to low threshold Ca^{2+} spikes. On top of these, a high-frequency burst discharge can occur. HVA Ca^{2+} channels contribute to the intracellular Ca^{2+} rising phase. Calcium entry into the cell is responsible for a Ca^{2+}-dependent K^+-current activation and burst termination. The modulatory effects induced by acetylcholine (ACh) and D-ala^2-D-leu^5-enkephalin (DADLE) on voltage-dependent Ca^{2+} channels have been investigated. The experiments were carried out on acutely dissociated neurons from the ventrobasal thalamus, of 2-14 day-old Wistar rats. The currents were recorded by means of whole-cell patch-clamp technique. ACh (20 μM) extracellularly perfused inhibited the peak amplitude of HVA Ca^{2+} currents of an average -54% in 26 out of the 36 cells tested (+10 mV). DADLE (400 nM) reduced HVA Ca^{2+} currents -58% in 31 out of 38 cells (+10 mV). The two neurotransmitters induced a voltage-dependent inhibition, relieved at more positive potentials and/or a voltage-independent effect persistent at all the potentials tested [*Formenti et al. 1993 Biophys J. 64:1029-1037*]. LVA Ca^{2+} currents were less sensitive to the two neurotransmitters (DADLE -40% in 4 out of 11 cells ACh -40% in 2 out 33). In conclusion, the modulation of the voltage-dependent Ca^{2+} channels may play a role in the production and control of the rhythmic discharge during slow-wave sleep and pathological seizures. The action of DADLE suggests a postsynaptic inhibitory mechanism on nociceptive pathways.

619.5

DYNORPHIN MODULATES DISTINCT CALCIUM CHANNEL SUBTYPES IN THE SOMATA AND AXON TERMINALS OF A MAMMALIAN CENTRAL NEURON. T.E. Fisher* and C.W. Bourque. Centre for Research in Neuroscience, Montreal General Hospital and McGill University, Montreal, Canada, H3G 1A4.

Although much is known about the modulation of calcium channels in neuronal somata, less is known about effects in nerve terminals. This is of particular importance since effects there are likely to influence transmitter release. The magnocellular neurosecretory cells (MNCs) of the rat supraoptic nucleus offer an opportunity to compare effects in the somata and axon terminals since both can be isolated and subjected to whole cell patch clamp. Dynorphin A (1-13), a κ-opioid selective agonist, may modulate influx through voltage-gated calcium channels in both locations since it has been shown to decrease the calcium component of action potentials in MNC somata as well as oxytocin release from MNC terminals. Addition of 1 μM dynorphin A to terminals selectively and reversibly decreased an inactivating (τ= 132±31 ms at -10 mV; n= 7), ω-CTX-sensitive component of the calcium current. In the somata, dynorphin A inhibited two ω-CTX-insensitive currents: a low-threshold, rapidly-inactivating current (τ= 62±6 ms at -10 mV; n= 8), likely a T-type, and a high-threshold, non- or slowly-inactivating current. These data demonstrate that dynorphin A modulates multiple calcium channel subtypes and that these actions are segregated in different cellular locations. *Supported by the Heart & Stroke Foundation and the Medical Research Council of Canada.*

619.6

SOMATOSTATIN TARGETS BOTH N- AND L-TYPE CALCIUM CHANNELS IN CHICK PARASYMPATHETIC NEURONS. Mark A. Crumling & Stephen D. Meriney* Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

Somatostatin (SOM) is endogenous to choroid neurons in the chick ciliary ganglion and is a potent inhibitor of transmitter release at choroid nerve terminals. At nerve terminals *in vivo*, the calcium channel types that regulate transmitter release switch during development from L-type before hatching to N-type after hatching (Gray et al., 1992 *Neuron* 8:1-20). Both ciliary and choroid neuron cell bodies express SOM receptors that can mediate a ~50% reduction in calcium current and an ~80% reduction in potassium-evoked somal ACh release. Here, we report the use of perforated patch recordings (R_{access} = 11 MΩ) from acutely dissociated ST 40 ciliary ganglion neurons to test the hypothesis that SOM targets both N- and L-type calcium channels. Ciliary ganglion neurons expressed calcium current that was comprised of 60% N (sensitive to 1 μM *w*-CgTX GVIA), 31% L (sensitive to 5 μM nitrendipine) and 9% non-N or L (as yet unidentified). SOM (100 nM) inhibited 25 pA/pF (49%) of current from control cells, 15 pA/pF (37% of control current) from cells pretreated with 5 μM nitrendipine, 5 pA/pF (9%) from cells pretreated with 1 μM *w*-CgTX GVIA, and 2 pA/pF (4%) from cells pretreated with both nitrendipine and *w*-CgTX GVIA. SOM affected Bay K 8644-induced tail currents in a complex manner suggesting either multiple L- channel subtypes or voltage-dependent effects at hyperpolarized potentials. These data indicate that SOM targets both N- and L-type calcium channels. Supported by Univ. of Pitt. Small Grants Program, a Winters Foundation Award, and NIH NS 32345.

619.7

RECEPTOR REGULATION OF Ca^{2+}-CHANNELS IN HEK 293 CELLS. Lee R. Shekter*, Arthur A. Simen, Peter T. Toth & Richard J. Miller. Dept. of Pharmacology and Physiology, University of Chicago, Chicago, IL 60637

Receptors have been shown to regulate voltage sensitive Ca^{2+}-channels through G-proteins and second messengers. We compared receptor regulation of N (α1B) and R (α1E) currents in HEK 293 cells. N-type and R-type Ca^{2+}-channels consisted of $\alpha_{1B\text{-}1}\alpha_{2b}\beta_{1\text{-}3}$ and $\alpha_{1E\text{-}3}\alpha_{2b}\beta_{1\text{-}3}$ subunits respectively. Perfusion of both types of cells with GTP-γ-S produced inhibition of the Ca^{2+}-currents which was relieved by prepulse depolarization. N-channel activity in on cell and excised patches could be inhibited by F^- and GTP-γ-S respectively. The somatostatin analogue ([D-Trp^8]-Somatostatin, 300nM) inhibited the whole cell N-current by 17.6% (n=23). Prepulse depolarization also relieved the inhibitory effect of somatostatin. The somatostatin analogue (300nM) inhibited the R-current to a lesser extent. The somatostatin effect was also tested in HEK 293 cells expressing N-type Ca^{2+}-channels with different subunit compositions. Somatostatin was still effective when the β-subunit was replaced with a different splice variant ($\beta_{1\text{-}2}$). HEK 293 cells also express muscarinic receptors. Muscarinic receptor activation released Ca^{2+} from intracellular stores. However it did not inhibit the whole-cell Ca^{2+}-current. The above experiments suggest the heterogeneity of the signalling pathways for different Ca^{2+}-currents.

619.8

SOMATOSTATIN (SS) MODULATION OF HVA CALCIUM CURRENTS IN ACUTELY ISOLATED NEURONS OF RAT AMYGDALA. F.Viana* and B. Hille University of Washington, Dept. of Physiology & Biophysics, Seattle, WA 98195.

Immunohistochemical and autoradiographic studies have revealed the presence of the neuropeptide SS and SS receptors (SSR) in the amygdala. We used the whole-cell configuration of the patch-clamp technique to investigate the effects of SS and SS-analogs on calcium currents in acutely dissociated, pyramidally-shaped, rat amygdala neurons (P7-P21). Depolarizing voltage steps from -90 mV revealed a robust, Cd^{2+}-sensitive, inward current (1821 ± 659 pA, n=23) that peaked at -10 mV and inactivated partially within 100 ms. Application of SS reduced peak inward currents (5-30%) in a reversible and dose-dependent manner. During prolonged applications (3 min) the inhibition showed little desensitization (<20%). The inhibition was voltage-dependent and accompanied by a marked slowing in activation kinetics. Brief prepulses to +70 mV produced a partial relief of the inhibition and slowing. Application of BIM-23060 (2-200 nM), a SSR2 agonist, but not BIM-23058, a SSR3 agonist, mimicked all the effects of SS. Cell dialysis with GTPγS also mimicked and occluded the actions of subsequent SS application. A 2 min pre-incubation with the alkylating agent N-ethylmaleimide (50 μM) prevented the modulatory effects of SS. A double pulse protocol isolated a transient LVA calcium current that was not inhibited by SS.

In the presence of ω-CgTX GVIA (2-4 μM) or ω-AgTx IVA (100 nM), the % inhibition was reduced, while the combined application of these toxins nearly blocked the modulation, suggesting the involvement of N- and P-type calcium channels. The L-type channel blocker nifedipine (10 μM) reduced the peak inward about 20% without affecting the modulation by SS. The slow tail currents induced by +202-791 were slightly reduced by SS, but also by ω-CgTX GVIA.

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619.9

VARIOUS NEUROTRANSMITTERS INHIBITED N- AND Q-TYPE CA^{2+} CHANNELS *VIA* DIFFERENT G-PROTEINS IN RAT SYMPATHETIC NEURONS. M. Miyake[1], R. Shibata[1] and A.Urano[2]*. [1]Faculty of Pharmaceu. Sci. and [2]Grad. School of Sci., Hokkaido Univ., Sapporo 060, Japan.

Recent progress in pharmacological studies reveals that at least six classes (T-, L-, N-, P-, Q- and R-types) of functional Ca^{2+} channels were present in the central and peripheral nervous system. Furthermore, various neurotransmitters modulate some Ca^{2+} channels through intracellular signaling pathways coupled to separate G-proteins.

In this study, we measured Ba^{2+} currents through Ca^{2+} channels in cultured neurons (up to 5 weeks) dissociated from superior cervical ganglia of a new born rat. Whole-cell recordings showed that these Ba^{2+} currents were approximately composed of 60% of the ω-CTX-GVIA sensitive- (N-type), 20% of the nifedipine sensitive- (L-type), less than 2% of the ω-Aga-IVA (30 nM) sensitive- (P-type), 10% of the ω-CTX-MVIIC sensitive- (Q-type) and the residual components. Norepinephrine (NE) and somatostatin (SST) caused modulating effects (inhibition) on the N- and Q-type current components through pertussis-toxin sensitive intracellular pathway(s). Vasoactive intestinal peptide (VIP) and bradykinin (BK) brought about a similar inhibition of the two components through a cholera-toxin sensitive pathway and through that resistant to both toxins, respectively. The inhibition of the N-type currents was voltage-dependent, whereas the inhibition of the Q-type currents was voltage-independent.

619.10

CALCIUM CURRENTS AND CORTICOTROPIN-RELEASING FACTOR (CRF) IN PYRAMIDAL AND NON-PYRAMIDAL NEURONS OF THE BASOLATERAL AMYGDALA (BLA). P. Shinnick-Gallagher,* F-C. Chang, P.W. Gean and B. Yu. Dept. of Pharmacology, University of Texas Medical Branch, Galveston, TX 77555-1031.

We have previously shown that in the central amygdala (CeA), CRF enhances calcium currents (I_{Ca}) and that the I_{Ca} is composed of L-(22%), N-(30%), and Q-(12%) type currents and dihydropyridine (DHP) and neurotoxin-resistant (37-53%) currents. In the present study we analyzed I_{Ca} and CRF actions in the BLA using whole cell patch recording in young rats. DHP-sensitive (L-type) currents comprised ~34%, while *w*-conotoxin GVIA (N-type) account for 15% of I_{Ca} in pyramidal cells (n = 17). Q-type, *w*-conotoxin MVIIC- or P-type, *w*-agatoxin IVA-sensitive, currents contributed to 3% and 5% of I_{Ca} respectively. I_{Ca} in non-pyramidal neurons was composed of L-(33%), N-(13%), Q-(3%) and P-(8%) type currents. A resistant current comprised ~43% and 43% of the total I_{Ca}, in the respective neurons. CRF (50 mM) inhibited I_{Ca} ~17% in 10/15 pyramidal and ~12% in 6/7 non-pyramidal neurons while it enhanced I_{Ca} ~23% in 5/15 pyramidal neurons. These data suggest that the composition of I_{Ca} in the CeA and BLA are different and that the excitatory and inhibitory actions of CRF in the amygdaloid nuclei may underlie the differential function of these structures in the stress response (supported by NS29265).

619.11

CORTICOSTEROID ACTIONS ON IONIC CONDUCTANCES IN ACUTELY DISSOCIATED RAT HIPPOCAMPAL NEURONS. T. R. Werkman*, S. van der Linden, M. Struik and M. Joëls. Dept. Exp. Zoology, University of Amsterdam, Kruislaan 320, 1098 SM Amsterdam, The Netherlands.

Previous *in situ* whole-cell voltage clamp studies have shown that steroid hormones are involved in the regulation of the excitability of hippocampal neurons by acting at mineralocorticoid (MR) and glucocorticoid (GR) receptors. In the present study whole-cell voltage clamp recordings of acutely dissociated CA1 neurons were made. As in the *in situ* experiments it was found that in neurons of adrenalectomized Wistar rats (weight 100-200 gram) the amplitude of voltage-dependent calcium currents was increased as compared with currents in neurons treated with 30 nM corticosterone (occupying both MR and GR) or neurons of sham-operated rats (peak current amplitudes at 0 mV: 1.12 ± 0.09 nA (n=56), 0.66 ± 0.03 nA (n=50), 0 72 ± 0.06 nA (n=33), respectively). However, selective activation of the MR after treatment with 30 nM corticosterone and 500 nM of the GR antagonist RU38486, did not decrease calcium current amplitudes (peak current amplitude: 0.72 ± 0.06 nA (n=25)), as was observed in the *in situ* experiments. Similar observations were made in acutely dissociated neurons of adrenalectomized rats injected with steroid and antagonist. Since in the acutely dissociated cell preparation the neurons have lost most of their dendritic processes, this indicates that the current involved in the MR effect in the slice preparation has a dendritic origin. Other electrophysiological characteristics of the calcium currents like threshold of activation, activation time, time-course of inactivation and steady-state inactivation were not affected by steroid treatments. In old animals (weight ± 400 gram) the difference in calcium current amplitudes between adrenalectomized and sham-operated was not observed anymore. To know whether the calcium currents are the only voltage-dependent ionic conductance affected by corticosteroid receptor activation, we are currently investigating the effects of steroid treatments on sodium currents in acutely dissociated neurons

619.12

CHRONIC GLUCOCORTICOID TREATMENT ENHANCES HIGH VOLTAGE-ACTIVATED CALCIUM CURRENTS IN RAT HIPPOCAMPAL NEURONS IN CULTURE. N.M. Porter*, V. Thibault and P.W. Landfield. Dept. Pharmacology, Univ. of Kentucky, Lexington, KY 40536.

Corticosterone has been shown to enhance the slow Ca^{2+}-dependent afterhyperpolarization in rat hippocampal slice neurons. In addition, specific glucocorticoid receptor agonists increase Ca^{2+} spikes and currents through a cycloheximide-sensitive process in the same preparation. However, prior studies have used relatively high concentrations of glucocorticoids and the hippocampal slice is a preparation generally not well suited for long-term pharmacological manipulation or concentration-response analyses. In the present studies, therefore, we chose to study the actions of a glucocorticoid receptor type II agonist, dexamethasone (DEX), on Ca^{2+} currents in hippocampal neurons in culture using more physiologically relevant concentrations.

Primary hippocampal neurons were isolated from fetuses of pregnant (E18) Fischer 344 rats. Cells were subsequently treated with either ethanol vehicle (0.05%) or DEX (0.1 to 1000 nM) on days 3 and 6 in culture. High voltage-activated Ca^{2+} currents were recorded on day 7 using the whole cell patch clamp technique.

The peak current amplitude was similar for pyramidal neurons chronically treated with either ethanol vehicle or concentrations of DEX (0.1 and 1 nM) below the Kd for the receptor. However, peak currents were increased by 40-60% in cells which had been treated with higher concentrations of DEX. The present results indicate that chronic treatment with physiologically relevant concentrations of glucocorticoids increases Ca^{2+} currents. Thus, adrenal steroid hormones may modulate Ca^{2+} channel function in mammalian neurons. (Supported by AG10836)

619.13

DUAL PATCH PIPETTE RECORDINGS IN HIPPOCAMPAL NEURONS: EVIDENCE THAT LONG Ca^{2+} TAIL CURRENTS REFLECT Ca^{2+} CHANNEL ACTIVITY AT RESTING POTENTIAL. O. Thibault*, N.M. Porter, M.L. Mazzanti-Rose, L.W. Campbell, E.M. Blalock and P.W. Landfield. Dept. Pharmacology, Univ. Kentucky Med. Center, Lexington, KY 40536.

We have previously reported the presence of a long-lasting Ca^{2+} or Ba^{2+} tail current which outlasts a depolarization pulse by several hundred to thousand milliseconds in cultured and adult hippocampal neurons. This tail current could arise from Ca^{2+} channel activity at resting potential or, alternatively, from inadequate space clamp of the large apical dendrite or from non-Ca^{2+} currents.

To resolve this issue, we conducted experiments in cultured neurons using dual patch pipette recordings. Simultaneous recordings from the soma and from mid-way along the dendrite were performed on the same cells. Current clamp recordings at the dendritic pipette showed that the dendritic voltage response mirrored the depolarization command step given at the somal pipette, including a rapid return to -70 mV. Further, simultaneous voltage clamp of the dendrite and soma did not selectively reduce the somal tail current, indicating that the Ca^{2+} tail is not of dendritic origin. In addition, severing or applying Cd^{2+} to the dendrite did not selectively reduce the tail at the soma. A series of pharmacologic/ionic manipulations ruled out non-Ca^{2+} currents as the basis for the tail.

Thus, the long Ca^{2+} tail currents reflect true Ca^{2+} channel activity at resting potential levels, apparently due to a reversible voltage-shifted mode of the channel, and may mediate a major unrecognized pathway of Ca^{2+} entry. (Supported by AG04542, AG10836 and Bayer Corp).

CALCIUM CHANNELS: CHARACTERIZATION AND ELECTROPHYSIOLOGY

620.1

CALCIUM CURRENTS IN HUMAN POLYMORPHONUCLEAR NEUTROPHIL LEUKOCYTES. M.A. SCHUMANN*, Y. OKA, M. NAKAYAMA, N. HASEGAWA, and T. A. RAFFIN. *Division of Pulmonary and Critical Care Medicine, Stanford University School of Medicine, Stanford, California 94305-5236.*

Calcium involvement in neutrophil activation is underscored by its mediation of a variety of cytoplamic processes and changes in membrane potential. Our laboratory has investigated Ca^{2+} oscillations and activation of membrane ion channels in human neutrophils. Although nonselective cation, potassium, and chloride channels in these cells have been found to be Ca^{2+}-activated, the presence of Ca^{2+}-selective channels linking transient changes in membrane conductance to neutrophil signaling have not been characterized. In the present study, we recorded Ca^{2+} currents in human neutrophils using barium, as the major charge carrier, and the electrophysiological whole-cell patch-clamp technique. The sodium-free bath solution contained $Ba(CH_3CO_2)_2$ 100 mM, $MgCl_2$ 2 mM, HEPES 10 mM, and glucose 15 mM (pH 7.4). The pipette solution included $CsCH_3CO_2$ 140 mM, EGTA 0.5 mM, $MgCl_2$ 2 mM, ATP (Mg salt) 2 mM, and HEPES 10 mM (pH 7.3). Transient inward currents were elicited at room temperature by depolarizing 25-mV steps (between -75 and +75 mV) delivered from holding potentials ranging from -60 to -100 mV. Currents reached a peak value between -40 and -10 mV (n = 15). As the depolarization was raised the current eventually became outward with a reversal potential of ≈ +40 mV. Currents were activated at holding potentials < -60 mV, and fully inactivated at holding potentials > -60 mV. In addition, currents decreased when Ba^{2+} decreased, and were significantly blocked by 1 mM Cd^{2+}. These electrophysiological observations suggest that these currents are contributed by Ca^{2+} channels of low-voltage-activated "T"-type. This work was supported by National Institutes of Health Grant HL455330.

620.2

Differential Ontogeny of Calcium Currents in the Rat Nigro-Striatal System. T. DeFazio* and J.P. Walsh, Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089-0191

Thick slice patch clamp techniques were used to examine changes in morphology and calcium channel physiology during rat postnatal development. Biocytin-filled dopaminergic Substantia Nigra pars Compacta (SNc) neurons at 1 day postnatal (1d) displayed extensive dendritic arbors comparable to those observed in adult tissue. At birth, striatal medium spiny (MS) neurons were aspiny and bipolar with tufts of dendrites at each end and a tip-to-tip diameter of ~100μm. During the first two postnatal weeks these tufts extended, the two primary dendrites began to split, and a few spines were present. By adulthood these neurons exhibited many spiny dendrites extending hundreds of microns.

Whole cell calcium currents recorded from 0-30d rats revealed both different types and developmental time courses of calcium currents. 0-2d SNc neurons exhibited typical ~100pA LVA and sustained HVA inward currents. Unclamped 100-500pA HVA currents appeared after 2d. After 7d LVA currents were no longer observed and the runaway HVA currents increased to adult levels (>500pA). ~20% of the HVA current was blocked by 5μM SNX-124 (ω-conotoxin GVIA, N-type calcium channel antagonist).

0-2d MS neurons also exhibited Ni^{2+}-sensitive ~60pA LVA currents (although with a more depolarized activation profile); but, very little HVA current was observed (<50pA). After 3d, LVA currents disappeared and runaway 100-300pA HVA currents appeared. ~30% of the HVA current was blocked by 5μM SNX-124.

Peptide toxin kindly provided by Louis Brogley at Neurex. Supported by NIA Grants 5PO1 AG0979 and AG00093.

620.3

AN ANALYSIS OF THE LOW THRESHOLD CALCIUM CONDUCTANCE IN THE SUBSTANTIA NIGRA. L. R. Johnson and S.A. Greenfield* University Department of Pharmacology, Oxford, UK. OX1 3QT

The low threshold calcium conductance (LTS, T type channel) has been well documented in the thalamus, the hippocampus CA3 and the anterior SNpc (Nedergaard and Greenfield, 1992) where in all cases its activation leads to a burst of action potentials. However, more recently an apparent LTS has been described in some dopaminergic neurons of the rat SNpc (Kang and Kitai, 1993) which does not lead to a burst of action potentials *in vitro*.

Using the *in vitro* intracellular current clamp technique we have explored this apparent difference in the LTS in two populations of guinea-pig SNpc neurons with similar morphology. We identified the LTS in 10% of posterior SNpc neurons and all of the anterior SNpc neurons. Activating the LTS with a depolarising current pulse or rebound hyperpolarisation led to bursts of action potentials in the anterior SNpc neurons; however a burst was never activated in the posterior SNpc. The presence of the LTS was later confirmed by blocking action potentials with TTX.

A further difference between the LTS in anterior and posterior SNpc populations was found in sensitivity to pharmacological blockade. For example in the presence of TTX equimolar concentrations of cadmium was applied: It was found that the LTS in anterior SNpc cells was abolished while the LTS in the posterior SNpc cells was not.

These results suggest that two distinct T channels may operate in different populations of substantia nigra neurons. Although the function of that seen in the anterior SNpc appears to be to favour generation of burst firing, that in the posterior SNpc is still unknown; perhaps it could play a role in dendritic dopamine release.

Nedergaard, S. and Greenfield, S. A. (1992) Neuroscience 48, 423-437.
Kang, Y and Kitai, S. (1993) Neuroscience Research, 18 195-207.

620.4

MULTIPLE COMPONENTS OF THE LOW-THRESHOLD CALCIUM CURRENT IN HIPPOCAMPAL CA3 PYRAMIDAL NEURONS. R.B. Avery* and D. Johnston, Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

In recent years there has been compounding evidence that low-threshold Ca^{2+} currents contribute to sub-threshold signalling in central neurons. Because they may be particularly crucial to the physiological functions of hippocampal CA3 pyramidal neurons, we have studied the low-threshold Ca^{2+} current in these cells.

We used 2 mM Ca^{2+} to record whole-cell Ca^{2+} currents from acutely isolated CA3 pyramidal cells from rats age 7–14 days. We elicited low-threshold currents by giving test pulses to -50 mV from a holding potential $\leq$ -80 mV. High-threshold currents were isolated by holding at -50 mV and stepping to 0 mV. Long (600 ms) pulses to -50 mV revealed two kinetically distinct components to the low-threshold Ca^{2+} current: one inactivating (mean amplitude 38±3 pA) and one non-inactivating (23±7 pA). The inactivating component decayed with a time constant of 55±6 ms. Half-maximal steady-state inactivation of the component occurred at -86 mV. The inactivating current was preferentially blocked by nickel and amiloride.

The non-inactivating component persisted throughout the step, even for depolarizations lasting several seconds. As measured at the end of a 600 ms pulse, the sustained component was enhanced by switching to equimolar Ba^{2+} (2 mM, 113±9%), a manipulation that reduced the amplitude of the inactivating component (66±6%). The sustained component was inhibited by nimodipine (10 μM, 75±4%) and potentiated by BayK8644 (1 μM, 126±6%). It was also inhibited by the L-channel toxin calciseptine (5 μM, 75±2%). The inactivating component was not affected by these agents.

These data suggest that at least two-types of Ca^{2+} channels can be operative at potentials near rest; the inactivating T-type channel and a non-inactivating, dihydropyridine-sensitive channel (MH10473, MH44754, MH48432, NS11535).

620.5

CALCIUM CHANNELS OF EMBRYONIC RAT SPINAL MOTONEURONS B. Hivert and D. Pietrobon* Dept. of Biomedical Sciences, Univ. of Padova, 35121 Padova, Italy.

Single channel patch-clamp recordings (with 90 mM Ba^{2+} as charge carrier) were used to characterize the calcium channels expressed by E15 rat spinal motoneurons purified by immunopanning and maintained in culture for 1-2 days. The Ca^{2+} channels most frequently observed (in 20 out of 40 patches with 1-3 channels) were dihydropyridine (DHP)-sensitive L-type channels, as judged from the prolongation of openings in the presence of the DHP agonist (+)-S-202-791. Three different DHP-sensitive gating patterns were distinguished. The most frequent L-type activity was characterized (in the presence of (+)-S-202-791) by long closings and relatively short openings (average open time, t_O = 2.5 ms at +20 mV), and by a rather peculiar fast inactivation (time constant of decay of the average single channel current: 100 ms at +20 mV). Single channel currents and conductance (24 pS) were indistinguishible from those of a different DHP-sensitive gating pattern with t_O = 10 ms and negligible inactivation during 700 ms (in the presence of DHP agonist at +10 mV). Two patches contained L-type channels with smaller conductance (20 pS) and anomalous gating similar to those recently characterized in cerebellar granule cells (Forti and Pietrobon, 1993, Neuron 10, 437). The DHP-insensitive Ca^{2+} channels observed most frequently (in 40% of patches) had functional properties similar to those of T-type channels (conductance: 7 pS, activation threshold: -40 mV, time constant of decay of average current: 28 ms at -10 mV). 20% of the patches showed DHP-insensitive Ca^{2+} channels (most likely N-type) with 20 pS conductance, 0 mV activation threshold, almost complete steady-state inactivation at -40 mV and biexponential time course of inactivation (with time constants of 27 and 253 ms at +20 mV). Several other DHP-insensitive Ca^{2+} channels were observed less frequently and remain to be characterized.

620.6

EXPRESSION AND SUBCELLULAR DISTRIBUTION OF L-TYPE CALCIUM CHANNELS IN GLIA *IN VITRO*. Sylvie VANDAELE* and Philippe PIERRET, Unité de neurocytologie moléculaire, Département de pathologie, Université de Montréal, Montréal, Québec, Canada.

Glial L-type calcium channels are involved in a variety of cellular functions, such as the astrocytic response to variations of extracellular K+, or in oligodendrocytic myelinization process. The aim of this work is to examine the expression and the sub-cellular distribution of L-type channel subunits on primary culture of glial cells from new-born rats.

Primary cultures of astrocytes (> 98%) from cerebral cortex were characterized by their phenotype, which is vimentin+/GFAP- at one week, vimentin-/GFAP+ at 2-3 weeks, and A2B5- at all stages. In certain cell culture conditions, oligodendrocytes are present either at a progenitor stage (GD3+), either at preoligodendrocyte stage (Rmab+, O4+).

On all these cells, antibodies directed against α_1 or α_2 subunits reveal a punctiform immunoreactivity. The similarity of this immunoreactivity with that of clathrin and caveolin suggests that these channels could be concentrated in invaginated regions of the membrane.

Calcium channel subunit expression varies depending on the batch of horse serum used in the culture. It is significantly increased following dbcAMP treatments. This suggests that seric factors and/or cell processes modulating cAMP may be involved in the regulation of the synthesis of L-type calcium channel subunits. *Supported by the Savoy Foundation and the CAFIR.*

620.7

IN THE PRESENCE OF BAY K 8644, LONG DEPOLARIZATIONS SLOW DEACTIVATION OF L-TYPE Ca^{2+} CHANNELS IN GH_3 CLONAL PITUITARY CELLS BY PROMOTING ACCESS TO A THIRD OPEN STATE. D. M. Fass* & E. S. Levitan. Departments of Neuroscience and Pharmacology, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Application of BAY K 8644 (1 μM) produces a dramatic slowing of deactivation of L-type Ca^{2+} channel currents in GH_3 cells. Specifically, whole-cell tail current decay after brief (10 ms) depolarizations is fit by a single exponential with a τ of ~150 μsec in drug-free conditions and two exponentials with τ_{fast} = ~2 ms and τ_{slow} = ~8 ms in the presence of the drug. On the single channel level, we have found that these two exponentials arise from two open states visited by each L-channel. In the presence of 1 μM BAY K 8644, deactivation is further slowed when recorded upon repolarization after long (200 ms) depolarizing pulses. Analysis of whole-cell tail currents recorded in the presence of BAY K 8644 from 5 cells indicates that both τs are increased in magnitude (τ_{fast} increases from 2.0 $\pm$ 0.7 to 3.7 $\pm$ 0.5 and τ_{slow} increases from 7.7 $\pm$ 1.4 to 9.9 $\pm$ 1.2, $p < 0.05$, data are mean $\pm$ SD), and also that the relative amount of current deactivating with the slow τ increases (from 64 $\pm$ 19 % to 80 $\pm$ 20 %, $p < 0.05$). In cell-attached patch recordings performed in the presence of BAY K 8644 on patches containing single L-channels, ensemble average tail currents from patches subjected to long (100 ms) depolarizations showed slower deactivation than those recorded from separate patches subjected to brief (10 ms) depolarizations. In 4 of 5 patches subject to the long depolarizations, this effect appeared to be due in part to a time-dependent increase in access to a third, slower open state (open time τ = 14.4 $\pm$ 1.3).

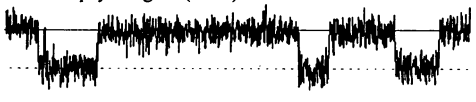
620.8

SINGLE CALCIUM CHANNEL CURRENTS RECORDED AT PHYSIOLOGICAL CALCIUM LEVELS. P.J. Church* and E.F. Stanley SMS, NINDS, NIH, Bethesda, MD 20892.

The influx of calcium ions through voltage sensitive ion channels is critical for the gating of several cellular processes. However, little is known about the conductance of these channels at physiological Ca^{2+} levels. We used quartz glass electrodes to record single neuronal Ca^{2+} currents in external Ca^{2+} down to 0.5mM.

Ciliary ganglia from 15-day-old chick embryos were enzymatically dissociated, triturated to single neurons and plated onto coverslips. 140 KAsp was used to collapse the membrane potential. Single channel current at -40mV was approximately 1pA with 80mM $CaCl_2$ in the pipette and approached 0.1pA with 0.5mM $CaCl_2$. Even at these low concentrations, channel conductance was not linearly related to external divalent cation concentration.

Shown below is an on-cell patch recording exhibiting a single open level of 0.203pA (dashed line) in response to a 300ms step to -40mV in physiological (2mM) Ca^{2+}.



620.9

ASYMMETRIC INTRAMEMBRANE CHARGE MOVEMENT IN MICE HIPPOCAMPAL NEURONS. R. Boumaud, P. Chameau and T. Shimahara*. Lab. Neurobiol. Cell. & Mol., CNRS. 91198 Gif sur Yvette Cedex. France.

Intramembrane charge movement elicited by membrane depolarization is belived to be electrical manifestation of the conformational change in the channel protein.The highly conserved S4 region of voltage gated channels is assumed: 1-to be a voltage sensor because of the presence of repeated motifs of positively charged amino acid residues (arginine and lysine) followed by non polar residues. 2-to carry a part of intramembrane charge movement. So far, asymmetric currents related to charge movement in the central nervous system were recorded in molluscan neurons and recently in Purkinje neurons frommice cerebellum. In the present work, we studied intramembrane charge movement in hippocampal pyramidal neurons using the whole cell clamp technique. The hippocampal cells were enzymatically dissociated from 14 to 18 day-old mice. The relationship between the amount of charge movement and pulse potential is described by a two state Bolzmann equation (Qmax=10.87±0.62nC/μF, V*=1.72±2.91mV, k=18.84±1.20mV, n=16). The arginine modifying reagent phenylglyoxal (10mM) decreases Qmax to about 14% of control. The charge movement was partially immobilized by nifedipine in a dose dependant manner with a IC_{50} of 350nM. Nifedipine (5μM) reduces Qmax to about 46% of the control value. Our results show that asymmetric currents can be elicited in pyramidal cells; these current are related to the conformational changes involved in the gating mechanism of the voltage sensitive channels. A nifedipine sensitive component of charge movement can be isolated. The nifedipine sensitive charge movement presumably originates the gating current of the L type calcium channels expressed in pyramidal neurons. Supported by AFM and DRED.

620.10

LEAD MEDIATED RELEASE OF CALCIUM FROM INTRACELLULAR STORES IN ISOLATED HIPPOCAMPAL NERVE TERMINALS. T.E. Rhodes* and D.K. Bartschat. Dept. of Physiology, Eastern Virginia Medical School, Norfolk, VA 23501.

Isolated presynaptic nerve terminals were prepared from rat hippocampus and loaded with the intracellular calcium indicator Fura-2. The changes in cytoplasmic free calcium ($[Ca^{2+}]_i$) were measured by stopped-flow fluorescence spectroscopy following depolarization with elevated $[K^+]_o$ on a millisecond time scale (Lentzner et al.,J. Physiol. 450:613-628, 1992). Depolarization promoted a rapid increase in intracellular $[Ca^{2+}]$ which occurred in two phases: a fast component, representing the activity of rapidly inactivating Ca^{2+} channels ($\tau \cong 30$ - 60 msec), and a slow component, which is comprised of slowly inactivating Ca^{2+} channels ($\tau \cong 1$ - 2 sec) and Na^+/Ca^{2+} exchange operating in reverse. Low Pb^{2+} (<1 μM) competitively blocked both the fast and slow channels ($K_i \cong 0.1$ μM, 0.2 μM, respectively). At ≥ 1μM, Pb^{2+} permeated the fast channels. Pb^{2+} did not permeate the slow channels and Na^+/Ca^{2+} exchange did not promote Pb^{2+} transport. Lead permeation led to a subsequent rise in intracellular Ca^{2+} even in the absence of extracellular calcium. The rise in Ca^{2+} was reduced by thapsigargin, which depletes non-mitochondrial Ca^{2+} stores, suggesting Pb^{2+} activates Ca^{2+} release from Ca^{2+} storing organelles. The Pb^{2+} mediated rise in Ca^{2+} occurred in terminals treated with ryanodine and caffeine suggesting Pb^{2+} does not act upon these stores. Furthermore, it appears that Pb^{2+} mediates release from 2 distinct pools, a thapsigargin sensitive pool, and a Ca^{2+} sensitive or labile pool which fills when the nerve terminals are incubated with Ca^{2+} (100 μM). The release of Ca^{2+} by Pb^{2+} was greater in younger animals (35g) than in older (350g) rats for both pools, and the ability to load the labile store also diminishes during development. These results suggest age dependent changes in intracellular Ca^{2+} storage and Pb^{2+} sensitivity and that the neurotoxic effects of Pb^{2+} may be due in part to interference with Ca^{2+} metabolism in the presynaptic terminal.

620.11

Pb^{+2} ACTIVATES THE RELEASE OF Ca^{+2} FROM ISOLATED ENDOPLASMIC RETICULUM (ER) VESICLES FROM RAT CEREBELLUM. D.K. Bartschat, T.E. Rhodes, and D.C. Meyer.* Dept. of Physiology, Eastern Virginia Medical School, Norfolk, VA 23501.

Pb^{+2} influx activates the release of Ca^{+2} from an intracellular thapsigargin-sensitive, ryanodine- and caffeine-insensitive store in hippocampal synaptosomes (see acompanying abstract). We isolated microsomes from rat cerebellum, a preparation rich in ER vesicles containing Type I IP_3 receptors, in order to determine if Pb^{+2} activates IP_3-sensitive Ca^{+2} channels. Ca^{+2} flux was monitored spectrophotometrically using fluo-3 or chlotetracycline (CTC) as extravesicular (fluo-3) or intravesicular (CTC) Ca^{+2} indicators.

Incubation of the ER vesicles with Ca^{+2} (1 - 50 μM) resulted in accumulation of Ca^{+2} that was dependent on ATP, was largely blocked by thapsigargin, and was reversed by ionomycin. Addition of IP_3 ($IC_{50} \approx 0.1$ - 0.5 μM) resulted in the prompt discharge of ¼ - ½ of the accumulated Ca^{+2}, an effect blocked by preincubation with heparin. Addition of heparin after IP_3 resulted in re-accumulation of Ca^{+2}, which could subsequently be discharged again by further IP_3 addition. In stopped-flow experiments, mixing of the vesicles with 1 μM IP_3 resulted in two kinetically resolvable phases of release, with $k_1 \approx 15$ sec^{-1} and $k_2 \approx 1$ sec^{-1}. Elevated (> 10 μM) extravesicular Ca^{+2} reduced the IP_3 effect.

Addition of Pb^{+2} (1 - 100 μM) induced a rapid release of Ca^{+2} from ER vesicles in the absence of IP_3. Pb^{+2} addition after Ca^{+2} release by 1 μM IP_3 resulted in no further release, and IP_3 addition after Pb^{+2}-induced release resulted in no further Ca^{+2} release. The effect of Pb^{+2} was reduced in solutions containing > 10 μM Ca^{+2}. The apparent IC_{50} for Pb^{+2}, ≈ 5 μM (added Pb^{+2}), is probably orders of magnitude too high owing to the substantial complexation of Pb^{+2} with components of our solutions (*e.g.*, ATP, PO_4^{-2}). These results indicate that Pb^{+2} directly activates IP_3-activated Ca^{+2} channels, possibly through interaction with a Ca^{+2} binding/modulatory site, and suggest inappropriate activation of IP_3-activated Ca^{+2} channels may underly some aspects of Pb^{+2} neurotoxicity.

620.12

EXTRACELLULAR IONIZED Mg ($[Mg^{2+}]_o$) REGULATES INTRACELLULAR Ca^{2+} CONCENTRATION ($[Ca^{2+}]_i$), NOT INTRACELLULAR Mg^{2+} CONCENTRATION ($[Mg^{2+}]_i$), IN ACUTELY ISOLATED HIPPOCAMPAL CA1 PYRAMIDAL CELLS OF THE GUINEA-PIG. A.Zhang, S.Fan, T.P.O.Cheng, B.T.Altura,R.K.S. Wong, and B.M. Altura*. SUNY Health Science Center at Brooklyn, NY 11203

Hypo- or hypermagnesemia are associated with manifestations of central nervous system dysfunction. The mechanism(s) by which $[Mg^{2+}]_o$ affects neuronal excitability is not fully understood. The present studies, using digital imaging microscopy and ion selective electrodes, determined whether $[Mg^{2+}]_o$ could affect $[Ca^{2+}]_i$ and $[Mg^{2+}]_i$ in single, acutely isolated pyramidal cells from the hippocampal CA1 region of the guinea-pig. After loading with fura-2 or mag-fura-2, the cells were perfused 15 min with HEPES buffer solutions containing 0.3 0.6, 1.2 or 4.8 mM $[Mg^{2+}]_o$, respectively, and then $[Ca^{2+}]_i$ and $[Mg^{2+}]_i$ in these CA1 neurons were measured. Lowering $[Mg^{2+}]_o$ increases $Ca^{2+}]_i$, but exerts no significant effects on$[Mg^{2+}]_i$. However, 1 μM MK-801 and 5 mM Ni^{2+} inhibited 0.3 mM $[Mg^{2+}]_o$-induced Ca^{2+} increase by 39.7% and 99.5%, respectively. The subcellular distributions of both $[Ca^{2+}]_i$ and $[Mg^{2+}]_i$ appeared to be heterogeneous. These data suggest that: 1) $[Mg^{2+}]_o$ regulates $[Ca^{2+}]_i$ in hippocampal pyramidal cells, probably by modulating Ca^{2+} entry via voltage-sensitive Ca^{2+} channels and N-methyl D-aspartate channels; 2) $[Mg^{2+}]_i$ is not acutely sensitive to $[Mg^{2+}]_o$ in these cells; and 3) the neurons seem to have active Mg^{2+} transport system(s) which maintains steep concentration gradients of Mg^{2+} across membranes.

$[Mg^{2+}]_o$ (mM)	N	$[Ca^{2+}]_i$ (nM)	N	$[Mg^{2+}]_i$ (mM)
0.3	32	304 ± 23*	62	549 ± 32
0.6	37	158 ± 27	68	618 ± 11*
1.2	34	118 ± 10	72	529 ± 15
4.8	21	100 ± 7	80	506 ± 20

* *P<0.05 compared to all other* $[Mg^{2+}]_o$; # N=*number of cells tested.*

620.13

EXTERNAL AND INTERNAL pH SHIFTS MODULATE VOLTAGE-GATED CALCIUM CURRENTS IN ISOLATED RAT CA1 NEURONS. G.C. Tombaugh* and G.G. Somjen, Dept. of Cell Biology, Duke Univ. Med. Ctr., Durham, NC 27710.

The effects of extracellular and intracellular H^+ (pH_O, pH_i) on voltage-gated Ca^{2+} currents were examined in acutely dissociated rat hippocampal CA1 neurons using the whole-cell patch clamp technique (21-24°C). Ca currents could be carried by Ba^{2+}, blocked by Cd^{2+}, and were found to be primarily of the high-voltage activated (HVA) type. Moderate external acidosis (pH 6.9-6.0) reversibly depressed HVA Ca current amplitude and caused a depolarizing (+8mV) I-V shift compared to control (pH 7.4); alkaline treatment (pH 8.0) had the opposite effect. The depression of HVA Ca currents by low pH_O was unaffected when the the internal H^+ buffering was raised from 10 to 50mM HEPES. A Hill plot of the effect of pH_O on HVA current amplitude revealed a pK of 7.1 and a slope of 0.625. Neither HVA current decay kinetics nor steady-state inactivation (h_∞) were significantly affected by external H^+ within the pH range tested (6-8). Internal alkalinization by exposure to the weak acid NH_4Cl (20mM) caused a rapid increase in HVA current amplitude and a positive I-V shift, followed by a more modest depression and a slight negative I-V shift upon washout. Internal acidification by exposure to Na-acetate (20mM) depressed the HVA current by 50% while causing a 10-20mV hyperpolarizing I-V shift. In the presence of acetate HVA currents could be further depressed by external acidosis, indicating that physically separate and independent H^+-sensitive sites exist on each face of the cell membrane. Low threshold Ca currents, though observed less frequently, were also depressed by external H^+ but appeared insensitive to internal acidosis (acetate). These results demonstrate that pH shifts within the pathophysiological range are capable of modulating voltage-gated Ca channels in CA1 neurons and strengthen the idea that endogenous pH shifts may modulate both neuronal activity and vulnerability in situ.
Supported by the National Stroke Association and NS18670 (NINDS).

620.14

EFFECTS OF pH ON CALCIUM CURRENTS AND SURFACE CHARGE IN BULLFROG SYMPATHETIC NEURONS. W. Zhou and S. W. Jones*. Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH 44106.

H^+ can affect gating and permeation in ion channels, in part by binding to negative "surface charges" on or near the channel. Whole-cell currents through calcium channels (2 mM Ba^{2+}) are increased at high extracellular pH (8-9), and reduced at low pH (buffered with 5-10 mM HEPES and TAPS or MES extracellularly and 60 mM HEPES intracellularly). There are corresponding shifts in the activation curve, as expected for titration of a surface charge associated with gating. The shift of activation is best described with 2 classes of surface charge (1 e^- per 100 $Å^2$ with pK=4.9, and 1 e^- per 300 $Å^2$ with pK=6.7). The estimated net surface charge at pH 7.2 is 1 e^- per 112 $Å^2$. In contrast, there is no detectable effect of increased pH on the instantaneous I-V relation for fully activated channels, implying that no surface charge associated with permeation can be titrated in that region. The increased currents at high pH can be explained by the shifted activation curve. The instantaneous I-V is reduced at low pH, possibly reflecting channel block. Effects of low pH are complex, as both fast (< 1 s) and slow (seconds) effects are observed. The conclusion that the surface charge associated with permeation is much lower than that associated with gating, and the estimate of the surface charge for gating, are in good agreement with our previous work, based on effects of Ba^{2+} and ionic strength (*J. Gen. Physiol.* 105:441-462, 1995).

620.15

VOLTAGE-DEPENDENT FACILITATION OF CALCIUM CONDUCTANCES IN RAT NEOSTRIATAL NEURONS. W.-J. Song* and D. J. Surmeier. Dept. of Anatomy and Neurobiology, College of Medicine, U. of Tennessee, Memphis, TN 38163.

In a variety of cell types, a large depolarizing prepulse enhances calcium currents evoked by weaker stimuli. While this voltage-dependent facilitation has been intensively studied in non-neuronal cells and peripheral neurons, few studies have focused on central neurons. This type of facilitation is potentially important in the understanding of repetitive activity and neuromodulatory processes. We sought to determine whether similar facilitation currents were present in neostriatal neurons and, if so, what were their characteristics.

To this end, calcium currents were recorded in acutely-isolated and cultured neostriatal neurons with the whole-cell form of patch clamp recording with Ba^{2+} as the charge carrier. In some neurons, amphotericin perforated patch recording was used to determine the impact of cellular dialysis on the facilitation process.

In acutely isolated neostriatal neurons, a 30 msec prepulse to 100 mV typically increased the currents evoked by a -20 mV test pulse by 40-60%. Similar results were obtained in cultured neurons and in acutely isolated neurons recorded with perforated patch. This facilitation was voltage- and time-dependent. Pharmacological experiments suggested that N-, P-, Q- and L-type currents contributed to the facilitation currents. Although dialysis with GTP analogs (GTP-β-S, GTP-γ-S) failed to alter their magnitude, inhibition of protein kinase A/protein kinase G with H-89 eliminated the resting facilitation currents.

Supported by NIH grant NS26473 to D.J.S. and S.T. Kitai.

620.16

RUN-UP OF P-TYPE CACIUM CHANNEL CURRENT IN ACUTELY DISSOCIATED PURKINJE NEURONS DURING WHOLE CELL RECORDINGS. B. Lükensmejer and A.J. Hansen*, Novo Nordisk A/S, 2760 Måløv, Denmark.

The function of many ion channels is dependent upon soluble factors in the cytoplasma. Usually ion currents are diminished with time after establishment of the whole cell configuration - the socalled run down phenomenon. Run up has also been described, but less frequently.

We used acutely dissociated Purkinje cells from cerebellar vermis of 5-10 day-old rats. Whole cell calcium currents were recorded with 1-3 MΩ electrodes and filled with, in mM: 100 CsCl, 40 HEPES, 10 EGTA, 2.9 CaCl2, 2.5 MgCl2, 1 ATP and 0.3 GTP (pH = 7.2). The external medium was: 10 BaCl2, 130 TEACl, 10 glucose, 10 HEPES, 1 MgCl2 (pH = 7.3). In whole cell mode most of the cells had the P-type current (90% inhibitable by Aga IVa). When the current was evoked from a holding potential of -80 mV to 0 mV, the amplitude increased 5-10 fold during the following 3-5 min after going whole cell. The phenomenon was observed whether or not the currents were elicited. The voltage-activation of the current was unchanged during this period. The prolonged time course of the run up suggests that rather small molecules like GTP are not involved, but points to a large intracellular factor (eg phosphatase) that is being washed out.

620.17

EXPRESSION AND REGULATION OF VOLTAGE-GATED SODIUM AND CALCIUM CHANNELS IN RAT RETINAL GANGLION CELLS DURING DEVELOPMENT. Susanne Schmid and Elke Guenther. Dept. Exp. Ophthalmology, Univ. Eye Hospital Tübingen, Germany. **SPON: European Neuroscience Association**

Purpose: The aim of this study was to characterize the expression of voltage-gated sodium channels and of different subtypes of voltage-gated calcium channels in retinal ganglion cells (RGCs) during the development of the retina.

Methods: Patch-clamp recordings of RGCs were performed in the perforated patch mode. Retinas were obtained from pigmented rats (*Brown Norway*) of different ages and cut into slices of 200 µm. Extracellular solution contained (in mM): NaCl (130), KCl (5), $BaCl_2$ (2), $MgCl_2$ (1), Hepes (10) and Glucose (20), continuosly bubbeled with oxygen. Calcium currents were isolated replacing sodium by choline chloride and adding (in mM) TEA-Cl (20) and 4-AP (0,1) to block potassium currents. The pipette solution contained CsCl (130), $MgCl_2$ (2), EGTA (10), Hepes (10) and ATP (2). Nystatin (100µM) was added to perforate the patch.

Results: Amplitude and activation threshold of sodium currents increase during development. RGCs of fully differentiated retinas show an action potential always larger than 1 nA. Additionally they show a large voltage-activated calcium inward current, of which one fraction (30%) can be blocked by the N-type channel blocker ω-conotoxin GVIA and another fraction (17%) can be blocked by the L-type channel blocker Nifedipine, Diltiazem and Verapamil (n=50). There is no detectable T-type calcium current. More than 50% of the voltage-gated calcium current is resistant against any known selective calcium channel antagonist.

Whereas N- and L-type currents are present in most RGCs of neonatal rats, the resistant fraction of the calcium current is expressed only after the second postnatal week, resulting in a strong increase of total I_{Ca} at that time.

Conclusion: RGCs express N- and L-type calcium channels and at least one more type of calcium channel which is resistant to all known calcium channel antagonists. Expression of N- and L- type channels and the "unknown" channel is differentially regulated during development.

620.18

SLOW CURRENT-DEPENDENT INACTIVATION EXPLAINS CHANGES IN BASAL FOREBRAIN HVA Ca^{2+} CURRENTS DURING AGING. D.A. Murchison and W. H. Griffith*, Dept. Medical Pharmacol. & Toxicol., Texas A&M University Health Science Center, College Station, TX 77843-1114.

Age-related increases in high voltage-activated (HVA) calcium (Ca^{2+}) currents have been proposed to explain possible changes in Ca^{2+} homeostasis during aging. We now provide evidence that changes in the inactivation properties of this current rather than the absolute current magnitude may explain the age-related changes. Conventional whole-cell and nystatin perforated-patch clamp recordings were made from acutely dissociated neurons from male Fischer 344 rats of 1-2 months (young) and 20-26 months (aged). Barium (Ba^{2+}, 2 mM) was used as the charge carrier across the calcium channel and interfering conductances were blocked. Current-voltage relationships following ramp step changes showed no difference in peak current-density or voltage-dependence in young (n=94) and aged (n=76) cells. Conversely,voltage-steps delivered at 6 sec intervals, revealed a larger current-density in aged cells. This difference between age groups was explained by a greater current-dependent inactivation in young cells. When currents were generated at varying frequencies (0.5 - 0.05 Hz), current-dependent inactivation was statistically greater in young cells ($p < 0.05$). Using the perforated-patch recording techniques inactivation was always reversible with appropriate recovery periods (3-4 min). Little current-dependent inactivation was generated if slow stimulation rates ($\geq$ 0.033 Hz, or every 30 s) were used. These results suggest that subtle rather than global changes in HVA Ca^{2+} currents may control changes in Ca^{2+} homeostasis during aging.

(Supported by NIH grant AG07805)

ACETYLCHOLINE RECEPTOR: NICOTINIC II

621.1

PRONOUNCED CELLULAR DIVERSITY AND EXTRASYNAPTIC LOCATION OF NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNIT IMMUNOREACTIVITIES IN THE CHICKEN DIENCEPHALON. E.M. Ullian* and P.B. Sargent. Neuroscience Graduate Program and Depts. of Stomatology and Physiology, UCSF, San Francisco, California 94143

The diversity of nicotinic acetylcholine receptor (AChR) expression in the chick lateral spiriform nucleus (SpL) was assessed using subunit-specific monoclonal antibodies (mAbs) and laser scanning confocal microscopy. The late embryonic SpL was immunoreactive for mAbs against the α2, α5, α7, α8, and β2 AChR subunits. Distinct neuronal cell classes were identified using pair-wise staining of mAbs. Approximately 90% of the neurons in the SpL contained both α5-like immunoreactivity (LI) and β2-LI, with no neurons having only one of these subunit-LIs. Approximately 70% of the neurons contained α2-LI. All α2-LI neurons contained α5/β2-LI; thus, neurons having α2-LI are a subset of those having α5- and β2-LI. Fewer neurons, approximately 20%, contained α7-LI. A subset of α7-positive neurons were immunoreactive for other subunits; for example, some α7-positive neurons also contained α2-LI. Fewer than 15% of the neurons contained α8-LI. Some of the α8-LI-containing neurons contained α7-LI. The 14 week post-hatch SpL resembles the late embryonic nucleus in the percentage of neurons immunoreactive for α2, α5, α7, α8, and β2 AChR subunits, and in the presence of multiple classes based on AChR subunit immunoreactivity. In addition, α4-LI was found in about 20% of the 14 week SpL neurons. Double-label immunofluorescence experiments with mAbs to AChRs and to synaptic vesicle antigens showed that most clusters of α5-LI and β2-LI are extrasynaptic. The pronounced diversity of AChR subunit expression and the extrasynaptic location of AChR-LI suggest that AChR-like molecules in the SpL do not function solely to respond to transmitter focally released from presynaptic terminals.

Supported by NIH grants GM07449, NS 24207, and RR 07131.

621.2

PERISYNAPTIC SURFACE DISTRIBUTION OF MULTIPLE CLASSES OF NICOTINIC ACETYLCHOLINE RECEPTORS ON NEURONS IN THE CHICKEN CILIARY GANGLION. H.L. Wilson Horch and P.B. Sargent*. Depts. of Stomatology and Physiology and the Neuroscience Graduate Program, UCSF, San Francisco, CA 94143

Most nicotinic acetylcholine receptors (AChRs) studied to date in the embryonic chicken ciliary ganglion are recognized either by monoclonal antibody (mAb) 35 or by α-bungarotoxin (α-Bgt). Previous studies found that mAb 35-AChRs are found at both synaptic and extrasynaptic sites, while α-Bgt-AChRs are exclusively extrasynaptic [Loring et al., Neurosci. 14(1983)645-660; Jacob and Berg, J. Neurosci. 3(1983)260-271; Jacob et al., Proc. Natl. Acad. Sci. USA, 81(1984)3223-3227; Loring and Zigmond, J. Neurosci., 7(1987) 2153-2162]. To gain a three-dimensional understanding of the spatial relationship of these two AChR classes to one another and to synaptic sites, we visualized clusters of mAb 35-AChRs and α-Bgt-AChRs immunofluorescently using laser scanning confocal microscopy and compared their distribution with that of the synaptic vesicle antigen SV2. Both mAb 35-AChR and α-Bgt-AChR clusters are widely distributed over the neuronal surface. Some mAb 35-AChR clusters are located at synaptic sites, but the bulk of them are located extrasynaptically in well- defined patches measuring 1-4 µm in diameter. α-Bgt-AChRs are found almost exclusively in these extrasynaptic sites, which thus contain both AChR types. These sites are often surrounded by elements of the synaptic calyx but are themselves largely free of synaptic antigen. In 14 week chickens the relationship between mAb 35-AChRs, α-Bgt-AChRs, and synaptic sites is similar to that in embryos except that in this instance, individual synaptic boutons are often partly surrounded by AChR-containing patches. These results suggest that most surface AChRs on chicken ciliary neurons are perisynaptic, and they raise questions about the function of these AChRs.

Supported by NIH grants NS24207 and RR07131.

621.3

MAPPING THE LOCATION OF FUNCTIONAL NICOTINIC ACETYLCHOLINE RECEPTORS (nAChRs) ON HIPPOCAMPAL NEURONS OF THE RAT. M. Alkondon[1]*, E.F.R. Pereira[1,2] and E.X. Albuquerque[1,2]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. of Med., Baltimore, MD 21201, USA; [2]Lab. Mol. Pharmacol., IBCCF, UFRJ, RJ 21944, Brazil.

Hippocampal neurons of the rat express multiple subtypes of functional nAChRs (*J. Pharmacol. Exp. Ther.* 265:1455, 1993). In the present study, we investigated the distribution and location of the nAChRs along the somatic and dendritic membranes of hippocampal neurons. To this end, either neurons obtained from the hippocampi of fetal rats and grown in culture or 200-μm slices obtained from the hippocampi of postnatal rats (P8-P18) were used. The presence of nAChRs was assessed from the whole-cell currents elicited by either focal or U-tube application of ACh. Infrared video microscopy was used to view the neurons in hippocampal slices and a 'nanorobot' micromanipulator was used to position the ACh-containing pipette at locations near the soma or the dendrites. Exposure of cultured hippocampal neurons to the microtubule-destabilizing agent colchicine (100 nM for 3 days) caused a marked decrease in the dendritic branching pattern and a significant decrease in the peak amplitude of α-bungarotoxin-sensitive nicotinic currents. Nicotinic currents could be recorded from the whole-cell pipet positioned at the cell soma when ACh (1 mM) was applied to the soma itself or to various regions of the apical dendrites (even up to 100 μm from the soma). NMDA-evoked currents were also detected at the soma when NMDA (20 μM) and glycine (10 μM) were coapplied focally either at the soma or at different parts of the dendrites. Similar results were obtained when the pyramidal cells of the CA1 region of hippocampal slices were used. The present results indicate that the α-bungarotoxin-sensitive nAChR is located both on the soma and along the dendritic membranes, suggesting a synaptic role for this nAChR subtype. (USPHS Grants NS25296, ES05730 & T32-ES07263).

621.4

EFFECTS OF PRENATAL NICOTINE EXPOSURE ON LOCOMOTOR ACTIVITY, SENSORY GATING, AND CENTRAL NICOTINIC RECEPTORS. Y. Tizabi*, E. J. Popke, M. A. Rahman, S. M. Nespor and N. E. Grunberg. Dept. of Pharmacology, Coll. of Med. Howard Univ., Washington, DC 20059, and Uniformed Services Univ. of the Health Sciences, Bethesda, MD 20814

Prenatal exposure to nicotine may be a contributing factor in the manifestation of attention-deficit hyperactivity disorder. To evaluate possible involvement of neuronal nicotinic receptors in a model of this disorder, pregnant rats were implanted with osmotic minipumps to receive nicotine (6 mg/kg/day) or saline throughout gestation. The offspring were tested for locomotor activity on postnatal days 19-24, and prepulse inhibition (PPI) on days 31-36. Hyperactive and non-hyperactive offspring as well as high and low (impaired) PPI responders of both sexes were analyzed for nicotinic receptor densities utilizing ^{125}I-cytisine binding to homogenates of cerebral cortex (left hemisphere: LCX), striatum, hippocampus, thalamus, colliculi, substantia nigra region and cerebellum. Prenatal nicotine exposure resulted in approximately 50% more hyperactive offspring for both sexes. Prenatal nicotine also impaired PPI response in the offspring and the effect was more dramatic in females. Male offspring with low PPI, regardless of prenatal nicotine exposure, exhibited an upregulation of nicotinic receptors in LCX compared to high PPI responders. The LCX nicotinic receptors were also significantly elevated in the male hyperactive (vs non-hyperactive) offspring that were prenatally exposed to nicotine. Additionally, there was an upregulation of nicotinic receptors in the striatum of PPI impaired male offspring that were exposed to nicotine prenatally. It is concluded that hyperactivity and impaired PPI in male offspring that were exposed to nicotine prenatally are associated with upregulation of nicotinic receptors in specific brain regions. Since nicotinic upregulation is associated with a functional impairment of these receptors, it is postulated that such offspring could experience a reversal or reduction of their symptoms with administration of nicotine.

621.5

CHARACTERIZATION OF α-BUNGAROTOXIN SENSITIVE NICOTINIC RECEPTORS IN AN IMMORTALIZED HIPPOCAMPAL CELL LINE. J. Komourian* and M. Quik, Dept. of Pharmacology, McGill University, Montreal, Canada, H3G 1Y6.

The functional significance of neuronal nicotinic α-bungarotoxin (α-BGT) receptors is not fully understood. As a model for studying this receptor in the CNS, we have selected an immortalized rat hippocampal cell line H19-7 (courtesy Dr. B. Wainer). Our previous work demonstrated that when differentiated, these cells express saturable high affinity α-BGT receptors with characteristics similar to rat brain α-BGT sites. In the present study, we show that these receptors are located extracellularly; exposure of the cells to α-BGT to block receptors show that 92.4 ± 2.6% (n=3) of the sites are located on the cell surface. The neuronal nicotinic receptor subunit α7 has been linked to α-BGT binding sites and here we show using the RNase protection assay that the α7 mRNA transcript is present in differentiated H19-7 cells, as well as α4 and ß2 mRNAs. These receptor subunit mRNAs are not present in undifferentiated cells. No RNA transcripts were identified encoding the nicotinic receptor subunits α2, α3, α5 and ß3 in either differentiated or undifferentiated H19-7 cells. Initial results indicate that nicotinic receptor activation increases the intracellular calcium concentration in these cells. The present results suggest that H19-7 cells may represent a useful model system to study the identity and role of CNS α-BGT receptors.

621.6

POSTNATAL EXPRESSION OF NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNIT mRNAs IN RAT VISUAL CORTEX. B.S. Stoveken*, U.H. Winzer-Serhan, F.M. Leslie. Dept. Pharmacology, Univ. of California, Irvine, CA 92717.

A transient increased binding of [^{3}H]nicotine and [^{125}I]α-bungarotoxin has been reported in primary visual cortex (V-1) during postnatal development. These two ligands bind to nicotinic acetylcholine receptors (nAChR) of different subunit compositions in rat brain. In order to elucidate possible subunit contributing to this binding, we studied the mRNA expression of α3, α4, α5, α7, β2, and β4 nAChR subunits in V-1. Transverse sections of rat brains collected from postnatal (P)0 to P30 were processed for *in situ* hybridization with [^{35}S]labeled riboprobes. All nAChR subunit mRNAs were were found to be expressed in V-1 at P0 and throughout postnatal development. However, expression intensity and distribution varied greatly among subunits. The highest levels of mRNA expression throughout development were seen for α4 and β2 which were both expressed in layers II-VI. The α7 nAChR subunit was moderately expressed in all layers, and showed transient increased mRNA expression in layer IV and VI during the first two postnatal weeks. α3 nAChR mRNA was expressed at low levels of intensity in several cortical layers during development. By P3 an increase in α3 mRNA expression was detected in layer IV of V-1 which remained at moderate levels into adulthood. The expression of β4 exhibited a similar distribution pattern to that of α3 during the first and second postnatal week, but then declined at P15 to very low levels of expression. The α5 subunit showed the most discrete expression pattern, and was almost exclusively found in the subplate or layer VIb during development and into adulthood. This study confirms that a number of nAChR subunit mRNAs are expressed in V-1 during development. The transiently increased expression of α3 and β4 mRNAs in layer IV suggest that these subunits may be responsible for the increased nicotine binding in this layer. The lack of increased expression of other nAChR subunits in layer VI besides α7 supports previous findings that α7 forms functional homomeric receptors and is not associated with other known nAChR subunits. *Supported by PHS grant # NS30109*

621.7

IN VITRO SYNCHRONIZATION OF AChR DEGRADATION RATES BY EXOGENOUS ATP OR ADENOSINE. J.P.O'Malley* & M.M. Salpeter
Section of Neurobiology and Behavior, Cornell University, Ithaca NY 14867.

Acetylcholine receptors (AChRs) can be classified as two distinct physiological entities based on their metabolic degradation rates. The two forms have been called Rr and Rs to designate rapidly degrading and slowly degrading AChRs respectively. The Rr degrades with a half-life (t1/2) of 1 d while the Rs degrades with a t1/2 of 10 d in innervated muscle and 3 days in denervated or cultured muscle. Furthermore the 3-day-Rs of denervated or cultured muscle can be converted to a 10-day-Rs by elevating intracellular cAMP. To date, we have not been able to alter the t1/2 of the Rr from that of 1 day.

However evidence exist which suggests that the Rr and the Rs may share a common t1/2 of 3 days during reinnervation (see Liu, Szabo and Salpeter abstract this meeting). To determine if such Rr stabilization can be achieved, and may be due to the motor nerve, we tested the effect of putative neurotrophic factors on Rr degradation. We report that Rr of cultured rat myotubes are stabilized from their default t1/2 of 1 d to a t1/2 of 3 days within a 24 h period by exogenously applied ATP. Furthermore after this 24 h period the newly inserted and existing AChRs (both the Rr and Rs) all decayed with the same t1/2 of ≈ 3 in the presence of ATP. This degradation profile is very similar to that of the AChRs inserted into the membrane of reinnervating muscle (Liu et al abstract this meeting). The same results were also obtained with Adenosine but not with Inosine.

We also report that treatment of AChRs with dibutyryl cAMP converted the 3-day-Rs, but not the ATP stabilized 3-day-Rr, to a 10-day Rs. Thus the Rr can exist with a t1/2 of 1 or 3 days and the Rs with a t1/2 of 3 or 10 days. We suggest that this ability to share a common t1/2 of 3 days may be important during periods in which the Rr and Rs content of the muscle is changing such as during normal development or reinnervation. If both Rr and Rs share a common t1/2 during these periods it would allow for a smooth transition of receptor type without altering the postsynaptic AChR content and thus ensuring the integrity of neuromuscular communication. Supported by NIH grants GM10422 and NS21767.

621.8

ACETYLCHOLINE RECEPTOR DEGRADATION AND mRNA LEVELS DURING REINNERVATION OF DENERVATED MOUSE MUSCLE
Enchi Liu*, Maria Szabo, Miriam M. Salpeter. Section of Neurobiology & Behavior, Cornell University, Ithaca, NY.

At innervated neuromuscular junctions, (nmjs) acetylcholine receptors (Rs AchRs) have a stable degradation half-life (t1/2) of ~8-10 days (10-day Rs). After denervation the Rs degrade with a t1/2 of ~3 days (3-day Rs) and, as they degrade are replaced by embryonic-like AChRs (Rr) with a rapid degradation t1/2 of ~1 day (1-day Rr). To understand the molecular structure of the Rs and Rr, we examined events during reinnervation. When mouse sternomastoid muscles are denervated by multiple nerve crushes, regeneration begins within 3 to 4 days after the last crush and is complete by day 7. EM autoradiography established that AChRs inserted at the nmj during and after reinnervation degraded with 3 and 8 day half-lives, respectively. In addition, the AChR site density decreased only slightly during this reinnervation period. In situ hybridization was used to quantify g and e subunit mRNAs. We report that at the nmj the g subunit mRNA is increased over non-specific levels by about 6 fold after denervation, by 4.5 to 3.5 fold during reinnervation and is back to innervated levels within four days after full reinnervation. The junctional e subunit is increased only about 1.5 fold after denervation, remaining so throughout the reinnervation period and decreasing to innervated values by one day after full reinnervation. Assuming that mRNA levels reflect AChR content (Witzemann et al FEBs 223:104, 1987), our data suggest that in denervated muscle the 1-day Rr are predominantly g containing which are replaced by e-containing Rs as the g mRNA drops after reinnervation. Finally, our data on AChR degradation rate, site density and g mRNA levels fit a model suggesting that the 1-day Rr may stablilize to a 3-day Rr during reinnervation (see abstract by O'Malley & Salpeter) before being replaced by e-containing 3- or 10-day Rs. Such a mechanism would have the advantage of preventing a major decrease in AChR site density expected if replacement of 1-day Rr by 10-day Rs would occur without a 3 day intermediate.
Supported by NIH NS 09315

621.9

ANALYSIS OF BRAIN NICOTINIC ACETYLCHOLINE RECEPTORS WITH SUBUNIT-SPECIFIC ANTIBODIES AND [^{3}H]EPIBATIDINE. W.G. Conroy* and D.K. Berg, Dept. of Biology, UC San Diego, La Jolla, CA. 92093.

Although eleven neuronal nicotinic acetylcholine receptor (AChR) genes have been identified, the combinations of subunits forming native AChRs in the CNS remain uncertain. Previous work distinguished two classes of AChRs in chick brain based on high affinity nicotine binding (AChRs with α4 and β2 subunits) or α-bungarotoxin binding (AChRs with α7 and/or α8 subunits). We report that additional AChRs can be identified using the nicotinic ligand [^{3}H]epibatidine and subunit-specific monoclonal antibodies (mAbs). A filter assay for [^{3}H]epibatidine binding yields values of about 140 fmol/mg protein in brain extracts prepared from 18 day chick embryos. About 90% of the sites can be immunoprecipitated with either α4- or β2-specific mAbs. [^{3}H]Epibatidine does not bind detectably to αBgt-AChRs. A solid-phase immunotethering assay demonstrates that mAbs to α3, α5, and β4 also are able to retain brain AChRs labeled with [^{3}H]epibatidine. Performing sequential immunodepletions indicate that some AChRs containing α3, β4, or α5 subunits also have α4 and/or β2 subunits. These results confirm the abundance of α4β2 AChRs in brain and go on to identify novel AChRs that may possess distinctive properties. (NS 12601 & 25916)

621.10

αBGT-SENSITIVE ACH-INDUCED CURRENTS IN PC12 CELLS. E.M. Blumenthal*, W.G. Conroy, S.J. Romano, and D.K. Berg. Dept. of Biol., UCSD, La Jolla, CA. 92093.

PC12 cells have been widely used to study neuronal nicotinic ACh receptors (AChRs). Despite having α7 transcripts and α-bungarotoxin (αBgt)-binding sites, the cells have not been found to express αBgt-sensitive ACh-induced currents. Using whole-cell patch-clamp and rapid application of agonist (500 μM ACh), we examined three PC12 variants. One (vI) expressed small, rapidly desensitizing currents that were completely blocked by 60 nM αBgt; the cells also displayed high-affinity αBgt-binding sites on the cell surface. The second (vII) expressed slow, αBgt-insensitive currents and had no αBgt surface binding. The third (vIII) expressed both the rapid, toxin-sensitive current and a slow, insensitive current; vIII cells had αBgt-binding sites on the cell surface. While levels of αBgt-binding among the three variants correlated approximately with the αBgt-sensitive currents, they did not correlate with α7 transcript levels. RNase protection showed, surprisingly, that α7 transcript levels are close to 10-fold higher in vII than in either vI or vIII. Discrepancies were also found between levels of other AChR subunit mRNAs and the amplitude of the slow current. The results demonstrate that transcript levels are not a reliable indicator of functional receptor abundance, and suggest that important regulatory controls may exist at posttranscriptional steps. In addition, the results are the first to demonstrate functional αBgt-AChRs on PC12 cells. (NS 12601 & 25916)

621.11

TYROSINE KINASE INHIBITORS SELECTIVELY DOWN-REGULATE A SUBPOPULATION OF NEURONAL ACH RECEPTORS. R.C. Haselbeck, E.M. Blumenthal, and D.K. Berg*, Dept. of Biology, UCSD, La Jolla, CA 92093.

Protein tyrosine kinases (PTKs) play key roles in signal transduction pathways for a wide range of cellular processes and are highly expressed in the nervous system. We used PTK inhibitors to examine the possible involvement of PTKs in regulating ACh receptors in chick ciliary ganglion neurons. The neurons express several classes of ACh receptors including a class (mAb 35-AChRs) that binds mAb 35 and is responsible for synaptic transmission through the ganglion. They have a second class (αBgt-AChRs) that binds α-bungaratoxin and lies predominantly in non-synaptic membrane. Treatment of cultured neurons with the membrane-permeant PTK inhibitor herbimycin (0.5 μg/ml) reduces surface expression of mAb 35-AChRs by two-fold but does not affect αBgt-AChRs. Little change is seen in the large intracellular pool of mAb 35-AChRs. Lavendustin, a second PTK inhibitor, also reduces surface expression of mAb 35-AChRs. The herbimycin-induced decrease in mAb 35-AChRs requires protein synthesis, and therefore is unlikely to represent increased receptor turnover. The findings implicate a PTK in the regulation of AChR surface number. It may increase synthesis of certain AChR gene products, though one cannot yet exclude a posttranslational effect that depends indirectly on protein synthesis. (NS12601 & 25916; AHA93-72)

621.12

REGULATION OF THE NEURONAL ACETYLCHOLINE RECEPTOR GENE α7 IN VERTEBRATE SKELETAL MUSCLE. S.J. Romano* and D.K. Berg, Dept. of Biology, UC San Diego, La Jolla, CA 92093.

Several of the neuronal nicotinic ACh receptor (AChR) genes are expressed in embryonic chick skeletal muscle. One of the most abundant, α7, produces a component having the size expected for a fully assembled AChR. Because α7 and α1 gene expression rise and fall roughly in parallel during muscle development, experiments were designed to determine if the two are co-regulated. Hatchling chicks were unilaterally denervated by transecting the sciatic nerve, and total RNA extracted from leg muscles 2-14 days later. RNase protections revealed the expected large increase in α1 mRNA; in contrast, α7 message levels increased only modestly and with a slower time course. The regulatory role of electrical activity was examined by treating cultured myotubes with TTX to block spontaneous contractions. Consistent with the denervation results, TTX increased the α1-containing AChRs and α1 transcripts in cultured muscle without appreciably increasing α7 protein or mRNA levels. To investigate potential regulation by neuronal factors, muscle cultures were treated with ARIA. α1 message levels were increased by ARIA as expected, but α7 mRNA expression was not enhanced. Though both α1 and α7 mRNA levels decline markedly in vivo around embyronic days 15-17, the results suggest that different regulatory mechanisms may be responsible. (NS12601 & NS25916)

621.13

RESIDUES FROM NON-ALPHA SUBUNITS OF NICOTINIC ACETYLCHOLINE RECEPTOR PARTICIPATE IN AGONIST BINDING - EVIDENCE FROM SINGLE CHANNEL STUDIES. Y. Zhang, J. Chen, G. Akk, M. Slaughter*, S. Sine and A. Auerbach. Dept. of Biophysics, SUNY Buffalo NY 14214, and Dept. Physiology, Mayo Foundation, Rochester, MN 55905.

Macroscopic recording and photolabelling studies revealed that residues from δ and γ subunits of nAChR contribute to agonist binding. In δ-subunit these residues include W57, D180, and E189 ([1,2]). We have mutated the δW57 into aromatic (Y, F) and non-aromatic (T, E) residues. We have also changed the negatively charged D175 and E184 in the ε-subunit to their uncharged counterparts, N and Q. Receptors were expressed in oocytes and HEK 293 cells. Single channel data indicate that the aromatic nature of W57 is important to ACh binding and channel gating. The EC_{50} values are (in μM): 11 (wt $\alpha_2\beta\gamma\delta$), 19 (Y), 9 (F), 543 (T) measured as intracluster P_{open} *vs.* ACh concentration. Kinetic analysis indicates that both ligand binding and channel gating steps are affected by the mutations. The mutant receptors open at a rate that is about 10 times slower than the wild type. The channel closing rate is unchanged. Our data indicate that channel block by ACh and QX-222 is enhanced by the mutation suggesting that structural changes in agonist binding site caused by mutation may have propagated to the pore region.

Mutants D175N and E184Q have their dose response curves shifted towards higher agonist concentrations. The EC_{50} values are (in μM): 29 (wt $\alpha_2\beta\delta\varepsilon$), 246 (D175N), and 81 (E184Q). Single-channel analysis shows that i) D175N has slower opening rate ($2000s^{-1}$) which accounts for most of the effect on the dose-response curve, ii) E184Q has similar gating rates as wt receptor but drastically increased dissociation rate suggesting its participation in the ACh binding.

1. Chiara, D.C. and Cohen, J.B.(1992) Biophysical J. 61 A106.
2. Czajkowski, C. et al., (1993) *PNAS*. 90: 6285.

621.14

BIOTINYLATED ARSENICALS: PROBES FOR THE AGONIST BINDING SITE OF TORPEDO NICOTINIC RECEPTORS. R.H. Loring*, X.G. Zhang, A. Sharma, A. Baaj, R. Moaddel, and G.S. Jones, Jr. Dept. Pharmaceut. Sci., Northeastern University, Boston, MA 02115.

Unwin (Nature 373:6509, 1995) proposes that the agonist binding site on Torpedo nicotinic receptors is located within a pit visualized on the α subunit. Adjacent cysteine residues Cys^{192}-Cys^{193} on the α subunit are part of the agonist binding site, and we previously demonstrated (Loring et al., Mol. Brain Res. 15:113, 1992) that aromatic trivalent arsenicals, which recognize paired but not single thiols, will covalently bond to reduced Torpedo receptors and block α-bungarotoxin (ABT) binding. We therefore investigated the properties of N-biotinyl-p-aminophenyl arsonous acid (Bio-APA) as a labeling agent for nicotinic receptors. Bio-APA blocks ABT binding to Torpedo receptors treated with dithiothreitol with an IC_{50} of 30 nM, but has no effect on untreated receptors. The effect of Bio-APA is long-lasting (> 9 d), but can be reversed at any time with the anti-arsenical agent dimercaptopropane sulfonate (DMPS). However, ^{125}I-streptavidin, which binds to biotin, does not bind to intact Torpedo receptors in which ABT binding is blocked by Bio-APA. Streptavidin does bind to Bio-APA when covalently bonded to an 18mer peptide corresponding to the portion of Torpedo α subunit containing Cys^{192}-Cys^{193}. The labeling of peptide blotted onto PVDF membranes is prevented if the peptide is alkylated with N-ethylmaleimide prior to Bio-APA treatment, or if DMPS is used after labeling. Taken together, these data suggest that the 3D protein structure of intact Torpedo receptors prevents streptavidin binding to covalently attached Bio-APA. We are examining analogs of Bio-APA with varying spacer groups between the arsenical and biotin moieties to map the agonist binding site. Supported by NS22472 and Smokeless Tobacco Research Council.

621.15

EFFECTS OF A BIOTINYLATED ARSENICAL ON NEURONAL NICOTINIC RECEPTORS. X.G. Zhang*, T. McHugh, R. Moaddel, G.S. Jones, Jr. and R.H. Loring. Dept. Pharmaceut. Sci., Northeastern U., Boston, MA 02115.

Since N-biotinyl-p-aminophenyl arsonous acid (Bio-APA) covalently bonds near the agonist binding site of reduced Torpedo nicotinic receptors (Loring et al., Soc. Neurosci. Abstr., 1995), we investigated whether Bio-APA has similar effects on neuronal nicotinic receptors. Nicotinic responses in intact chick retina are blocked by treatment with dithiothreitol (DTT), but this effect is readily reversed by reoxidation by dithiobis-nitrobenzoate (DTNB). Bio-APA (20 μM) completely blocks the ability of DNTB to restore function to DTT-treated retina, but subsequent treatment with the anti-arsenical reagent dimercaptopropane sulfonate (DMPS) restores nicotinic function to arsenylated receptors. Similarly, Bio-APA treatment (20 μM) protects DTT-treated ciliary ganglia receptors against irreversible alkylation by bromoacetylcholine. Bio-APA blocks ^{125}I-α-bungarotoxin binding to DTT-treated immunoprecipitated α7-containing receptors from chick brain with an IC_{50} of 100 nM, but had no effect on untreated receptors or receptors reoxidized with DTNB. The effect of Bio-APA was long lasting (> 1 d), but could be reversed at any time by DMPS. Arsenylation with Bio-APA also blocks the binding of ^{3}H-cytisine to immunoprecipitated α4-containing receptors from chick brain. The ability of ^{125}I-streptavidin to bind to arsenylated receptors (immunoprecipitated α3, α4, α7, or α8 receptors, antibodies from Dr. J. Lindstrom) was also determined. In no case did ^{125}I-streptavidin bind to neuronal receptors arsenylated with Bio-APA. These data suggest that neuronal nicotinic receptors are similar to Torpedo α1 receptors in that the thiols arsenylated by Bio-APA are not located at sites on the receptor surfaces easily accessible to streptavidin. Supported by NS22472 and STRC.

621.16

ACETYLCHOLINE RECEPTOR γ SUBUNIT ASP^{174} CONTRIBUTES TO THE BINDING OF ACETYLCHOLINE AND CURARE. M. Martin, C. Czajkowski and A. Karlin*. Center for Molecular Recognition, Columbia University, New York, NY 10032.

Muscle-type nicotinic acetylcholine receptors have the composition $\alpha_2\beta\gamma\delta$. One of the two acetylcholine binding sites is formed between an α subunit and the δ subunit, and the other binding site between the second α subunit and the γ subunit. δAsp^{180} is within 1 nm of the disulfide-linked cysteines, αCys^{192} and αCys^{193}, and the mutation of δAsp^{180} to Asn decreased the affinity for ACh by two orders of magnitude(Czajkowski and Karlin, J. Biol. Chem. 270: 3160-64, 1995). The residue that aligns with δAsp^{180} in mouse-muscle γ subunit is γAsp^{174}. We mutated this residue and each of the other nine negatively charged residues contained in the extracellular segment from Glu163 to Glu203. The mutants were co-expressed in *Xenopus* oocytes with wild-type α, β, and δ subunits, or with just α and β. In the latter case, the mutation of γAsp^{174} to Asn (γD174N) decreased the affinity for ACh 170 times and for d-tubocurarine 10 times, as measured by their competition with α-bungarotoxin. Mutation of the other nine negatively charged residues to Asn or Gln did not significantly change the affinity for ACh. No detectable ACh-induced current was obtained with oocytes co-expressing γD174N and wild-type α and β, but with the additional co-expression of wild-type δ, ACh-induced currents were obtained and were characterized by an apparent affinity for acetylcholine one-seventh that of wild-type receptor. Mutation of γAsp^{174} to Glu had no effect on the affinity for ACh, or on ACh-induced currents. γAsp^{174}, which aligns with δAsp^{180}, is likely to interact electrostatically with ACh at the binding site formed between α and γ.
Supported by T32-NS07258, RO1-NS07065, and MDA Research Grant.

621.17

EXPRESSION OF A SOLUBLE 62 AMINO ACID PEPTIDE FRAGMENT FROM THE EXTRACELLULAR DOMAIN OF THE α–SUBUNIT OF THE NICOTINIC ACETYLCHOLINE RECEPTOR. E. Hawrot, M.A. Grant, Q-L. Shi, R. Patrick*, L.N. Gentile. Department of Molecular Pharmacology and Biotechnology and Department of Neuroscience, Brown University, Providence, RI 02912.

We have constructed a synthetic gene for a 62 amino acid fragment (α143-204) of the N-terminal portion of the nicotinic acetylcholine receptor (nAChR) using the protein sequence of the α-subunit from *Torpedo californica*. This gene was attached to the C-terminus of thioredoxin and expressed in plasmid pTrxFus as a soluble fusion protein. The polylinker region separating thioredoxin and the receptor fragment was re-designed to include a thrombin cleavage site. The fusion protein was over-expressed in *E. coli* GI724 cells, purified by 35% ammonium sulfate precipitation, and treated with thrombin to liberate the desired 62mer. Following purification by ion exchange and gel filtration chromatography a calculated yield of 6.86 mg of the 62mer protein was obtained from a 2 L prep in M-9 media.

Both the fusion protein and the cleaved 62mer were shown to have excellent binding affinity for a variety of nAChR ligands by ^{125}I-bungarotoxin (BGTX) competition binding assays. The apparent affinity of both the fusion protein and of the isolated 62mer for BGTX was within a factor of five of the affinity obtained with native ACh receptor (*Torpedo* electric organ membranes). Furthermore, competition binding experiments with the 62mer indicate an apparent IC_{50} of 35 μM for curare. The CD spectrum of the isolated 62mer is suggestive of a protein with β-structure. Metabolic labelling to incorporate the ^{15}N and/or ^{13}C labels needed for high resolution NMR analysis of the receptor fragment as well as of its stoichiometric complex with BGTX is currently underway.
(Supported by NIH GM32629.)

621.18

SUBUNIT COUPLING IN THE ACETYLCHOLINE RECEPTOR IS AFFECTED BY MUTATIONS THAT DESTROY POST-TRANSLATIONAL MODIFICATION SITES. E.C. Walcott* and K. Sumikawa. Dept. of Psychobiology, University of California, Irvine, CA 92717.

We have further investigated the role of two post-translational modifications, disulfide bond formation and N-glycosylation, in the biosynthesis of the nicotinic acetylcholine receptor (AChR). These modifications are thought to occur on each of the four types of AChR subunits (α,β,γ,δ) after translation in the endoplasmic reticulum. Site-directed mutant subunits were generated lacking one of the conserved cysteines (Cys -> Ser) or one residue in the conserved N-glycosylation site (Ser->Ala), and tested in *Xenopus* oocytes. Oocytes were injected with three wildtype mRNAs ($\alpha\beta\gamma$, $\alpha\beta\delta$) with or without a mutant subunit mRNA lacking a conserved post-translational modification site (mγC128S, mδC130S, mγS143A, mδ145A). Dose-response curves generated from whole cell ACh responses revealed that the maximal currents (I_{max}) from these groups were generally less than 5% of wildtype responses. Other physiological activation parameters (EC_{50}, and Hill coefficients) were also changed. When either mutant γ subunit (mγC128S or mγS143A) was co-expressed with $\alpha\beta\delta$ compared to $\alpha\beta\delta$ alone, EC_{50} values and I_{max} values shifted indicating that mutant γ subunits are capable of assembling into functional receptors. In contrast, few differences were observed between mutant δ subunits co-expressed with $\alpha\beta\gamma$ and $\alpha\beta\gamma$ alone, indicating that mutant δ subunits may not be incorporated into the correctly assembling receptor. Biochemical evidence suggests that subunits containing each of these mutations associate with other subunits, but the efficiency of correct subunit coupling is decreased and may at least partly account for the reduction in physiological responses. (Supported by NIMH-NRSA 14599-19 and MDA).

621.19

CHARACTERIZATION OF HUMAN NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNITS α3, β2, β4, and α5 EXPRESSED IN *XENOPUS* OOCYTES

E. Wang, V. Gerzanich, R. Anand,* X. Peng, and J. Lindstrom. Dept. of Neuroscience, Univ. of Pennsylvania Medical School, Philadelphia, PA 19104-6074.

We have isolated cDNAs for the human neuronal nicotinic acetylcholine receptor (AChR) subunits α3, β2, β4 and α5 from a library prepared from the human neuroblastoma cell line SH-SY5Y. The subunit compositions of functional human AChRs assembled in *Xenopus* oocytes have been studied by using subunit-specific mAbs and tagging of subunits with reporter epitopes. By using voltage clamp functional assays and radioimmunoassays, both electrophysiological and pharmacological properties of human α3 AChRs expressed *in vitro* have been characterized.

When expressed in *Xenopus* oocytes, both human AChR subunit combinations $\alpha3\beta2$ and $\alpha3\beta4$ form functional ligand-gated ion channels with strong inward rectification. $\alpha3\beta2$ and $\alpha3\beta4$ AChRs show different ligand binding and ion channel properties. For example, $\alpha3\beta2$ AChRs exhibit significantly faster desensitization than do $\alpha3\beta4$ AChRs; radioimmunoassay shows $\alpha3\beta2$ AChRs have higher affinity by at least 10 fold than $\alpha3\beta4$ AChRs do for epibatidine (a potent agonist for neuronal AChRs). $\alpha5$ efficiently co-assembles with both $\alpha3\beta2$ and $\alpha3\beta4$ in oocytes; but in both cases, no substantial change in either ligand binding or channel properties of the resulting AChRs was detected. According to results with subunit specific mAbs and comparisons of their binding affinities for epibatidine, the subunit compositions and pharmacological properties of the heterologously expressed α3-type AChRs from *Xenopus* oocytes resemble those of native α3-type AChRs from the human neuroblastoma cell line SH-SY5Y. (Supported by grants from NINDS, STRC, CTR, and MDA).

621.20

THE NICOTINIC ACETYLCHOLINE RECEPTOR IN CEREBELLUM IS COMPOSED OF FOUR DIFFERENT SUBUNITS J. R. Forsayeth*, E. Kobrin, B. D. Winegar and D. J. Fitzgerald. Dept. Anesthesia, University of California San Francisco, CA 94143-0542

Nicotine has well described effects on motor coordination exerted in part through receptors in the cerebellum. We investigated the distribution of nicotinic receptor subunits in rat cerebellum and brain-stem with immunohistochemistry on free-floating sections. Two rabbit polyclonal antibodies were generated with bacterially synthesized cytoplasmic loop fragments encoded by the β3 and β4 subunits. In order to ensure maximum specificity, antisera were further purified with immobilized unique peptides. Immunoprecipitation of subunits from extracts of transfected COS cells showed that the antibodies were specific. Immunoperoxidase staining with sections of fixed rat brain showed strong staining with both antibodies in the granular layer and in Purkinje cells with both antibodies. Staining was also seen in cell bodies in Trigeminal nuclei and Vestibular nuclei in the medulla. Staining in Pons by the β3 antibody and the β4 subunit antibody was strong. Receptor composition was assayed by making detergent extracts from rat cerebellum and precipitating receptors with the anti-β2 antibody, mAb270. The immunoprecipitate contained the α4, β3 and β4 subunits (Western). All four antibodies could precipitate acetylcholine binding activity in the detergent extracts of COS cells transfected with a combination of the four subunit cDNAs. We conclude, therefore, that the nicotinic receptor in the cerebellum is composed largely of a receptor with the stoichiometry $\alpha4\beta2\beta3\beta4$. This study was supported by a grant to J.R.F. from the NIH.

622.1

HYPOXIA-INDUCED NEURONAL INJURY IN CORTICAL-PIAL COCULTURES. S.-I. Chi*, T.-L. Bai, T.-I. Wong and T.N. Lin. Department of Physiology, Tzu-Chi College of Medicine, Hualien and Institute of Biomedical Sciences Academia Sinica, Taipei, Taiwan.

The role of cerebral vessels and extracellular acidity in hypoxic-ischemic neuronal injury have been controversial. We studied the interaction between cultured cortical neurons and pial vessels under oxygen-glucose deprivation conditions typical of hypoxia-ischemia in vivo. A progressive neuronal loss in neuronal-pial cocultures induced by oxygen-glucose deprivation was in a time-dependent manner, assessed by phase-contrast microscopy and release of lactate dehydrogenase into medium. No significant injury was found in cultured pial vessel cells after deprivation for 6 hours. In neuronal-astrocyte cocultures, neuronal injury was induced by deprivation for 60 minutes. While to produce significant neuronal injury in neuronal-pial cocultures required longer deprivation for 100 minutes even with higher neuronal density. The neuronal death was partially attenuated by both NMDA antagonist MK-801 (10 uM) and extracellular acidity (pH 6.4).

These observations suggest that hypoxic neuronal injury in the neuronal-vessel mix cultures may be mediated partly by NMDA receptors and cerebral vessels and extracellular acidity may have neuroprotective effects during hypoxia-ischemia.

622.2

ISCHEMIA-INDUCED CHANGES IN EXCITATORY AMINO ACID CONCENTRATIONS AFTER TRAUMATIC BRAIN INJURY. L.W. Jenkins, M.H. Zornow*, D.S. Prough, D.J. McAdoo, D.S. DeWitt. Department of Anesthesiology and Marine Biomedical Institute, The University of Texas Medical Branch. Galveston, TX 77555-0591.

Introduction: Both laboratory and clinical evidence suggest that traumatic brain injury (TBI) sensitizes the brain to secondary insults (1). Laboratory studies suggest that excitotoxicity may play a role in this sensitization; one possible mechanism for this hypersensitivity is an enhanced release or failure of reuptake of excitatory amino acids (EAA) (2). To study this possibility, the technique of in vivo microdialysis was used to measure glutamate and aspartate levels in the periischemic period after fluid-percussion TBI.

Methods: Fasted male Wistar rats (N=6) were anesthetized with isoflurane. A femoral arterial catheter was inserted and snares were placed around the carotid arteries before the production of TBI (0.8 atm). Sixty minutes after TBI, a microdialysis catheter was stereotactically inserted into the dorsal hippocampus and perfused at 2 µl/min for an additional 30 minutes before the collection of a preischemic sample. Six minutes of forebrain ischemia was then produced by carotid occlusion and hypotension to 40 mmHg. Dialysis samples were collected throughout the ischemic period and for 30 minutes of reperfusion. Body temperature was maintained at 38°C. Ischemia-only rats (n=6) were treated in an identical fashion, but were not subjected to TBI.

Results and Conclusions: Forebrain ischemia resulted in a 50-100% increase in EAA concentrations in both groups. Although EAA levels were higher in the TBI group throughout the duration of the study, this finding reached statistical significance only for aspartate concentrations. These results suggest that EAA levels may be tonically elevated after TBI thereby increasing the brain's duration of exposure to these potential excitotoxins.

1. J Trauma 34:216, 1993. 2. J Neurosurg 79:369, 1993

622.3

FIMBRIA-FORNIX (FF) LESIONS AND EXCITOTOXIC INJURY PRODUCE SIMILAR DAMAGE TO SEPTAL NEURONS. S.D. Ginsberg*[1,2], C. Portera-Cailliau[1,2,4], D.L. Price[1,4], and L.J. Martin[1,2,4]. [1]Neuropathology Lab., Dept. of [2]Pathology, [3]Neurology, and [4]Neuroscience, The Johns Hopkins Univ. Sch. Med., Balt., MD 21205.

The FF transection paradigm has been used primarily as a model of retrograde degeneration within the medial septal nucleus (MSN). We tested the hypothesis that anterograde and transynaptic degeneration, as well as retrograde degeneration, occurs following unilateral FF transections in adult rats. The MSN, dorsolateral septal nucleus (LSNd), and medial mammillary nucleus (MMN) were analyzed ultrastructurally at 3, 7, 14, and 30 days postlesion. Terminal degeneration was observed predominantly at 3 and 7 days postlesion within the MMN and at 7 and 14 days postlesion within the MSN and LSNd. Somatodendritic changes, including membrane- bound vacuoles and loss of cytoplasmic matrix, occurred mainly at 7 and 14 days postlesion within the ipsilateral MSN, LSNd, and MMN, suggesting both retrograde and transynaptic degeneration. The somatodendritic pathology resembled some of the morphological changes following excitotoxic damage to forebrain neurons. We tested whether excitotoxic injections of quinolinate (60 nmol) or kainate (25 nmol) into the septal complex of adult rats would mimic the transynaptic ultrastructural damage observed following FF transections. Similar patterns of somatodendritic injury were observed between the axotomy and excitotoxicity models, suggesting that an excitotoxic-like event occurs transynaptically as a result of FF transections. Because glutamate is the principal transmitter of hippocampal/subicular efferents within the FF, axotomy of these fibers may cause a traumatic release of glutamate and resultant excitotoxic damage to postsynaptic targets expressing functional glutamate receptors.

622.4

CHARACTERIZATION OF DELAYED NEURODEGENERATION INDUCED BY OXYGEN/GLUCOSE DEPRIVATION IN ISOLATED RETINA. C. Romano*[1], M.T. Price[2], T. Almli[2] and J.W. Olney[2] Depts. of Ophthalmology[1] and Psychiatry[2], Washington University Medical School, St. Louis, MO 63110.

The isolated embryonic chick retina is an excellent preparation for studying the acute pathomorphological changes resulting from excitotoxic stimulation. We have recently adapted this preparation for studying delayed excitotoxicity by maintaining isolated chick embryo retinas in isolation for 24 hours and assessing the health of the tissue by both histological and biochemical means (LDH release). Studies of neurotoxicity induced by brief exposure to excitatory amino acid agonists in this system have indicated that non-NMDA receptor activation, as well as NMDA receptor activation, leads to delayed neurodegeneration (*J. Neurochem.* **65:** 59-67). This is in marked distinction to other *in vitro* systems, such as cultures of cortical or hippocampal neurons from embryonic rodent brain, in which prolonged stimulation of non-NMDA receptors with high concentratons of agonists is necessary to cause neuronal death.

Neurodegeneration was induced by simulated ischemia (SI, i.e. combined oxygen/glucose deprivation) in this preparation. The retinas were subjected to varying periods of SI, then placed into glucose-containing, oxygenated media and the incubation continued until 24 h from the start of the experiment. Two hours of SI led to substantial but submaximal retinal LDH release by the next day, and was chosen as a standard lesion. LDH release ocurred gradually after two hours of SI, requiring about 12 hours for half of the amount eventually released to appear in the medium. Providing low concentrations of glucose during SI could prevent LDH release: 100 µM glucose prevented half that caused by oxygen/glucose deprivation.

Glutamate receptor antagonists were effective neuroprotectants. Maximally effective concentrations of the NMDA receptor antagonist MK-801 (10 µM) and the non-NMDA receptor antagonist GYKI 52446 (100 µM) lowered LDH release to 23± 3 and 42±2 %, respectively, of that observed in their absence. Combining the antagonists resulted in near complete protection (2± 0.2%). These results indicate that neurodegeneration induced by SI in this preparation is an excitotoxic process mediated by both classes of ionotropic glutamate receptors.

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622.5

GLUTAMATE MEDIATED EXCITOTOXICITY IS MACROMOLECULAR SYNTHESIS DEPENDENT IN CULTURED CEREBELLAR GRANULE NEURONS. J. Rollins and J. Holliday.* Department of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY 14642.

There is evidence that several types of cellular mechanisms can be involved in neuronal glutamate toxicity depending upon the cell type, age, and glutamate challenge paradigm. However, toxicity usually depends upon activation of the NMDA-subtype of glutamate receptor with a subsequent increase in intracellular calcium. We have tested the hypothesis that excitotoxicity is a result a glutamate-activated apoptosis, or "programmed" cell death, in a cellularly defined culture system of purified cerebellar granule neurons.

Exposure of neuron cultures (11 days *in vitro*) to glutamate under conditions that permit the activation of the NMDA receptor (low magnesium) for 15 minutes causes approximately 15% of the cells to undergo glutamate-induced death after 12 hours. Control neurons were treated with the low magnesium solution without added glutamate. Application of transcription or translation inhibitors significantly reduces the death due to glutamate exposure by nearly 50%. In addition, glutamate challenge specifically increases the expression of key proteins, despite the decline in neuronal viability. These data support the notion that glutamate-mediated cell death utilizes mechanisms common to other types of apoptotic neuron death, such as nerve growth factor deprivation (Martin et al, 1988).

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622.6

THREE TYPES OF EXCITOTOXICITY EVALUATED FOR "APOPTOSIS" SIGNALS. M. Ishimaru, T. C. Der, T. I. Tenkova, M.A. Sesma, J. H. Thurston* and J. W. Olney. Dept of Psychiatry, Washington Univ Med School, St. Louis MO.

Using available tools for diagnosing apoptosis, several authors have recently reported that excitotoxicity can trigger an apoptotic cell death process. This prompted us to use Apop Tag, a commercially available *In Situ* Apoptosis Detection Kit ® (Oncor) to see if apoptotic signals occur in the following types of excitotoxic neurodegeneration: 1. Seizure-mediated brain damage induced by sc administration of kainic acid (KA). 2. Subacute degeneration of posterior cingulate cerebrocortical neurons induced by the NMDA antagonist, phencyclidine (PCP). 3. Acute degeneration of arcuate hypothalamic (AH) neurons induced by sc administration of glutamate (Glu) to infant rats. Following KA treatment, Apop Tag signals started to appear at 12 h post-treatment (pt) and were detectable for up to 7 days pt in various brain regions subject to seizure-mediated damage. Following PCP treatment, there were no Apop Tag signals detected in the posterior cingulate cortex over a 4 day period during which abundant cell death was occuring in this brain region. Following Glu treatment, robust Apop Tag signals began to appear in AH at 4 hr pt and became increasingly more abundant at 8 and 16 hr. Interestingly, Apop Tag positivity in AH was not only associated with individual dying neurons but with macrophages that were engorged with degeneration products. It is also of interest that individual "necrobiotic" neurons scattered over other parts of the infant CNS were Apop Tag positive.

Many years ago, when excitotoxic neuronal necrosis was first described (Olney,1969), it was contrasted with "necrobiosis", a type of necrosis occurring as a programmed biological event during development. Today, "necrobiosis" has been supplanted with "apoptosis", and "necrosis" is an abused word that no longer connotes cell death but only non-apoptotic cell death. This is confusing; would it not be better to let necrosis mean cell death and recognize two categories of cell necrosis: apoptotic and non-apoptotic? But, if cell necrosis is to be subdivided into apoptotic and non-apoptotic categories, we need valid tools for defining apoptosis. Apop Tag may not be a valid tool since it marks both excitotoxic "necrosis" and "necrobiosis" as if both were "apoptosis". Supported by DA 05072, AG 11355 & RSA MH 38894 (JWO).

622.7

CALCIUM IONOPHORES CAN INDUCE EITHER APOPTOSIS OR NECROSIS DEATH OF CULTURED CORTICAL NEURONS. B.J. Gwag*, S.L. Sensi, L.M.T. Canzoniero, J.A. DeMaro, J. Koh, M.P. Goldberg, M.F. Jacquin, and D.W. Choi. Dept. of Neurology and the Center for the Study of Nervous System Injury, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Cultured murine cortical neurons exposed to low concentrations of the Ca^{2+} ionophores, ionomycin (250 nM) or A23187 (100 nM), for 24 hours underwent apoptosis, characterized by early degeneration of neurites, cell body shrinkage, chromatin condensation, and internucleosomal DNA fragmentation (laddering). This apoptosis death could be attenuated by inhibitors of protein or RNA synthesis, or certain growth factors - BDNF or IGF-I, but not bFGF. If the ionomycin concentration was increased to 1 - 3 μM, then neurons underwent necrosis, characterized by early cell body swelling without DNA laddering, and lacking sensitivity to cycloheximide or growth factors. Calcium imaging with fura-2 suggested a possible basis for this differential effect of low and high concentrations of ionomycin. At low concentrations, ionomycin induced greater increases of $[Ca^{2+}]_i$ in neurites than in neuronal cell bodies, whereas at high concentrations, ionomycin induced high levels of $[Ca^{2+}]_i$ simultaneously in both neurites and cell bodies. In addition, high concentrations of ionomycin also produced a marked increase in $[Na^+]_i$ detected with SBFI. The ability of low concentrations of Ca^{2+} ionophores to raise $[Ca^{2+}]_i$ preferentially in neurites may be responsible for early neurite degeneration, leading to loss of growth factor availability to the cell body and consequent apoptosis. High concentrations of ionomycin may induce overwhelming Ca^{2+} overload resembling that induced by NMDA receptor overactivation, and consequent necrosis.

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622.8

EVEN SLOWLY-TRIGGERED EXCITOTOXIC NEURONAL DEATH OF CULTURED CORTICAL NEURONS OCCURS BY NECROSIS, NOT APOPTOSIS. D.W. Choi*, J. Koh, J.A. DeMaro, H.S. Ying, M.F. Jacquin and B.J. Gwag. Dept. of Neurology and Center for the Study of Nervous System Injury, Washington Univ. School of Medicine, St. Louis, MO 63110.

We have previously reported that the rapidly-triggered death of cultured murine cortical neurons induced by brief intense exposure to NMDA or glutamate occurs by necrosis. Here we examined graded levels of slowly-triggered excitotoxic neuronal death induced by NMDA, AMPA, or kainate to determine whether it occurred by necrosis or apoptosis. As previously reported, exposure of murine cortical cell for 24 hours to 10-50 μM concentrations of NMDA, AMPA, or kainate produced degeneration of neurons without glial death. Neuronal cell body swelling was evident after 2 hr of agonist exposure, associated at the electron microscope level with disruption of cytoplasmic membrane integrity and organelles, and nuclear chromatin condensation in a dispersed pattern. Death was not attenuated by cycloheximide, or the neurotrophic factors BDNF or IGF-I, agents that are effective in blocking several types of apoptosis neuronal death in the same cultures. However, agarose gel electrophoresis revealed some internucleosomal DNA fragmentation (ladders) appearing transiently 8 hr following excitotoxin exposure. Despite these transient DNA ladders (and acknowledging the somewhat vague boundaries of the necrosis and apoptosis categories), we view the above data in sum as most supportive of necrosis death.

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622.9

Stimulation of NMDA but not AMPA/KA Receptors Induces Internuleosomal DNA Fragmentation in Rat Striatum

Zheng-Hong Qin, Yumei Wang and Thomas N. Chase*, Experimental Therapeutics Branch, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD 20892

Previous studies have indicated that the instrastriatal administration of the excitotoxin quinolinic acid induced apoptosis in rat striatum. To evaluate whether glutamate receptor agonists generally act by apoptotic mechanism to induce cell death, and to characterize the subtypes of glutamate receptors which are involved in inducing apoptosis, we examined the biochemical and morphological effects of intrastriatal administered various doses of N-methyl-D-aspartate (NMDA) receptor agonist NMDA and quinolinic acid (QA); AMPA receptor agonist quisqualate (QUIS); and kainate receptor agonist kainic acid (KA). All these agonists induced profound striatal cell death as indicated by the significant loss of D1 and NMDA receptors. Agarose gel electrophoresis revealed that only NMDA (75-300 nmol) and QA (60-240 nmol) but not QUIS (75-300 nmol) and KA (1.25-5 nmol) induced internucleosomal DNA fragmentation. TUNEL labeled nuclei were observed in all four agonists treated striatum. Many fragmentated nuclei were seen in NMDA or QA-treated striatum but are rarely detected in QUIS or KA-treated striatum. The NMDA or QA-induced internucleosomal DNA fragmentation and positive TUNEL labeling were abolished by the NMDA receptor antagonist MK-801 but not by the AMPA/KA receptor antagonist NBQX. Treatment with MK-801 but not NBQX also prevented NMDA or QA-induced cell death in striatum.

These results demonstrate that excessive stimulation of NMDA receptors leads to apoptotic cell death but stimulation of AMPA/KA receptors may lead to necrosis, suggesting that different cellular mechanisms are involved in neurodegeneration induced by different glutamate receptor agonists.

622.10

TYROSINE KINASE INHIBITORS INDUCE NEURONAL APOPTOSIS, BUT ATTENUATE NMDA-MEDIATED NEUROTOXICITY IN CORTICAL CELL CULTURES. M.M. Behrens*, B.J. Gwag, J. Koh and D.W. Choi. Dept. of Neurology and Center for the Study of Nervous System Injury, Washington Univ. School of Medicine, St. Louis, MO 63110.

Protein tyrosine kinase (PTK) inhibitors, genistein or lavendustin A, produced a concentration-dependent, slowly evolving neuronal death in cortical cell cultures containing both neurons and glia. This neuronal death was associated with cell body shrinkage and attenuated by cycloheximide, consistent with apoptosis. Cotreatment with 100 μM vanadate, a phosphotyrosine phosphatase inhibitor, substantially reversed the neuronal death induced by genistein or lavendustin A. In contrast, vanadate did not attenuate apoptosis induced by staurosporine (a nonspecific protein kinase inhibitor; 100 nM) or GF 109203X (a selective PKC inhibitor; 15 μM).

Since several studies have implicated protein kinases as downstream mediators of excitotoxicity, we performed additional experiments to see if inhibitors of PTK or PKC would block excitotoxicity in the same cultures. Indeed, the $^{45}Ca^{2+}$ accumulation or the excitotoxic neuronal necrosis induced by exposure to 10 - 30 μM NMDA for 24 hr was attenuated by cotreatment with 10 uM lavendustin A or 100 μM genistein; slowly-triggered AMPA or kainate toxicity was not reduced. Although cycloheximide itself did not reduce neuronal death following NMDA exposures, the combination of cycloheximide together with a tyrosine kinase inhibitor almost completely blocked NMDA induced neuronal death. Vanadate reversed the protective effect of PTK inhibitors against NMDA neurotoxicity. These data suggest that tyrosine kinase activity may be a two-edged sword relative to neuronal cell death: needed to suppress apoptosis, but capable of promoting NMDA receptor-mediated excitotoxic necrosis. Supported by NIH grant NS 30337 (DWC); MMB was a recipient of a fellowship from the Spanish Ministry of Science and Education

622.11

ANTI-EXCITOTOXIC EFFECT OF MILD HEAT STRESS ON CULTURED CORTICAL NEURONS IS NOT DEPENDENT ON HSP70 EXPRESSION. B. J. Snider* and D.W. Choi. Dept. of Neurology and Center for the Study of Nervous System Injury, Washington Univ. School of Medicine, St. Louis, MO 63110.

The cytoprotective effects of heat stress in many systems are correlated with expression of the 70 kDa heat shock protein (hsp70). Heat stress leading to hsp70 expression protects cerebellar or cortical neurons from excitotoxic insults (Lowenstein et al., Neuron 7 :1053,1991; Rordorf et al., Neuron 7 :1043,1991). Last year we reported that high level heat stress (44 °C for 25 min), a paradigm that produces hsp70 expression in astrocytes but not neurons, protected neurons in our murine cortical cell cultures from oxygen-glucose deprivation-induced injury, but not excitotoxic injury (Soc. Neurosci. Abstr. 20: 1479, 1994). Here, we examined the possibility that more gentle heat stress would have an anti-excitotoxic effect.

Mixed neuronal-glial or neuronally-enriched (>95% neurons) murine cortical cultures were heat stressed at 42.5 °C for 1 hr or at 44°C for 25 min. Immunocyto-chemistry and Western blotting showed that both treatments induced hsp70 in the glia but not neurons. Mixed cortical cultures that were heat stressed at 42.5 °C exhibited 20-50% less neuronal death after 24 hr exposure to 17.5 μM NMDA, or 10 min. exposure to 10-1000 μM glutamate in low chloride medium, than control cultures or cultures heat stressed at 44°C. Neuronally-enriched cultures that were heat stressed at 42.5°C exhibited less $^{45}Ca^{2+}$influx and neuronal death after a subsequent 10 min exposure to glutamate (10-1000 μM) in low chloride media, than control cultures. These data confirm earlier work suggesting that heat stress can reduce neuronal vulnerability to excitotoxicity, but raise the possibility that this neuroprotection need not be due to hsp70 induction.

Supported by NIH grant NS 30337 (DWC).

622.12

IN SITU NICK TRANSLATION DEMONSTRATES WIDESPREAD DNA FRAGMENTATION IN RAT BRAIN AFTER KAINIC ACID-INDUCED STATUS EPILEPTICUS. L.S. Dure IV*, M.G. Kautzman, and A.J. Newman. Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL 35294-0021.

Kainic acid (KA), a rigid analog of glutamate, is a potent neurotoxin which produces a syndrome of limbic status epilepticus after systemic administration in the rat. Previous studies have revealed significant losses of CA3 and CA1 pyramidal neurons after KA exposure, with concomitant neuropathology in the amygdala, midline thalamus, and septal regions. To elucidate the anatomical distribution of KA-induced neuronal loss, we utilized the method of *in situ* nick translation to incorporate biotinylated dUTP into fragmented DNA in rat brain after limbic status epilepticus. A time course of labeling was also performed to examine the temporal distribution of DNA fragmentation. Our results indicate that DNA fragmentation does not appear before 12 hours post-KA, and the earliest regions to demonstrate DNA fragmentation were the amygdala and midline thalamic nuclei. By 24 hours, CA1 pyramidal neurons were strongly labeled, and labeling persisted for some days after KA administration. Anatomic distribution of DNA fragmentation was compared to regions of neuronal loss as determined by silver impregnation and found to be correspondent. *In situ* nick translation is an extremely sensitive marker for DNA fragmentation, and is a powerful tool for assessing the extent of neuronal loss after excitotoxic lesions.

622.13

GLUTAMATE AND DOMOATE EXCITOTOXICITY IN CULTURED RAT CEREBELLAR GRANULE NEURONS IS COMPOSED OF ACUTE OSMOTICALLY MEDIATED AND DELAYED NEURODEGENERATIVE COMPONENTS. F.W. Berman, P.H. Franklin* and T.F. Murray. College of Pharmacy and Toxicology Program. Oregon State University, Corvallis, OR 97331

The effect of osmotically mediated neuronal swelling on the acute and delayed neurotoxicity induced by excitatory amino acids in vitro was assessed in cerebellar granule neurons. Neurons were exposed to glutamate (300μM) or domoate (10μM) for two hours in physiologic buffer at 22°C followed by a 22hr incubation in conditioned growth media at 37°C. Excitotoxicity was monitored at several timepoints by analysis of lactate dehydrogenase (LDH) activity in the exposure buffer and conditioned media. Under these conditions, 50% and 65% of the 24hr LDH efflux was produced after 2hrs by glutamate and domoate, respectively. This acute toxicity was completely prevented by the presence of 100mM sucrose, indicating that osmotic swelling contributed significantly to neuronal injury induced by these neurotoxins. Subsequent delayed neurodegeneration however, was not affected by the hyperosmolar medium. The competitive NMDA receptor antagonist D-AP5 reduced total LDH efflux by 75% and 70%, whereas the non-NMDA receptor antagonist NBQX reduced total LDH efflux by 25% and 100% for glutamate and domoate, respectively. The acute osmotically mediated LDH efflux appeared to be due primarily to NMDA receptor activation. These results suggest that glutamate toxicity is mediated primarily by activation of NMDA receptors, however stimulation of non-NMDA receptors by glutamate produces significant toxicity in the presence of an NMDA receptor antagonist. Although domoate toxicity is mediated by activation of non-NMDA receptors, domoate-induced release of glutamate and subsequent activation of NMDA receptors underlies the majority of domoate-induced LDH efflux.

622.14

Differential Effects of Kainic Acid at The Caudal and Rostral Ventrolateral Medulla. R.M. Douglas*, C.O. Trouth, L.M. Sexcius, E.T. Anderson, T. Cofield, and P. Khansari. Dept. of Physiol. and Biophysics, Coll. Med., Howard University, Washington, D. C. 20059.

The caudal (CVLM)- and rostral (RVLM)-ventrolateral medulla are involved in cardio-respiratory regulation. In this investigation, kainic acid(KA)(1%; 47 mM) was applied to the CVLM and RVLM in spontaneously breathing, chloralose-urethane anesthetized rats. Extracellular medullary neuronal activity(MNA) and diaphragmatic electromyo-graphic activity (DEA) were recorded. Unilateral KA application to the RVLM, near the retro-trapezoid nucleus, produces a transient decrease in MNA and DEA and a loss of CO_2 responsiveness- recovery occurring within 60 min. Bilateral application abolishes MNA and DEA. KA applied uni-laterally to CVLM evokes a dramatic transient increase in MNA and DEA followed by a decrease to extinction and irreversible apnea. The CVLM appears to exert a greater influence on the respiratory motor output within a hierarchy of brainstem chemo-sensory elements. Support:NIMH 5T32M19547 and ONR N00014-94-1-0523.

622.15

A COMPARISON OF THE NEUROTOXIC EFFECTS OF DIFFERENT EXCITOTOXINS IN THE RAT LATERODORSAL TEGMENTAL NUCLEUS. W.L. Inglis* and K. Semba. Department of Anatomy and Neurobiology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.

Excitotoxins are valuable tools in neuroscience research as they can help us to discover the extent to which certain neurons are necessary for different types of behavior. They have distinctive neurotoxic effects depending on where they are infused: for instance, AMPA is able to make relatively selective lesions of basal forebrain cholinergic neurons (Robbins *et al.*, Neuroscience 28: 337), but has indiscriminate effects in the pedunculopontine tegmental nucleus (PPTg) where quinolinate is fairly selective under certain anaesthetic conditions (Rugg *et al.*, Brain Res. 589: 181; Inglis *et al.*, Neurosci. Lett. 156: 78). This study was conducted to determine whether the neurotoxic profile of excitotoxins in the laterodorsal tegmental nucleus (LDTg) is similar to that which has been described in the PPTg. Two 0.1 μl infusions of 0.1 M ibotenate, 0.1 M quinolinate, 0.04-0.1 M NMDA, or 0.05-0.015 M AMPA, were made unilaterally into the LDTg in 48 rats (300-320 g) under either pentobarbitone or Avertin anaesthesia. Co-ordinates were AP +0.1 IAL, ML ±3.2, DV -5.8 SkS and AP -0.4 IAL, ML ±3.6, DV -6.4 SkS with the injection needle oriented at an angle of 24° in the ML plane. After 23-27 days, sections through the mesopontine tegmentum were processed using standard histochemical procedures for ChAT, NADPH diaphorase, TH and Nissl substance. Lesions were assessed by the size of the damaged area (identified by reactive gliosis) and degree of cholinergic and other cell loss. Ibotenate induced compact lesions in the LDTg (80-90% cholinergic loss) but did not affect locus coeruleus. Quinolinate and low doses of AMPA and NMDA made very small lesions (<20% cholinergic loss), while at higher doses AMPA and NMDA had destructive effects over very large areas but killed only some of the neurons within this. These data are consistent with neurotoxic effects of the same excitotoxins which have been previously documented for the PPTg. *Supported by The Wellcome Trust (038117/Z/93) and the MRC of Canada (MT-11312).*

622.16

IMAGING CELL SWELLING & TOXICITY IN THE HIPPOCAMPAL SLICE PRODUCED BY DOMOIC ACID AND ANTAGONIZED BY GYKI 52466. *Trevor M. Polischuk* and R. David Andrew* Department of Anatomy & Cell Biology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Excitotoxic processes are implicated in many neurodegenerative disorders. Early excitotoxic events are characterized by an acute phase of cell swelling. Cell swelling alters intrinsic optical signals of brain slices such that changes in light transmittance (ΔT/T) correlate with changes in cell volume. We imaged the effects of domoic acid (DOM), a seafood contaminant and potent glutamate agonist, on light transmittance through the rat hippocampal slice. At 22°C, 1 min exposure to 10 μM DOM elicited *reversible* changes in transmittance in CA1 dendritic regions. DOM maximally elevated ΔT/T by 56±2% in CA1 stratum radiatum (RAD), returning to baseline levels by 30 min (n=10). At 37°C, DOM elevated ΔT/T by 58±2% and rapidly reversed within 13 min (n=5). Prolonged exposure to 10 μM DOM (10 min) at 37°C resulted in an *irreversible* transmittance change in CA1 RAD. A peak response of 57±2% in CA1 RAD was followed by a latent and pronounced decrease in light transmittance (-34±4%) which did not return to baseline levels (n=10). This irreversible "swell/shrink" phenomenon was regarded as an indicator of neuronal death. GYKI 52466 (100 μM), a highly selective antagonist of AMPA/kainate receptor responses, reduced the peak response of 10 μM DOM (1 min/22°C and 10 min/37°C) to 6±1% and 21±3% respectively. Interestingly, GYKI blocked the irreversible "swell/shrink" event of the prolonged DOM exposure. Transmittance changes evoked by 10 μM AMPA (1 min/22°C) were reduced from 53±2% to 2±1%. Reduction of $[Cl^-]_o$ completely blocked DOM-evoked changes in light transmittance from 56±2% to 0±2%. We conclude that exposure to DOM evokes Cl^--dependent dendritic swelling in the CA1 region mediated by AMPA receptors. Prolonged exposure at body temperature leads to irreversible damage to CA1 neurons. This response can be inhibited by hypothermia or specific glutamate antagonists and can be imaged in real time. *Supported by the Canadian MRC*

622.17

HIGH CONCENTRATIONS OF GLYCINE CAUSE NEUROTOXICITY IN RAT HIPPOCAMPAL SLICE CULTURES BY ACTIVATION OF NMDA RECEPTORS. A. Barth, D.W. Newell*, T.N. Ricciardi, A.T. Malouf. Dept. of Neurological Surgery, Sch. of. Med., Univ. of Washington, Seattle, WA 98195

Glycine can act both as an inhibitory neurotransmitter and as a modulator of the NMDA receptor. Glycine (3-4 mM) induced neurotoxicity in hippocampal slice cultures. CA1 pyramidal neurons were significantly more susceptible to glycine toxicity than CA3 pyramidal neurons and dentate granule cells. All subregions of the hippocampus were equally damaged by 10 mM glycine. Glycine neurotoxicity developed within minutes after administration and was complete after 5-6 hours. Intracellular recordings from CA1 pyramidal neurons showed that 10 mM glycine initially produced an 11 mV hyperpolarization followed by repolarization and tonic spiking (n=6). Large depolarizations and sustained bursts of action potentials were observed in 2/6 cultures. Glycine abolished all synaptic transmission after 5 to 20 min. Neuronal damage and increased excitability caused by 10 mM glycine were completely blocked by 10 μM MK-801 or 100 μM D-APV. DNQX (100 μM) provided significantly less protection than the NMDA antagonists. These results demonstrate that high concentrations of glycine are neurotoxic and that glycine-induced neurotoxicity is mediated by activation of NMDA receptors. Supported by: NIH Grants 1P50NS30305-1 (DWN,ATM), NS28650 (ATM), Ciba-Geigy-Jubilee-Foundation (AB), CIDA 1K08NS01596901 (DWN), and T32NS-07144-15 (TNR).

622.18

PERSISTENT DEPRESSION OF SYNAPTIC RESPONSES IN QUISQUALATE-SENSITIZED HIPPOCAMPAL SLICES AFTER EXPOSURE TO L-ASPARTATE-β-HYDROXAMATE. J.F. Koerner and R.J. Roon*. Department of Biochemistry, Medical School, University of Minnesota, Minneapolis, MN 55455.

Neurons of the rat hippocampal CA1 pathway are sensitized at least 100-fold to depolarization by certain phosphonate compounds after brief exposure to L-quisqualic acid (QUIS). Three classes of compounds with distinct modes of action interact with this system: the prototypic agonist for sensitization, QUIS; phosphonate compounds such as L-AP4, L-AP5 and L-AP6, to which neurons become sensitized; and compounds such as L-α-aminoadipic acid (αAA) which pre-block or reverse sensitization. The phosphonates appear to act at extracellular receptors to induce neuronal depolarization, and their effects are rapidly reversed by washout. We now report a novel class of compounds which behave in a similar manner to the phosphonates except that their effects persist after washout. Exposure of QUIS-sensitized slices to 400 μM L-aspartate-β-hydroxamate for eight minutes results in complete depression of extracellular synaptic field potentials. This depression persists for at least one hour after washout. Complete restoration of the field potentials can be achieved by a 10 minute application of the reverser αAA, suggesting that there is some mechanistic commonality between the effect of phosphonates and L-aspartate-β-hydroxamate. L-Glutamate-γ-hydroxamate, D-aspartate-β-hydroxamate, and L-AP3 exhibit similar but weaker depressive activity. (Supported by NIH NS 17944).

622.19

QUISQUALATE SENSITIZATION AS A PUTATIVE EARLY STEP FOR QUISQUALATE NEUROTOXICITY OF HIPPOCAMPAL CA1 PYRAMIDAL NEURONS. L.A. Chase*, B. Rupert, R.J. Roon and J.F. Koerner. Department of Biochemistry, Medical School, University of Minnesota, Minneapolis, MN 55455.

L-Quisqualic acid (QUIS) sensitizes hippocampal pyramidal cells to depolarization by phosphonates.[1] Sensitization involves uptake of QUIS into a subset of hippocampal interneurons[2,3] and subsequent release of QUIS or an endogenous excitatory amino acid which acts postsynaptically as both an NMDA and a non-NMDA receptor agonist.[4] Pharmacological similarities of QUIS sensitization in hippocampal slices and of QUIS toxicity on cultured pyramidal cells[5,6] suggest that the mechanism which mediates QUIS sensitization also initiates QUIS neurotoxicity. As is true for QUIS-mediated neurotoxicity,[5] we observed sensitization to occur in low Ca^{2+} (0.5 mM) medium and in medium in which Na^+ was replaced by choline. Sensitization also occurred in the presence of L-*trans*-pyrrolidine-2,4-dicarboxylic acid (1 mM). This suggests that synaptic function and concentrative Na^+-dependent uptake of QUIS may not be required for sensitization. Addition of 0.5 mM Cd^{2+} blocks L- and N-type Ca^{2+} channels and inhibits orthodromic field potentials, but QUIS sensitization is observed antidromically. CNQX prevents QUIS toxicity when maintained in the culture medium after QUIS exposure.[6] Antidromic recording demonstrated that CNQX prevents depolarization by phosphonates in QUIS-sensitized slices. This supports previous intracellular observations with organotypic slice cultures.[6] (Support: NIH NS 17944).

[1]Robinson *et al.*, Brain Res. **381** 187 (1986) [2]Schulte *et al.*, Brain Res. **605** 85 (1993)
[3]Price *et al.*, Brain Res. **663** 317 (1994) [4]Charpak *et al.*, Eur. J. Neurosci. **4** 491 (1992)
[5]Zinkand *et al.*, Eur. J. Pharmacol. **212** 129 (1992)
[6]Garthwaite & Garthwaite, Neurosci. Lett. **99** 113 (1989)

EXCITATORY AMINO ACID RECEPTORS XII

623.1

ATTENUATION OF MK-801-INDUCED LEARNING IMPAIRMENT BY THE NEUROSTEROID DHEAS: IMPLICATION OF σ_1 RECEPTORS. T. Maurice*[1], J.-L. Junien[2] and A. Privat[1].
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We previously reported that high affinity σ_1 ligands attenuate the learning impairment induced in mice by MK-801, a non-competitive N-methyl-D-aspartate (NMDA) antagonist, in a similar manner as they modulate several NMDA-evoked responses in vitro and in vivo in rats. Neurosteroids, such as pregnenolone sulfate, progesterone and dehydroepiandrosterone sulfate (DHEAS), modulate some NMDA-evoked responses. Furthermore, some of them were reported to interact with σ receptors. We thus investigated whether DHEAS, a neurosteroid with memory enhancing effects, attenuates the MK-801-induced learning impairment in mice and, if so, by a mechanism involving σ_1 receptors. Behavioural tests included spontaneous alternation behaviour in the Y-maze, for spatial working memory, and step-down passive avoidance, for long-term memory. DHEAS (5-20 mg/kg sc) dose-dependently and significantly attenuated the impairment of alternation and decrease in step-down latency induced by MK-801 (0.15 mg/kg ip). These effects were suppressed by the simultaneous administration of the σ_1 antagonist BMY-14802 (5 mg/kg ip), and significantly reduced in mice sub-chronically treated with haloperidol (4 mg/kg/day sc for 7 days), a treatment down-regulating the σ_1 receptors. Furthermore, at the same dose range, DHEAS significantly decreased the in vivo [^{3}H]SKF-10,047 binding to σ receptors in the mouse cortex and hippocampus. These results confirm that neurosteroids, such as DHEAS, are positive modulator of the NMDA receptor activation, and modulate the NMDA dependent learning processes. This effect appears to be mediated through an interaction with σ_1 receptors.

623.2

CO-ADMINISTRATION OF MONOSODIUM GLUTAMATE (MSG) AND MONOSODIUM ASPARTATE (MSA) FOLLOWING PRETREATMENT WITH MK-801 HYDROGEN MALEATE IN PRE-WEANLING RATS. T. Bambrick and M. Saari*. Neuroscience Research Unit, Nipissing University, 100 College Dr., North Bay, Ontario, Canada, P1B 8L7.

Neonatal male rat pups were randomly allocated to eight treatment groups in a 2 X 2 X 2 factorial design. Half of the rats were injected with either MK-801 or saline (SAL) on postnatal day (PND) 4. Two hours following the initial injection the rats in each group were injected with either MSG (4g/kg; sc.) or SAL and MSA (2g/kg; sc.) or SAL. Following weaning at PND 25 the rats were housed in standard individual Nalgene cages in reversed light, controlled humidity and temperature until PND 62. Behavioral testing in the water maze commenced on PND 63. The rats were sacrificed on PND 72, carcasses dissected and organ weights taken. The experiment was replicated in a smaller study without MK-801 pretreatment. Analysis of the water maze data revealed the expected main effects as well as an unexpected MSG X MSA interaction. A similar pattern of results was found in both the body weight and organ weight data. Maximal MK-801 "protective" effects were observed in the groups receiving both MSG and MSA.

623.3

BIOCHEMICAL AND BEHAVIORAL ACTIONS OF INHIBITORS OF KYNURENINE-HYDROXYLASE AND/OR KYNURENINASE. A. Chiarugi, R. Carpenedo and F. Moroni*. Department of Preclinical and Clinical Pharmacology, University of Florence, Viale Morgagni 65, 50134 Florence, Italy.

Kynurenic acid (KYNA), an endogenous antagonist of the glutamate receptors, is synthesized from kynurenine (KYN) by the enzyme kynurenine-aminotransferase. KYN is also a substrate of the enzymes kynurenine hydroxylase, which catalyzes the formation of 3-OH-KYN, and kynureninase, which metabolizes both KYN and 3OH-KYN into anthranilic acid (ANA) and 3-OH-ANA, respectively. Further metabolism of these molecules may lead to quinolinic acid, an endogenous NMDA agonist and an excitotoxin. In order to switch KYN metabolism toward KYNA formation, we previously studied several inhibitors of kynurenine hydroxylase and kynureninase (*Neuroscience* **61**: 237, 1994; *J.Neurochem.*, in press). We report now the effects of systemic administration of *m*-nitrobenzoylalanine (*m*NBA, an inhibitor of kynurenine hydroxylase) and of *o*-metoxybenzoylalanine (*o*MBA, an inhibitor of kynureninase) on the content of KYNA, 3-OH-KYN, ANA, 3-OH-ANA in brain and blood of mice treated with KYN (0.3 mmol/kg i.p.). Both *m*NBA (200-400 mg/kg i.p.) and *o*MBA (400-800 mg/kg i.p.) increased the concentration of KYNA in brain and blood, but *m*NBA was more potent than *o*MBA, thus confirming that the hydroxylation is the main pathway of KYN metabolism in mice. As expected, *m*NBA decreased the content of 3-OH-KYN in the brain and periphery, while *o*MBA increased the content of this potentially toxic metabolite. Interestingly, while *m*NBA decreased brain content of 3-OH-ANA, *o*MBA did not change the brain content of this metabolite, thus suggesting that *o*MBA inhibits kynureninase when the substrate is KYN but does not affect the metabolism of 3OH-KYN. This observation was confirmed in *in vitro* experiments. Finally, the increased synthesis of KYNA obtained by inhibiting KYN metabolism was associated with behavioral effects such as sedation and antagonism of amphetamine actions, compatible with the expected functional antagonism of excitatory amino acid receptors.

623.4

EFFECTS OF SYSTEMIC MK-801 ADMINISTRATION ON THE EXPRESSION OF GLUTAMINE SYNTHETASE mRNA. T. Kakigi*, R. Kamidate, M. Hatakoshi, Y. Yamamoto, M. Yasuda, K. Maeda and C. Tanaka. Neuropharmacology Lab., Dept. of Neuroscience, Hyogo Institute for Aging Brain and Cognitive Disorders. 520 Saisho- ko, Himeji 670, Japan.

Glutamine synthetase (GS), which is specifically located in astrocytes, but not in neurons, convert synaptic glutamate to glutamine. Glutamine is then sent back to the pre-synaptic neuron for conversion to glutamate. This is one of the protective mechanisms to remove highly excitatory glutamate from the synapse. MK-801 is one of the dissociative anesthetics and acts by interacting with a so-called PCP receptor in the ion channel as potent non-competitive antagonist of the NMDA receptor. In this study, to investigate the effect of MK-801 on the elimination mechanism of glutamate, we measured the levels of GS mRNA in rat brain after subcutaneous injection of MK-801 (1.0mg/kg) at 4 time points (0 hour before injection and 1, 4 and 10 hour after injection). *In situ* hybridization technique using ^{35}S-dATP labeled oligodeoxynucleotide probe was used to determine the GS mRNA levels. Densities of the interested regions were measured by BAS2000 image analyzing system. Results were shown as % of value of 0 time point and statistically analyzed by ANOVA. Results were as follows: density of dorsolateral frontal cortex was significantly reduced (13.8% reduction compared to the value of 0 time point) after 1 hour, significantly elevated in hippocampus (21.4% increase), amygdala (20.4% increase), piriform cortex (18.6% increase) after 10 hour and had no changes after 4 hour of injection. Results showed that non-competitive NMDA receptor antagonist, MK-801, altered the expression of GS mRNA and suggests the alteration of the elimination system of the transmitter glutamate.

623.5

UNCOMPETITIVE NMDA RECEPTOR ANTAGONISTS (MK-801 AND MEMANTINE) IMPAIR OR ENHANCE LEARNING DEPENDING ON DOSE AND EXPERIMENTAL DESIGN - COMPARISON WITH EFFECTS ON LTP *IN VITRO*.
C.G. Parsons[1], G.L. Collingridge,[2] B. Potier[2], T. Frankiewicz[1], W. Zajaczkowski[1], M. Misztal[1] and W. Danysz*[1], Dept. Pharmacol., Merz + Co, 60318 Frankfurt / Main, Germany[1], Dept. Anatomy, The Medical School, Bristol BS8 1TD, England[2].

It is accepted that NMDA receptor antagonists inhibit learning and LTP. In a dark avoidance task uncompetitive NMDA receptor antagonists (+)-MK-801 and memantine impaired learning but starting at relatively high doses (0.2 and 20 mg/kg respectively) leading to serum (and predicted CSF) concentrations far above the respective IC_{50}s for NMDA receptor antagonism (patch clamp studies). Similarly, relatively high concentrations of (+)-MK-801 and memantine were required to inhibit LTP in CA1 of hippocampal slices (threshold 0.1 and 10μM respectively). To maintain *in vivo* steady-state concentrations close to the IC_{50}s assessed in patch clamp studies, these antagonists were infused s.c. for two weeks by osmotic minipumps. Infusion of memantine (20 mg/kg/day) failed to change LTP *ex vivo* or radial maze learning (4/8 design) but in the same test improved reference memory in rats with lesions of the entorhinal cortex (EC). In contrast, (+)-MK-801 (0.31 mg/kg/day) impaired reference memory in naive rats and failed to improve it in rats with EC lesions. It has been suggested (Jones et al. *Pharmacol. Biochem. Behav.* (1989) 34:181) that direct tonic, i.e. non-temporal, activation of NMDA receptors - in contrast to learning - may lead to an increase in synaptic "noise" and in turn to a loss of association detection. In fact, we also observed disruption of dark avoidance learning by NMDA (25 mg/kg) that was not related to toxic effects. NMDA-induced amnesia was antagonised by (+)-MK-801 and memantine at low doses of 0.05 and 2.5 mg/kg respectively. Thus, under some conditions NMDA receptor antagonists can paradoxically enhance learning, and this effect may be related to a restoration o signal to noise ratio following tonic activation of NMDA receptors.

623.6

Ethanol effects on GLU/GLY stimulated changes in the intracellular concentration of magnesium.
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It is well established that NMDA-stimulated increases in intracellular calcium are sensitive to inhibition by physiologically relevant concentrations of ethanol; however, the mechanism of ethanol inhibition is still unknown. NMDA-like (GLU/GLY) stimulated increases in intracellular magnesium have also been reported. The effects of ethanol on this response have not yet been examined.

Acutely dissociated neurons isolated from day old rat pups and loaded with the magnesium sensitive fluorescent probe, furaptra, demonstrated GLU/GLY-stimulated increases in intracellular magnesium that were independent of the presence of magnesium in the extracellular buffer. In the presence of 50mM ethanol the GLU/GLY-stimulated response was not significantly affected. Interestingly, ethanol alone caused an apparent dose-dependent increase in the intracellular concentration of magnesium.

These findings demonstrate that intracellular magnesium can be imaged in acutely dissociated neurons and NMDA-stimulated repsonses can be monitored. This GLU/GLY-stimulated response does not appear to display the expected inhibition by ethanol, but the effect of ethanol on the homeostasis of intracellular magnesium merits further attention.
(This work was supported by R37 AA05809, R01 AA09337, T32 AA07471).

623.7

NEUROPROTECTION FROM NMDA WITHIN THE NUCLEUS BASALIS MAGNOCELLULARIS.
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The activation of glutamate receptors has been implicated in the processes that underlie the development of some neurodegenerative diseases. The antagonism of N-methyl-D-aspartate (NMDA)-sensitive glutamate receptors may therefore have therapeutic applications. The present study compared the neuroprotective potency of MK-801, memantine, amantadine, and the tachykinin Neurokinin B (NKB), against NMDA injected directly into the nucleus basalis magnocellularis of rats. Each drug significantly attenuated the loss of nucleus basalis magnocellularis cholinergic cells. The ED_{50}s were respectively 0.077, 2.81 and 43.5 mg/kg for (+)-MK-801, memantine and amantadine, giving a relative potency ratio of 1:36:565. The results indicate a potential neuroprotective action of these noncompetitive NMDA receptor antagonists. In the case of memantine and amantadine, ED_{50}s are expected to lead to serum levels close to a therapeutic range. When NKB was co-injected with NMDA, it attenuated cholinergic cell loss by 67%, as compared to rats that were coinjected with saline. The neuroprotective actions of the NKB are potentially important inasmuch as a significant proportion of basal forebrain cholinergic cells in humans also contain this tachykinin. NIA RO1 AG10546 & Merz+Co.

623.8

Intrathecal NMDA evokes pain behavior and structural alteration of neurons that express the substance P receptor (SPR) via an action on SP-containing primary afferent terminals in the spinal cord dorsal horn.
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Intrathecal (i.t.) injection of SP or NMDA evokes a short-lived pain behavior. Since i.t. injection of SP, or noxious stimulation, also evokes profound internalization of the SP receptor and a structural reorganization of neurons that express the SPR and since the NMDA receptor is expressed on small diameter primary afferents we hypothesized that SP contributes to the NMDA-evoked effects. Our first study demonstrated that the duration of pain behavior produced by i.t. NMDA (6.8nM) was significantly reduced by prior injection of selective SPR antagonists (CP-99,994 and RP-67,580) or by neonatal injection of capsaicin, to destroy C-fibers. NMDA injection also resulted in extensive internalization of the SPR in neurons of lamina I. EM immunogold analysis revealed that in uninjected rats, approximately 73% of the SPR is associated with plasma membrane of neurons; within 5 minutes of NMDA injection, 85% of the SPR was internalized into cytoplasmic endosomes. NMDA also evoked dramatic structural reorganization of the dendrites that express the SPR. Within 5 minutes, 68% of SPR-immunoreactive dendrites in lamina I and in dorsally-directed dendrites of labelled neurons in lamina III became highly varicose. EM established that the varicosites (2.3-15 μm in diameter) were packed with SPR-immunoreactive endosomes; the intervaricose segments (0.4-1.6 μm in diameter) contained little label. Within one hour of injection most of the SPR was recycled to the plasma membrane and the morphology of these neurons returned to normal. 24 hours later i.t. NMDA again evoked pain behavior and structural changes, indicating that its effects are reversible. I.t. injection of SPR antagonists, or neonatal capsaicin treatment, also significantly reduced these structural changes. We suggest that the effects of NMDA are in part mediated via an action on a presynaptic receptor located on SP-containing small diameter primary afferents. Supported by: NS 21445, 14627 and DA 08377.

623.9

IRREVERSIBLE INHIBITION OF ^{3}H-KAINATE BINDING BY A PHOTOACTIVATED KAINATE ANALOGUE
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In the present study we examine the binding properties of trifluoromethyl-diazokainate (DKA), a putative photoaffinity label for the high-affinity kainate class of EAA receptors. Synaptic plasma membranes (SPMs) were prepared from the forebrains of male Sprague-Dawley rats and assayed for ^{3}H-kainate (KA), ^{3}H-AMPA, and NMDA sensitive ^{3}H-L-glutamate binding. As a competitive inhibitor (in the absence of UV irradiation) DKA preferentially blocked high-affinity ^{3}H-KA binding ($IC_{50} \approx 1\mu M$). At higher concentrations of DKA, some cross reactivity was observed with the AMPA and NMDA sites. For example, inclusion of 100 μM DKA reduced control levels of binding to the kainate, AMPA, and NMDA sites by 98%, 46%, and 38%, respectively. Irreversible inactivation of the binding sites was examined by exposing SPMs to UV irradiation in the presence of DKA, reisolating the SPMs to remove unbound DKA, and then assaying for radioligand binding. Incubation of the SPMs with 50 μM DKA in the presence of UV resulted in the irreversible loss of about 40% of the high-affinity KA sites. Control experiments demonstrated that neither UV nor DKA alone significantly reduced ^{3}H-kainate binding. The irreversible loss of the binding sites was considerably more selective for the kainate sites than would have been predicted from the initial binding studies. Thus, 250 μM DKA in the presence of UV led to an irreversible loss of only 25% of the NMDA sensitive glutamate sites and an insignificant amount of AMPA sites. Consistent with the action of DKA at the kainate ligand binding site, the irreversible loss of the KA sites could be prevented by including an excessive level of L-glutamate (250 μM). These results identify DKA as a useful tool in studying-structure function relationships of KA receptors. This work was supported in part by NIH NS27600.

623.10

THE TREATMENT OF CHRONIC NERVE INJURY PAIN WITH EXCITATORY AMINO ACID ANTAGONISTS AND A NITRIC OXIDE SYNTHASE INHIBITOR IN YOUNG, MATURE AND AGED MALE RATS.
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Unilateral ligation of the rat sciatic nerve is used as an animal model of neuropathic pain. Recent studies have shown that excitatory amino acid antagonists (EAA) and nitric oxide synthase (NOS) inhibitors reverse the behavioral hyperalgesic response to sciatic nerve ligation. These studies were designed to investigate: (1) the effects of aging on the thermal hyperalgesic response to sciatic nerve ligation; and (2) age-related differences in the spinal antinociceptive properties of EAA antagonists, (e.g., D-APV or MK-801) or a NOS inhibitor (L-NAME) administered intrathecally (i.t.). Male Fischer 344 rats (5-6 mos., 15-16 mos. and 25-26 mos.) were used in all experiments, and thermal hyperalgesia was measured using a Plantar Analgesic Meter. No age-related differences were observed in the ability of the sciatic nerve ligation surgery to induce hyperalgesia to a thermal stimulus in young, mature and aged rats. Additionally, D-APV, L-NAME and MK-801 reversed the thermal hyperalgesic response to sciatic nerve ligation in a dose-dependent manner.

623.11

DIFFERENTIAL CHANGES IN GLUTAMATE RECEPTOR SUBUNITS WITHIN SPINAL MOTONEURONS AFTER PERIPHERAL NERVE INJURY.

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Immunoreactivity for the AMPA-type glutamate receptor subunits GluR1, GluR2/3 and GluR4 and the NMDA-type receptor subunit NMDAR1 was studied after sciatic nerve lesion or crush using 2, 6, 10, 14 and 20 days survival. For each survival time, 2 rats were operated: in one rat the sciatic nerve was cut on one side, with the other side as control, while in the other rat the sciatic nerve was cut on one side and crushed on the other side. The rats were perfused (4% paraformaldehyde), and the spinal cords processed for immunocytochemistry using antibodies against the GluR1, GluR2/3, GluR4 (Chemicon) and NMDAR1 (Pharmagen) receptor subunits.

In the L3-S1 spinal segments, immunoreactivity in the motoneurons of the sciatic nerve was assessed. In all animals GluR1 labeling was weak and variable without differences between axotomized, crushed and control motoneurons. GluR2/3 labeling was strong and consistent on the control side. However, axotomized motoneurons showed a strong decrease in immunoreactivty, starting at 2 days. After 6, 10, 14 and 20 days GluR2/3 labeling was very low, while femoral nerve motoneurons in the same ventral horn, showed strong immunoreactivity like the control side. Motoneurons with crushed axons also showed a decrease in immunoreactivity, which was most apparent after 6 days, increasing again after 10 days survival. In all animals GluR4 labeling of motoneurons was strong and consistent without differences between axotomized, crushed and control motoneurons. NMDAR1 labeling was weak but consistent in the sciatic motoneurons, without significant differences between axotomized, crushed and control motoneurons.

It is concluded that after peripheral axotomy, motoneurons show a strong decrease in the expression of GluR2 and 3 subunits, while GluR1, GluR4 and NMDAR1 remain unchanged. After nerve crush, GluR2 and 3 subunit expression also decreased but may return to normal. Thus after axotomy AMPA receptors may be assembled without the GluR2 subunit. As a result, these AMPA receptors may be calcium permeable.

GABA RECEPTORS IV

624.1

GENOMIC REGULATION OF A PROMOTER FOR THE HUMAN $GABA_A$ RECEPTOR $\alpha 1$ SUBUNIT GENE. M. D. Leach* and D. H. Farb. Laboratory of Molecular Neurobiology, Department of Pharmacology, Boston University School of Medicine, Boston MA 02118.

The regulation of $GABA_A R$ subunit gene expression is of great interest as there are 17 distinct subunit genes that have distinct patterns of expression within the CNS as determined by *in situ* hybridization. The mechanisms that lead to the precise expression of the $GABA_A R$ subunit genes are unknown. We have reported previously that the immediate 5'-flanking region of the human $GABA_A R$ $\alpha 1$ subunit gene contains an active promoter as measured by transiently transfecting luciferase reporter constructs into primary embryonic cell cultures. Here, we report that the 5'-flanking region of the human $\alpha 1$ subunit gene confers tissue-specific gene expression. The transcriptional start sites have been identified by primer extension analysis. To map the minimal core promoter, we made a series of 5'-deletion constructs. Results show that the minimal core promoter is located within the immediate 5'-flanking region. A similar deletion strategy was used to identify the region involved in transcriptional initiation, demonstrating that a 76 bp region is important for transcriptional activation. Interestingly, comparison of our results with those of Bateson *et al.* (Gene (1995) 153:243-47) show that the 5'-flanking region for the human and chick $\alpha 1$ subunit genes exhibit a high degree of sequence similarity. Finally, we have examined regulation of the $\alpha 1$ promoter by GABA, demonstrating the existence of autologous regulation of the $\alpha 1$ promoter by $GABA_A R$ activation.

624.2

REGULATORY ELEMENTS IN THE $\beta 2$ $GABA_A$ RECEPTOR GENE. H.Y. Kim and A.J. Tobin*. Departments of Physiological Science and Neurology, Brain Research Institute and Molecular Biology Institute, University of California, Los Angeles, CA 90095.

The predominant form of $GABA_A$ receptors in the adult rat brain contains $\alpha 1$, $\beta 2$, and $\gamma 2$ subunits. We have isolated and characterized the rat $\beta 2$ gene promoter. Sequence analysis revealed several regulatory sequence elements, which include: (1) multiple TATA elements; (2) a CAAT box element; (3) a Neuron-Restrictive Sequence Element; (4) Zif268 protein binding site; (5) multiple AP-1 and SP-1 sites; (6) and a cAMP responsive element. Functional analysis from transient transfection assays of $\beta 2$ promoter reporter constructs revealed at least three separate functional promoter domains: (A) a region containing the "minimal" $\beta 2$ promoter; (B) an activation domain; (C) and a silencer domain. Within domain B, a stretch of alternating G and T residues is important for maximal expression of the $\beta 2$ subunit gene. Gel mobility shift assays suggest that the $(GT)_n$ sequence enhances transcription by interacting with sequence-specific DNA-binding proteins. A $(GT)_n$-binding factor is present in extracts from several different cell lines, as well as in an extract from neonatal rat cerebella. Together, our data suggest that expression of the $\beta 2$ $GABA_A$ receptor gene depends upon the concerted efforts of multiple regulatory elements.

624.3

REGIONAL CHANGES IN $GABA_A$ RECEPTOR (GABAR) α_1 SUBUNIT PROTEIN IN FLURAZEPAM (FZP) TOLERANT RATS: IMMUNOCYTOCHEMICAL DETECTION USING COMPUTER-ASSISTED IMAGE ANALYSIS. X.G. Huang*, S. Chen, W. Sieghart† and E.I. Tietz, Dept. of Pharmacology, Med. Coll. of Ohio, Toledo, OH 43699 and Dept. of Biochem. Psychiatry†, Univ. Clinic of Psychiatry, A-1090 Vienna, Austria.

Changes in GABAR α_1 subunit protein were examined in FZP-treated rats using immunocytochemical methods on thin brain sections. Several approaches were first used to assess the reliability and sensitivity of computer-assisted densitometry as a quantitative method to detect regional changes in antigen concentration as a function of immunohistochemical staining intensity. First, a model system was designed to mimic variations in antigen concentration in post-fixed, slide-mounted sections by varying the ratios of conjugated (biotinylated) to unconjugated 2° antibody (Ab). Antigen concentration was also varied in tissue discs made from rat brain homogenates with increasing amounts of tissue embedding compound. The monoclonal Ab to the GABAR $\beta_{2/3}$ subunit was used as the 1° Ab in these studies. Immunostaining was visualized with diaminobenzidine and staining density was measured over images acquired with NIH Image software. A significant, positive linear relationship (r=.97-.99) was found between immunostaining intensity and antigen concentration. Using this approach changes in antigen of $<<$ 20% were detectable. Since α_1 subunit mRNA is decreased in the CA1 region of hippocampus and cortex after chronic benzodiazepine treatment, this method was then used to analyze changes in GABAR α_1 subunit protein in 10 μM sections from 1 week FZP-treated and control rats using a polyclonal Ab to the α_1 subunit (Sieghart et al.). A significant decrease in α_1 staining was detected between groups (p=.004). Individual comparisons showed reductions (p<.01-.03) in stratum oriens (26%), radiatum (21%) and lancanosum (35%) of CA1, as well as layer IV (28%) and V (30%) of cortex. The regional changes in α_1 subunit staining in chronic FZP-treated rat brain are consistent with the changes in mRNA detected previously and confirm the reliability and sensitivity of computer-assisted image analysis immunostaining as a quantitative technique for detecting changes in antigen content changes in brain tissue. R01-DA-04075 and RSDA K02-DA00180 to EIT.

624.4

DECREASED AMPLITUDE AND FREQUENCY OF SPONTANEOUS IPSCS RECORDED IN HIPPOCAMPAL CA1 PYRAMIDAL NEURONS AFTER CHRONIC FLURAZEPAM (FZP) TREATMENT. Xu Zeng* and E.I. Tietz, Department of Pharmacology, Medical College of Ohio, Toledo, OH 43699

Intracellular recording from CA1 neurons in *in vitro* hippocampal slices from chronic FZP-treated rats showed a reduction in the amplitude of $GABA_A$ and $GABA_B$-mediated IPSPs evoked by direct stimulation of GABAergic interneurons. These findings implied that changes in both pre- and postsynaptic GABA function may contribute to reduced GABA inhibition benzodiazepine tolerant rats. The present experiment was designed to examine this possibility by recording spontaneous IPSCs in hippocampal slices (500 μM) from rats sacrificed 2 days after FZP treatment (100 mg/kg X 3 days; 150 mg/kg X 4 days, p.o. in .02% saccharin H_20). Control rats received saccharin H_20. The experimenter was blind to rat's treatment histories. Spontaneous IPSCs were recorded in CA1 neurons using whole-cell patch clamp techniques (Vh=-70 mV). Patch pipettes (6-10 MΩ) filled with 135 mM CsCl (mM: 130 CsCl, 1.0 EGTA, 0.5 $CaCl_2$, 2.0 $MgCl_2$, 2.0 ATP, 10.0 HEPES, pH 7.2). Inward currents, recorded at -70 mV, were reversed at positive potentials (E_{Cl}=0 mV) and could be inhibited by 5 μM bicuculline in a reversible manner. The amplitude and frequency of spontaneous events was analyzed from 2 min segments using Strathclyde software. Detection threshold was 10 pA. There was a significant difference between the FZP-treated and control groups in the cumulative relative frequency of sIPSC inter-event intervals (p=.02) and amplitudes (p=.005). A decrease in the frequency (1/inter-event interval) and amplitude of events suggests a decrease in pre- and postsynaptic GABA function, respectively, in the CA1 region of FZP tolerant rats. Support: RO1-DA04075 and RSDA K02-DA00180.

624.5

TEMPORAL REGULATION OF $GABA_A$ RECEPTOR (GABAR) BINDING AND COUPLING IN FLURAZEPAM (FZP) TOLERANT RATS. S. Chen, A. Cox, and E.I. Tietz*. Dept. of Pharmacology, Med. Coll. of Ohio, Toledo OH 43699.

GABA-mediated IPSCs are decreased in the CA1 region of hippocampus (HIP). Our recent *in situ* hybridization studies in FZP tolerant rats revealed discrete changes in the regulation of GABAR subunit mRNAs. The α_1 and β_3, but not the α_2, α_5 or γ_2 subunits were decreased in CA1 region and cortex (CTX) immediately (0 days) and 2, but not 7 days after FZP treatment, coinciding with recovery of anticonvulsant tolerance in vivo. These findings prompted the systematic re-examination of BZ and GABA binding and coupling, using autoradiographic techniques, at several time points after 1 week FZP treatment. Rats were treated with FZP (100 mg/3 days; 150 mg/kg X 4 days) in 0.02% saccharin H_2O and sacrificed 0, 2 or 7 days after treatment. Sagittal brain sections (10 μM) were cut from FZP-treated rats and their matched controls (n=8/group). As previously reported, [^{3}H]flunitrazepam (1 nM FNP) binding was significantly decreased in all areas of HIP and CTX 0 days after FZP treatment. BZ/GABA coupling, measured by the ability of 1 μM GABA to potentiate [^{3}H]FNP binding, was decreased in FZP-treated rats in stratum oriens (SO), lanconosum (SL) and moleculare (SM), but not stratum pyramidale (SP) of CA1, as well as CER molecular layer. There were significant differences in coupling at 0 days with 8 μM GABA in CA3 SO, SM and CER. BZ receptor downregulation was reversed in all brain regions 2 days after ending FZP treatment, as previously reported, and 7 days after treatment. The impaired coupling persisted in SO, but not SR of CA1, 2 days after treatment. In the absence of changes in BZ binding 2 days after FZP treatment, coupling was decreased with 8 μM GABA in SO, SR and SP of CA1; SO of CA3 and SM and granule layer of dentate gyrus. Uncoupling of BZ and GABA sites did not persist 7 days after treatment when rats are no longer tolerant. [^{3}H]GABA binding (50 nM) in the presence of 100 μM baclofen was not altered between groups 2 days after treatment. Since EPSP amplitude is also increased in FZP-treated rats, binding at NMDA receptors was studied with [^{3}H]glutamate and [^{3}H]MK-801 2 days after treatment. There were no differences in NMDA receptor binding between groups. Changes in GABA/BZ coupling, which may relate to specific changes in GABAR subunit composition following chronic BZ treatment, may contribute to decreased BZ and GABA potency in the HIP CA1 region and to BZ tolerance. Support: R01-DA04075 and KO2-DA00180.

624.6

CHRONIC DIAZEPAM TREATMENT DECREASES $BZ_1(\omega_1)$-SITE MEDIATED POTENTIATION OF GABA-INDUCED CURRENTS IN ACUTELY DISSOCIATED HIPPOCAMPAL NEURONS. P. Avenet*, V. Itier, P. Granger, H. Depoortere and B. Scatton. CNS Research Department, Synthélabo Recherche, 31 Ave. P. Vaillant-Couturier, 92220 Bagneux, France.

We compared, using the whole-cell patch-clamp technique, the functional and pharmacological properties of $GABA_A$ receptors in hippocampal pyramidal neurons (HPN) acutely dissociated from adult rats in control conditions and after repeated p.o administration of diazepam (15 mg/kg twice a day for 10 days). A significant decrease in the potency of diazepam to prevent pentylenetetrazole-induced convulsions was observed 48 hours after withdrawal indicating occurrence of tolerance. HPN were acutely dissociated 24 hours (HPN-24) and 48 hours (HPN-48) after diazepam withdrawal using an enzymatic/low Ca^{2+} protocol and were patch-clamped. In control HPN, HPN-24 and HPN-48, no significant difference was observed in the EC_{50} values for GABA-induced responses (6.32, 6.95 and 7.14 μM, respectively), in the density (at -20 mV) of 1 μM GABA-induced currents (8.75, 8.38 and 10.55 μA/cm^2, respectively) or in the EC_{50} values of diazepam at potentiating 1 μM GABA-induced currents (32.3, 52.8 and 37.5 nM, respectively). However, the EC_{50} value of zolpidem at potentiating GABA-induced currents in HPN-48 (276 nM) was significantly higher than in control HPN (130 nM). These results show that $GABA_A$ receptor sensitivity and density do not change following chronic diazepam treatment but that the BZ_1-site mediated allosteric modulation of this receptor is decreased, suggesting a modification in the $GABA_A$ receptor subunit assembly.

624.7

$GABA_A$ RECEPTOR SUBUNIT mRNA LEVELS ARE DIFFERENTIALLY AFFECTED BY CHRONIC DIAZEPAM AND ABECARNIL EXPOSURE. R.A. Holt*, A.N. Bateson and I.L. Martin. Department of Pharmacology, University of Alberta, Edmonton, AB, T6G 2H7, Canada.

Diazepam and abecarnil produce their anxiolytic and anticonvulsant effects by interaction with the $GABA_A$ receptor (1,2). Chronic treatment with abecarnil, however, does not induce diazepam-like withdrawal effects in experimental animals (3). This study investigates the effects of chronic diazepam and abecarnil treatment on expression of the $GABA_A$ receptor α1-, β2- and γ2-subunit isoforms in rat cortex and cerebellum. It is possible that changes in receptor expression underly the development of withdrawal.

Male Sprague-Dawley rats, 250 to 300g, were injected sub-cutaneously once daily for 14 days with 15mg/kg diazepam or 6mg/kg abecarnil in a "slow release" sesame oil vehicle. These doses have previously been reported to be equivalent in terms of kinetics and receptor occupancy in mice (3). The animals were killed 24 hours following their final injection and CNS drug levels were assessed by competition radioligand binding. Steady-state levels of $GABA_A$ receptor α1-, β2- and γ2-subunit mRNA were quantitated by solution hybridisation using oligonucleotide probes specific for these subunits and the results were normalized to the levels of β-actin mRNA (4). A significant decrease in γ2-subunit mRNA was found in the cortex but not in the cerebellum of both diazepam (73.7% of control) and abecarnil (58.5% of control) treated rats. The levels of α1-subunit mRNA were increased significantly in the cerebellum of diazepam treated rats (121.4% of control) while β2-subunit mRNA levels were decreased significantly in the cortex of abecarnil treated animals (72.9% of control). No other significant changes were found. The changes observed in $GABA_A$ receptor subunit mRNA levels are, therefore, both drug and region specific.

(1) Mohler H and Okada T. (1977) *Science* **198**;849. (2) Stephens et al. (1990) *J. Pharm. Exp. Ther.* **253**;334. (3) Steppuhn et al. (1993) *J. Pharm. Exp. Ther.* **264**. (4) O'Donovan MC and Buckland PR. (1991) *Nuc. Acids. Res.* 19:3466.

624.8

CHRONIC BENZODIAZEPINE EFFECTS ON THE $GABA_A$ RECEPTOR COMPLEX OBSERVED *IN VIVO* IS REPLICATED IN AN *IN VITRO* RECOMBINANT EXPRESSION SYSTEM. D.W. Gallager*, J. Yu, J. Xu, T.V.Ramabhadran, C. Hartnett, M. Brown, M. Meyyappan, C. Kostas, J. Tallman and R.J. Primus, Neurogen Corporation, 35 NE Industrial Road, Branford, CT 06405.

Recent advances in our knowledge of the molecular biology of the $GABA_A$ receptor system have yielded insight into the heterogeneity of receptor subtypes. The development of efficient recombinant expression systems has permitted the physiological and pharmacological characterization of the multiple subunits and various combinations of those subunits which are believed to make up the native $GABA_A$ receptor complex. In our earlier studies, different human GABA subunit constructs expressed in baculovirus infected insect cells were characterized by their ligand binding properties.

The present studies have compared the effects of chronic exposure to nonselective agonists (diazepam, bretazenil) and subtype selective agonists (e.g. zolpidem) on the binding characteristics of various recombinant human constructs of the $GABA_A$ receptor complex expressed in insect (Sf9) cells. These experiments demonstrate that phenomena observed following chronic exposure to diazepam *in vivo* in rats (including 'uncoupling' or a decrease in the allosteric GABA potentiation of benzodiazepine binding without a change in benzodiazepine density or affinity) can be replicated in the Sf9 expression system *in vitro*. As reported for the *in vivo* system, changes observed in *in vitro* reconstitution experiments are ligand specific and appear to be subtype selective as well. Reversal of the chronic agonist induced uncoupling process in the Sf9 expression system is produced by brief exposure to the antagonist, Ro15-1788 (flumanzenil), in further analogy to effects observed *in vivo*. These data suggest a utility for recombinant expression systems in studies examining pharmacodynamic changes at the receptor complex in addition to subtype characterizations.

624.9

EFFECTS OF CHRONIC ETHANOL ADMINISTRATION ON $GABA_A$ RECEPTOR SUBUNIT mRNAs: A COMPETITIVE, QUANTITATIVE RT-PCR ANALYSIS. F.D. Smith[1], L.L. Devaud[1]*, D.R. Grayson[2], and A.L. Morrow[1], [1]Bowles Center for Alcohol Studies, UNC School of Medicine, Chapel Hill, NC, and [2]Allegheny Singer Research Institute, Pittsburgh, PA.

$GABA_A$ receptors are thought to play a major role in the development of ethanol tolerance and dependence. Chronic ethanol exposure significantly alters $GABA_A$ receptor function through an undetermined mechanism, possibly through alterations in subunit assembly. Absolute messenger RNA levels of several $GABA_A$ receptor subunits in rat cerebral cortex were determined after chronic ethanol consumption using a competitive, quantitative RT-PCR assay. Ethanol was administered for two weeks by liquid diet to adult male rats in a pair fed design. An increase of 89% in α4 subunit mRNA levels (77.5 ± 15.9 vs. 146.2 ± 41.3 pg/µg total RNA) was detected, while α1 subunit mRNA levels decreased by 43% (86.5 ± 17.8 vs. 49.1 ±7.8 pg/µg total RNA). The increase in α4 subunit mRNA was equal to the decrease in α1 subunit mRNA. The γ2s splice variant increased 32%, from 23.5 ± 3.1 to 31.1 ± 4.2 pg/µg total RNA, while the γ2L splice variant showed no change in absolute mRNA levels. The γ1 subunit message levels increased 70%, from 3.5 ± 0.6 to 6.0 ± 1.3 pg/µg total RNA. No changes were found in α5, β1, β2, β3, γ3, and δ subunits. These data provide evidence for the hypothesis that chronic ethanol exposure alters the subunit composition of $GABA_A$ receptors, which may account for alterations in $GABA_A$ receptor function. (Supported by AA09013 and AA00191).

624.10

$GABA_A$ SITE ISOFORM GENE mRNA EXPRESSION IN PATHOLOGICALLY ALTERED CORTICAL REGIONS OF HUMAN CHRONIC ALCOHOLICS. P. R. Dodd* and G. J. Thomas *Clinical Research Laboratory, Royal Brisbane Hospital Research Foundation, Brisbane Q4029, AUSTRALIA.*

Although pathological changes such as loss of dendritic arborization are widespread in the brains of long-term chronic alcoholics, neuronal loss and reactive gliosis are marked in superior frontal cortex (SFC) but generally not apparent in most areas (e.g., primary motor cortex: PMC). Cirrhosis of the liver associated with the alcoholism may exacerbate the pathological changes. Our binding studies have shown that $GABA_A$ receptor sites are selectively altered in SFC synaptic membranes, on indices of both the form and quantity of the receptor. Since variations in $GABA_A$ isoform gene expression may provide a mechanism to explain changes in its receptor subtype, we assayed the mRNA expression of the seven isoform genes for which human sequence data were available, using S_1 protection assays and phosphorimager quantitation. Combined GAPDH and 18S mRNA expression values were used to normalize the data; SFC values were then adjusted to those in PMC, to control for the effects of age, post mortem delay, etc. Tissue samples were obtained at autopsy from 9 non-cirrhotic and 7 cirrhotic alcoholics (ethanol intake > 80 g/day), 10 controls, and 5 non-alcoholic cirrhotic cases (< 20 g/day). α_1 expression was reduced *cf* controls, slightly in both alcoholic groups but markedly (to 74%; $P \approx 0.05$, *t* test) in non-alcoholic cirrhotics. β_3 expression was reduced *cf* controls in uncomplicated alcoholics (81%) but raised in both alcoholic (134%) and non-alcoholic (180%) cirrhotics ($P \approx 0.05$, ANOVA). β_1 expression was slightly lower in both alcoholic groups, but unchanged in non-alcoholic cirrhotics. Recombinant studies suggest these changes have implications for $GABA_A$ receptor functionality. The variations were further defined by using *in situ* hybridization histochemistry in the two cortical regions.

624.11

PENTOBARBITAL TREATMENT ALTERS THE $GABA_A$ RECEPTOR SUBUNIT GAMMA 2 L/S RATIO. R.F. Tyndale*, S.V. Bhave, P.L. Hoffman, B. Tabakoff, A.J. Tobin and R.W. Olsen. Addiction Research Foundation and Department of Pharmacology, University of Toronto, Toronto, Canada; Department of Pharmacology, University of Colorado, Denver; and Departments of Biology and Pharmacology, UCLA, Los Angeles.

Cerebellar granule cells in primary culture were treated with pentobarbital (PB) which caused a change in the γ-aminobutyric acid $(GABA)_A$ receptor subunit mRNA γ2L/γ2S ratio. The long form of the γ2 subunit mRNA contains a 24 base-pair exon which codes for an 8 amino acid insertion in the cytoplasmic domain between the putative third and fourth transmembrane regions. This 8 amino acid insertion contains a protein kinase C consensus phosphorylation site which is phosphorylated in vitro. In the present study cerebellar granule neurons were cultured from postnatal day 7 rats for 4 days before treatment with PB (500 μM) or ethanol (100 mM) in the medium for 3 days. RT-PCR and Southern blotting were used to detect the $GABA_A$ receptor subunit mRNAs in a blind study, with non-neuronal enolase as an internal standard. γ2L/γ2S Ratios were consistent from 22 to 30 cycles of amplification and over serial dilutions of PCR product. PB decreased the γ2L/γ2S ratio up to 65 percent, while ethanol did not. The change in the γ2L/γ2S ratio was dependent on dose and on duration of the treatment. The PB did not affect the total amount of γ2 subunit mRNA suggesting that the mechanism of action involves a change in alternate splicing of a common pre-messenger γ2 mRNA. We are currently investigating whether PB is acting via $GABA_A$ receptor activation or through alternate mechanisms. It is interesting that while barbiturates do not require a γ2 subunit for binding to the $GABA_A$ receptor, pentobarbital alters this subunit in a very specific manner, which may modify the phosphorylation of the γ2 subunit. Supported by NIAAA and MRC of Canada and the Addiction Research Foundation of Ontario.

624.12

THE EFFECT OF CHRONIC PENTOBARBITAL TREATMENT ON $GABA_A$ RECEPTOR δ SUBUNIT mRNA LEVEL IN MOUSE BRAIN. L.-H. Lin* and L.-H. Wang. Department of Pharmacology, Chang Gung College of Medicine and Technology, Tao-Yuan 333, Taiwan, R.O.C.

Previous studies have suggested that chronic pentobarbital exposure decreases the high affinity GABA binding. It has also been shown that the δ subunit may constitute the high affinity $GABA_A$ receptor. The present study was set out to address the issue whether chronic pentobarbital treatment results in a change in δ subunit expression. The male ICR mice were injected with pentobarbital (75 mg/kg i.p.) three times per day, 7 days in a row. We found that in comparison with the first day, duration of pentobarbital-induced loss of righting reflex was significantly reduced by the end of the 7th day of injection (paired t test, $p < .0001$). This suggests that tolerance has been developed by the treatment. To assess the change of mRNA level, we isolated cerebellar RNA from pentobarbital- and saline-injected mice. RNase protection assay was used to quantify δ subunit mRNA level. To minimize experimental variation, the amount of δ subunit mRNA was normalized to the internal standard of house keeping genes (β-actin or glyceraldehyde-3-phosphate dehydrogenase) within each RNA sample. We found that pentobarbital-treated mice showed significantly higher δ subunit mRNA level than that of saline-treated mice (unpaired t test, $p < .05$). The result suggests that increase of δ subunit expression may contribute to or be resulted from the development of tolerance to pentobarbital.

624.13

CHRONIC NEUROSTEROID TREATMENT DECREASES THE EFFICACY OF GABA AND NEUROSTEROIDS AT THE $GABA_A$ RECEPTOR COMPLEX IN CORTICAL NEURONS. R. Yu* and M.K. Ticku. Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284-7764

In previous studies we have observed that chronic neurosteroid 5α-pregnan-3α-ol-20-one (5α3α) (1or 2 μM) treatment produced downregulation of $GABA_A$ receptors and heterologous uncoupling at the $GABA_A$ receptor complex in cultured mammalian cortical neurons (Yu and Ticku, Mol. Brain Res. 47:603-610, 1995). In the present study, we examined if chronic neurosteroid treatment decreased the efficacy of GABA and 5α3α to potentiate GABA responses, utilizing $[^{36}Cl^-]$ influx and patch clamp assays. Chronic 5α3α (1μM; 5 days) treatment decreased the efficacy of GABA and 5α3α to potentiate GABA responses in these neurons. In $[^{36}Cl^-]$ influx assay, the chronic 5α3α, while not altering the EC_{50} values of GABA, decreased its E_{max} values from 84 ± 2% to 26 ± 2% (-69%). Furthermore, chronic 5α3α treatment decreased the E_{max} value of 5α3α to potentiate GABA-induced $[^{36}Cl^-]$ influx, without altering its EC_{50} value. Since the cortical neurons used for $[^{36}Cl^-]$ influx represent a heterogeneous population of neurons, we examined the effect of GABA and 5α3α on GABA potentiation of single cell responses in control and chronically treated neurons, using patch clamp technique. We found that following chronic 5α3α treatment, GABA (3 μM) induced current was decreased from 513 pA ± 144 to 112 pA ± 33 (-78%). We also observed a decrease in 5α3α (150nM) potentiation of GABA current from 62 ± 9% in control to 30 ± 10% in chronically treated neurons (-52%). These findings support the notion that both GABA response and its potentiation by neurosteroid 5α3α are attenuated after chronic 5α3α treatment.

624.14

CHRONIC NEUROSTEROID TREATMENT DECREASES THE EFFICACY OF BENZODIAZEPINE LIGANDS AND NEUROSTEROIDS AT THE $GABA_A$ RECEPTOR COMPLEX IN MAMMALIAN CORTICAL NEURONS. M.K. Ticku* and R. Yu. Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284-7764

The effect of chronic 5a-pregnane-3a-ol-20-one (5a3a; neurosteroid) treatment was investigated on the GABA, 5a3a, and ligands that bind to the benzodiazepine (BZ) site on GABA-induced $[^{36}Cl^-]$influx in intact cultured mammalian cortical neurons. Chronic 5a3a treatment (1 μM; 5 days) decreased the efficacy of GABA, because its E_{max} (maximal response) value was decreased, whereas the EC_{50} (potency) value was not altered. Chronic 5a3a treatment also decreased the E_{max} value of BZ agonists like diazepam to potentiate GABA-induced $[^{36}Cl^-]$ influx, and decreased the $-E_{max}$ (maximal inhibitory response) value of inverse agonists like DMCM (methyl-6,7-dimethoxy-4-ethyl-ß-carboline-3'-carboxylate) to inhibit GABA-induced $[^{36}Cl^-]$ influx, while not altering their EC_{50}/IC_{50} values. Furthermore, chronic 5a3a treatment decreased the E_{max} value of 5a3a to potentiate GABA-induced $[^{36}Cl^-]$ influx, without altering its EC_{50} value. The decreased efficacy of GABA and 5a3a were reversed by concomitant exposure of the neurons to R 5135 (a competitive GABA antagonist). Taken together, these findings suggest that chronic 5a3a treatment produces decreased efficacy of GABA, ligands that bind to the BZ site, and neurosteroids at the $GABA_ABZ$ receptor complex. The decreased efficacy is heterologous in nature and involves mediation via the $GABA_A$ receptor site.

624.15

TESTOSTERONE REGULATES $GABA_A$ α2 SUBUNIT mRNA EXPRESSION IN NEWBORN FEMALE CNS

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In the CNS testosterone is aromatized to estrogen, which binds to intracellular receptors and organizes male brain function during a critical in postnatal CNS development. A single injection of testosterone given to a female rat within the first few days after birth induces in male behavior in the mature animal. Thus, the sex hormones may play a very important role in the developing CNS. Here we have examined whether circulating testosterone regulates $GABA_A$ and $5\text{-}HT_{1A}$ and $5\text{-}HT_{2A}$ receptor mRNA expression in the newborn female CNS. *In situ* hybridization using ^{35}S-labeled oligonucleotides against α1 and α2 subunit mRNAs of $GABA_A$, $5\text{-}HT_{1A}$ and $5\text{-}HT_{2A}$ receptors followed by densitometry was used to demonstrate that α2 mRNA levels were increased 30-60% in olfactory bulbs, cortex, hippocampus, septum and hypothalamus, but decreased in preoptic areas by 60% 4 hrs after single injection of testosterone (150 μg, ip). Testosterone exposure did not effect GABA α1, $5\text{-}HT_{1A}$ and $5\text{-}HT_{2A}$ receptor mRNA expression anywhere. The results indicate that specific receptor mRNAs are regulated differently by testosterone in the new born female. These data suggest that acute effects on GABA receptor expression may contribute to the organizing effects of testosterone on capacity for masculine behavior.

624.16

$GABA_A$/BENZODIAZEPINE RECEPTOR CHANGES IN GLOBUS PALLIDUS AFTER STRIATAL LESIONS.

L.C. Yang, Z. Hollingsworth*, A.B. Young and J.B. Penney, Neurology Service, Massachusetts General Hospital, Boston, MA 02114.

Globus pallidus (GP) γ-aminobutyric $acid_A$/ benzodiazepine ($GABA_A$/BZ) receptors have predominantly high BZ affinity (BZ1) and are composed of α1, ß2 and γ2 subtypes. We examined the time course of GP $GABA_A$/BZ receptor ligand binding (using quantitative [3H]muscimol and [3H]flumazenil autoradiography) and gene expression (using in situ hybridization of [35S]-labelled oligonucleotide probes) changes in rat brain after unilateral striatal ibotenic acid lesions. Ligand binding was lower on the lesioned than on the unlesioned side at 3 days but returned to normal at 7 days. Muscimol binding remained normal therafter while flumazenil binding kept rising through 90 days. The IC50 of alpidem displacement was unchanged indicating no shift in BZ receptor subtype. The binding changes dramatically corresponded to α1 and ß2 gene expression changes. Film optical density and grain counting results indicated that both α1 and ß2 expression were decreased at 3 days and increased at 7 days. At 30 and 90 days, ß2 expression returned to normal, a pattern similar to that of muscimol which predominantly binds ß peptides. α1 expression continued to rise at 30 and 90 days, identical with the changes in flumazenil binding to receptor α peptides. Our study implys that, after striatal lesions, $GABA_A$/BZ receptor peptides in the ipsilateral GP increase in different patterns but these increases correspond well to changes in the expression of their respective mRNAs.

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624.17

EXPRESSION OF $GABA_A$ RECEPTOR δ SUBUNIT mRNA IN CULTURED CEREBELLAR GRANULE NEURONS IS REGULATED BY DEPOLARIZATION. L.M. Gault and R.E. Siegel*. Dept. of Pharmacology, Case Western Reserve University, Cleveland, OH 44106-4965

The $GABA_A$ receptor is a multisubunit, ligand-gated ion channel that mediates the inhibitory actions of GABA. The regional and temporal expression of $GABA_A$ receptor subunit mRNAs has been well-characterized, but little is known about the factors that regulate their expression. To address this issue, we focused on the expression of the δ subunit mRNA in cultured cerebellar granule neurons. In cultures maintained in complete medium containing fetal bovine serum and 25 mM KCl (CM), the δ subunit transcript level increases several-fold between 4 and 8 days in culture. In contrast, the transcript is undetectable in neurons cultured in a chemically defined, serum-free medium containing only 5 mM KCl (DM). The lack of δ subunit mRNA expression in DM is not due to a permanent change in neuronal properties; expression returns within 2 days after switching cultures from DM to CM. Furthermore, supplementing DM with 25 mM KCl results in an increase in δ subunit mRNA expression comparable to that observed in CM. This effect of KCl is dose-dependent, is maintained in the presence of 1 μM TTX and is not mimicked by the addition of glutamate to DM. Thus, KCl-induced depolarization alone is sufficient to induce δ subunit mRNA expression; additional serum factors are not required. The signalling pathways involved in this cascade are currently under investigation.

624.18

γ-HYDROXYBUTYRATE (GHB)-INDUCED ABSENCE-LIKE SEIZURES DECREASES THE EXPRESSION OF $GABA_A$ RECEPTOR α4 mRNA IN THALAMUS. P.K. Banerjee *[1], N.J.K. Tillakaratne [4], S. Brailowsky [1,3,4], R.W. Olsen [3,5,6], O.C. Snead [1], and A.J. Tobin [2,3,4,5,6], [1]Department of Neurology, University of Southern California, Los Angeles, CA 90027; Departments of [2]Neurology, [3]Pharmacology, and [4]Physiological Science, [5]Molecular Biology Institute, and [6]Brain Research Institute, University of California, Los Angeles, CA 90095.

During the course of absence-like seizures induced by γ-hydroxybutyrate (GHB), neurosteroids continue to modulate ligand binding to thalamic $GABA_A$ receptors, except in mediodorsal, intralaminar, and ventrobasal thalamic relay nuclei, where GHB-induced spike and wave discharges originate. (Snead and Banerjee, SFN Abstract, 1994.) Since α4 mRNA is present at higher levels in these nuclei than other $GABA_A$ receptor mRNAs, we hypothesized that the altered expression of this subunit might account for the suppression of neurosteroid modulation. To look for such alterations, we used *in situ* hybridization with digoxigenin-labeled cRNAs to determine the levels of the mRNAs for the α4 subunit of $GABA_A$ receptors and for the GAD_{67} form of glutamate decarboxylase in the thalami of rats with GHB-induced seizures. In GHB-naive animals, both darkly and lightly labeled cells were most abundant in the thalamic relay nuclei, while α4 mRNA expression was low in the reticular nucleus (RT). In contrast, GAD_{67} cRNA hybridized strongly to the RT, but not to the relay nuclei. In GHB-treated animals, α4 mRNA levels decreased with time of GHB treatment, while GAD_{67} mRNA levels were unaffected. The observed decrease in α4 mRNA may underlie the observed suppression of neurosteroid modulation in the relay nuclei during GHB seizures.

624.19

RAPID DOWN-REGULATION OF $GABA_A$RECEPTORS IN THE GERBIL HIPPOCAMPUS FOLLOWING TRANSIENT CEREBRAL ISCHEMIA. Bruno Alicke* and Rochelle D. Schwartz-Bloom. Department of Pharmacology, Duke University Medical Center, Durham, NC 27710.

During transient cerebral ischemia, there is a temporary, but robust, accumulation of extracellular GABA in the hippocampus. Within an hour following ischemia, GABA levels return to baseline. We examined whether the acute exposure of $GABA_A$ receptors to high concentrations of GABA results in down-regulation in vivo, as observed in vitro. Gerbils were subjected to 5 min bilateral carotid occlusion and sacrificed 30 or 60 min later. Brain sections were prepared for receptor autoradiography using the hydrophilic $GABA_A$ receptor antagonist, [^{3}H]SR95531 and the hydrophobic benzodiazepine agonist, [^{3}H]flunitrazepam, to distinguish between cell surface and internalized receptors. The oil:water partition coefficients were determined for [^{3}H]SR95531and [^{3}H]flunitrazepam to be 0.037 and 21.0, respectively. Ischemia significantly decreased [^{3}H]SR95531 binding to $GABA_A$ receptors in the dendritic fields of hippocampal areas CA1, CA3 and dentate gyrus 30 and 60 min after the onset of reperfusion (p=0.001). Scatchard analysis of [^{3}H]SR95531 binding revealed that ischemia caused a 51% decrease in the Bmax. The affinity of the remaining sites was increased substantially (69% decrease in Kd). In contrast, no changes were observed in the binding of [^{3}H]flunitrazepam at 30 and 60 min following ischemia. This is expected since the benzodiazepine has access to the cell interior where it can bind to internalized receptors as well as those on the cell surface. We hypothesize that prolonged exposure (~30-45 min) of $GABA_A$ receptors to high concentrations of synaptic GABA in vivo causes receptor down-regulation, via receptor internalization. Supported by NIH grant NS28791 & Am. Heart Association.

624.20

ANTISENSE OLIGONUCLEOTIDE MODULATION OF GAD ALTERS COCAINE-INDUCED SEIZURES IN MICE. M.S. Abel* and T. J. Kirages. Dept. of Cell Biology & Anatomy, FUHS/The Chicago Medical School, North Chicago, IL 60064.

The administration of synthetic antisense oligonucleotides (oligo) is an elegant method of regulating gene expression. In this study, we undertook to decrease cerebral GABA levels by inhibiting translation of glutamic acid decarboxylase (GAD), the rate-limiting enzyme for GABA synthesis, and determine susceptibility to seizures by the acute administration of the ED_{20} dose of cocaine. The lateral ventricles of BALB mice (20-25g) were unilaterally infused with 2 μl [0.5 ng] of either 1) antisense oligonucleotide in artificial CSF, 2) artificial CSF alone, or 3) random oligonucleotide in artificial CSF. After surgery, animals were allowed to recover for 15 hours before analysis of GABA levels and/or behavioral testing. Mice were injected i.p. with the ED_{20} dose (55 mg/kg) of cocaine, and seizure behavior was evaluated. Antisense oligonucleotide to GAD significantly increased the number of cocaine-induced seizures. 33.3% of mice in the CSF group exhibited cocaine-induced seizures. 0% of mice in the random-oligo group exhibited cocaine-induced seizures. 75% of mice in the antisense-oligo group exhibited cocaine-induced seizures. The seizures consisted of severe tremors initially, followed by tonic-clonic extensions and contractions. Seizure latency and duration were not significantly different between groups. These data suggest that 15 hours after ICV injection, GABA levels are decreased sufficiently to reduce either a) widespread tonic inhibition by GABA, or b) specific inhibitory GABAergic pathways.

624.21

ANTISENSE KNOCKOUT OF α6 SUBUNIT ALTERS PHARMACOLOGICAL PROPERTIES OF $GABA_A$ RECEPTORS IN CEREBELLAR GRANULE NEURONS. **W.J.Zhu*, J.F. Wang, S.Vicini, & D.R.Grayson[1] Georgetown University, Washington D.C.; [1]Med. College of Pennsylvania, Pittsburgh, PA.**

Analysis of the profile of $GABA_A$ receptor subunit gene expression in cerebellar granule neurons have indicated that several forms of α subunit, including α1, α6, together with β2, γ2 and δ subunit, are expressed to form unique subtypes of $GABA_A$ receptor channels. To characterize the role of the α6 subunit in the native $GABA_A$ receptor, which are exclusively expressed in the granule cells, primary cerebellar cultures from 7 days old rats were treated with antisense oligodeoxynucleotides (aODNs) spanning the initial ATG codon of the α6 subunit mRNA. ODNs were applied for 48 hours in culture medium at the concentration of 5 μM. Sister cultures were treated with mismatch (scrambled) mODNs. Inhibition of GABA-gated currents by furosemide, a selective inhibitor of $GABA_A$ receptors containing α6 subunit, was attenuated following aODNs treatment. Furthermore, aODNs specifically reduced immunocytochemical staining for α6 subunits. We observed a concomitant alteration of the α6 subunit expression and pharmacological properties of $GABA_A$ receptors in granule cells. Treatment with α6 aODNs increased EC_{50} and caused an inhibition of GABA-induced maximal currents when compared with control or mODNs treated cultures. Furthermore, the depletion of α6 subunits from cerebellar granule cells enhanced flunitrazepam-induced potentiation of GABA-activated currents. These results show that α6 subunit expression can be blocked by means of synthetic aODNs complementary to the initial coding exon, and that α6 subunits in the $GABA_A$ receptor form a part of native neuronal Cl^- channels and modify their pharmacology, suggesting that α6 subunit expression during in vivo development contributes the plasticity of $GABA_A$ receptors in the cerebellar development. Supported by NINDS grants R01 NS30537 and K04 NS01647 to D.R.G. and K04 NS01680 to S.V.

624.22

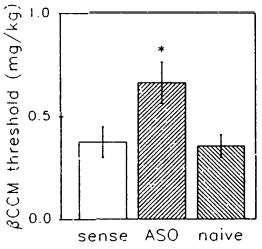
ALTERED GABAERGIC FUNCTIONS IN MICE LACKING THE γ2 SUBUNIT OF $GABA_A$ RECEPTORS. B. Lüscher*, J.-M. Fritschy, U. Günther, J. Benson, D. Benke, F. Crestani, F. Knoflach, A. Aguzzi, M. Arigoni, Y. Lang, H. Bluethmann, H. Möhler. Institute of Pharmacology, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland.

Vigilance, anxiety, epileptic activity, and muscle tone can be modulated by drugs acting at the benzodiazepine (BZ) site of γ-aminobutyric acid type A ($GABA_A$) receptors. *In vivo*, BZ sites are potential targets for endogenous ligands regulating the corresponding CNS states. To assess the functional properties of $GABA_A$ receptors devoid of BZ sites, mutant mice lacking the γ2 subunit were generated by gene targeting. Newborn mutant mice were morphologically indistinguishable from wildtype littermates. The size and cytoarchitecture of their brain were conserved, and, except for the γ2 subunit, the level of expression and the regional and cellular distribution of the major subunits were unchanged. Biochemical analysis revealed a loss of 94% of BZ sites while the number of GABA sites was only slightly changed, suggesting that the γ2 subunit was dispensable for proper expression and assembly of $GABA_A$ receptors during embryogenesis. Patch clamp analysis revealed that the single channel main conductance level and the Hill coefficient were reduced to values consistent with recombinant $GABA_A$ receptors composed of α and β subunits. The GABA response was potentiated by pentobarbital but not by flunitrazepam. Diazepam was inactive behaviorally. Thus, in newborn mice, the γ2 subunit is dispensable for the assembly of functional $GABA_A$ receptors, but is required for normal channel conductance and the formation of BZ sites *in vivo*. Postnatally, however, the reduced $GABA_A$ receptor function was associated with retarded growth, sensorimotor dysfunction, and drastically reduced life span. In addition, abnormal dendritic targeting of $GABA_A$ receptor subunits was observed immunohistochemically in postnatal brain, which might exacerbate the impairment of inhibitory neurotransmission and contribute to the mutant phenotype.

624.23

TREATMENT WITH AN ANTISENSE OLIGODEOXYNUCLEOTIDE TO THE GABA-A RECEPTOR γ2 SUBUNIT INCREASES CONVULSIVE THRESHOLD FOR βCCM, A BENZODIAZEPINE "INVERSE AGONIST", IN RATS. T.-J. Zhao, T.H. Chiu* and H.C. Rosenberg. Dept. of Pharmacology, Medical College of Ohio, Toledo, OH 43699.

Modulation of GABA actions by benzodiazepines and related drugs is associated with the presence of a γ2 subunit in the GABA-A receptor. Male Sprague-Dawley rats were surgically implanted with guide cannulae to allow unilateral intracerebroventricular (ICV) injections of phosphorothioate-modified antisense oligo (ASO; 18 μg in 2 μl saline) every 12 hr for 3 days. Other rats received the corresponding sense oligo. 6 hr after the last ICV treatment, rats were given a slow IV infusion of βCCM, a benzodiazepine "inverse agonist". The time to onset of myoclonic jerking was recorded, and the threshold convulsant dose determined. Compared to naive rats (n=5), the rats that had received sense oligo (n=4) had no significant change in βCCM threshold. Rats that had been treated with ASO (n=5) required an 87% larger dose of βCCM to induce convulsions. These results suggest that ASO treatment can alter GABA-A receptor composition in vivo and may provide a tool to study regulation of receptor structure and function. Supported by DA02194.



624.24

SMALL REDUCTION OF BENZODIAZEPINE RECEPTOR RADIOLIGAND BINDING IN RAT BRAIN FOLLOWING INTRACEREBROVENTRICULAR ADMINISTRATION OF ANTISENSE OLIGODEOXYNUCLEOTIDE TO $GABA_A$ RECEPTOR γ2 SUBUNIT SUBTYPE. J. Karle, S.E.W. Hansen, M.R. Witt*and M. Nielsen . The Research Institute of Biological Psychiatry, St. Hans Psychiatric Hospital, DK-4000 Roskilde, Denmark.

Brain $GABA_A$ receptor chloride channel complexes are probably pentamers of different polypeptide subunits. Five different subunit families with several subunit subtypes (α1-6; β1-4; γ1-3; δ and ρ1-2) have been characterised. There is evidence indicating that the γ2 subunit subtype is a functionally integral part of the benzodiazepine binding site of the $GABA_A$ receptor complex, important for optimal binding of benzodiazepines. By means of benzodiazepine receptor radioligand binding assays we have evaluated the possibility of specifically reducing benzodiazepine receptor binding properties *in vivo* using phosphorothioate antisense oligodeoxynucleotides to $GABA_A$ receptor γ2 subunit subtype. Infusions of an 18-mer antisense oligonucleotide into the right lateral cerebral ventricle once daily (2 μg/μl; 5 μl infusion volume) for two days induced a significant and reproducible reduction of [^{3}H]flunitrazepam (11%; $p<0.005$, Mann-Whitney U test, n=8) and [^{3}H]Ro 15-1788 (15%; $p<0.05$, n=4) binding in preparations of right cortex compared to preparations from animals treated with saline. In preliminary experiments the corresponding sense oligonucleotide did not reduce radioligand binding compared to saline. There were no differences in [^{3}H]QNB binding between antisense oligonucleotide and saline treated animals. These results illustrate that an antisense oligonucleotide to $GABA_A$ receptor γ2 subunit can selectively reduce benzodiazepine receptor radioligand binding *in vivo*.

PEPTIDE RECEPTOR STRUCTURE AND FUNCTION III

625.1

Characterization of tachykinin NK1/NK3 chimeric receptors constructed by exon shuffling Fu-Zon Chung*, Lan-Hsin Wu, and Ye Tian; Department of Biotechnology, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105

All three tachykinin receptor genes contain five exons which are separated by introns of variable length. Interestingly, the location of all exon/intron junctions are completely conserved among these receptor genes. To explore the possibility that these evolutionary conserved exons may serve as functional units, three chimeric receptors were constructed by shuffling exons between tachykinin NK1 and NK3 receptors. In NKC-1, NKC-2, and NKC-3 chimeric receptors, various portions of the N-terminal sequences of the human NK3 receptor encoded by exon 1(transmembrane domains 1-3) , exon 1-2 (transmembrane domains 1-4), and exon 1-3 (transmembrane domains 1-5), respectively, were systematically replaced by their counterparts of the human NK1 receptor. All three chimeric receptors retained relatively high affinity binding for the natural ligand substance P, indicating that sequences located at the N-terminal end of the NK1 receptor are important for substance P binding. On the contrary, in the NKC1 receptor, in which the first exon (transmembrane domains 1-3) of the human NK3 receptor was replaced by its counterpart of the human NK1 receptor, the binding affinity of the natural ligand neurokinin B is almost the same as that in the wild type human NK3 receptor. It is concluded that sequences located at the N-terminal end of the human NK3 receptor are not important for binding to neurokinin B. These data indicate that chimeric receptors constructed by exon shuffling may be useful tools for accessing the functional importance of individual exons of these tachykinin receptors.

625.2

Asp^{131} of the Neurokinin-3 receptor is important for G-protein coupling but not for ligand binding: a mutagenesis study Ye Tian*, Lan-Hsin Wu and Fu-Zon Chung . Department of Biotechnology, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105

Neurokinin receptors belong to the G-protein coupled receptor superfamily. One of the common features of this receptor superfamily is the conservation of an aspartate residue in the TM II domain which has been hypothesized to be important for receptor G-protein coupling. The NK3 receptor is highly expressed in neuronal cells but its biological function has not yet been elucidated. Similar to other G-protein coupled receptors, human NK3 receptor contains an Asp^{131} in the TM II. In the present study, the involvement of the Asp^{131} of the human NK3 receptor in receptor-ligand interaction was examined. Two mutant receptors were constructed from the human NK3 receptor with Asp^{131} being mutated to either Glu or Asn. Receptor binding and agonist-induced receptor activation were measured to evaluate the interactions between mutant NK3 receptors and their ligands. Here we report that mutation of Asp^{131} to either Glu or Asn residue conserves high affinity binding to selective NK3 ligands such as [MePhe^7]NKB. The conversion of Asp^{131} to Glu fully conserved the ability for the receptor to conduct agonist-induced phosphatidylinositol turnover. In contrast, the conversion of Asp^{131} to Asn which lost side chain charge but preserved the size of the residue resulted in a total loss of receptor activation as evaluated by agonist-induced phosphatidylinositol turnover. These results suggest that the Asp^{131} is crucially involved in the receptor-G-protein coupling, but not in ligand binding.

625.3

NOVEL HIGH-AFFINITY NPY ANTAGONIST IDENTIFIES DIFFERENT SUBCLASSES OF Y2 TYPE OF NPY RECEPTORS. J.E. Matthews, M. Jansen, R.J. Slepetis, V.A. Rash and A.J. Daniels*. Burroughs Wellcome Company, Research Triangle Park, North Carolina 27709.

Identification of NPY receptor subtypes has relied on the differential binding and activity of C-terminal fragments (NPY 13-16) and C-terminally modified analogs of NPY (Leu^{31}, Pro^{34} NPY). At least two NPY receptor subtypes have been described based on the relative affinity of different NPY agonists: NPY-Y1 receptors require essentially the full NPY sequence of amino acids for activation and have high affinity for the analog [Leu^{31}, Pro^{34}]-NPY, whereas NPY-Y2 receptors can be activated by both NPY and NPY 13-36 but have low affinity for [Leu^{31}, Pro^{34}]-NPY. We have found a high-affinity, apparently non-selective, NPY receptor antagonist (1229U91). 1229U91 is a very potent Y1 antagonist when tested in three models used for the characterization of Y1 receptors: SK-N-MC cell binding (IC_{50} 0.2nM), HEL cell intracellular calcium release (IC_{50} 0.25nM), and perfused isolated rat kidney (IC_{50} 3nM). 1229U91 displays an even higher affinity for the Y2 rat brain receptor (IC_{50} 21pM), being 700 fold more potent than NPY 13-16 (IC_{50} 15nM). However, 1229U91 displays lower potency than NPY 13-36 on other Y2 binding and functional assays: In the human neuroblastoma cell line, KAN-TS, the affinity of 1229U91 is 300 fold lower than for NPY 13-36 (IC_{50} 2.7μM and 8nM respectively). Likewise, while NPY 13-36 antagonized the Y2 mediated inhibition of the electrically stimulated twitch in the rat vas deferens (ED_{50} 60nM), 1229U91 at 1μM does not show agonistic or antagonistic activity. The different relative affinities for NPY 13-36 and 1229U91 for NPY receptors previously characterized as being of the Y2 class, leads us to propose the existence of subclasses of Y2 receptors.

625.4

BINDING OF [^{125}I]-[LEU^{31}-PRO^{34}]-PEPTIDE YY (LP-PYY) TO RECEPTORS FOR NEUROPEPTIDE Y (Y-1) AND PANCREATIC POLYPEPTIDE (PP1). S.L. Gackenheimer*, J. Lundell[1], R. Schmidt, L. Beavers, R.A. Gadski, M. Berglund[1], D.A. Schober, N.L. Mayne, J.P. Burnett, D. Larhammar[1] and D.R. Gehlert. Lilly Research Laboratories, Indianapolis, IN and [1]Dept. Pharmacol., Uppsala University, Uppsala, Sweden.

Neuropeptide Y (NPY) is the predominant brain peptide of the PP-fold peptide family. The other members, peptide YY (PYY) and pancreatic polypeptide (PP) are primarily circulating endocrine peptides though receptor sites for these peptides have been found in brain. Receptor populations for NPY were subdivided into Y1 (postsynaptic) and Y2 (presynaptic). NPY and PYY have similar affinity for Y1 and Y2 but may have their own distinct receptor subtypes as well. PP was believed to have its own distinct receptor population. The exchange of the Pro^{34} found in PP for the Gln34 in NPY and PYY produces NPY and PYY analogs with selectivity for Y-1 over Y-2 receptors. In the present study, we have characterized the binding of [^{125}I]-LP-PYY to Y1 and the newly identified PP1 receptors. In addition we have examined the distribution of binding sites for this radioligand in the rat brain.

The DNA encoding for the Y1 receptor and the PP1 receptor were subcloned into the pHD vector and expressed in AV-12 cells. [^{125}I]-LP-PYY bound to the Y1 receptor with a K_d of 320 pM and a B_{max} of 2700 fmoles/mg protein. This ligand also bound to the PP1 receptor with a Kd of 120 pM and a B_{max} of 2050 fmoles/mg protein. The pharmacology of the binding was consistent with the pharmacology of Y1 and PP1 binding in the respective cell lines. The localization of [^{125}I]-LP-PYY binding sites was compared with the localization of Y-2 and PP receptors in rat and guinea pig brain using receptor autoradiography. In general, [^{125}I]-LP-PYY bound to Y1 receptors in most brain regions. However, in areas that contained high densities of PP receptors such at the nucleus of the solitary tract and interpeduncular nucleus, binding of [^{125}I]-LP-PYY was also observed.

Therefore, [^{125}I]-LP-PYY binds to both Y1 and PP receptors. Caution needs to be exercised when using this peptide analog in in vitro and in vivo assays.

625.5

DIFFERENTIAL DISTRIBUTION OF NEUROPEPTIDE Y1 AND Y2 RECEPTORS IN HUMAN AND RAT BRAIN. M.A. Statnick*, D.A. Schober, J.P. Burnett, N.G. Mayne, R. Sharp, Y. Snyder, and D.R. Gehlert, Central Nervous System Research, Lilly Research Labs, Eli Lilly and Company, Indianapolis, IN 46285.

Neuropeptide Y (NPY) is a 36 amino acid amidated peptide present in both peripheral and central nervous system (CNS) neurons. Receptor subtypes for NPY have been elucidated by the differing affinity of [Leu^{31}, Pro^{34}]NPY and NPY$_{13-36}$ to displace ^{125}I-NPY or ^{125}I-Peptide YY (^{125}I-PYY) binding from Y1 and Y2 receptors, respectively. The CNS distribution of these receptor subtypes has been extensively studied in the rodent. In general, Y1 receptors are found in the cerebral cortex, while Y2 receptors are found in the hippocampus and hypothalamus (Gehlert et al., *Neurochem Int* 21: 45-67, 1992). Recently, species differences in the distribution of Y1 and Y2 receptors have been described in human and rat brain (Widdowson, *Brain Res* 631: 27-38, 1993). To further characterize these findings, we examined the pharmacology of NPY receptors in the human and rat cerebral cortex and hypothalamus by radioligand binding. Moreover, the phamacology of a cell line stably expressing a cloned human Y1 receptor gene (hNPY1/AV12) and of a Y2 receptor expressing cell line (SMS-KAN) were examined for comparative purposes. In hNPY1/AV12 cells, analysis of saturation curves with ^{125}I-PYY showed a single binding site with a K_d = 172 pmol and a B_{max} = 3.2 pmol/mg protein. Displacement of 100 pM ^{125}I-PYY binding and inhibition of forskolin stimulated adenylate cyclase displayed a Y1 profile in hNPY1/AV12 cells, while in SMS-KAN cells displacement of ^{125}I-PYY binding was Y2. In human cortex homogenates, displacement of ^{125}I-PYY binding with various peptides displayed a distinct Y2 receptor profile (PYY > NPY ≥ NPY$_{2-36}$ > PYY$_{13-36}$ ≥ NPY$_{3-36}$ ≥ NPY$_{13-36}$ >>> h[Pro^{34}]NPY = h[Leu^{31}, Pro^{34}]PYY). Conversely, displacement of ^{125}I-PYY binding from rat cortical membranes was Y1 in nature (h[Pro^{34}]NPY > NPY > h[Leu^{31}, Pro^{34}]PYY > NPY$_{2-36}$ >> NPY$_{13-36}$ > NPY$_{3-36}$ ≥ PYY$_{13-36}$). NPY receptor binding in the hypothalamus was Y2 for both species (PYY > NPY = NPY$_{2-36}$ > NPY$_{13-36}$ >>> [Leu^{31}, Pro^{34}]NPY = hPP = [dTrp^{32}]NPY). Therefore, it appears that species differences exist in the distribution of NPY receptor subtypes expressed in the cerebral cortex, while in the hypothalamus the receptor subtype appears to be similar between species. The functional consequences of these differences await further study.

625.6

PHARMACOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF A NOVEL MEMBER OF THE NEUROPEPTIDE Y (NPY) RECEPTOR FAMILY, PP1. D.A. Schober*, I. Lundell[1], R. Schmidt, L. Beavers, R.A. Gadski, M.A. Statnick, M. Berglund[1], J.A. Hoffmann, R. Chance, D. Larhammar[1], and D.R. Gehlert, CNS Research, Lilly Research Laboratories, Indianapolis, IN and [1]Dept. Pharmacology, Uppsala University , Uppsala, Sweden.

Receptors for NPY have been traditionally divided into Y1 and Y2 subtypes based on peptide pharmacology and synaptic localization. Other receptors have been proposed based on preferences for NPY, peptide YY (PYY) or pancreatic polypeptide (PP). Until recently, only the Y1 receptor has been cloned and pharmacologically evaluated. At this meeting (see Lundell et al.) we have disclosed a novel member of this receptor family that exhibits high affinity for PP and PYY. In the present study, we have stable expression of the PP1 receptor in CHO cells and evaluate its pharmacological properties with an array of peptides.

When expressed in CHO cells, the PP1 receptor bound both [^{125}I]-pPYY and [^{125}I]-bPP with Kd's in the 30-50 pM range. Human, rat and bovine PP inhibited binding with similar affinities while chicken PP was less potent with a Ki of 1 nM. PYY inhibited binding with a Ki of 300 pM while NPY was much less potent with a Ki of 10 nM. Examination of a variety of PP fragments indicated the importance of the N- and C-terminus of PP in the binding to the receptor. Deletion of the first 4 amino acids reduced affinity of bPP to 1 nM. Deletion of Tyr^{36} produced a substantial reduction in affinity. Leu^{31}-Pro^{34} substitued NPY and PYY had increased affinity when compared to the native peptides. In biochemical assays, PP and PYY were able to produce a concentration dependent reduction in forskolin stimulated adenylate cyclase activity while NPY did not affect activity at concentrations of up to 1 μM.

Thus, PP1 is a novel member of the PP-fold receptor family and has a distinctive pharmacology. Like Y1 and Y2 it is coupled negatively to adenylate cyclase.

625.7

CLONING OF A HUMAN RECEPTOR OF THE NPY RECEPTOR FAMILY WITH HIGH AFFINITY FOR PANCREATIC POLYPEPTIDE AND PEPTIDE YY I. Lundell*, A. G. Blomqvist, M. Berglund, D. A. Schober[1], D. Johnson[1], R. A. Gadski[1], D. R. Gehlert[1], and D. Larhammar. Dept. of Medical Pharmacology, Uppsala University, Box 593, S-751 24 Uppsala, Sweden, 1) Lilly Research Laboratories, Eli Lilly and Company, Indianapolis.

Neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP) form a family of structurally related peptides. NPY is expressed exclusively in neurons whereas PYY is produced in endocrine cells of colon and pancreas as well as in some brainstem neurons. PP is exclusively expressed in pancreas. Several receptor subtypes have been identified pharmacologically, but only the NPY/PYY receptor of subtype Y1 has been cloned. We have isolated the gene for a novel human receptor, called PP1, with 43% overall amino acid sequence identity to Y1 and 53% identity in the transmembrane regions. The PP1 gene lacks the intron present in the Y1 gene. When transiently expressed in COS cells, the PP1 receptor displays a pharmacological profile that distinguishes it from all previously described NPY-family receptors. It binds PP with an affinity (Ki) of 16 pM, PYY with 100 pM and NPY with 36 nM. The Y1-selective NPY analogue [Leu^{31},Pro^{34}]NPY has a Ki of 10 nM. Northern hybridization to human organ and brain-region mRNA panels detected mRNA in colon and pancreas. Because all three endogenous peptides may be present in these organs through either endocrine release or innervation, all three peptides may be physiological ligands. Thus, PP1 represents a novel peripheral subtype of the PP-fold receptor family. This sequence provides not only a novel pharmacological entity, but should facilitate identification of other family members.

625.8

MUTAGENESIS OF CONSERVED AMINO ACID RESIDUES IN THE RAT Y1 RECEPTOR Magnus Berglund*, Anders G. Blomqvist$ & Dan Larhammar, Dept of Medical Pharmacol., Uppsala Univ., Sweden, $Lab. for Mol. Endocrinol., Rigshosp., Copenhagen, Denmark

Neuropeptide Y (NPY) and peptide YY (PYY) are structurally related peptides that function as a neurotransmitter and gastrointestinal hormone, respectively. At least four distinct NPY and/or PYY receptors, called Y1, Y2, Y3, and "feeding receptor" have been described. The cloning of the Y1 receptor in several species (rat, mouse, human and Xenopus laevis) as well as the cloning of a Y1-like receptor (Lundell et al, submitted) allows the identification of well conserved amino acid residues in the Y1-family of receptors. We have mutated conserved positions in the rat Y1 receptor to study the effects on ligand interactions.

Amino acids that have been chosen for site-directed mutagenesis are polar and charged residues located in the transmembrane regions and in the extracellular loops. Mutations have been introduced by using a two step PCR method with mutant primers. Upon sequence confirmation the mutated receptors have been cloned into the expression vector pTEJ8 (kindly provided by T.W. Schwartz, Denmark) and CHO-K1 cells have been stably transfected. Ten mutant receptor clones are being studied with regard to their ability to bind iodinated NPY and PYY. A number of different peptide analogs are used to displace these radioligands.

Mutant receptors that bind neither NPY nor PYY will be checked with an antibody to the carboxy terminus of the receptor to investigate cell-surface expression.

625.9

PANCREATIC POLYPEPTIDE POTENTIATES CA^{2+} CHANNEL CURRENTS IN CULTURED RAT HIPPOCAMPAL NEURONS. D.L. Czilli*, W.Y. Li and L.K. Simmons. CNS Research, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

Pancreatic polypeptide (PP) belongs to a family of structurally related peptides which includes neuropeptide Y (NPY) and peptide YY (PYY). Although cells containing PP are localized primarily in the gut and pancreas, there is evidence suggesting that PP receptors depress Ca^{2+} channel activity in rat superior cervical ganglia. Here we describe a PP-mediated potentiation of voltage-sensitive Ca^{2+} channel currents in embryonic day 18 rat hippocampal neurons. Using whole-cell voltage clamp protocols with 10 mM Ba^{2+} in the bath as the charge carrier, PP (3 - 100 nM) enhanced the peak Ba^{2+} currents (I_{Ba}) elicited with voltage steps to +10 mV from a membrane holding potential of -70 mV. In response to 100 nM PP, I_{Ba} was enhanced (relative to control values) by a mean of 56±3 %. Therefore, unlike NPY, which depresses excitatory transmission presynaptically in hippocampal neurons via a Y_2 receptor subtype, PP enhances the activity of post-synaptic Ca^{2+} channels. Future studies will investigate whether PP is acting at a distinct receptor in this neuronal population.

625.10

CYSTEINE 199 IN TM V OF THE SUBSTANCE P RECEPTOR IS THE SITE FOR MODULATION OF PEPTIDE BINDING BY SULFHYDRYL SPECIFIC REAGENTS. H. Li[1], B. S. Sachias[2], J. E. Krause[2], S. E. Leeman*[1], N. D. Boyd[1], [1]Dept of Pharmacology, Boston Univ. Sch. of Med, Boston, MA 02118 and [2]Dept. of Anat. & Neurobiol., Washington Univ. Sch. of Med., St. Louis, MO 63110.

Substance P (SP) is a peptide neurotransmitter that produces multiple responses in both the central and the peripheral nervous system through a G-protein coupled receptor. The primary structure of the rat SP receptor contains a number of cysteine residues. To study the role of these cysteines in the structure-function of the SP receptor, we have examined the effect of sulfhydryl specific reagents on radiolabeled SP (^{125}I-(BH)-SP) binding to the rat SP receptor stably expressed in CHO cells. The membrane-permeable reagent N-ethyl-maleimide (NEM) inhibits ^{125}I-(BH)-SP binding in a concentration-dependent manner, while charged and thus membrane-impermeable reagents had no effect on binding. These results indicate that the site of NEM modulation is located within the transmembrane (TM) domain of the receptor. To locate the reactive sulfhydryl group, we used amphipathic maleimide analogues which penetrate to different depths within the plane of membrane. The results obtained suggest that the maleimide-sensitive sulfhydryl group is about 11 Å from the extracellular membrane boundary. The residue cys199 is predicted to be close to the extracellular end of the fifth TM helix of the SP receptor. The receptor mutant C199S, in which cys199 has been replaced by a serine, is no longer sensitive to modulation by NEM treatment, indicating that cys199 of the receptor is the target of NEM modulation. In addition, the occupation of the receptor by SP protects cysteine 199 from modification by NEM. Thus, this residue is either located at or conformationally linked to the SP binding site.

625.11

EXPRESSION OF THE RAT B_2 BRADYKININ RECEPTOR IN STABLY TRANSFORMED CHINESE HAMSTER OVARY CELLS. S. Yokoyama*, Y. Kimura, M. Taketo, K. Iki and H. Higashida. Dept. of Biophysics, Neuroinformation Research Institute, Kanazawa University School of Medicine, Kanazawa 920, Japan

In the previous study we demonstrated that both the rat and mouse B_2 bradykinin receptors (BKRs) of the smooth muscle type are expressed in NG108-15 mouse neuroblastoma x rat glioma hybrid cells (BBRC 200: 634-641, 1994). For further analysis, we have established transformed Chinese hamster ovary (CHO) cells which stably express the rat B_2 BKR and developed a polyclonal antibody. Rat B_2 BKR cDNA was amplified from brain mRNA by reverse transcription polymerase chain reaction (RT-PCR) and cloned into pRc/CMV. The expression plasmid was transfected into CHO cells and two cell lines, designated as CHO-RBKR8 and CHO-RBKR11, were isolated. RNA blot hybridization analysis identified a proper transcript in these transformed cells. Upon application of bradykinin, a transient increase of intracellular calcium was observed in both cells, but not in parental CHO cells. Next, cDNA corresponding to the *C*-terminal region of the rat B_2 BKR was subcloned into pGEX2T, a bacterial expression vector, and the fusion protein expressed in E. coli was used to generate rabbit antisera. The transformed cells exhibited intense immunoreactivity with the antibody. In contrast, the occasional faint staining was observed in parental CHO cells. These transformed cells may provide a useful system to study the properties of the B_2 BKR.

625.12

CLONING AND EXPRESSION OF THE HUMAN NEURONAL B2 BRADYKININ RECEPTOR. J. Baumgold* and J. S. Handen. Receptor Biology Inc., Baltimore, MD 21227 and Neuroscience Program, The George Washington University, Washington, DC 20007.

Two B2 bradykinin receptor subtypes have been described in the literature: a "smooth-muscle" B2 receptor, and a "neuronal" B2 receptor which differ pharmacologically. The "smooth-muscle" B2 receptor has been cloned from several species, whereas this is the first report of cloning the "neuronal" B2 receptor.

We used the published sequence of the smooth muscle B2 receptor to design PCR primers which were used for RT-PCR. The resulting DNA fragment was then used to screen a cDNA library from a human cell line. One of the resulting positive clones contained a sequence that has 83% homology to the human "smooth muscle" B2 receptor. We transfected this cDNA into COS-7 cells, and found that transfected cells exhibit bradykinin-mediated PI turnover (Kact = 4 nM), whereas untransfected cells do not. Furthermore, NPC431 which has been previously described as a full agonist at "neuronal" B2 receptors and as an antagonist at "smooth muscle B2 receptors", acted as a full agonist in the transfected cells. Stably-transfected cell lines expressing this novel bradykinin receptor are currently being established, and will be used to characterize this receptor further. Taken together, our data indicate that we cloned the human "neuronal" B2 receptor.

625.13

CONSERVED CYSTEINE RESIDUES IN THE CYTOPLASMIC TAIL OF THE HUMAN NEUROKININ A RECEPTOR ARE INVOLVED IN RECEPTOR DESENSITIZATION C.R. Cyr, S. Josiah, B. Rudy#, L. Devi* and R.M. Kris Department of Pharmacology and Physiology# New York University Medical Center,550 First Avenue, New York, New York 10016

Neurokinin A receptor, a member of the tachykinin family of G protein coupled receptors, produces vasoconstriction of smooth muscle and a number of other effects. When neurokinin A receptors were expressed in *Xenopus laevis* oocytes, stimulation by neurokinin A produced a calcium dependent chloride response that desensitized for 25-35 minutes. In an effort to characterize the role of two cysteine residues within the cytoplasmic tail of the neurokinin A receptor on desensitization, three palmitoylation mutants and two chimeric receptors were expressed and analyzed in the *Xenopus* oocyte system. Two single point mutations and a double mutation of adjacent cysteines within the cytoplasmic tail were constructed. Both of the single mutations desensitized for only 5 minutes while the double mutation was not functional. Substitution of 61 amino acids of the neurokinin A receptor by an equivalent region of the endothelin A receptor produced a chimeric receptor that desensitized for a period equal to that of wild type neurokinin A receptor, while substitution of 81 amino acids resulted in a chimeric receptor that does not undergo desensitization. This data demonstrates that alteration of the two cysteine residues within the cytoplasmic tail affects normal desensitization of the neurokinin A receptor and supports a role for palmitoylation in receptor desensitization. C.R. Cyr is supported by Postdoctoral Training Grant DA 7254 and NIH NS30989.

625.14

ELUCIDATION OF STRUCTURAL REQUIREMENTS OF NEUROPEPTIDE Y FOR RECOGNITION BY Y_2 RECEPTOR USING ACYLATED AND CROSS-LINKED ANALOGS. N. Yumoto*, S. Murase, Y. Shigeri and S. Yoshikawa. Osaka National Research Institute, AIST, Ikeda, Osaka 563, Japan.

Neuropeptide Y (NPY) is an amidated peptide of 36 amino acids which has an α-helical part between residues 13 and 32. Among the subtypes of receptor for NPY, the Y_2 receptor is thought to mediate several important central activities such as memory retention. The Y_2 receptor can effectively bind long *C*-terminal fragments, in which the critical region for receptor recognition is the *C*-terminal hexapeptide. By comparing IC_{50} values of a series of analogs ([Xaa^{11}]NPY(11-36), [Xaa^{12}]NPY(12-36) and [Xaa^{13}]NPY(13-36)) in binding assays, we found, in the present study, that removal of residue 11 caused a significant decrease in the binding affinity for the Y_2 receptor. Acetylation or succinylation of the α-amino group of [Xaa^{12}]NPY(12-36) largely increased the binding affinity without any exceptions. Upon such modification, the binding affinity returned to a level similar to that of [Xaa^{11}]NPY(11-36), an indication that the region around the peptide bond between residues 11 and 12 interacts directly with the receptor. Next, we investigated whether or not the affinity of the NPY analogs for binding to the Y_2 receptor is affected by cross-linking of a dimer. By oxidation of an analog of NPY(12-36), in which Ala-12 and Ile-31 were substituted with Cys residues, we prepared parallel and antiparallel dimers. The IC_{50} value of the antiparallel dimer was of the same order as that of the reduced molecule, whereas the binding affinity was greatly decreased by the formation of parallel dimer.

625.15

CLONED RECEPTORS FOR NPY, PYY AND PP: RAT Y2 AND Y4 SUBTYPES. K.E. Smith*, M.W. Walker, C. Gerald, J.A. Bard, S. Daouti, P.J.-J. Vaysse, T.A. Branchek and R.L. Weinshank. Synaptic Pharmaceutical Corporation, Paramus, NJ 07652.

Multiple receptor subtypes are proposed to mediate the diverse physiological effects of the related peptides neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP). Using expression cloning and homology approaches, we recently isolated DNAs encoding two novel human Y-type receptors, designated Y2 and Y4 based on their pharmacological properties (Gerald et al., Bard et al., and Walker et al., Soc. Neurosci Abst., 1995). We report here the cloning of the rat Y2 and Y4 receptors using the nucleotide sequences of the corresponding human receptors.

The predicted protein sequence of the rat Y2 receptor exhibits 95% identity with the human Y2 receptor. The pharmacological properties of the rat Y2 receptor transiently expressed in COS-7 cells parallel those of the human Y2 receptor and are characterized by high-affinity binding of NPY, PYY and PYY 13-36 (K_i values 0.28-1.5 nM). Human [Leu^{31},Pro^{34}]-NPY and rat PP were inactive at concentrations up to 1μM. Thus, the rat Y2 receptor, like the human Y2, is relatively tolerant of N-terminal ligand deletions and intolerant of Pro^{34} or C-terminal substitutions. We recently isolated DNA encoding a second isoform of the rat Y2 receptor that differs by two amino acids and may represent an allelic variant of the Y2 receptor gene.

The amino acid sequence of the rat Y4 receptor displays 75% identity with the human Y4 receptor (84% in TM domains); the third intracellular loop is particularly divergent (56%). The pharmacological profile of the rat Y4 receptor transiently expressed in COS-7 cells, when tested with human peptides, is similar to that of the human Y4 receptor: PP>[Leu^{31},Pro^{34}]-NPY>NPY. However, certain nonmammalian PP homologs (avian, frog PP) bind selectively to rat or human Y4 receptors. The relatively low degree of sequence identity between rat and human Y4 receptors, combined with their contrasting affinities for PP-related peptides, may reflect the fact that PP itself is not highly conserved across species.

Comparison of rat Y2 and Y4 receptors with their human homologs will help to elucidate the roles of NPY, PYY and PP in rat and human physiology.

625.16

IDENTIFICATION OF RESIDUES INVOLVED IN THE BINDING OF THE ANTAGONIST BIBP3226[1] TO THE HUMAN Y1 RECEPTOR. J.A. Salon*, J.A. Tamm, C. Hou, P. Du, P.J.-J. Vaysse, B. Dowling, Y. Shifman, M.W. Walker, N. Adham, N. Boyle, D. Dhanoa, W. Cui, T.A. Branchek, R.L. Weinshank and C. Gluchowski. Synaptic Pharmaceutical Corporation, Paramus, NJ 07652.

The recently reported compound BIBP3226 is a non-peptide antagonist of neuropeptide Y (NPY) with high affinity and selectivity for Y1 over Y2 receptors. The compound was designed to mimic the C-terminus of the agonist NPY. To investigate antagonist/receptor interactions we performed site-directed mutagenesis on the human Y1 receptor and compared the binding affinity of BIBP3226 with that of NPY by measuring displacement of [^{125}I]-PYY. Mutation of residues Y47, F173, Y196, Y211, Q219, F282, F286 and H306 in the human Y1 receptor was found to decrease the binding affinity for BIBP3226 by 5 to 25-fold. Computer modeling and docking studies suggest that these residues may interact with the ligand through hydrophobic or polar contacts. Mutation of five of these residues (F173, Y196, Y211, F286 and H306) had little or no effect on NPY binding affinity indicating that these amino acids were selectively interacting with the antagonist. Conversely, mutation of several residues important for NPY binding (Y100, D190 and Y192) had little effect on antagonist binding affinity. Taken together, the data suggest that certain residues are common to the NPY and BIBP3226 binding sites, while other residues are used exclusively by the antagonist. These findings support the premise that this antagonist acts, in part, by competing for specific residues important for agonist attachment. (Note[1] BIBP3226 was synthesized and supplied by Ciba-Geigy Limited, Basel, Switzerland.)

625.17

NPY, PYY, OR PP ALTER cAMP AND Ca^{++} IN CELLS EXPRESSING CLONED HUMAN Y2 OR Y4 RECEPTORS. P.J.-J. Vaysse*, M.W. Walker, C. Gerald, J.A. Bard, R.L. Weinshank and T.A. Branchek. Synaptic Pharmaceutical Corporation, Paramus, NJ 07652.

Pancreatic polypeptide family members neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP) function as neurotransmitters or hormones through activation of membrane bound receptors. At least 5 receptor subtypes are proposed: Y1 (previously cloned from vertebrates), Y2, Y3, PP, and Y1-like "feeding" receptors. We recently isolated human cDNA for two novel Y-type receptors and classified them as Y2 and Y4 subtypes based on peptide binding profiles of transiently transfected COS-7 preparations (Gerald et al., Bard et al., Walker et al., Soc. Neurosci. Abst. 1995). Y2 receptor function was tested using 293 cells stably transfected with Y2 cDNA (293-Y2). Forskolin-stimulated [cAMP] detected by RIA decreased $\geq$ 70% when 293-Y2 cells were incubated with human PYY (EC50 = 0.31 nM) or NPY (EC50 = 0.25 nM), but not [Pro34]-PYY (up to 100 nM). Y2-selective analogs were potent, including the cyclic disulfide analog C2-NPY (EC50 = 0.14 nM) plus C-terminal fragments PYY 13-36 (EC50 = 0.13 nM) and NPY20-36 (EC50 = 3.2 nM). Intracellular free [Ca^{++}] detected by Fura-2 fluorescence increased in 293-Y2 cells incubated with human PYY: Emax = 41nM, EC50 = 39 nM. [cAMP] reduction is consistent with reports for pharmacologically defined Y2 receptors; Ca^{++} mobilization is less often noted. Y4 receptor function was tested using LMtk- cells stably transfected with Y4 cDNA (LM-Y4). Forskolin-stimulated [cAMP] decreased $\geq$ 65% when LM-Y4 cells were incubated with human PP (EC50 = 0.09 nM) or PYY analogs (EC50 = 47 nM for PYY, 1.1 nM for [Pro34]-PYY) or NPY (EC50 = 20 nM). Intracellular free [Ca^{++}] increased when LM-Y4 cells were incubated with human PP: Emax = 334 nM, EC50 = 35 nM. Y4 preference for PP > PYY, NPY resembles that for pharmacologically defined PP receptors and supports a role for cAMP and Ca^{++} in PP-mediated cell signaling. We propose the name "Y4" to account for activity of all PP family members and to extend the Y-type nomenclature.

625.18

CLONING OF RECEPTOR GENES THAT SHARE IDENTITY WITH PEPTIDE BINDING GPCR GENES. A. Marchese, T. Nguyen, B. P. Jung, Y. Shen, V. R. Saldivia, P. Seeman*, S. R George, B. F. O'Dowd. Addiction Research Foundation, Dept. of Pharmacology, Univ. of Toronto, Toronto, ON. M5S 1A8.

G protein-coupled receptors (GPCR) are involved in a variety of brain mechanisms, including reward, pain, mood and learning. We have cloned 11 human genes for which the endogenous ligands are unknown (GPR1 to 10 and APJ). These genes share identity to known peptide binding GPCRs (Genomics **23**: 609; DNA and Cell Biol. **14**: 25; Gene **136**: 355). Gene GPR1 is abundantly expressed in hippocampus in human but not in rat, and is expressed in rat heart, kidney and spleen. Primers designed based on sequences of GPR1 were used to search for related genes, and from PCR amplification of human DNA we isolated 4 novel genes (including GPR14) and each shares identity with genes encoding peptide binding GPCRs. GPR14 shares significant identity to opioid and somatostatin receptors (41% in TM domains). Also, in our search for novel opioid receptors we used primers based on the sequences encoding the opioid receptor genes and the related genes, GPR7 and 8, to amplify human DNA. This resulted in the isolation of 2 genes encoding novel receptors and each shares greatest identity with the ATP receptor. We also designed primers based on the sequences of μ, δ and κ opioid receptors, contained on a single exon (encoding TM2 to TM3), to amplify human genomic DNA. This PCR resulted in the isolation of 2 other novel genes. One of these genes contained sequence identical to an EST fragment (GenBank Accession Number: F03704) isolated from human brain and which showed high identity to the ATP receptor. Ultimately, it is hoped that the discovery of novel GPCR genes and their categorization into distinct families of receptors will advance our understanding of the complex actions of peptide binding receptors.

625.19

EXPRESSION OF VIP-R mRNA IN MURINE LYMPHOCYTES. M.C. Johnson , B. Wortham, R.I. McCormack, D. Ganea*. Dept. of Biological Sciences, Rutgers University, Newark, NJ 07102.

Our laboratory has previously reported that the neuropeptide vasoactive intestinal peptide (VIP) inhibits IL-2 and IL-4 production in activated murine T lymphocytes. This response appears to be mediated through specific receptors since structurally-related peptides such as secretin and glucagon have little or no activity. To determine whether the inhibitory effect of VIP on cytokine production by T cells is indeed mediated through specific receptors, we investigated the expression of VIP-R mRNA in murine lymphocytes by RT-PCR. Specific primers were designed based upon the published rat lung and human intestine VIP-R cDNA sequences. RT-PCR was performed on both unstimulated and stimulated murine Balb/c spleen cell cultures and the predicted 453 bp fragment was amplified. The 453 bp fragment has also been amplified from murine brain, lung, highly purified $CD4^+$ T cells, and thymocytes. The specificity of these fragments has been confirmed by Southern blots. The amplified fragment from unstimulated and stimulated spleen cells was cloned and sequenced. The murine VIP-R cDNA sequence, which spans the IV, V, VI, and VII transmembrane domains of the receptor, shares greater homology with the rat lung than with the human intestine VIP-R cDNA sequence. Quantitative PCR needs to be performed to investigate the possible upregulation of VIP-R expression in murine lymphocytes. These preliminary results indicate that VIP-R mRNA is expressed in murine lymphoid organs, as well as brain and lung tissues.

625.20

PHARMACOLOGICAL PROFILES OF NEWLY SYNTHESIZED NEUROTENSIN(8-13) ANALOGS. B.M. Cusack, D. McCormick, C-T Phung, R. Perry, Y-P Pang, A. Fauq, and E. Richelson*. Neuropsychopharmacology Research, Mayo Foundation for Medical Education and Research, Mayo Clinic, Jacksonville, FL 32224.

We recently reported results with 33 newly synthesized NT(8-13) analogs at both the human and rat neurotensin receptors expressed in CHO cells. We derived equilibrium dissociation constants (K_d's) and EC_{50}'s for phosphatidylinositol turnover. We found that side chain length at position 9 and steric bulk/pi electron density at position 11 were important for binding and functional NT parameters. We have synthesized 11 additional compounds, which explore further permutations of these variables. Specifically, by increasing side chain length at position 8 and 9 (with homoarginine), we obtained a K_d of 0.27 ± 0.05 nM (geo. mean ± SEM). For another compound, we shortened the position 9 side chain by substituting diaminopropionic acid for this residue. The K_d derived for this analog was 8.6 ± 0.8 nM, which was a decrease in binding affinity over our previously synthesized compound with diaminobutyric acid at position 9 (Kd = 0.27). For position 11 substitutions, we further explored steric bulk effects by substitution with both D- and L-forms of 3,1-β naptha-alanine and also the conformationally constrained analog of phenylalanine, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid. The results with these 11 compounds present further evidence of the importance of side chain length at position 8 and 9 as well as steric bulk effects at position 11. [Support: Mayo Foundation & USPHS Grant MH27692 to E.R.]

NEUROTRANSMITTER RELEASE

626.1

EFFECT OF HALOPERIDOL ON NEUROTENSIN RELEASE IN THE RAT BASAL GANGLIA: A MICRODIALYSIS STUDY

J.M. Radke, M.D. Stipetic*, M.J. Owens and C.B. Nemeroff. Dept. Psychiat. & Behav. Sci., Emory Univ. Sch. of Med., Atlanta GA.

Neurotensin (NT) is an endogenous CNS tridecapeptide which has been implicated in the mechanism of action of antipsychotic drugs. Administration of haloperidol has repeatedly been shown to increase both NT concentrations and NT mRNA expression in the rat striatum and nucleus accumbens. The purpose of these experiments was to ascertain the effect of haloperidol on NT release using in vivo microdialysis. This method has been used extensively to measure neurotransmitter release, however few have examined NT release due to the low concentrations found in the brain. Using a 'loop' designed probe, coupled with a solid phase radioimmunoassay, we have successfully measured both striatal (4-5 pgrams/ 25 min sample) and nucleus accumens (1-2 pgrams/sample) NT release. Both regions showed 2-3 fold increases in NT release following depolarizing concentrations of potassium and these increases were shown to be calcium dependent. Following these studies, we examined whether the acute administration of haloperidol alters NT release. No differences in NT release were observed over a 30 hour period in control animals nor were changes in NT release observed following the acute administration of haloperiol (2.0 mg/kg). These results indicate that acute haloperidol administration has no effect on the basal release of neurotensin in the rat striatum. Future work analyzing the effects of chronic administration of haloperidol and other antipsychotics on NT release in both the nucleus accumbens and the striatum are in progress.

Supported by NIMH-39415

626.2

STRUCTURE SPECIFIC DETECTION OF NEUROTENSIN IN THE RAT BRAIN BY UTILIZING MICRODIALYSIS AND MICRO-ELECTROSPRAY MASS SPECTROMETRY. P.E. Andrén* and R.M. Caprioli. Analytical Chemistry Ctr., Dept. of Biochemistry & Molecular Biology, Univ. of Texas Medical School, 6431 Fannin, Houston TX 77030

The structure specific molecular detection of the endogenous neuropeptide neurotensin has been accomplished in *in vivo* microdialysates and brain tissue homogenates by using capillary liquid chromatography (LC) and mass spectrometry. Microdialysis probes implanted in specific brain regions of unanesthetized freely moving rats were used to collect samples from brain extracellular fluids. Micro-electrospray/tandem mass spectrometry (micro-ES MS/MS) was used to achieve the molecular weight/specific identification of the neurotensin with a sensitivity in the attomole/μL range. Basal levels of neurotensin in microdialysates were approximately similar in hypothalamus (73±12 attomoles/10 μL) and the globus pallidus/ventral pallidum region (84±27 attomoles/10 μL). A 7-8 fold increase in neurotensin in microdialysates from these regions of the brain was observed after KCl-stimulation.

In other experiments, rat brain tissue was homogenized and purified using Sep-Pak columns and HPLC, and neurotensin was detected from striatum, globus pallidus and substantia nigra by subsequent analysis by capillary LC/micro-ES MS/MS.

The present study shows that the mass and molecular structure specific detection of neurotensin by mass spectrometry can be used to measure baseline and stimulated release in *in vivo* microdialysates in different brain regions.

626.3

CO-INDUCTION OF c-JUN AND NEUROTENSIN mRNAs IN THE DORSOLATERAL STRIATUM FOLLOWING HALOPERIDOL TREATMENT R. J. Harrison*[1,2], P. R. Dobner[2] and R. H. Melloni Jr[1] [1]Behav. Neurosci. Prog., Dept. Pysch., and [2] Dept. of Mol. Genetics and Micro. and Prog. in Neurosci. Univ. of Mass. Med. Ctr., Worcester, MA 01655. The dopamine (DA), D2 receptor antagonist, haloperidol causes a transient induction of neurotensin/neuromedin N (NT/N) mRNA in the dorsolateral striatum (DLSt). We have previously detailed the AP-1 dependent activation of the NT/N gene in neuroendocrine PC12 cells and the potent ability of c-Jun to drive NT/N gene expression. Neuroleptic blockade of DA receptors causes induction of c-Jun, c-Fos and Jun B in the DLSt. c-Fos mRNA expression has been co-localized to striatal neurons expressing NT/N mRNA following haloperidol, and antisense c-Fos oligonucleotides decrease NT/N mRNA induction by haloperidol in this region suggesting a functional correlation. c-Fos, however is unable to bind to the AP-1 elements within the NT/N gene *cis*-regulatory region in the absence of a dimerization partner. We report here on the induction of c-Jun in the DLS following DA D2 receptor blockade by haloperidol. The c-Jun hybridization signal was restricted to the DLSt and paralleled the induction of NT/N mRNA in this region. c-Jun hybridization signals were also prominent in other regions of the CNS where NT/N mRNA expression is observed. Taken together with our previous functional studies in PC12 cells and additional antisense data presented here, the temporal and spatial localization c-Jun mRNA in the DLSt following haloperidol blockade of DA receptors suggests that c-Jun serves as a key regulator of NT/N gene expression in the CNS.

626.4

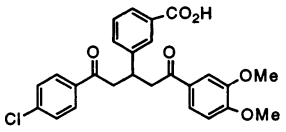
FUNCTIONAL ACTIVITY OF C-TERMINAL CYCLIC NEUROTENSIN FRAGMENT ANALOGS. Hyacinth C. Akunne1*, Kim T. Zoski1, Selina Darling1, Andrea M. Sefler2, Wayne L. Cody2, Thomas A. Pugsley1. Psychiatric Disorders Therapeutics1 and Department of Chemistry2, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105.

Neurotensin (NT, -pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ileu-Leu) is a tridecapeptide that displays a wide spectrum of biological actions both in the central and peripheral nervous systems of different mammalian species. Identification of functionally active and metabolically stable small molecule peptides or non-peptides has been difficult. Cyclic derivatives of a hexapeptide NT(8-13) (NαMeArg-Lys-Pro-Trp-tLeu-Leu) which is active upon systemic administration and was metabolically stable were synthesized by a combination of solution and solid-phase peptide synthetic methodologies. As reported previously, several analogs had nanomolar binding affinities both in newborn (10-day-old) mouse brain and HT-29 (human colonic adenocarcinoma) cell membrane preparations. The major signal transduction pathway for NT receptors is the G-protein dependent stimulation of phospholipase C, leading to the mobilization of intracellular free calcium, [Ca2+]i. We tested agonist or antagonist effects of these analogs in this *in vitro* assay using the Ca2+-sensitive dye fura-2/AM for quantitative measurement of the cytosolic free Ca2+ concentration in HT-29 cells. Of most interest were PD 149966, PD 151482, PD 151149 which had respective Ki values of 16, 28 and 83 nM in mouse brain membranes and 194, 3499 and 4175 nM in the HT-29 cell membranes, respectively. In the functional assay, PD 149966 and PD 151482 exhibited agonist effects with EC50 values of 130 nM and 20 μM, respectively. PD 151149 exhibited an antagonist profile in blocking the NT-induced increase in intracellular Ca2+ mobilized, with an IC50 of 1700 nM. The present findings indicate that small molecule cyclic analogs with functional activities can be designed which might be of potential use in the treatment of schizophrenia and possibly other disorders.

626.5

THE DISCOVERY AND STRUCTURE ACTIVITY RELATIONSHIP OF A NOVEL SERIES OF SMALL MOLECULE NEUROTENSIN ANTAGONISTS.
S. Johnson, S. Kesten, H. Akunne, D. Wustrow, T. Pugsley T. Heffner, L. Wise* Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company; Ann Arbor MI, 48105

Neurotensin (NT) is a tridecapeptide thought to modulate dopamine neurotransmission in the CNS. As part of a program to identify novel nonpeptide ligand for the NT receptor we carried out a high volume screen against the Parke-Davis compound library. Structure activity relationship (SAR) studies on an initial lead will be presented. These studies led to the discovery of PD 156425 which has affinity for the NT receptor in an immature mouse brain preparation (Ki = 42 nM). It has also been shown to be an antagonist *in vitro* , inhibiting the effects of NT on the mobilization of calcium in HT-29 cells. SAR studies indicate that the carboxylic acid and the appropriately substituted phenone residues are required for compounds of this type to exhibit good binding to the NT receptor



PD 156425

626.6

NEURAL REGULATION OF TRH BIOSYNTHESIS.
R.M.Uribe, L.Pérez-Martínez, M.L.Covarrubias, B.O.Gómez, J.L.Charli and P. Joseph-Bravo. Instituto de Biotecnología, UNAM.(A.P. 510-3, Cuernavac Mor., Mexico 62271)

Thyrotropin releasing hormone (TRH) controls the biosynthesis and release of thyrotropin and prolactin from the hypophysis. TRH mRNA levels are negatively controled by thyroid hormones in the paraventricular hypothalamic nucleus and increased, in a fast and transient manner (30-60min), in conditions where TRH release is stimulated, such as cold exposure or suckling (Neuroendocrinology *58*,140,1993). In order to get insight if TRH mRNA increase is due to transsynaptic stimulation we studied the effect of second messenger pathways'stimulation on primary cell culture of rat hypothalamus. Embryonic hypothalami (17 day gestation) were dispersed with trypsin-DNAse and incubated in complete DMEM-FBS for 18 days. Culture conditions have been optimized to express TRH mRNA and TRH utilizing autologous conditioning medium. Cultures were treated with phorbol ester (TPA,100 nM) or dibutiryl cAMP (1 mM) for various times. TRH mRNA quantified by Northern blot. Levels of TRH-mRNA raised from the 1st hr of dBcAMP or 2h of TPA treatment. An increase was still observed at 24h with both drugs. These results provide evidence for a neural regulation of TRH biosynthesis. *Financed by DGAPA (IN206094) and CONACYT (0776 N9110).*

626.7

KINDLING INDUCED CHANGES IN mRNA EXPRESSION OF PRO-TRH AND PROHORMONE CONVERTASES IN DENTATE GYRUS L. P. Pu*[1] and I.B. Rosen[2]. [1]LDN, NICHD, [2]Biol. Psychiatry Br., NIMH, NIH, Bethesda, MD 20892.

The mRNA encoding several pro-neuropeptides and the neuropeptides themselves are dramatically increased in the granule cells of the dentate gyrus following an amygdala kindled seizure. It is not known whether the enzymes related to the maturation of these neuropeptides are also altered after kindling. The prohormone convertases PC1 and PC2 have been shown to be able to process several pro-neuropeptides including pro-TRH at paired-basic residue cleavage sites, a key step involved in neuropeptide maturation. In this study, we have examined the co-regulation of expression of PC1 and PC2 mRNAs with pro-TRH mRNA in the dentate gyrus after amygdala kindling. Rats were kindled daily until a fully generalized (stage 5) seizure was elicited. The rats were sacrificed either 4 or 24 hrs following the last seizure. *In situ* hybridization using cRNA probes for PC1, PC2 and pro-TRH mRNAs was performed on brain sections containing the dentate gyrus. High levels of pro-TRH mRNA were expressed in the dentate gyrus 4 hrs after the seizure, while at 24 hrs they were diminished but still elevated compared to non-kindled sham controls. PC1 mRNA expression was also increased 4 hrs after the seizure in the dentate gyrus, although it was below control levels at 24 hrs. Similarly, an increase in PC2 mRNA expression levels was observed at 4 hrs, but was significantly decreased at 24 hrs following the seizure. These data demonstrate that in the dentate gyrus, there was co-ordinate expression of prohormone convertase mRNAs with pro-neuropeptide mRNA expression, suggesting that these enzymes play a role in pro-TRH processing *in vivo*.

626.8

NEUROPEPTIDE Y BIOSYNTHESIS AND METABOLISM IN THE HIPPOCAMPAL MOSSY FIBER SYSTEM J.B. McCarthy* and J.D. White[1] Dept. Neurobiology & Behavior, SUNY Stony Brook, Stony Brook, NY 11974; [1] Trophix Pharmaceuticals, Inc., S. Plainfield, NJ 07080

As a first step in understanding the physiological role(s) for neuropeptide Y in specific projection systems in the CNS, neuropeptide Y biosynthesis was investigated in the hippocampal granule cell mossy fiber projection. Previous studies demonstrated increased hippocampal NPY gene expression in response to both recurrent and single seizure episodes. To study NPY biosynthesis and transport in the mossy fiber system, male Sprague Dawley rats were subjected to a single seizure with pentylenetetrazole (57 mg/kg ip). To radiolabel newly synthesized NPY, ^{35}S- methioine was delivered unilaterally to the dentate gyrus at a rate of 1μl/hr with osmotic mini-pumps via an indwelling cannula. *In vivo* radiolabeling was performed at a post-seizure time point coincident with maximal preproNPY mRNA expression. Following labeling, the ipsilateral hippocampus was dissected and sliced (500μm) with a McIlwain tissue chopper. CA3 subfields were then collected from each slice and the resulting tissue pool was homogenized in acid extraction buffer. This homogenate was then subjected to immuno-affinity purification followed by sequential reverse phase HPLC purification. The resulting radio-chemical purification of bona fide NPY from the CA3 subfield supports the hypothesis that there is a novel post-seizure expression of NPY in granule cells, and that this peptide is transported to mossy fiber boutons. Current experiments are examining the *in vitro* metabolism of NPY by synaptosome peptidases to assess the possibility that bioactive fragments of NPY can be generated extracellularly following release from the mossy fibers.

626.9

HUMAN FETAL BRAIN CELLS IN AGGREGATE CULTURE: A MODEL SYSTEM TO STUDY REGULATORY PROCESSES OF NEUROPEPTIDE Y (NPY) PRODUCING NEURONS. N. Aguila-Mansilla, G. Kozlowski* and A. Barnea. Dept. of OB/GYN, UT Southwestern, Dallas, TX 75235-9032.

NPY, a 36 amino acid peptide, is one of the most abundant neuropeptides in the brain and it has been implicated in a wide range of brain functions including mentation. The aim of this study was to establish a culture system of human fetal brain cells expressing NPY in a regulated manner. NPY production in response to forskolin (For) and phorbol 12-myristate 13-acetate (PMA) was taken as a criterion for regulated expression of NPY. Aggregates were formed from dissociated cells derived from the cerebral hemispheres of human fetuses (12-19 wks gestation) by constant rotation and were maintained in serum-free medium. A 24 h exposure to 10 μM For + 20 nM PMA led to a 3-5-fold increase in NPY content of the cultures, most (80-90 %) of which was secreted into the medium. The latter consisted of two substances differing in size: one corresponding to proNPY and the other to NPY. Thus, For + PMA led to an increased production of NPY. Exposure to PMA alone led to an increase in NPY production comparable to that seen after For + PMA; this effect of PMA was dose-dependent. In contrast, For alone did not induce NPY production. Eliminating the dividing cells from the aggregates totally abolished For + PMA induction of NPY production. Thus, expression of the NPY neurons in this culture system is a regulated process: activation of the protein kinase C pathway enhances NPY production and input from glial cells is involved. These results support the proposition that this culture system is an excellent model to study regulatory processes of the human fetal NPY neuron.

626.10

NEUROPEPTIDE Y (NPY) GENE EXPRESSION AND INTRACELLULAR TRANSDUCTION MECHANISMS IN PHEOCHROMOCYTOMA (PC12) CELLS. X.L. Chen*, S.P. Han, M. Weidman, Y.M. Wu, L. Naes and T.C. Westfall. Dept. of Pharmacol. and Physiol. Sci., Saint Louis Univ. Health Sciences Center, St. Louis, MO 63104.

NPY is a vasoconstrictor peptide primarily co-synthesized and co-released with catecholamine from sympathetic nerve terminals and the adrenal medulla. NPY is thought to play an important role in blood pressure regulation and may participate in development and/or maintenance of hypertension. We have previously used rat pheochromocytoma (PC12) cells differentiated with NGF or dexamethasone as model systems of peripheral sympathetic neurons or the adrenal medulla cells, respectively. In the present study, we examined the mechanisms mediating NPY gene expression in PC12 cells using Northern hybridization techniques. Depolarization of NGF-differentiated PC12 cells with nicotine produced a concentration-dependent (10-300 μM) and time dependent (4-48 hours) increase in NPY mRNA abundance. In contrast, the increase in NPY mRNA abundance in undifferentiated PC12 cells was much smaller. Nifedipine, attenuated nicotine-induced NPY gene expression in NGF-differentiated PC12 cells, suggesting that the response is mediated by L-type calcium channel. Additional experiments have examined the intracellular transduction mechanisms mediating NPY gene expression in PC12 cells. Calmidazolium attenuated NPY gene expression response to nicotine suggesting that calcium/calmodulin-dependent protein kinases may be involved in mediating NPY gene expression. Both forskolin and PMA produced an increase in NPY mRNA abundance and had a synergistic effect on NPY gene expression. The results suggest that multiple protein kinase pathways are involved in regulating NPY gene expression in PC12 cells. (Supported by NIH HL-26319 and HL-35202).

626.11

EXPRESSION, SECRETION AND PLASTICITY OF ENDOGENOUS PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDES (PACAP) IN RAT SUPERIOR CERVICAL GANGLION NEURONS. C. A. Brandenburg*, K. M. Braas and V. May. Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

Neurons of the superior cervical ganglion (SCG) express a wide variety of neuropeptides as well as classical neurotransmitters, and the specific complement of signaling molecules present in individual cells depends on developmental and environmental cues. This phenomenon contributes to the neurophenotypic plasticity and functional diversity of the nervous system. Many SCG target tissues respond to PACAP27 and PACAP38, members of the VIP/secretin/glucagon family of bioactive peptides. The current studies examined whether PACAP peptides are expressed by rat SCG. Endogenous PACAP38 and PACAP27 were identified in adult, neonatal and cultured SCG neurons. PACAP38 levels were approximately 5 fmol/adult SCG, 2 fmol/ neonatal SCG and 2 fmol/10^4 cultured neurons; comparable PACAP27 levels were identified. Authentic peptide immunoreactivity was verified by coelution with synthetic PACAP in reversed phase HPLC. Reverse transcription PCR and sequence-specific hybridization revealed PACAP mRNA in adult, neonatal and cultured SCG neurons. Endogenous neuronal PACAP peptides must be released to function as important signaling molecules and influence target tissues. Assay of conditioned medium established basal PACAP38 secretion from cultured SCG neurons. Furthermore, depolarization with 40 mM potassium stimulated the secretory rate approximately 10-fold; cellular peptide content and PACAP mRNA expression were also increased. Axotomized and decentralized adult SCG exhibited a dramatic over 40-fold increase in PACAP38 content. These studies identify PACAPs as important peptides contributing to the functional diversity and phenotypic plasticity of sympathetic neurons. Supported by HD27468 and NS01636 (VM) and AHA94015540 (KMB).

626.12

WITHDRAWN

626.13

DELAYED HIGH POTASSIUM-EVOKED RELEASE OF GLUTATHIONE AND γ-GLUTAMYL-GLUTAMATE FROM RAT HIPPOCAMPAL SLICES Sandberg, M.*, Li, X., Orwar, O. and Revesjö, C. Institute of Anatomy and Cellbiology, Univ. of Gothenburg, S-413 90 Göteborg, SWEDEN

The potassium-evoked release of amino acids and γ-glutamyl di- and tripeptides was studied from rat hippocampal slices. The concentrations of amino acids and peptides in the incubation media were determined by precolumn derivatisation with OPA followed by reversed phase HPLC and fluorescence detection. The release of glutathione (analyzed as GSH) and γ-glutamyl-glutamate was delayed in comparison to glutamate release. Preincubation with acivicin, an enzyme blocker of γ-glutamyl transpeptidase, inhibited the release of γ-glutamyl-glutamate and increased the release of GSH while the release of glutamate was unaffected. Our results imply: i) delayed release of GSH from slices may be coupled to prior reuptake of released glutamate. ii) γ-glutamyl transpeptidase may utilize a fraction of released glutamate as an acceptor amino acid during situations with high extracellular glutamate concentrations. iii) γ-glutamyl transpeptidase does not appear to contribute to uptake of glutamate even when glutamate-carriers are reversed by high potassium.

626.14

LIPOPOLYSACCHARIDE (LPS) FACILITATES RELEASE OF CGRP FROM THE RAT TRACHEA X.-Y. HUA*, P. CHEN & T.L. YAKSH Dept. of Anesthesiology, Univ. of California, San Diego, CA 92093-0818, USA.

LPS, an endotoxin, produces pain behavior, inflammation and changes of immune function. Many of these effects of LPS are secondary to the production of cytokines. LPS and cytokines upregulate expression of neuropeptide such as substance P and its receptor. In the present study, we investigate the effect of LPS treatment on tracheal calcitonin gene-related peptide (CGRP)/neurokinin A (NKA) release as well as changes of the peptide content in the trachea and vagus nerve. Application of capsaicin 0.1 μM to an intralumenally perfused *in vitro* rat trachea induced an increase in CGRP outflow (ΔCGRP 131±24 fmol/fraction, n=10), which was significantly enhanced in the tracheas from the LPS-treated rats (0.3 mg/kg iv or 0.75 mg/kg ip, -5hrs) (ΔCGRP 295±59 fmol/fraction, n=8, $P<0.05$). This enhancement was blocked by IL-1β antagonist, Lys-D-Pro-Thr, at concentration of 10 μM (ΔCGRP 137±12 fmol/fraction, n=4). LPS 10 μg/ml added directly to tracheal perfusates did not induce any release of CGRP or NKA. CGRP level in the vagus nerve, but not in the trachea, was significantly increased by LPS treatment (LPS vs Saline: 623±94 vs 407±4 CGRP fmol/mg protein, n=5, $P<0.05$). In contrast, neither NKA release caused by capsaicin nor NKA level in trachea or vagus nerve was altered by LPS treatment at 5 hrs, although LPS treatment at 2 hrs caused a moderate but significant reduction of NKA content in the rat trachea (LPS vs Saline: 4.1±0.2 vs 6.1±0.5 NKA fmol/mg tissue, n=5, $P<0.05$). These data suggest that LPS may not have direct stimulatory effect on sensory neurons, but sensitize CGRP-containing sensory nerves via a mechanism requiring production of cytokines such as IL-1β. LPS may have a different interaction with NKA processing system as compared with CGRP.
(This work was supported by NIH R29 HL 50403, X.-Y. H.)

626.15

CHARACTERIZATION OF SUBSTANCE P RELEASE FROM THE INTERMEDIATE AREA OF RAT THORACIC SPINAL CORD. L.Yang[1*], N.D.Thomas[2] and C.J.Helke[1,3]. [1]Dept. of Pharmacol. & [3]Neurosci. Program, Uniformed Services Univ. of the Health Sci., Bethesda, MD 20814; [2] NIMH, Bethesda, MD 20892.

Substance P (SP) coexists with serotonin (5-HT), neurokinin A (NKA) and thyrotropin-releasing hormone (TRH) in nerve terminals of the intermediolateral cell column (IML), site of sympathetic preganglionic neurons. Neither the depolarization-induced release of SP nor the regulation of SP release has been studied in this system. Basal and K^+-evoked release of SP from the microdissected intermediate area (includes the IML) of the rat thoracic spinal cord and the regulation of SP release by 5-HT, NKA and TRH were studied using an in vitro superfusion system. Elevated K^+ evoked a concentration- and extracellular Ca^{2+}-dependent release of SP. In rats pretreated with serotoninergic neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), both SP content and the absolute amount of SP released from the intermediate area were decreased. However, the fraction of the remaining tissue content of SP released by K^+-depolarization was not changed subsequent to 5,7-DHT treatment. Moreover, 5-HT, 5-HT_{1B} agonists (CGS-12066B and RU 24969) and a 5-HT_3 agonist (2-methyl-5-HT) did not alter the K^+-evoked release of SP. A neurokinin-1 (NK_1) antagonist (GR 82334) dose-dependently (10^{-9}-10^{-7} M) increased the K^+-stimulated release of SP suggesting the presence of presynaptic inhibitory NK_1 autoreceptors. However, NK_1 agonist GR 73632 (10^{-9}-10^{-6}M) increased both the basal and K^+-stimulated release of SP and the excitatory effects were not blocked by the NK_1 antagonist. Neither NKA, a NK_2 agonist (GR 64349) nor a TRH analog (MK-771) changed the K^+-evoked release of SP. These data demonstrate the depolarization-induced release of SP and the release (at least in part) comes from serotoninergic nerve terminals in the intermediate area of the rat thoracic spinal cord. Although 5-HT, NKA and TRH coexist with SP in the IML, they are devoid of activity in the regulation of SP release (NIH NS24876).

626.16

NOVEL POTENT INHIBITORS OF N-ACETYLATED-α-LINKED ACIDIC DIPEPTIDASE ACTIVITY FAIL TO REDUCE EXTRACELLULAR GLUTAMATE EFFLUX OR STIMULATED RELEASE. B.A. Donzanti*, L.E. Ross, S.L. Stetz, B.S. Slusher, R.A. Keith, D.C. Cole, P.F. Jackson. Deptartments of Pharmacology and Medicinal Chemistry, Zeneca Pharmaceuticals, 1800 Concord Pike, Wilmington, DE 19850-5437.

N-Acetyl-aspartyl-glutamate (NAAG) is an acidic dipeptide which has been proposed to be a storage form of synaptically available glutamate due to its neuronal metabolism by N-acetylated-α-linked acidic dipeptidase (NAALADase) to N-acetyl-aspartate and glutamate. To test this hypothesis *in vivo*, we developed two novel NAALADase inhibitors with low nanomolar potency and evaluated their ability, along with β-NAAG (a competitive but weak NAALADase inhibitor), to alter extracellular basal glutamate efflux and K^+-evoked glutamate release using microdialysis. Male Sprague-Dawley rats were anesthetized with urethane (1.5 g/kg, i.p.) and stereotaxically implanted with microdialysis probes into the ventroposterior thalamus or striatum. Artificial CSF was perfused at a flow rate of 2.5 µl/min and samples were collected at 10 minute intervals. Following the collection of 3 consecutive basal samples, an inhibitor was perfused directly into the brain through the probe for 30 minutes during which 3 additional dialysate samples were obtained. Finally, at the 30-40 minute collection period, high K^+ (100 mM) CSF containing an inhibitor was perfused to observe local effects on evoked glutamate release. Samples were analyzed using LC-EC methods. All three NAALADase inhibitors failed to reduce basal or evoked glutamate release as predicted by the working hypothesis. In fact, all of the compounds had a tendency to elevate both basal and stimulated glutamate release for reasons which are not completely understood. Since NAALADase inhibitors do not reduce extracellular glutamate *in vivo*, it is concluded the NAAG does not serve as a major depot of synaptic glutamate under normal physiological conditions.

626.17

LOCALIZATION OF PROOPIOMELANOCORTIN (POMC) mRNA AND PEPTIDES IN DEVELOPING AND ADULT RAT HEART. C. Nyquist-Battie*, C. B. Unal, S. A. Sands, B. M. Chronwall, W. R. Millington. School of Biol. Sciences, Univ. of Missouri, Kansas City, MO 64108.

β-Endorphin, α-MSH and other POMC peptides have been identified in mammalian heart. In the present study, the regional distribution of POMC mRNA and peptides, and the physiological regulation of POMC peptides were examined in adult and developing rat heart. POMC mRNA was demonstrated in atria by *in situ* hybridization. Polymerase chain reaction amplification confirmed that full-length POMC mRNA transcripts are present in atrial extracts. The distribution of POMC peptides was examined immunohistochemically using antisera against α-MSH and N-acetyl-ß-endorphin, which we showed previously to be the major end-products of POMC processing in heart. Atrial myocytes, especially in the atrial appendages, contained α-MSH immunoreactivity (IR) and N-acetyl-β-endorphin-IR. Ventricular myocytes and cardiac nerves were not stained. Atrial natriuretic factor (ANF)-IR was similarly distributed. During renovascular hypertension produced by constriction of the abdominal aorta, α-MSH- and ANF-IR were again detectable in ventricular myocytes. Neonatal atrial and ventricular myocytes exhibited α-MSH- and ANF-IR, but by postnatal day 21, ventricular staining was reduced. In correlative studies, α-MSH was detectable by RIA in neonatal ventricular myocyte cultures (37 ± 3.3 fmol/10^4, n = 6 cultures). These results demonstrate that POMC peptides are synthesized by cardiac myocytes, and further indicate that POMC peptides and ANF are co-regulated in heart during development and pathophysiological states. (Supported by a grant-in- aid from American Heart Association-Kansas Affiliate).

626.18

LOCALIZATION OF VASOPRESSIN mRNA AND IMMUNOREACTIVITY IN PITUICYTES OF PITUITARY STALK-TRANSECTED RATS AFTER OSMOTIC STIMULATION Y. P. Loh*, L. P. Pu, H.L. Tracer, M.A.F. Sonnemans, and F.W. Van Leeuwen. LDN., NICHD/NIH, Bethesda, MD 20892. Netherlands Institute for Brain Res, Amsterdam, The Netherlands.

The presence of vasopressin (AVP) mRNA and AVP immunoreactivity (AVPi) in pituicytes of the neural lobe (NL) of intact and pituitary stalk-transected rats, with and without osmotic stimulation was examined. AVP mRNA was analyzed by Northern blot and *in situ* ybridization in combination with immunocyto-chemistry using anti-GFAP as a marker for pituicytes. In intact rats, a 0.62 kb AVP mRNA with a truncated poly A tail was detected in the NL and increased 10-fold with 7 days of continuous salt-loading. Morphological analysis of these 7 day salt-loaded rats revealed the presence of AVP mRNA in a significant number of GFAP positive pituicytes in the NL . Eight days after stalk-transection the NL AVP mRNA disappeared in animals given water to drink, whereas in those given 2% saline for 18 h followed by 6 h of water, a treatment repeated on 6 successive days beginning 2 days post-surgery, the 0.62 kb AVP mRNA was present. The AVP mRNA in the stalk-transected, salt-loaded rats showed an exclusive cellular distribution in the NL, indicative of localization in pituicytes. Furthermore, immunoelectron microscopic examination showed the presence of AVPi in a subpopulation of pituicytes 7 and 10 days after pituitary stalk transection in salt-loaded animals. In these animals, almost all AVP fibers had disappeared from the NL. These data indicate that the expression of AVP mRNA and AVP is upregulated in pituicytes in response to osmotic stimulation.

PEPTIDES: PHYSIOLOGICAL EFFECTS II

627.1

PHARMACOLOGY OF VIP-MEDIATED RELEASE OF CYTOKINES FROM ASTROGLIAL CULTURES. D.E. Brenneman*, J.M. Hill, I. Gozes, M. Fridkin and T.M. Phillips. Lab. Dev. Neurbiol., NICHD, NIH, Bethesda, MD; Dept. Clinical Biochem., Tel Aviv Univ., Israel; Weizmann Inst. Science, Rehovot, Israel; Immunochem. Lab. George Washington Univ. Med. Ctr., Washington, D.C.

The neuroprotective action of vasoactive intestinal peptide (VIP) is mediated through the release of astroglial factors. VIP treatment produces an increase in the secretion of 8 cytokines from astroglial cultures derived from rat cerebral cortex. To investigate the pharmacology of the VIP receptor(s) mediating this secretion, the actions of VIP-related substances and VIP analogues were compared. Stearyl norleucine$_{17}$ VIP (SNV), an analogue shown to be 100 times more effective than VIP in neuroprotection, produced secretion equivalent to that of VIP. Peptide T also increased secretion, but was 3-5 fold less efficacious than equimolar VIP. Neither SNV or peptide T had a detectable increase on cAMP. An antagonist to VIP inhibited VIP-stimulated secretion of cytokines. Reduced extracellular calcium attentuated the release of 3 of the 8 cytokines. Pituitary adenylate cyclase activating peptide (PACAP-38), a homologue peptide to VIP, produced an increase in the same cytokines as VIP; however, equimolar PACAP released 60-80% less than VIP. Except for TNF α, 10 nM PACAP released the same amount of the 8 cytokines as 1 mM 8-bromo cAMP. Stimulation of microglial cultures with 0.1 nM VIP produced no detectable release of the 8 cytokines observed with astrocytes. Although cAMP-associated mechanisms can result in the release of cytokines, these data suggest VIP-stimulated secretion of cytokines from astroctyes was mediated by a receptor that was not linked to adenylate cyclase and by a process that was relatively insensitive to extracellular calcium.

627.2

EFFECT OF PROCTOLIN ON CONTRACTILE PROPERTIES OF CRUSTACEAN SKELETAL MUSCLE. A. Romero-Vásquez, G. Maynard-Salgado, S. Soto-Morales and C. Zuazaga*. Institute of Neurobiology, University of Puerto Rico, Medical Sciences Campus, San Juan, PR 00901.

Mechanical activation of tonic flexor muscle fibers of the crustacean Atya lanipes is strictly dependent on extracellular Ca^{2+}, even though the fibers do not generate Ca^{2+} action potentials. Proctolin's action on this muscle results on an increase of the isometric tension in a dose- and $[Ca^{2+}]_o$ -dependent manner, although action potentials are not induced (Romero et al., Biophys. J. 64:A24, 1993). We have been investigating the mechanisms by which proctolin enhances tension without changing the electrical properties of the muscle fiber. Using immunocytochemistry we found that proctolin-like immunoreactivity is present at the neuromuscular system in two neurons in each abdominal ganglia of the central nerve cord and in axons localized in the motor root that innervates the muscle. K^+ - contractures shows two phases of tension development. A phasic component that contributes mostly to the maximal tension is partially blocked by Cd^{2+} ions (2 mM, 15 min), and is strongly dependent on $[Ca^{2+}]_o$. The subsequent tonic component is highly reduced in presence of the Na-Ca exchanger inhibitor 3',4'-dichlorobenzamil (DCB, 10 µM). When exposed to DCB, the resting tension increases slowly and spontaneous contractile activity was also observed; K^+ - contractures were reduced and prolonged in a reversible manner. The effect of proctolin on the activity of the Na-Ca exchanger was also studied by examining Na - withdrawal contractures ($[Na^+]_o$ substituted with NMG or Li^+) and K^+ - contractures. In both cases, the tension generated was potentiated in the presence of proctolin (1 µM). We conclude that proctolin alters $[Ca^{2+}]_i$ homeostatic regulatory systems in this muscle. Supported by NIH NS07464, RR 03051.

627.3

ACCUMBAL MODULATION OF VENTRAL PALLIDAL RESPONSES TO ACTIVATION OF THE AMYGDALA. I. Mitrovic* and T.C. Napier. Dept. of Pharmacol., Loyola Univ. Chicago, Sch. of Med., Maywood, IL 60153.

The nucleus accumbens (NA) is a primary source of opioid and substance P (SP) input to the ventral pallidum (VP). We have previously demonstrated that VP neurons decrease firing during microiontophoresis of DAMGO (a μ opioid receptor-selective agonist) and increase firing with SP iontophoresis. We also have shown that VP neurons respond to electrical stimulation of the basal lateral amygdala (BLA) which provides glutamatergic innervation of the VP. This study investigated the ability of DAMGO and SP to modulate BLA-evoked neuronal responses in chloral hydrate-anesthetized rats. BLA stimulation (1 Hz, 0.4-1.2mA, 0.1ms) altered firing by more than 20% of baseline in 25 of 28 VP neurons tested. Nine neurons exhibited an early-onset excitation (latency $\leq$12ms, putative monosynaptic input) with a magnitude of 651 ± 170%. Four neurons exhibited an early-onset inhibition. Late-onset (latency >12ms, putative polysynaptic input) excitation and inhibition were observed in 10 and 7 VP neurons, respectively. Five neurons demonstrated more than one type of evoked response. Of 22 neurons evoked by the BLA stimulation and tested with iontophoresis of DAMGO, 14 (63%) were sensitive to the opioid. Of 14 BLA-evoked neurons tested with SP, 8 (57%) were sensitive to the tachykinin. Iontophoretic ejection current levels that did not alter spontaneous firing were used to study peptide effects on BLA-evoked responses. DAMGO and SP altered at least one evoked component in 93% and 88% of the neurons tested, respectively. Both peptides exhibited the most prominent effects on early-onset excitation. DAMGO potentiated this evoked component in 5 of 6 neurons tested, to 232 ± 86% of the pre-DAMGO excitation level. SP decreased the magnitude of this component in 2 of 3 neurons, to 62 ± 18% of pre-SP excitation. These data suggest that peptide projections from the NA may be able to alter the "gain" of the excitatory input from the BLA to the VP. Work supported by DA05255 to TCN.

627.4

NEUROMODULATION BY ACHE IN IDENTIFIED CHOLINERGIC AND NON-CHOLINERGIC NEURONS. B. Peretz*, D. R. Brown and M. Srivatsan. Dept. of Physiology, Univ. of Kentucky Col. of Medicine, Lexington, KY 40536-0084.

AChE (acetylcholinesterase) is released by neurons and is found circulating within nervous tissue, yet the function of this unbound AChE is poorly understood. When superfused over neurons, AChE appears to have direct neuromodulatory actions on both vertebrate and invertebrate neurons (Webb & Greenfield, 1992; Peretz, 1994); no function has been found for this action.We report here that in *Aplysia,* AChE modulates the activity of both cholinergic (R2 and LDG1) and non-cholinergic neurons (L7, L9 and R15); three sources of AChE were used from: human - (from T. Rosenberry), *Aplysia* hemolymph and eel (Sigma). In the isolated and partially desheathed abdominal ganglion, each neuron under study (impaled with two electrodes) had a distinct spike signature; all five neurons exhibited inward rectification (IR) when their membrane potentials were hyperpolarized ca 30mV below resting levels of 50-60 mV. After a latency of 15 min., AChE (ca 30 U/ml in *Aplysia* saline) elicited changes of the following in the non-cholinergic neurons, compared to control values: spike rate increased 50 to 90%, spike width increased 10-15% and IR decreased 30-40%. In cholinergic neurons, AChE elicited decreased IR, with no detectable changes in spike rate and spike broadening. After washout of AChE, the spike rate returned to control levels as did spike width. The decrease of IR persisted for at least 1 hour after washout in all five neurons. These actions of AChE were tested when the catalytic activity of AChE in the superfusate was inhibited with BW284c51, blocking the catalytic sites and a peripheral site, and edrophonium, blocking just the catalytic sites. BW284c51 blocks AChE's actions in both types of neurons, yet inhibition with edrophonium fails to block the actions. The three actions are not dependent on the enzymatic sites. Our findings show that AChE's modulatory actions are non-cholinergic and have a differential effect on cholinergic vs non-cholinergic neurons. By modulating spike width and IR, unbound AChE may be regulating synaptic input and output of non-cholinergic neurons.

627.5

GLUTATHIONE AS A NEUROACTIVE PEPTIDE. B. A. Pasqualotto*[1] and C. A. Shaw[2,3,1]. Depts. of Physiology[1], Ophthalmology[2], and Neuroscience Program[3], University of British Columbia, Vancouver, B.C., Canada.

Glutathione is a tripeptide comprised of glutamate, cysteine, and glycine which is abundantly distributed in the CNS. In its reduced form, glutathione (GSH) plays an important role in antioxidant defense mechanisms and free radical scavenging. However, evidence is accumulating which suggests a role for GSH in signal transduction as well. Specific, high affinity [^{35}S]GSH binding sites have been characterized previously in cultures of rat cortical astrocytes (Guo & Shaw, Mol. Brain Res. 15, 207, 1992) and in thin sections of human spinal cord (Lanius et al., Neurosci. Lett. 163, 89, 1993). GSH binding to synaptic membranes from rat cortex was assayed using [^{35}S]GSH. Homologous competition experiments reveal the presence of two binding sites for [^{35}S]GSH (k_{d1}=693nM, k_{d2}=10.8μM). These results are in good agreement with previous experiments characterizing the binding of [^{3}H]GSH to rat cortical synaptic membranes (Ogita & Yoneda, Neurosci. Res. 4, 486, 1987).

Cortical slices perfused with 100μM GSH responded with rapid depolarizing potential shifts as assayed by the cortical wedge recording preparation. Binding interactions between GSH and certain glutamate receptors have been reported previously. GSH may compete for binding at some glutamate receptor types (Ogita et al. Life Sciences, 39, 2411, 1986) and some glutamate receptor ligands may compete for GSH binding sites (Ogita & Yoneda, Neurosci. Res. 4, 486, 1987). Experiments to determine whether GSH depolarizes cortical slices through interaction with its own binding sites or through interaction with glutamate receptors are currently underway.

627.6

A COMPARISON OF THE EFFECTS OF A GnRH AGONIST AND ANTAGONIST, WITH AND WITHOUT SUPPLEMENTAL ANDROGEN, ON PITUITARY GnRH RECEPTOR mRNA LEVELS. A. Robbins*, A. Moo-Young, I. Huhtaniemi[1], C.W. Bardin and M.M. McCarthy[2]. The Center for Biomedical Research, The Population Council, New York, NY 10021; [1]Dept. Physiology, University of Turku, Turku, Finland; [2]Dept. Physiology, University of Maryland, Baltimore MD 21201.

Gonadotrophin Releasing Hormone receptors (GnRH-R) are regulated by endogenous GnRH and androgen levels in male rats. In the present study, the effects of continuous administration of 100 μg/day of the GnRH agonist, Histrelin, or the GnRH antagonist, Azaline B on pituitary GnRH-R mRNA and serum FSH, LH and testosterone (T) levels in intact male rats were compared to untreated control rats at 1, 2, 3, 4 or 8 weeks. During weeks 5 through 8, half of the rats in the 8 week group received the synthetic androgen, 7α-methyl 19-nortestosterone (MENT; 30 μg/day). A castrated control group at week 8 was also included (n=7 rats for all groups). A GnRH-R riboprobe was used in a lysate version of the RNase protection assay to measure pituitary GnRH receptor mRNA levels. Data were quantified by densitometry and expressed as the ratio of signal for GnRH-R to GAPDH mRNA. LH was measured by an immunofluorometric assay (Delfia) and FSH and testosterone by standard RIA.

At one week, Histrelin and Azaline B significantly suppressed T compared to intact rats. Serum LH was completely suppressed by Azaline B, whereas FSH levels were lower in the Histrelin group compared to intact rats. There were no significant differences in GnRH-R mRNA levels. At 8 weeks, in rats without supplemental androgen, serum LH, FSH and T were significantly suppressed in both the agonist- and antagonist-treated rats compared to intact levels. Histrelin, but not Azaline B, suppressed pituitary GnRH-R mRNA levels compared to intacts. Castration significantly increased LH and FSH, and decreased GnRH mRNA, compared to intact rats. In the rats receiving Histrelin or Azaline B with supplemental MENT, levels of T, FSH and LH remained low, and GnRH-R mRNA levels in both groups were significantly suppressed compared to Intact+MENT. MENT given to intact or castrated rats decreased serum LH, FSH and T compared to non-MENT treated rats, but increased GnRH-R mRNA.

627.7

CHARACTERIZATION OF CP-212,454: A SELECTIVE, CCK-B RECEPTOR ANTAGONIST. S.H. Zorn*, J.M. Morrone, S. McLean, D.K. Bryce, R.T. Crawford, A.A. Fossa, M.J. DePasquale and J.A. Lowe, III. Department of Neuroscience and General Pharmacology, Pfizer Inc., Central Research Division, Groton, CT 06340.

The CCK-B receptor has been implicated as a molecular target involved in the etiology of panic. CCK-B receptor antagonists have been shown to be active in anxiety models in rodents and may have anxiolytic activity in man. We have identified CP-212,454 as a selective ligand with high affinity for the CCK-B receptor (IC50 = 0.45 nM). The present study was undertaken to characterize CP-212,454 by examining its effects in various assays reflective of CCK receptor function. In AR 4-2J cells where pentagastrin (PG) stimulates phosphatidylinositol (PI) turnover via CCK-B receptors, CP-212,454 was a more potent antagonist of the PG-induced response than the CCK-B receptor antagonists CI-988 and L-365,260 (IC50's = 0.65 nM, 2.03 nM, and 4.14 nM, respectively). In this system, CP-212,454 was found to be a competitive antagonist with a pA2 = 9.7 (Kb = 0.18). When evaluated for functional activity at CCK-A receptors in rat pancreas, CP-212,454 only weakly antagonized CCK-stimulated PI turnover (IC50 > 200 nM). CP-212,454 was also found to be an antagonist *in vivo* in three animal models of CCK-B receptor action. Thus, in the rat, CP-212,454 was a potent antagonist of PG-induced acid secretion after s.c. administration. In the conscious guinea pig, CP-212,454 administered orally blocked the CCK-4 (i.v. bolus)- induced decrease in heart rate and increase in blood pressure. And finally, in the conscious dog, orally adminstered CP-212,454 antagonized CCK-4 (i.v. bolus)- induced increases in both heart rate and blood pressure. Thus, CP-212,454 is a potent, selective and orally active CCK-B receptor antagonist which may prove useful in studying the pharmacology of this receptor.

627.8

DIURETIC AND NATRIURETIC ACTIONS OF MELANIN CONCENTRATING HORMONE (MCH) IN CONSCIOUS SHEEP. D. G. Parkes*. Howard Florey Institute of Experimental Physiology and Medicine, Parkville 3052, Australia.

MCH is a 19 amino-acid peptide expressed in high concentrations within the dorso-lateral hypothalamus of rats, sheep and man. MCH regulates skin color and ACTH release in teleost fish, however its physiological relevance in mammals is unclear. The present study examined the cardiovascular and metabolic actions of intracerebroventricular (icv) infusion of MCH in conscious, chronically instrumented sheep. Human MCH (1-19) was infused icv for 24 hours at 10μg/h into 6 sheep, and measurements were made every 10min of arterial pressure, heart rate, cardiac output, stroke volume and peripheral conductance. Recordings of water intake (H20in), urine volume (Uv), urinary Na (UNaV) and K excretion (UKV) were made, as well as hematocrit, plasma Na, K, osmolality, protein, glucose, ACTH, vasopressin, renin, endothelin, ANF, cortisol and aldosterone.

After 24 hours of infusion, MCH produced a significant increase in Uv from 0.8±0.2 to 1.4±0.3 l/day (P<0.05), together with an increase in UNaV from 56±8 to 107±14 mmol/day (P<0.01), and in UKV from 202±18 to 369±38 mmol/day (P<0.001). H20in was unchanged. There was no change in any cardiovascular parameter, although hematocrit exhibited a large decrease from 26±1 to 22±1% (P<0.01) after 24h infusion. Plasma osmolality increased from 291±1 to 295±1 mosm/kg (P<0.05), whereas total protein and plasma Na and K were unchanged. Plasma glucose increased from 3.4±0.2 to 3.8±0.2 mmol/l (P<0.05). All other plasma concentrations did not change. This study has shown that icv infusion of MCH can produce diuretic, natriuretic and kaliuretic changes in conscious sheep, triggered by a possible increase in plasma volume as indicated by the changes in hematocrit. These results, together with anatomical data reporting the presence of MCH in fluid regulatory areas of the brain, indicate that MCH may be an important peptide involved in the central control of fluid homeostasis in mammals.

627.9

VASODILATOR RESPONSE TO BRADYKININ (BK) AND [desArg^9]-BK IN CYTOKINE TREATED RABBIT KNEE JOINT SYNOVIUM. H. Cambridge, M.P. Caulfield*[1] & S.D. Brain Dept.Pharmacology, Kings College, London SW36LX & [1]Dept.Pharmacology, University College, London WC16BT.

[Des-Arg^9]-BK is a selective BK B_1 receptor agonist which produces hyperalgesia and oedema formation in inflamed but not normal joints, implying induction of B_1 receptors. We hypothesised that B_1 receptor activation would result in vasodilation of the synovial microcirculation in cytokine treated joints. Rabbits (NZW, 2.5-3.5kg, both sexes, n=18) were anaesthetised (thiopentone i.v.; 1.5-2.5% halothane in O_2) and the femoral artery retrogradely cannulated, allowing close intra-arterial infusion into the synovial circulation. Blood flow (arbitrary flux units) was continuously monitored by laser-Doppler flowmetry using a needle probe positioned in the joint adjacent to the medial synovium. Results are expressd as AUC ($x10^2$) of the change from baseline. Infusion of BK (10pmol) produced vasodilation in all joints co-injected (-5-7 h) with IL-1β plus TNFα (both 100ng) or vehicle (mean±s.e.mean, 306.6±40.0, n=8, 404.5±81.1, n=10). Infusion of the B_2 antagonist HOE140 (0.1nmol) abolished the response to BK in the 5 joints tested from each group. [DesArg^9]-BK (up to 10nmol) had no effect in 7 vehicle treated joints but in 3 animals 10-100nmol produced small responses (89.2, 131.5, 150.1). In cytokine treated joints [desArg^9]-BK (10 and 100nmol) produced vasodilation (258.5±44.2, n=4; 553.0±131.9, n=3) with no response obtained in 1 animal. HOE140 (0.1nmol) abolished the response in 1 animal whereas in the others the response after HOE140 was 51-73% (n=4) of control. The finding that cytokine treatment increased the number of animals in which a response to [desArg^9]BK could be measured provides some evidence for the induction of B_1 receptors, and the failure of HOE 140 to totally inhibit this response points to B_1 receptor mediated vasodilation, however the potency difference between BK and [desArg^9]-BK suggests that B_2 receptors remain of major physiological or pathological importance in this site.

627.10

INTRADERMAL INJECTION OF SUBSTANCE P INDUCES HIND-PAW SCRATCHING MEDIATED BY SENSORY STIMULATION OF TREATED SKIN. Y. Kuraishi*[1], T. Nagasawa[1], T. Andoh[1], K. Hayashi[1], M. Satoh[2]. [1]Dept. of Applied Pharmacol., Res. Inst. for Traditional Medicines, Toyama Med. & Pharm. Univ., Toyama 930-01, and [2]Dept. of Mol. Pharmacol., Fac. of Pharm. Sci., Kyoto Univ., Kyoto 606-01, Japan.

Substance P (SP), a pruritogenic peptide, elicits hind-paw scratching when applied to the mouse skin. The present experiments were conducted to determine whether scratching behaviors induced by intradermal SP would be due to a sensation, especially an itch sensation, of the treated skin. Male ICR mice were used unless otherwise mentioned, and SP was intradermally injected (50 μl) into the back. When injected into the rostral back, SP (10-135 μg) dose-dependently elicited scratching of the injected skin by the hind paws. The effects were not apparently different between doses of 135 μg (= 100 nmol) and 300 μg. When injected into the caudal back, a site that the mouse cannot scratch by the hind paws, SP (100 nmol) elicited few or no scratches of the rostral back. Pretreatment with capsaicin (50, 50, 100, 150 and 200 mg kg^{-1} day^{-1}, s.c. for 5 days) significantly inhibited SP (100 nmol)-induced scratching. SP (300 μg) elicited scratching either in the mast cell-deficient mice (WBB6F1 *W/W^v*) or in the wild-type mice (WBB6F1 +/+). Pretreatment with compound 48/80 (100 μg, s.c. for 2 or 3 h), an agent that inhibits SP-induced itching in humans, significantly suppressed scratching induced by SP (100 nmol) in the mast cell-deficient mice as well as in the wild-type and ICR mice. These results suggest that at least in the mouse, hind-paw scratching, an itch-related behavior, induced by intradermal SP is due to the sensory stimulation of the injected skin and that the cutaneous mast cells are not exclusively responsible for the SP-induced scratching.

627.11

HALOPERIDOL-RESISTANT REDUCTION OF SUBSTANCE P CONTENT INTHE PREFRONTAL CORTEX OF THE RAT BY AN ACUTE INJECTION OF PHENCYCLIDINE. Y. Shirayama*, T. Nishikawa, H. Mitsushio and K. Takahashi. Div. Mental Disorder Res., National Institute of Neuroscience, NCNP and Dep. Psychiatry, Kanto Teishin Hospital, Tokyo, Japan.

The effects of a schizophrenomimetic drug, phencyclidine (PCP), on substance P (SP) contents have been investigated in the discrete brain areas of the rat. To this end, a simple and sensitive enzyme-immunoassay for SP has been developed. An acute administration of PCP (10 mg/kg, intraperitoneally (i.p.)), which is a non-competitive antagonist of N-methyl-D-aspartate (NMDA) type excitatory amino acid receptor, reduced the concentration of SP in the prefrontal cortex, limbic forebrain (nucleus accumbens, septum, oifactory tubercule, etc.), striatum and substantia nigra at 60 min post-injection. A selective non-competitive NMDA antagonist, (+)-MK801 (1 mg/kg, i.p.) also caused a decrease in SP in the prefrontal cortex and limbic forebrain, but failed to alter the content in the other areas, 30 min thereafter. In contrast, dopamine (DA) agonists, methamphetamine (4.8 mg/kg, i.p.) and apomorphine (4.4 mg/kg, i.p.) reduced the content in the striatum and substantia nigra, but not in the other regions at 60 min after the injection. Furthermore, the pre-injection of DA antagonist, haloperidol (1 mg/kg, i.p.) blocked the ability of PCP to decrease SP content in the substantia nigra but not in the prefrontal cortex. The present results suggest that PCP may reduced SP content by blockade of the NMDA receptor in the prefrontal cortex and limbic forebrain and by activation of DA neurotransmission in the striatum and substantia nigra.

PEPTIDES: PHYSIOLOGICAL EFFECTS III

628.1

ANORECTIC AND NEUROCHEMICAL EFFECTS OF PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP) IN RATS. W.T. CHANCE*, H.L. THOMPSON and I. THOMAS. Dept. Surgery, Univ. Cincinnati Med. Ctr. and VA Med Ctr., Cincinnati, OH 45267.

Although PACAP reduces food intake in mice following intraventricular injection, no tests of this peptide have been reported for intra-hypothalamic (iht) administration in rats. Therefore we tested the anorectic and neuro-chemical effects of PACAP (1.25 & 2.5 ug) following perifornical iht injection. Pretreat-ment of rats with PACAP 10 minutes prior to the injection of 1 ug neuropeptide Y (NPY), signifi-cantly reduced food and water intake during the 1 to 4 hr measurement periods. PACAP treatment of schedule-fed rats also reduced food and water intake for 2 hrs. A smaller 1 hr reduction of water intake was observed in water-deprived rats, suggesting that the anticonsummatory effects of PACAP were primarily against food intake. PACAP treatment did not alter hypotha-lamic level of NPY, nor were neurotransmitters, precursors or metabolites altered in corpus striatum or nucleus accumbens regions. These results indicate primary anorectic effects of PACAP, and may suggest a role of cAMP activation and inhibition in the control of food intake.

628.2

PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE MODULATES INTRACELLULAR CALCIUM IN RAT PINEAL CELLS. N. Darvish, H. Jiang* and J. T. Russell LCMN and OSD NICHD, NIH, Bethesda, MD, 20892 USA .

In this study we have examined the effect of pituitary adenylate cyclase activating polypeptide (PACAP) on $[Ca^{++}]_i$ in adult rat pineal cells. Pinealocytes in primary culture were loaded with Fura-2 and than exposed to 100nM PACAP. This treatment resulted in an increase in $[Ca^{++}]_i$ in 51% of the cells examined. Similar treatment with norepinephrine (1μm), however, resulted in $[Ca^{++}]_i$ responses in 95% of the pinealocytes. The PACAP-induced $[Ca^{++}]_i$ signals were heterogeneous, ranging from rapid transients to a slow sustained elevation in Ca^{++} (1.5mM) containing medium. This response persisted when extracellular Ca^{++} was removed. Under this condition, the $[Ca^{++}]_i$ changes were rapid and transient followed by a sustained decrease to levels lower than before PACAP was applied. These findings suggest that the PACAP-induced $[Ca^{++}]_i$ signals in pineal cells are, at least in part, due to release of Ca^{++} from intracellular stores. The sustained decrease in $[Ca^{++}]_i$ in the absence of extracellular Ca^{++} remains to be explored.

628.3

A NOVEL PACAP ANTAGONIST, NEUROTENSIN$^{6-11}$PACAP$^{7-28}$: EFFECTS ON NEURONAL SURVIVAL AND SPERM MOTILITY: A. Davidson*, A. Reshef, O. Ashur-Fabian, R. Zamostiano, L. Schochat, L.M. Lewin, S. Rubinrout, M. Fridkin, D.E. Brenneman and I. Gozes. Dept. Clin. Biochem. Sackler Med. Sch. Tel Aviv Univ. Israel, 69978; Dept. Organic Chem. Weizmann Inst. Rehovot, Israel 76100; Section Develop. & Molec. Pharmacol. LDN, NIH, Bethesda, MD 20892, USA.

Pituitary stimulating adenylate cyclase (PACAP) is a major regulatory peptide with two active molecular forms: PACAP-27 and PACAP-38 (Regul. Pept. 37: 287, 1992). Both molecular forms promote neuronal survival and protect against neurotoxicity (N.Y. Acad. Sci. 739,228, 1994). By Northern blot hybridization utilizing synthetic oligodeoxynucleotides we have identified a 1464 base PACAP receptor mRNA in rat cerebral cortical cultures. Based on our previous hybrid peptide strategy in designing VIP antagonists (J. Pharmacol. Exp. Therap. 273:161, 1995), a novel PACAP analogue was synthesized (neurotensin$^{6-11}$PACAP$^{7-27}$). In addition to the hybrid modification, the methionine in position 17 was replaced by norleucine (Nle). Treatment of cerebral cortical cultures for five days with the putative PACAP antagonist (1 nM) resulted in a 47% reduction in neuronal cell counts as compared to controls and the EC50 of this effect was about 100 pM. Co-administration of the PACAP hybrid analog with picomolar amounts of PACAP-38 or PACAP-38 with the Met in position 17 replaced by Nle attenuated the reduction in neuronal cell counts. The putative PACAP antagonist also inhibited sperm motility in a dose dependent manner (Golden Hamster) as assessed *in vitro*. Complete inhibition was observed at 10 μM, suggesting a role for PACAP in sperm motility and sexual function. The availability of a novel PACAP antagonist should help to further delineate role of this neuropeptide in neurodevelopment and sexual activity.

628.4

BIOLOGICAL ACTIVITY OF THE CALCITONINE SOME FRAGMENTS. N.Balashov*, V.Akopian, S.Burov, G.Vlasov, Sechenov Instititute of Evolutionary Physiology and Biochemistry, 194223, St.Petersburg, Russia.

Calcitonin , gene-related peptide , is a neuropeptide. It has been shown that calcitonin to be a potent vasodilator. However not only does the whole molecule of calcitonin exhibit the biological activity but also its some fragments do.We studied the following fragments of calcitonin gene ralated neuropeptide (1-9), -(10-20), -(15-24), -(20-29), -(30-37).The fragment (20-29) was the single one to decrease arterial blood pressure. Effects of this fragment on the arterial, venous and lymphatic microvessels of the rat mesentery by method of the vital television microscopy has been investigated. It was found that this fragment can exhibit a vasodilator activity during experiments on the arterial and venous microvessels but to exert also some stimulating effect on the lymphatic ones, raising there tones and increasing spontaneous contractions. This fragment was applicated on the microvessels surface. Thus these findings suggest that fragment -(20-29) in polyfunctional molecule is responsible for the effects on the microcirculation bed.

628.5

THE ROLE OF CA^{2+} IN CHANGES OF MEMBRANE FUNCTION AND THE PROTECTION OF CGRP IN HIPPOCAMPAL SLICE DURING HYPOXIA. Fu-Zhuang Wang*, Zhen-Wei Liu and Ai-Shi Ding. Dep.of Neurobiology, Institute of Basic Medical Sciences, Beijing 100850, China

Recent findings suggest that elevation of free intracellular calcium([Ca^{2+}]i) is the important factor triggering cell injury in hypoxia. The role of Ca^{2+} in the mechanisms of acute hypoxia involved in neurons and synaptic transmission in CA1 area of rat hippocampal slice were studied by using extra-and intracellular recording techniques and biochemical methods. From extracellular recordings it was found that phasic changes showing initial disappearance, reappearance and re-disappearance of evoked population spike (PS) in CA1 pyramidal neurons were caused by hypoxia. After reoxygenation the recovery rate of PS was obviously higher in slices perfused with calcitonin gene-related peptide (CGRP, 4nM) or Ca^{2+}-free artificial cerebrospinal fluid (ACSF) than in controls. Intracellular recordings showed that hypoxia also caused phasic changes of membrane potential in CA1 pyramidal neurons, which included an initial hyperpolarization, a slow depolarization and a final rapid depolarization. Ca^{2+} -free ACSF reduced the amplitude of depolarization and delayed the occurence of depolarization in hypoxia. By measuring of [Ca^{2+}]i with fura-2 it was found that hypoxia induced a marked phasic elevation of [Ca^{2+}]i in hippocampal neurons. The phasic changes disappeaared when removing Ca^{2+} from extracellular fluid and CGRP can inhibit the hypoxic elevation of [Ca^{2+}]i. These results indicate that the phasic elevation of [Ca^{2+}]i induced by influx of Ca^{2+} play an important role in phasic changes of membrane function in hippocampal neurons and its synaptic transmission during hypoxia. CGRP may have a neuronal protective action against hypoxia.

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628.6

CALCITONIN GENE-RELATED PEPTIDE DOES NOT INHIBIT UTERINE CONTRACTION THROUGH ATP-SENSITIVE K^+ CHANNELS IN THE RAT UTERUS. R.L. Shew*, C. Gatto, J.S. Shenberger and D.E. Johnson, Departments of Cell Biology & Neuroanatomy and Pediatrics/Neonatology, University of Minnesota and Children's Health Care-St. Paul, Minneapolis, MN 55455 and St. Paul, MN 55102.

Calcitonin gene-related peptide (CGRP) is localized in nerves in the uterus and relaxes uterine contraction in a dose-related manner. While the mechanism of CGRP on uterine relaxation is unclear, K^+ channels have been suggested to play a role in the regulation of myometrial activity. In addition, the vasodialtory effect of CGRP on uterine arteries has been inhibited by ATP-sensitive K^+ channel blockers. Thus, we investigated the role of ATP-sensitive K^+ channels and CGRP relaxation in rat uterine tissue. Mature Sprague-Dawley rats (200-225 g) were treated with diethylstilbestrol and uterine horns removed 24 hours later and examined using an in vitro uterine assay. Both CGRP (10^{-7} M) and cromakalin (10^{-5} and 10^{-6} M), an ATP-sensitive K^+ channel activator, inhibited acetylcholine (Ach)-stimulated (10^{-6} M) uterine contraction by 73% and 41-38% respectively. Glybudride (10^{-4}-10^{-5} M), an ATP-sensitive K^+ channel blocker, blocked the ability of cromakalin to inhibit Ach-stimulated uterine contraction (38% versus 0%). However, glybudride at effective doses failed to block CGRP inhibition of Ach-stimulated uterine contraction. These data suggest that in our assay ATP-sensitive K^+ channels do not play a role in CGRP relaxation of Ach-stimulated uterine contraction.

628.7

EFFECTS OF CORTICOTROPIN-RELEASING FACTOR (CRF) ON SLEEP CYCLES IN THE RAT MEDIAL DIENCEPHALIC STRUCTURES. Y. Slisli, P. Denise and R. de Beaurepaire*. Lab. de Pharmacologie, Lab. de Physiologie, & INSERM U.320, CHRU, 14032 Caen, FRANCE.

We have previously shown that injections of salmon calcitonin into the rat hypothalamic paraventricular nucleus and perifornical area produce dramatic changes in sleep cycles, with suppression of slow wave sleep and predominant insomnia during approximately 18 hours (Soc Neurosci Abstr, Vol 20, Part 1, p 160, 1994). In the present work, we studied the effects of another peptide, CRF, on sleep. Sprague-Dawley female rats were implanted with electrodes for chronic EEG recordings, and with a cannula directed into several hypothalamic and thalamic sites. All animals were injected twice, once with CRF (0.5 μg in 0.3 μl) and once with the vehicle (0.3 μl) and recordings made over 3 hours post injection. An insomnia during the 3 hours period was observed after injections into the hypothalamic ventro- and dorso-medial nuclei, an area internal to the mamillo thalamic tract, the bed nucleus of the stria terminalis, the internal part of the zona incerta, the nucleus reuniens, and the nucleus paracentralis of the thalamus. CRF had no effects in the tuber cinereum, the lateral and posterior hypothalamus, and the centro-medial, ventro-medial and ventro-lateral nuclei of the thalamus. The effects into the paraventricular nucleus and perifornical area were not interpretable because of constant diffusion of the CRF to the above nucleus reuniens and zona incerta. Some rats were tested after 3 hours in the paraventricular nucleus/nucleus reuniens, and the insomnia never lasted more than 5 hours. In conclusion, CRF produces an insomnia when injected into several hypothalamic and thalamic sites, but the sleep abnormalities observed with CRF are always less dramatic than those observed with salmon calcitonin injected into the hypothalamic paraventricular nucleus and perifornical area.

628.8

EXPRESSION PATTERN OF CORTICOTROPIN-RELEASING FACTOR (CRF) BINDING SITES IN RAT BRAIN AND PITUITARY DURING THE 24-HOUR CYCLE. E. FUCHS*, J.-C. WASMUTH, G. FLÜGGE, G.HUETHER. German Primate Center and Department of Psychiatry, 37077 Göttingen.

CRF is thought to be involved in the regulation of the diurnal activity of the HPA-axis and to act as a neurotransmitter in the brain. To date it is unknown whether the cellular effectors of the central CRF system are subject to diurnal variations. We therefore measured the number of binding sites for iodinated ovine CRF over a complete 24-hr light-dark cycle in the pituitary, amygdala, bed nucleus of the stria terminalis (BNST), paraventricular nucleus of the hypothalamus, hippocampus, and locus coeruleus of rats by in vitro receptor autoradiography. A 24-hr time course was also established for plasma corticosterone. Within the brain, CRF binding in the basolateral nucleus of the amygdala showed a U-shaped curve with maximum levels in the morning and a wide hallow between 15:00 h and 19:00 h. A biphasic profile with a small depression in the afternoon and more pronounced depression in the second half of the activity period is characteristic for the other brain areas and for the pituitary. The profile for the pituitary correlates with those for the BNST and the area of the locus coeruleus. The diurnal pattern of CRF binding sites in the BNST correlates with that of the hippocampus. Since the CRF-binding profiles in the brain and the pituitary clearly differ from the profile of plasma corticosterone we conclude that the diurnal pattern of the central CRF binding sites is not directly coupled to the activity of the HPA-axis.

628.9

SLEEP DEPRIVATION INCREASES CRF LEVELS AND CRF mRNA EXPRESSION IN RAT BRAIN.

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Evidence has been accumulated suggesting that corticotropin-releasing factor (CRF) might play a physiological role in the regulation of sleep-wake cycle. EEG studies have shown that CRF administration induces EEG activation and decreased sleep time both in rat and humans. We have previously shown that i.c.v. administration of CRF increases paradoxical sleep rebound in sleep deprived rats (Marrosu et al., Brain Research 515, 315; 1990). In the present study we have investigated on the possible effect of sleep deprivation on CRF levels and CRF mRNA expression in different brain areas and pituitary. Sleep deprivation was induced by the water tank method in male Sprague-Dawley rats (175-200 gr.) for 72 hours. CRF levels were detected by radioimmunoassay; CRF mRNA was measured by RT-PCR method. The results obtained indicate that sleep deprivation induces a significant increase both in CRF levels and CRF mRNA in striatum (+225% and + 30%),limbic system (+144% and +28%) while in hypothalamus an increase in CRF mRNA (+30%) was concomitant to a decrease (-58%) of CRF levels. Such a decrease could reflect a CRF over-release from the hypothalamus to the pituitary, where CRF levels were found increased without any significant changes in CRF mRNA. These data indicate that CRF neuronal activity is enhanced by sleep deprivation and might strengthen the hypothesis of a functional role of CRF in the regulation of sleep-wake cycle.

628.10

ROLE OF CORTICOTROPIN-RELEASING HORMONE (CRH) AND EXCITATORY AMINO ACIDS (EAA) IN LOCUS COERULEUS (LC) ACTIVATION BY COLONIC DISTENTION.

S.M. Florin*, A.L. Curtis and R.J. Valentino, Dept. Psychiatry, Hahnemann University, Philadelphia, PA 19102.

The LC-noradrenergic system is activated by a variety of visceral stimuli including bladder and colon distention. The present study was designed to determine the neurochemical mediators of LC activation by colon distention. Extracellular LC spontaneous discharge was recorded from halothane-anesthetized rats during 2 min of sustained colon distention produced by injection of 0.5, 1.0 or 3.0 ml of water into a balloon catheter inserted in the distal colon. Consistent with previous reports (Elam, et al., Brain Res 375:117-125, 1986), colon distention increased LC spontaneous discharge rate in a volume dependent manner, with distention produced by 0.5, 1.0 and 3.0 ml injections resulting in a 16±3%, 35±5% and 52±3% increase in discharge rate, respectively (n≥6). I.C.V. administration of the CRH antagonist, D-PheCRH$_{12-41}$ (3.0 µg) completely prevented LC activation produced by 0.5 ml colon distention (n=5), and decreased LC activation produced by 1.0 ml colon distention by 64% (n=5; p<0.005). A higher dose of D-PheCRH$_{12-41}$ (10 µg) was no more effective than the 3.0 µg dose. Interestingly, D-PheCRH$_{12-41}$ did not attenuate LC activation associated with a 3 ml distention. EAA neurotransmission in the LC has been demonstrated to mediate LC activation by a variety of stimuli. In this study, the EAA antagonist, kynurenic acid (5.0 µmol, i.c.v.), significantly decreased LC activation associated with both a 1.0 ml and a 3.0 ml colon distention (n=5). These findings suggest that EAA neurotransmission is integral to LC activation by colonic distention and that CRH may be selectively involved in LC activation by low levels of colonic distention. Supported by PHS grants MH40008 and MH00840.

628.11

GLYCYL-L-GLUTAMINE ANTAGONISM OF IL-1ß. G.E. Resch* and W.R. Millington. Division of Molecular Biology & Biochemistry, Univ. Missouri, Kansas City MO 64108-2792.

Glycyl-L-glutamine (Gly-Gln; ß-endorphin-30-31) was previously shown to antagonize α-MSH and PGE2. Since PGE2 mediates some thermogenic responses to IL-1ß in the medial preoptic area (mPOA), the hypothesis was tested that Gly-Gln would also antagonize the thermogenic response to IL-1ß. Groups of 3 to 5 Sprague Dawley rats were stereotaxically implanted with guide tubes 0.5 mm lateral of the midline at bregma with tips 7 mm below the surface of the skull. Sites sensitive to PGE2 (1 pg/µl) in the mPOA were selected for a rise in colonic temperature (Tc) vs saline vehicle. Injection of IL-1ß (1 U in 1 µl) into these sites elicited a Tc rise of 1.02 ± 0.06 °C (Mean ± SEM). Injection of IL-1ß mixed with Gly-Gln (3 nmol) abolished the response (-0.18 ± 0.22 °C) vs saline controls (-0.03 ± 0.18 °C) in the same mPOA sites. The antagonism did not occur to equimolar Gly and Gln nor did Tc change to Gly-Gln injected alone (0.20 ± 0.20 °C). Gly-Gln also antagonized the PGE2-elicited rise in Tc (0.86 ± 0.10 °C vs 0.1 ± 0.03 °C). These data are consistent with PGE2 mediation of the IL-1ß-elicited fever. The data show Gly-Gln antagonizes the febrile responses to IL-1ß and PGE2. Since Gly-Gln is co-released with ß-endorphin, the data suggests a possible link between effects of ß-endorphin and IL-1ß.

628.12

MODULATION OF THE SPREAD OF EXCITATION IN RAT NEOCORTICAL SLICES BY INTERLEUKIN-1 BETA, VISUALIZED WITH INFRARED-DARKFIELD VIDEOMICROSCOPY.

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The combination of infrared videomicroscopy with darkfield techniques allows the visualization of the spread of excitation in neocortical slices without the use of voltage sensitive dyes. With this technique optical recordings were performed in neocortical slices of adult rats. Tetanic stimulation of white matter or Layer VI of the neocortex with trains of 50 Hz for 2 sec induced a column-like pattern of increased brightness, the "intrinsic optical signal" (i.o.s.). This signal is considered an indicator of the spread of neuronal excitation. To visualize the i.o.s. an averaged darkfield image was stored in computer memory and subsequently subtracted in real time from the incoming image during tetanic stimulation of the slice. The contrast of the resulting image was digitally enhanced and displayed in false colours.

The cytokine interleukin-1 beta (IL-1β) added to the bath at a concentration of 1 ng/ml for 60 min (n=8) induced a reduction of the i.o.s. to 88 ± 6% of control value. Application of IL-1β at a concentration of 10 ng/ml for 30 min (n=8) reduced the i.o.s. to 67 ± 10% with a recovery of the signal after 90 min of washout. No change of the i.o.s. was observed during control experiments (n=5) when heat-inactivated (100 °C for 30 min) IL-1β was applied. The action of IL-1β resembles the effect of GABAergic agonists like muscimol or the neuroactive steroid THDOC. These findings support the notion that IL-1β enhances GABAergic inhibition. Such enhancement of GABAergic inhibition may underly behavioural effects mediated by IL-1β, such as fever associated "sickness behaviour".

OPIOID RECEPTORS: MOLECULAR BIOLOGY

629.1

KOR-3: STRUCTURE AND CHARACTERIZATION OF A KAPPA3-RELATED OPIOID RECEPTOR GENE

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We have cloned and sequenced a kappa$_3$-related opioid receptor (KOR-3) gene from a mouse genomic library. The mouse KOR-3 gene is 8.3 kb in length containing five exons of 0.26, 0.25, 0.3, 0.91 and 1.14 kb. The first and fifth ones are noncoding while the second, third and fourth contain the complete coding region, with intron junctions at the position 522 and 878 of cDNA nucleotide sequence. The intron between second and third exons is 2.4 kb, and the intron between third and fourth is 81 bp. The transcription initiation site was determined by primer extension and 5'RACE. Several putative regulatory elements were found in the 5' flanking sequence, such as AP1 and GRE. A polyadenylation signal (AATAAA) was identified in the 3' flanking region. (YXP is supported by an Aaron Diamond Postdoctoral Fellowship.)

629.2

CHARACTERIZATION OF A HUMAN MU-OPIOID RECEPTOR GENE PROMOTER

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The human mu-opioid receptor gene, cloned in this laboratory, was studied to evaluate DNA sequences which may influence transcription. Portions of the gene which are upstream of the translation start codon were isolated and placed upstream of a reporter gene to assess transcription rate by assaying reporter protein level. These reporter constructs were transfected into a mu-opioid receptor expressing cell line and into non mu-opioid receptor expressing cell lines. A construct containing 61 basepairs (from -337 to -397 bases upstream of the translation start codon) significantly promoted transcription of the reporter gene in both mu-opioid receptor expressing and non-expressing cell lines. This minimal promoter lacks a TATA box sequence but does contain a sequence that matches the consensus for an initiator. Other upstream portions of the mu-opioid receptor gene were shown to influence the level of transcription of the reporter gene. (Supported by NIDA grant DA-00017 to E.J.S. and by an Aaron Diamond Foundation Fellowship to M.L.A.)

629.3

DIFFERENTIAL EXPRESSION OF RAT KAPPA OPIOID RECEPTOR GENE. A.G.Yakovlev * and A.I.Faden Departments of Neurology and Pharmacology Georgetown University Medical Center, Washington, DC 20007

Kappa opioid receptors mediate a number of physiological functions, including feeding behavior, fluid balance, cardiorespiratory activity and antinociception among others. They may also be involved in pathophysiological responses, such as in CNS injury. The existence of multiple kappa isoreceptors has been suggested by pharmacological studies. However, a number of cDNA cloning experiments from various laboratories have resulted in isolation of only a single sequence, one encoding for the kappa-1 isoreceptor, as demonstrated by transfection of COS-1, COS-7 and PC12 cells. Recently, we isolated a gene encoding the rat kappa opioid receptor (Yakovlev et al., 1995). Results of genomic library screening and southern blot analysis showed that the kappa receptor gene represented at one copy per haploid genome. RT-PCR analysis indicated that alternative splicing of KOR RNA did not occur. However, we demonstrated that two species of transcripts are differentially produced by a single KOR gene in tissue-specific manner. The first transcript (KOR1) is equivalent to the cDNA sequence characterized elsewhere for kappa-1 opioid receptor. The second transcript (KORx) begins in intron 1 and thereby retains the intronic sequence in the mature mRNA. Within this sequence there is a potential translation initiation codon that is in frame with the first ATG codon for KOR1. The results of comparative *in vitro* translation of KOR1 and KORx mRNA demonstrated that the extension of an open reading frame upstream in KORx mRNA leads to the translation of a larger variant of KOR protein having an additional peptide sequence at its amino terminus. Immunoprecipitation analysis showed that monoclonal antibodies KA8 directed against the kappa-2 opioid receptor enriched fraction of frog brain (Maderspach et al., 1991) recognized rat KOR1 but not KORx protein synthesized by the *in vitro* translation. Taken together the data suggest that the existence of multiple KOR isoreceptors might result from different posttranslational modifications of a single KOR protein and/or its interaction with different membrane proteins. Studies of a functional role of KORx protein in the modulation of kappa opioid receptor signaling system are in progress.

629.4

IDENTIFICATION OF REGIONS OF THE HUMAN DELTA OPIOID RECEPTOR INVOLVED IN THE SELECTIVE BINDING OF AGONISTS AND ANTAGONISTS: A CHIMERIC RECEPTOR STUDY. E. V. VARGA, X-P LI, D. STROPOVA, S. CALDERON, K. RICE, R. ROTHMAN, F. PORRECA, V. HRUBY, W. R. ROESKE, and H.I. YAMAMURA. U of Arizona, Tucson, AZ.

Three major types of the human opioid receptors have been cloned: the mu, delta and kappa receptors. They show unusually high homology in the putative transmembrane and intracellular domains. The hypothesis we tested in this study is that the signal transductory "message" part of the opioid peptides binds in the transmembrane domains, while the specificity determining "address" part interacts with the extracellular domains. To study the role of the extracellular loops in the ligand recognition we replaced the 2nd and 3rd extracellular loops of the human delta opioid receptor with those of the human mu opioid receptor. The chimeras were spliced together using a modified PCR overlap extension method. The chimeric PCR fragments were digested with the appropriate restriction enzymes and ligated into the digested human delta receptor fragment. The chimeras were transferred into the mammalian expression vector pcDNA3. COS-7 cells were transiently transfected with the constructs using the DEAE-dextran method. The binding experiments were performed using cell membranes two days after the transfection. The specific binding of ^{3}H-pCl-DPDPE and ^{3}H-SNC 121 decreased dramatically to both chimeras, while the binding of ^{3}H-DAMGO remained similar to that of the wild type (wt) delta receptor. Unexpectedly, the replacement of the 3rd extacellular loop conferred a wt mu affinity to the chimera against ^{3}H-Naltrindole (low) and ^{3}H-Naloxone (high). The chimeric receptors were labeled with ^{3}H-Naloxone and the inhibition constants of opiod ligands of different classes were measured. The affinity of NTI decreased more than 100-fold. The decrease in the affinity of the linear deltorphin was more robust than that of the cyclic peptide pCl-DPDPE. Supported by NIDA grants.

629.5

TRP^{284} IS IMPORTANT FOR THE BINDING OF THE NONPEPTIDE SNC121 TO THE HUMAN DELTA OPIOID RECEPTOR. X. Li, R.J. Knapp, D. Stropova, Y. Wang, E. Varga, E. Malatynska, S. Calderon[1], K. Rice[1], R. Rothman[2], F. Porreca, V. Hruby, W.R. Roeske and H.I. Yamamura. U. of Arizona, Tucson, AZ 85724, [1]NIH and [2]NIDA, MD.

Based on the comparison of the structure of the three (δ, μ and κ) opioid receptor proteins, three single amino acid mutations of hDOR were constructed to elucidate the ligand selectivity and binding domain of human delta opioid receptor. Here we report the Trp284 in extracellular loop III of the human δ-receptor, corresponding to Lys^{305} in hMOR and Glu^{297} in hKOR, is involved in binding of nonpeptide SNC121. Saturation binding studies revealed that (1) no difference was observed between W284L and wild-type receptors in the ligand binding affinity of the δ-selective antagonist [^{3}H]Naltrindole (Kd = 140 vs. 53 pM, respectively) and the δ-selective peptide agonist [^{3}H]pCl-DPDPE (Kd = 320 vs. 260 pM), (2) no specific binding was observed for 1.0 nM [^{3}H]SNC121 to the W284L mutation receptor. Inhibition binding studies of [^{3}H]Naltrindole to both W284L and wild-type receptors was inhibited by pCl-DPDPE (Ki = 1.1 vs. 3.6 nM and Deltorphin II (Ki = 11 vs. 14 nM) with high affinity. But SNC121 inhibited [^{3}H]pCl-DPDPE binding to the W284L mutant with a Ki of 144 nM in contrast to the Ki of 3.7 nM at the wild-type. These results indicate that Trp284 of hDOR plays an important role in the binding of SNC121, but not pCl-DPDPE, Deltorphin II and Naltrindole. Furthermore it suggests that nonpeptide and peptide agonists and antagonists have different binding domains. Supported by NIDA grants.

629.6

A CHANGE IN A SINGLE AMINO ACID RESIDUE ENABLES THE DELTA OPIOID RECEPTOR TO BIND DAMGO, A MU OPIOID RECEPTOR SELECTIVE LIGAND, WITH HIGH AFFINITY. M. Minami*, T. Nakagawa, Y. Ienaga, S. Katsumata and M. Satoh
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We have previously reported that DAMGO, a μ-opioid selective ligand, distinguishes between μ- and δ-opioid receptors (OPRs) in the region around the first extracellular loop, in which only seven amino acid residues are different between these receptors (*FEBS Lett.* **357** (1995) 93-97). In this study, we examined which amino acid residue(s) is involved in the distinction between the two OPRs by DAMGO. Each amino acid residue of the seven residues in the region around the first extracellular loop of the δ-OPR was changed to the corresponding residue found in the μ-OPR by site-directed mutagenesis. The mutated cDNAs were transfected to COS cells and binding assay was carried out with [^{3}H]DADLE and non-radioactive DAMGO. Among the seven mutated δ-OPRs, only the δK108N bound DAMGO with a high affinity. A replacement of lysine 108 of the δ-OPR with alanine (δK108A) also gave a high affinity for DAMGO to the mutated receptor, suggesting that lysine 108 of the δ-OPR is obstructive to the binding of DAMGO rather than that asparagine at the corresponding position of the μ-OPR is necessary to that. Furthermore, the mutated receptors δK108N and δK108A mediated the signals of agonist binding to the inhibitory system of adenylate cyclase in CHO cells. These results indicate that the difference in a single amino acid residue of the first extracellular loop between the μ- and δ-OPRs is critical for the subtype-specificity in the binding to DAMGO.

629.7

THE DELTA OPIOID RECEPTOR BINDING SITE. Katia Befort, Lina Tabbara, Dominique Kling, Bernard Maigret and Brigitte Kieffer*. UPR 9050, ESBS, Université Louis Pasteur, 67400 Illkirch, France.

Opiates act through three classes of membrane receptors known as mu, delta and kappa. We have recently cloned a mouse opioid receptor of delta subtype. Analysis of the primary sequence of the cloned receptor indicates that it belongs to the G protein-coupled receptors superfamily. Using 3D computer modeling, we have constructed a model for the delta receptor based on the resolved structure of bacteriorhodopsin. In this model, a hydrophylic site together with a strongly hydrophobic pocket are postulated to form the active site of the receptor. To test this model, we are using site-directed mutagenesis and examine the binding properties of single-amino-acid substituted mutant receptors expressed in Cos cells. Preliminary experiments partly support our model. We have also examined in details the possibility that aspartate 128 (Tm III), which is classically found also in catecholamine receptors, might provide a counterion for the positively charged opioids. We have found that this residue is differently involved in ligand binding as previously demonstrated for the beta-adrenergic receptor. Taken together our results should allow to establish a putative topology of the delta opioid receptor binding site

629.8

THREE DIMENSIONAL MODEL OF THE δ OPIOID RECEPTOR: INTERACTION OF AGONISTS AND ANTAGONISTS IN THE PUTATIVE BINDING SITE. P.A. Maguire*, I. Alcorta, P. Du and G.H. Loew. Molecular Research Institute, Palo Alto, CA 94304.

A 3D model of the transmembrane domain of the δ opioid receptor was constructed using Sequence Divergence Analysis of 42 homologous sequences of G-protein coupled receptors belonging to the opioid, somatostatin and angiotensin receptor families. No template was used in the prediction steps, which included multiple sequence alignment, identification of the boundaries of the transmembrane regions by calculation of a variability profile of the aligned sequences and optimization of the packing shape in the helix bundle. A candidate ligand binding site was identified using the δ-selective antagonist, naltrindole, as a probe. The interaction of the protonated amine nitrogen of naltrindole with the highly conserved Asp-127 in TM 3 was used as an anchoring point. This binding site was also used to dock other ligands, including the δ-selective peptide antagonist Tyr-Tic-Phe-Phe-NH$_2$ (TIPP-NH$_2$) and two agonists from different structural families, lofentanil and BW373U86. The resulting ligand-receptor complexes were then energy optimized. Common interaction points for recognition of these ligands were identified, involving at least two of three lipophilic moieties and two polar substituents of the ligands. In addition, comparisons of agonist and antagonist binding allowed preliminary identification of polar interactions that could be involved in activation. Specific amino acid residues interacting with these ligands were identified that could direct the design of mutation studies that would be invaluable in the efforts to validate and refine the model. **This work was supported by NIDA Grant (DA02622)**

629.9

SINGLE AMINO ACID MUTATION IN TRANSMEMBRANE REGION CONFERS AGONIST ACTIVITY TO ANTAGONISTS. P. Claude, L. Erickson, P. Prather*, H. Loh and P. Law. Dept. of Pharmacology, University of Minnesota, Minneapolis, Minnesota, 55455.

A chimeric receptor was created from the N-terminus to the C-terminus of the second transmembrane regions of the μ opioid receptor and the N-terminus of the first extracellular loop to the C-terminus of the δ2 opioid receptor (μδ2). In order to analyze ligand/receptor binding and subsequent signal transduction, the μδ2 chimeric receptor was stably expressed in CHO cells then screened for binding properties and the ability to inhibit adenylyl cyclase with a number of μ and δ selective agonists and antagonists. Binding and adenylyl cyclase assays determined that the μδ2 chimeric receptor was exhibiting δ-like characteristics. However, opioid antagonists (NTB, naloxone, naltrexone) did not reverse DPDPE inhibition of adenylyl cyclase nor did they produce a compensatory increase in cAMP production when assayed in μδ2 stably transfected CHO cells that had been chronically treated with DPDPE. Antagonist assayed on their own, though, did produce an inhibition of adenylyl cyclase activity. Sequencing of the μδ2 chimera uncovered a single amino acid mutation in a single transmembrane region. Mutation of this amino acid back to the wildtype sequence abolished the agonististic effects that the antagonists exhibited. Analogous mutations in wildtype δ2 and μ opioid receptors again conferred full-agonist properties to antagonists. None of the mutant receptors demonstrated significant differences in binding affinity as compared to wildtype for any of the ligands assayed. This implies that the mutated amino acid is not involved in ligand recognition but rather with signal transduction. (supported in part by NIDA grant #DA07339, 0564, 01583, 07234, 05695)

629.10

DETERMINING THE SPECIFIC INTERACTIONS BETWEEN OPIOID LIGANDS AND THEIR RECEPTORS: A COMPLEMENTARY STUDY F. Meng*, M. T. Hoversten, R. C. Thompson, L. Taylor, S. J. Watson and H. Akil MHRI, Univ. of Michigan, Ann Arbor, MI48109

To fully understand the working mechanism of a receptor, one need to learn how a ligand interact with its receptor. The combination of both receptor mutagenesis and ligand "mutagenesis"(comparing the binding properties of structurally similar compounds) may help us to empirically determine some of the specific interactions between a ligand and its receptor. The richness of opioid ligands creates a very good opportunity to implement such a complementary study. There are many well characterized ligands that only differ slightly in structure but differ greatly in binding properties. An interesting example is U63,639 and U63,640. While being mirror images to each other, U63,639 prefers the μ receptor whereas U63,640 binds the κ receptor with high affinity. Furthermore, a pair of structurally similar ligands, (+)U50,488 and (-)U50,488, show a different binding profile. Although (-)U50,488 is still κ-selective, (+)U50,488 exhibits similar affinities toward both the κ and the μ receptors. By analyzing their binding profiles on a series of κ/μ chimeric receptors as well as on the wild type κ and μ receptors, we are able to determine that (1) a single functional group on U63,639 is solely responsible for its μ selectivity (2) this particular functional group most likely interacts with a region in the μ receptor containing the sixth transmembrane domain(TM6) and the third extracellular loop (3) U63,640 and (-)U50,488 achieve κ-selectivity mainly through their interactions with a κ receptor region spanning from TM1 to the beginning of TM3. Site-directed mutagenesis studies are underway to pinpoint the individual residues that may directly interact with specific functional groups in these ligands.

629.11

THE μ OPIATE RECEPTOR TM 6 HISTIDINE RESIDUE IS CRITICAL FOR HIGH AFFINITY AGONIST BINDING. *C.K. Surratt*#, P.S. Johnson#, C.E. Spivak#, B.K. Seidleck#, L.D. Hirshbein#, C.J. Blaschak#, A. Moriwaki#, G.R. Uhl#$@* Molecular Neurobiology Branch#, Office of the Director$, Intramural Research Program/NIDA; Dept. of Neurology and Neuroscience@, Johns Hopkins School of Medicine.

A transmembrane (TM) 6 histidine residue is conserved among several G-coupled receptors including the rat and human μ, δ and κ opiate receptors, and represents the lone positive charge predicted to lie in the TM region of the μ receptor. To characterize the role of His-297 in μ receptor function, substitution mutants were tested using radioligand binding, adenylate cyclase inhibition and agonist-catalyzed opening of co-expressed K^+ channels. His-297 was replaced with amino acids of like or opposite charge or structure, and cell surface expression was verified immunocytochemically. Ligand binding B_{max} values quantitated expression levels for several mutants. Substitution with asparagine and glutamine retained high affinity binding of radiolabeled DAMGO and naloxone, while substitutions with alanine, aspartic acid, glutamic acid, arginine, lysine or phenylalanine resulted in dramatically reduced affinities for both peptide and alkaloid ligands. Application of high agonist concentrations mediated inhibition of adenylate cyclase and opening of inwardly-rectifying K^+ channels co-expressed in Xenopus oocytes for all mutant receptors. These results suggest that His-297 contributes prominently to both agonist and antagonist binding sites, and support a role of the His-297 sidechain in μ receptor function that cannot be duplicated by sidechains which merely donate π electrons or a full positive charge.

629.12

DETERMINATION OF THE REGION INVOLVED IN [^{3}H]ß-FUNALTREXAMINE COVALENT BINDING IN THE CLONED RAT μ OPIOID RECEPTOR BY PEPTIDE-MAPPING. C. Chen[1], L. Yu[2], Y-W Chen[1], J. Zhu[1] and L. Liu-Chen[1]*. [1]Dept. of Pharmacology, Temple Univ. Sch. of Med., Philadelphia, PA 19140. [2]Dept. of Med. & Mol. Genetics, Indiana Univ. Sch. Med., Indianapolis, IN 46202.

We previously demonstrated that [^{3}H]ß-funaltrexamine ([^{3}H]ß-FNA) specifically and irreversibly labeled rat brain μ-opioid receptors as well as the cloned rat μ opioid receptor expressed in COS or CHO cells. SDS-PAGE and fluorography showed that [^{3}H]ß-FNA-labeled receptors migrated as one broad band with Mr of 80 kDa in CHO or COS cells and as one band with Mr of 67 kDa in the rat brain. Upon removal of N-linked carbohydrates, labeled receptors became a sharp band with Mr of ~40kDa. In order to locate the covalent binding site of [^{3}H]ß-FNA within the μ receptor, we carried out peptide-mapping studies on the labeled cloned rat μ receptor. μ receptors expressed in CHO cells were labeled with [^{3}H]ß-FNA and then purified by wheat germ lectin affinity chromatography followed by immunoaffinity chromatography with antibodies against a C-terminal domain peptide. The partially purified receptor was treated with endoproteinase Glu-C or CNBr. Glu-C digestion of [^{3}H]ß-FNA labeled μ receptor generated a diffuse labeled band with Mr 53-69 kDa, which after deglycosylation with N-Glycanase, became a sharp band with Mr of ~25kDa. CNBr treatment yielded a labeled fragment with Mr of 4.5 kDa. The glycosylation nature of the labeled fragment generated by Glu-C and the size of the deglycosylated fragment suggest that this peptide corresponds to the one from Met1 to Glu229 (calc.mw 25016). In addition, the size of CNBr-generated labeled fragment suggests that the site is within Ser162-Met203 (calc.mw 4668) or Ala206 - Met243 (calc.mw 4546). We will determine more precisely the region and eventually the amino acid residue of ß-FNA covalent incorporation. Since [^{3}H]ß-FNA is a rigid molecule, the information will be very useful for computerized modeling of the μ receptor. (supported by NIDA grant 04745).

629.13

BINDING DOMAIN OF FENTANYL AND SUFENTANIL IN THE CLONED μ OPIOID RECEPTOR. J.Zhu*, J.Yin, P.-Y.Law[1], P.A.Claude[1], C.Chen, C.Evans[2], L. Yu[3] and L.-Y.Liu-Chen. Dept. Pharmacol., Temple Univ. Sch. Med., Philadel., PA 19140; [1]Dept. Pharmacol., Univ. Minnesota Sch. Med., Minneapolis, MN 55455; [2]Neuropsychiat. Inst., UCLA, Los Angeles, CA 90024; [3]Dept. Med. & Mol. Genetics, Indiana Univ. Sch. Med. Indianapolis, IN 46202.

Fentanyl and its analog sufentanil are narcotic analgesics with high potency and short duration of action. Both are highly selective μ opioid agonists and displayed morphine-like side effects clinically. Recently μ, δ and κ opioid receptors have been cloned, which permits identification of structural basis of binding selectivity of these receptors at the molecular level. In this study, we determined the binding domain of fentanyl and sufentanil in the μ receptor by examining their binding to μ and δ receptors and μ/δ chimeric receptors. Four chimeras constructed from the mouse δ and the rat μ receptors were used. For μ/δ1 and δ/μ1, the segments from the N-terminus to the beginning of the first intracellular loop were exchanged. For μ/δ4 and δ/μ4, the fragments from the N-terminus to the beginning of second extracellular loop were swapped. All four μ/δ chimeric receptors bound [^{3}H] diprenorphine with high affinity, similar to μ and δ receptors. Fentanyl and sufentanil inhibited [^{3}H] diprenorphine binding to μ, δ/μ1 and δ/μ4 with high affinity, but to δ, μ/δ1 and μ/δ4 with low affinity. Thus, the segment from the beginning of the second extracellular loop to the C-terminus of the μ receptor appears to confer the binding selectivity for fentanyl and sufentanil. (Supported by NIDA grants 04745 and 06650)

630.1

OPIATE BINDING AFTER TREATMENT WITH THE NONEQUILIBRIUM κ-ANTAGONIST DIPPA. W.E. Polgar,[1] J.Berzetei-Gurske,[1] A.-C. Chang,[2] P.S. Portoghese,[2] and L. Toll.* [1]SRI International, Menlo Park, CA 94025; [2]University of Minnesota, Minneapolis, MN 55455.

The benzeneacetamide DIPPA has been shown to cause long-lasting inhibition of antinociception induced by the selective κ-agonist U50,488 but was ineffective in inhibiting the actions of μ and δ agonists. This compound also caused wash-resistant inhibition of binding when tested *in vitro*, and so we examined binding parameters after intraperitoneal injection into guinea pigs. Guinea pigs were injected with 0.25, 0.5, or 1.0 mg/kg DIPPA. Brains were removed and binding was conducted 24, 48, or 72 hr after injection. DIPPA caused a dose- and time-dependent inhibition of binding of the κ-selective ligand [^{3}H]U69,593 without affecting the binding parameters of the μ-selective ligand [^{3}H]DAMGO or the δ-selective ligand [^{3}H]Cl-DPDPE. DIPPA caused maximal inhibition of binding at 0.5 mg/kg, inhibiting 35% of specific [^{3}H]U69,593 binding. DIPPA was most effective 48 hr after injection, but [^{3}H]U69,593 binding was significantly lower than control at both 24 and 72 hr after DIPPA injection. As expected for an irreversible inhibitor, DIPPA induced a decrease in B_{max} without significantly affecting the K_d of [^{3}H]U69,593. These results complement our studies showing a DIPPA-induced inhibition of κ-activity in the guinea pig ileum after intraperitoneal administration.

630.2

N-TERMINAL ALKYLATED DERIVATIVES OF [D-Pro^{10}]DYNORPHIN A-(1-11) ARE HIGH AFFINITY PARTIAL κ-OPIOID RECEPTOR AGONISTS. K.Soderstrom, H.Choi, J.V. Aldrich, and T.F.Murray*, College of Pharmacy, Oregon State University, Corvallis OR 97331.

As part of an effort to develop peptides with selective κ-opioid antagonist activity, a series of N-alkylated [D-Pro^{10}]Dyn A(1-11) derivatives were made through solid-phase peptide synthesis: R-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-D-Pro-LysOH, where R = monobenzyl (MB), monocyclopropyl (MCP), dicyclopropylmethyl (DCPM), or diallyl (Diallyl). These derivatives and dynorphin A(1-13)NH_3 (DynA) were evaluated for κ-opioid receptor binding affinity and potency as inhibitors of adenylyl cyclase (AC). Equilibrium competition binding experiments using [^{3}H]diprenorphine (0.5 nM) were performed on membranes prepared from CHO cells stably expressing the rat κ-opioid receptor. Cultures of these κ-receptor-expressing cells were used to evaluate opioid peptide inhibition of forskolin-stimulated (50μM) AC. Displacement of [^{3}H]diprenorphine specific binding by these peptides was observed with a rank order of affinity (Ki,nM) = DynA (0.84) > MCP (1.3) > DCPM (12.7) > MB (19.1) > Diallyl (25.7). A similar rank order was observed for potency of AC inhibition (EC_{50},nM): DynA (0.19) > MCP (2.9) > DCPM (10.2) ≈ Diallyl (11.2) > MB (51.6). The peptides differed in their maximal inhibition of AC: DynA (89%) ≈ MCP (84%) > MB (63%) > Diallyl (25%) ≈ DCPM (24%). As the DCPM and Diallyl derivatives were found to have only weak partial agonist activity with respect to AC inhibition, they were evaluated for their ability to reverse DynA (10 nM) inhibition of AC activity. DCPM and Diallyl reversed DynA inhibition to levels equal to the maximal inhibition produced by DCPM and Diallyl alone. This weak partial agonism combined with nanomolar potency render the DCPM and Diallyl compounds promising leads for further attempts to synthesize peptide κ-opioid receptor antagonists. (Supported by PHS grant DA05195 to J.V.A.)

630.3

N-CBM-TAMO: A SHORT-TERM OPIOID AGONIST AND A LONG-TERM OPIOID ANTAGONIST. J. Y. Xu*, S. Archer*, and J. M. Bidlack. University of Rochester, Rochester, NY 14642 and *Rensselaer Polytechnic Institute, Troy, NY 14181.

The antinociceptive and opioid binding properties of the N-cyclobutylmethyl analogue of normorphinone, 14α,14'ß-[dithiobis[(2-oxo-2,1-ethanediyl)imino]]bis[7,8-dihydro-N-(cyclobutylmethyl)-normorphinone] (N-CBM-TAMO) were investigated. In the mouse 55°C warm-water tail-flick test, N-CBM-TAMO given supraspinally acted as a short-term partial agonist, because only submaximal antinociception was observed at doses up to 100 nmol. Pretreatment of mice with i.c.v. N-CBM-TAMO at 10 nmol which exhibited moderate short-term antinociception produced a time- and dose-dependent long-term antagonism of morphine-induced antinociception. In the mouse writhing test, N-CBM-TAMO acted as a full agonist with a D_{∞} value of 0.08 (0.044-0.147) nmol and the antinociception was blocked by co-administration of the κ-selective antagonist, nor-BNI. These *in vivo* data are consistent with the *in vitro* binding study which demonstrated: 1) incubation of membranes with N-CBM-TAMO resulted in wash-resistant inhibition of μ and κ opioid binding; 2) the wash-resistant inhibition of μ, but not κ opioid binding was partially reversed by the addition of the disulfide reducing reagent dithiothreitol; 3) Scatchard analysis of saturation binding data showed that N-CBM-TAMO decreased the B_{max} value without affecting the K_d value for the μ-selective peptide [^{3}H]DAMGO. Whereas, N-CBM-TAMO increased the K_d value without altering the B_{max} value for the κ-selective ligand [^{3}H]U69,593. This study demonstrates that N-CBM-TAMO is a very potent κ agonist and at higher concentrations produces long-term antagonism of antinociception mediated by μ opioid receptors. (Supported by USPHS grant DA03742, DA07232 and DA01674)

630.4

LIGANAD BINDING PROFILES OF U-69,593-SENSITIVE AND -INSENSITIVE KAPPA OPIOID RECEPTORS IN HUMAN CEREBRAL CORTEX MEMBRANES. K. W. Kim[1], H. I. Kim[2], H. W. Rho[3], D. G. Kim[4*], and K. P. Cho[1] Dept. of Pharmacol.[1] and Neurosurg[2] and Biochem[3]. Chonbuk Univ. Med. Sch., Chonju 560-182, Chonbuk, Dept. of Pharmacol.[4], Yonsei Univ. Med. Sch., Seoul 120-752, Republic of Korea

In vitro competition studies were performed to characterize the properties of subtypes of κ opioid receptors in membrane preparations of epileptic human cortex. κ_1 and κ_2 sites are labeled by [^{3}H]U-69,593 ([^{3}H]U-69), a selective κ_1-agonist, and [^{3}H]diprenorphine ([^{3}H]DIP), a non-selective opioid antagonist, in the presence of 1 μM each of DAMGO, DPDPE and U-69 to block μ-, δ-, and κ_1 sites, respectively. [^{3}H]U-69 binding site is of single-population and low density (Kd= 3.1 nM, Bmax= 8 fmol/mg protein) and have a binding profile that corresponds to κ_1 opioid receptor. That is, dynorphine-A(1-13)(Dyn-A), bremazocine (BZC), U50,488H (U50), (-)ethylketocyclazocine (EKC) and nor-binaltorphimine (nor-BNI) bind to this site with high affinities, whereas μ- and δ-ligands do not. [^{3}H]DIP binding site is of single-population and high density (Kd= 9 nM, Bmax= 352 fmol/mg protein). [^{3}H]DIP binding was not influenced by U-50,488H, confirming it is not κ_1-site, but inhibited by BZC, EKC and nor-BNI ligands that possess affinities to κ_2 opioid receptors. The rank order of affinities of these ligands are correlated with that of κ_2 receptor originally reported in rat brain. These results show existence of both κ_1 and κ_2 opioid receptors in human cortex. (This work is supported by 1994 KOSEF Grant.)

630.5

IRREVERSIBLE BLOCKADE OF THE κ OPIOID RECEPTORS IN GUINEA PIG ILEUM. R.W. Schwartz, A.-C. Chang, P.S. Portoghese, and J. Berzetei-Gurske*. SRI International, Menlo Park, CA 94025

In studies by Chang et al. (J. Med. Chem. 37:4490, 1994) on a new κ-selective affinity label for opioid receptors, 2-(3,4-dichlorophenyl)-N-methyl-[(1S)-1-(3-isothiocyanatophenyl)-2-(1-pyrrolidinyl) ethyl] acetamide (DIPPA), the compound was shown to be a selective κ-opioid receptor antagonist with a long duration of action *in vivo* , but no antagonist activity was found in the *in vitro* bioassay on guinea pig ileum (GPI). However, our studies showed that DIPPA is a very powerful irreversible κ receptor blocker 24–72 hr after i.p. injection into guinea pigs.

Effects on GPI were evaluated 24–72 hr after male Hartley guinea pigs were injected i.p. with single doses of DIPPA ranging from 0.1 to 10 mg/kg. By 24–48 hr, doses as low as 0.5 mg/kg caused complete blockade of the κ-opioid receptors, with only a minimal shift (1.45–3.41) in the IC_{50} value for the μ receptor agonist [D-Ala^2,MePhe^4, Gly-ol] enkephalin (DAMGO). U69,593 ([5a,7a,8b]-(+)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro-(4,5)-dec-8-yl]), which has an IC_{50} of 1.9 nM for untreated GPI, turned out to be a partial agonist, with less than 30% agonist activity at 10^{-6} M; higher concentrations (5×10^{-5} and 10^{-4} M) caused enhancement, which could not be blocked by naloxone or the other selective opiate antagonists tested.

This new assay will be very useful for studying compounds with κ agonist/μ antagonist activity where determination of μ antagonism was hindered by the presence of a strong κ agonist activity.

630.6

RESOLUTION OF TWO SUBTYPES OF DELTA$_{ncx}$ BINDING SITES IN RAT BRAIN USING [3H]DADL: SELECTIVE EFFECTS OF DELTA ANTISENSE DNA. Q. Ni*, X.Y. Cha , H. Xu , F. Porreca†, J. Lai†, J.S. Partilla , K.C. Rice^, D. Matecka^ and R. B. Rothman . *CPS, DIR, NIDA and ^LMC, NIDDK, NIH, Baltimore and Bethesda, MD. †Department of Pharmacology, University of Arizona, Tucson, AZ 85724.

Quantitative binding studies resolved two high affinity [3H]DADL binding sites in rat brain membranes depleted of mu binding sites by pretreatment with BIT. Assays were conducted in 50 mM Tris-HCl, pH 7.4, containing 100 mM NaCl, 2 μM GTP, 3 mM MnCl2 and 5 mM 2-mercaptoethanol for 4-6 hr at 250 C. The two binding sites had lower (DELTAncx1, Ki=96.6 nM) and higher (DELTAncx2, Ki=1.55 nM) affinity for DPDPE. The ligand-selectivity profile of the DELTAncx2 site was that of a classic delta binding site. The ligand-selectivity profile of the DELTAncx1 site was neither mu- or delta-like. The Ki values of selected agents were: ([pCl]DPDPE, 3.91 nM), (DPLPE, 142 nM), (DAMGO, 2.65 nM). Under these assay conditions, [3H]DADL binding to the cells expressing the cloned mu receptor is very low and pretreatment of cell membranes with BIT almost completely inhibits [3H]DAMGO and [3H]DADL binding. Chronic administration of morphine pellets up-regulated [^{3}H]DAMGO binding to mu receptors, but decreased DELTAncx2 binding. I.c.v. administration of antisense DNA to the cloned delta receptor selectively decreased [3H]DADL binding to the DELTAncx1 site. Viewed collectively, these studies have identified a novel non-mu, delta-like binding site in rat brain.

630.7

ONE OF THE MOUSE BRAIN DELTA$_{NCX}$ BINDING SITES IS SIMILAR TO THE CLONED OPIOID DELTA RECEPTOR: FURTHER EVIDENCE FOR HETEROGENEITY OF DELTA RECEPTORS. H. Xu*, X.Y. Cha , J.S. Partilla , F. Porreca†, J. Lai†, J. Pinto^, S.N. Calderon^, D. Matecka^ , K.C. Rice^, S. Ananthan# and R.B. Rothman . *CPS, DIR, NIDA, NIH, Baltimore, MD 21224. †Department of Pharmacology, College of Medicine, University of Arizona, Tucson, AZ 85724. ^LMC, NIDDK, NIH, Bethesda, MD 20892. #Organic Chemistry Department, SORI, Birmingham Alabama 35255.

We recently presented evidence for subtypes of the delta$_{NCX}$ binding site in rat and mouse brain which we termed delta$_{NCX1}$ and delta$_{NCX2}$. Cha et al. (this meeting) report that i.c.v. administration of antisense DNA complementary to the cloned delta opioid receptor (CDOR) to rats decreased [3H]DADL binding to one of the binding sites. Here we compare the binding parameters, ligand selectivity profile and pharmacological properties of the mouse CDOR stably expressed in a cell line to the delta$_{NCX}$ binding sites of mouse brain. [3H]DADL labeled a single binding site in membranes prepared from these cells. BIT-pretreatment had no significant effect on [3H]DADL binding parameters. The DOR had high affinity for delta agonists and antagonists. [3H]DADL labeled two binding sites in mouse brain membranes depleted of mu receptors by pretreatment with BIT: the delta$_{NCX-1}$ site (high affinity for DPDPE and deltorphin) and the delta$_{NCX2}$ site (low affinity for DPDPE and deltorphin). Some agents were moderatley selective for the delta$_{NCX2}$ site: [pCl]DPDPE (10.9-fold), JP41 (5.9-fold) and JP45 (3.8-fold). The K_i values of 12 opioids at the mouse CDOR were determined. These values were highly correlated (r=0.996) with their values at the delta$_{NCX1}$ site but not the delta$_{NCX2}$ site (r=-0.05). These data suggest that the delta$_{NCX2}$ site may be distinct from the cloned delta opioid receptor.

630.8

EFFECT OF NONPEPTIDIC AGONISTS AT THE CLONED HUMAN DELTA OPIOID RECEPTOR. G. Santoro, R.J. Knapp, K.B. Lee, S. Edsall, I. De Leon, S. Waite, S.N. Calderon†, K.C. Rice†, F. Porreca, L. Stern*, W.R. Roeske and H.I. Yamamura. Dept. of Pharmacology and Medicine, College of Medicine, Univ. of Arizona, Tucson, AZ 85724 and †Laboratory of Medicinal Chemistry, NIDDK, NIH, Bethesda, Maryland.

Cloning of the three receptor types from different species including human, and the availability of recombinant cell lines expressing a homogeneous opioid receptor population present new research opportunities for the characterization of opioid ligands. Drugs acting at the delta opioid receptor may have therapeutic advantage over opiate narcotic acting primarily at the μ receptor. Nonpeptidic agonists are recently being developed given their capability to cross the blood-brain-barrier. BW 373U86 was the first nonpeptidic agonist selective for the delta opioid receptor. Due to the capability of this compound to induce convulsions in mice, a series of compounds were developed from the original structure of BW 373U86. We report here the characterization of SNC 67, SNC 79, SNC 80, SNC 83, SNC 85, SNC 86, SNC 88, SNC 89, SNC 121 and SNC 162 . All compounds were tested for their capability to inhibit specific [^{3}H]p-Cl-DPDPE binding measured on membrane homogenate obtained from CHO cells stably expressing the human delta opioid receptor (hDOR) and specific [^{3}H]DAMGO binding measured on membrane homogenates from B 82 cells stably expressing the human μ opioid receptor (hMOR). These compounds, with the exception of SNC 67, showed high selectivity for the delta receptor with K_i values for [^{3}H]p-Cl-DPDPE ranging from 0.4 nM to 15 nM and K_i values for [^{3}H]DAMGO ranging from 50 nM to 12,000 nM. The high selectivity of these agonists for the hDOR makes these compounds potential therapeutic agents for the management of pain.

Supported by NIDA grants.

630.9

INHIBITION OF FORSKOLIN-STIMULATED cAMP FORMATION BY PEPTIDE AND NON-PEPTIDE AGONISTS ACTING AT THE HUMAN δ OPIOID RECEPTOR. E. Malatynska*, Y.J. Wang, R.J. Knapp, G. Santoro, X. Li, S. Waite, H. Nagase, S. Calderon, K. Rice, W.R. Roeske and H.I. Yamamura. University of Arizona, Tucson, AZ 85724, Toray Inddustries, Japan and NIH, MD.

Stable expression of a human δ opioid receptor (hDOR) was established in Chinese hamster ovary (CHO) cells. This cell line has a high density of hDORs (137,000 ± 21,000 receptors per cell) that bind the δ receptor selective antagonist naltrindole (NTI) with a Kd of 139 ± 36 pM. The recombinant cells were used to characterize the potency of opioid agonists for the inhibition of cAMP accumulation. Ligands selective for μ (DAMGO) and κ (U50488) opioid receptors inhibit forskolin-stimulated cAMP formation with low potency (EC-50, 2.0 μM and 85 μM, respectively). DOR selective agonists inhibit forskolin-stimulated cAMP formation with high potency (EC-50, for the peptides DPDPE, 1.0 nM and pCl-DPDPE, 0.4 nM and for non-peptides, SNC80, 8.3 nM and TAN67, 1.8 nM). This inhibition was antagonised by NTI in a dose dependent manner. Schild analysis showed that NTI competitively antagonized (slope 0.9) pCl-DPDPE induced cAMP inhibition with a pA_2 value of 9.4 that is consistent with the high affinity of this antagonist.

Receptor desensitization is thought to be one of the reasons for the development of tolerance and/or dependence observed after prolonged opioid administration. However, DOR selective agonists are less effective at producing this effects than μ receptor selective agonists when tested in animals. We pretreated CHO cells expressing hDOR with non-peptide DOR agonist, SNC 80 for 24 hr. After such treatment the B_{max} value obtained for [^{3}H]NTI binding was reduced by 41% relative to untreated control cells. Cells exposed to SNC80 for 24 hr show a 10 fold reduction in the potency of SNC80 to inhibit forskolin-stimulated cAMP formation and decreased the E_{max} value to 50% inhibition compare to 85% for control. We conclude that the hDOR undergoes down-regulation after exposure to delta agonist. Supported by NIDA grants.

630.10

A NEW DIHYDROCODEINONE DERIVATIVE DISPLAYS OPIOID AGONISTIC AND ANTAGONISTIC PROPERTIES. J.P. McLaughlin*, J.P. Egamino, W. El-Hamouly, S. Archer, and J.M. Bidlack. Univ. of Rochester, Rochester, NY 14642 and Renesselaer Polytechnic Institute, Troy, NY 12181.

N-Cyclobutylmethylnor-14β-(p-nitrocinnamoylamino)-7,8-dihydrocodeinone (N-CBM-CACO) was characterized for opioid affinity, selectivity and activity with receptor binding and analgesic studies. In competition binding assays with bovine striatal membranes, nanomolar concentrations of N-CBM-CACO inhibited μ, δ and κ opioid binding. Like previously characterized cinnamoylamino opioid derivatives, N-CBM-CACO inhibited binding of the μ-selective peptide [^{3}H][D-Ala^2,(Me)Phe^4,Gly(ol)^5]enkephalin (DAMGO) in a wash-resistant manner, but produced no significant inhibition of δ- or κ-opioid binding. A concentration of 16 nM N-CBM-CACO produced a 50% wash-resistant inhibition of the binding of 0.25 nM [^{3}H]DAMGO, with complete inhibition occurring at 100 nM. Scatchard analysis of [^{3}H]DAMGO saturation binding revealed a two-fold reduction of the B_{max} value, suggesting N-CBM-CACO may bind covalently to the μ opioid receptor. In the mouse 55°C warm-water tail-flick test, N-CBM-CACO produced antinociception that lasted up to 80 min, and displayed a D_{50} value of 8 nmol after i.c.v. administration. However, 24-hr N-CBM-CACO pretreatment of mice inhibited the antinociceptive effect of 3-nmol morphine. These results suggest this compound possesses both short-term agonistic and long-term antagonistic effects at the opioid receptor. The receptor selectivity of N-CBM-CACO is currently being characterized in the mouse tail-flick and acetic-acid writhing tests. (Supported by USPHS grants DA03742 and DA06786).

630.11

Isolation and pharmacological characterization of a novel neuropeptide specific to an opioid-like receptor

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A cDNA encoding a G protein-coupled receptor which shows high homology to μ, δ and κ-opioid receptors has recently been cloned by several groups. However, none of the classical opioid ligands was able to bind to this receptor. We have now purified and characterized an endogenous ligand for this opioid-like G protein-coupled receptor. The ligand was isolated from porcine brain extracts and identified as a 17 amino acid peptide which bears structural similarity to the opioid peptides. The corresponding synthetic peptide was shown to potently inhibit forskolin-stimulated cAMP accumulation in CHO and HEK 293 cells stably transfected with the opioid-like receptor. Interestingly, this novel peptide was unable to activate opioid receptors. In addition, we found that opioid ligands were unable to antagonize binding of this novel peptide at its receptor. Preliminary studies indeed suggest that the mode of interaction of the peptide with its receptor may be different from that proposed for the opioid peptides. To further the characterization of this novel neuropeptide, we have developed a radioligand binding assay and began to correlate the tissue distribution of this peptide with that of its corresponding receptor and that of the opioid peptides. Taken together these results suggest that this peptide belongs to a peptidergic transmitter class reminiscent of but different from that of the opioid peptide family.

630.12

PHOLCODINE, AN ANTITUSSIVE OPIOID DRUG, SHOWS VERY LOW AFFINITY FOR μ, δ OR κ OPIOID RECEPTORS. M. Attila*, J. Waldén, T. Heikkinen and J. Aaltonen[1]. Div. of Pharmacology and Toxicology, Dept. of Pharmacy, Univ. of Helsinki and [1]Leiras Oy, Helsinki, Finland.

Pholcodine is used as an antitussive agent which does not possess analgesic properties although it is structurally related to morphine. Pholcodine is classified into narcotic drugs which restricts its marketing. Binding of pholcodine to opioid receptors has been only sporadically studied. Chen *et al.* (Life Sci. 48: 2165-2171, 1991) reported negligible binding of pholcodine to μ opioid receptors (about 4700 times lower affinity than morphine). In this study we have confirmed the low affinity of pholcodine to μ opioid receptors and report its even lower affinity to δ and κ opioid receptors. Synaptosomal (P_2 fraction) preparations from whole brain (minus cerebellum) of adult male Wistar rats (Lazarus *et al.*, J. Med. Chem. 35: 1222-1227, 1992) were used for the binding assays. Competition studies with pholcodine, morphine, DPDPE, DAMGO, or U-69593 were conducted using 6 nM [^{3}H]DPDPE, 3.5 nM [^{3}H]DAMGO or 6 nM [^{3}H]U-69593 to label δ, μ or κ sites, respectively. The non-specific binding was defined by displacing the label with cold DPDPE, DAMGO or U-69593 (15.5, 19.5 or 28.0 μM, respectively). Competition curves were calculated by using non-linear regression analysis each combined from 3 to 5 assays using different synaptosomal preparations. Pholcodine showed K_i values at μM or mM level - thus being 900 to 2000 times less potent than morphine. We conclude that due to the low affinity of pholcodine on μ, δ or κ opioid receptors this antitussive drug should not any more be classified into narcotic drugs.

630.13

NALTREXONE ANTAGONISM OF MORPHINE AND U50,488 IN A THREE-KEY DISCRIMINATION IN PIGEONS. M. Makhay* and A. Poling. Department of Psychology, Western Michigan University, Kalamazoo, MI 49008.

It has been demonstrated that morphine and U50,488 are discriminable in a three-key assay in pigeons (Makhay, 1994). To determine if the discriminative and rate-decreasing effects of U50,488 and morphine were differentially sensitive to opioid antagonists, naltrexone was administered in combination with morphine and U50,488. Five pigeons were tested with various doses of naltrexone (0.01 to 3.2 mg/kg) in combination with morphine and U50,488 in doses ranging from 3.2 to 32 mg/kg. Low doses of naltrexone administered in combination with the training doses of U50,488 and morphine (5.6 mg/kg) occasioned about 50% drug-appropriate responding, whereas higher doses of naltrexone completely blocked the drug cues. Naltrexone did not substantially affect rates when compared to control values. High doses of naltrexone completely antagonized high doses of morphine, but only reduced U50,488-appropriate responding by about 50%. These results are in contrast with those of Picker & Dykstra (1987) in that naltrexone did not differentially antagonize the stimulus and rate-reducing effects of the training doses of morphine and U50,488.

OPIOID RECEPTORS: SIGMA RECEPTORS

631.1

SIGMA2-MEDIATED INHIBITION OF ELECTRICALLY-EVOKED GUINEA-PIG ILEUM LONGITUDINAL MUSCLE/MYENTERIC PLEXUS (GPLMMP) CONTRACTIONS. G.G. Kinney*, E.W. Harris, R. Ray and T.J. Hudzik. Astra Pharm., Dept. of Biol., Box 1071, Rochester, NY 14603.

At least two subtypes of the sigma binding site are known to exist in the brain and periphery. Little is known, however, about any biological function these sites may subserve. Thus, the potency of a number of sigma ligands to inhibit electrically-stimulated contraction of the GPLMMP was compared to their relative affinities for $sigma_1$ and $sigma_2$ binding sites. The rank potencies (IC_{50}) of compounds to suppress contraction of the GPLMMP perfectly matched the rank affinities (IC_{50}) of these compounds for the $sigma_2$ site (r=1.0), while no significant correlation with $sigma_1$ (r=0.28, *P*>.46) binding was found. In addition, no significant correlation between GPLMMP efficacy and previously reported muscarinic cholinergic (r=0.04, *P*>.94), dopamine D_2 (r=-0.18, *P*>.67), and PCP (r=-0.58, *P*>.18) binding affinities were found. Thus, the GPLMMP appears to represent a functional biological assay linked to the $sigma_2$ binding site which has important implications for future research into the etiology of disorders in which sigma receptors have been implicated.

631.2

SIGMA LIGANDS DIMINISH THE ABILITY OF RIMCAZOLE TO INHIBIT PCP *hsp70* INDUCTION. J.W. Sharp* and D.S. Williams, Dept Anatomy & Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506-5602.

PCP(phencyclidine) binds at the NMDA receptor calcium channel PCP site and at the sigma receptor. PCP also induces the heat shock gene, *hsp70*, in cingulate and retrosplenial cortex neurons (Sharp *et al.*, 1991). PCP has also been implicated in drug induced schizophrenia-like behavior. Rimcazole, an antipsychotic drug, inhibits PCP *hsp70* induction (Sharp *et al.*, 1992). Rimcazole binds predominately to sigma-2 sites. It is hypothesized that sigma ligands without antipsychotic properties and with some sigma-2 affinity should partially reverse the effects of rimcazole.

(+)-3-PPP, (+)-cyclazocine, and (+)-pentazocine bind predominately to sigma-1 sites. (+)-3-PPP, and (+)-cyclazocine are modest sigma-2 ligands. Female Sprague Dawley rats(200-250g) were injected(ip) with (+)-3-PPP (50mg/kg), rimcazole (60mg/kg), and after 5 minutes with PCP (40mg/kg). Rats were anesthetized after 24hrs with halothane and perfused. Brains were sectioned (100μm) and reacted immunocytochemically for the protein product of the *hsp70* gene, *HSP70*, with the monoclonal anti-72kd heat shock protein antibody. Appropriate controls were conducted. Analogous experiments and controls employed either (+)-cyclazocine (15mg/kg ip) or (+)-pentazocine (50mg/kg ip) in the place of (+)-3-PPP.

(+)-3-PPP and (+)-cyclazocine diminished the ability of rimcazole to inhibit PCP *hsp70* induction in the cingulate and retrosplenial cortex. The number of *hsp70* induced neurons in rats given (+)-3-PPP (50mg/kg), rimcazole (60mg/kg), and PCP (40mg/kg) was significantly greater ($p<.05$) than rats given rimcazole (60mg/kg) and PCP (40mg/kg). (+)-Cyclazocine showed similar findings. (+)-Pentazocine did not diminished the ability of rimcazole to inhibit PCP *hsp70* induction.

631.3

DUAL MODULATION OF CELLULAR CALCIUM BY SIGMA RECEPTOR LIGANDS: RELEASE FROM INTRACELLULAR STORES AND BLOCKADE OF VOLTAGE-DEPENDENT INFLUX. B.J. Vilner* and W.D. Bowen. Unit on Receptor Biochemistry and Pharmacology, Laboratory of Medicinal Chemistry, NIDDK, NIH, Bethesda, MD 20892.

Changes in intracellular Ca^{++} [Ca^{++}]i were measured in single human SK-N-SH neuroblastoma cells and rat cerebellar granule cells by the indo fluorescence ratio method in PBS. Data taken from several cells were averaged and reported as maximal % increase over existing baseline. In SK-N-SH cells, 100 μM BD737, reduced haloperidol (RHAL), and CB64D produced small but significant increases in [Ca^{++}]i (23.2$\pm$1.4%, 38.0$\pm$3.5%, and 71.6$\pm$3.7%, respectively). The changes in [Ca^{++}]i occurred over a 5 min period, first increasing, followed by a decrease. Depolarization by addition of 55 mM KCl, produced a sharp "spike" of [Ca^{++}]i (~ 600%). Removal of extracellular Ca++ reduced the effect of added KCl on [Ca^{++}]i to 0%, but did not change the effect of sigma ligands. Thus, sigma ligands appear to release Ca++ from intracellular stores. This was confirmed by pretreatment of cells with thapsigargin for 10 min to deplete stores, which eliminated the rise in [Ca^{++}]i produced by 100 μM BD737. In cerebellar granule cells, the effect of sigma ligands on depolarization-induced Ca++ entry was examined. KCl (55 mM) produced a large "spike" of [Ca^{++}]i (2,302% increase). Sigma ligands (0.5 - 100 μM) were added to cells prior to addition of KCl. BD737, RHAL, CB64D, BD1008, and (+)-pentazocine, dose-dependently blocked the KCl-induced rise in [Ca^{++}]i, with most ligands having strong effects at 1 μM. Ligands lacking sigma affinity (BD1006, CB53D, (-)-sulpiride) had little effect at 30 - 100 μM. Nitrendipine completely blocked the Ca++ entry. However, the effect of sigma ligands did not appear to coincide with binding to channels since most exhibited IC50 values > 100 μM *vs.* 1 nM [^{3}H]nitrendipine. Sigma receptors may thus be capable of signalling intracellular Ca++ release and of modulating L-type Ca++ channels. (We thank Dr. Yael Eilam for helpful discussions.)

631.4

SIGMA-1 RECEPTOR-MEDIATED INHIBITION OF THE MUSCARINIC PHOSPHOINOSITIDE RESPONSE IS ASSOCIATED WITH M1 RECEPTOR-G PROTEIN UNCOUPLING. D.B. Joseph* and W. D. Bowen. Unit on Receptor Biochemistry and Pharmacology, Laboratory of Medicinal Chemistry, NIDDK, NIH, Bethesda, MD 20892.

Previous studies from this laboratory have shown that activation of σ_1 receptors in rat synaptoneurosomes (SN) reduced the maximal stimulation of phosphoinositide (PPI) turnover by muscarinic agonists. This was associated with a reduction in the number of surface muscarinic receptors. Here we have examined the possible role of muscarinic receptor/G-protein uncoupling. SN were treated with 50 μM (+)-pentazocine (a σ_1-selective agonist) for 30 min followed by centrifugal washing and challenge with the muscarinic agonist, oxotremorine-M. Maximal stimulation of PPI turnover was reduced by 50% in (+)-pentazocine-treated SN compared to control tissue. Binding studies were carried out in treated SN after lysis to allow access to the total receptor pool. Competition of oxotremorine-M against the M1-selective antagonist [^{3}H]pirenzepine in untreated lysed SN revealed the presence of a guanine-nucleotide sensitive high affinity state (IC_{50} = 8 $\pm$ 3 nM, 24%) and a low affinity state (IC_{50} = 5,590 $\pm$ 360 nM, 76%) (n_H of one-site fit = 0.49 $\pm$ 0.01). In the presence of the guanine-nucleotide GppNHp, only the low affinity state was observed (n_H = 0.72 $\pm$ 0.02), indicative of G-protein uncoupling. (+)-Pentazocine pretreatment caused a reduction in the population of high affinity sites to undetectable levels and a significant increase in n_H (n_H = 0.64 $\pm$ 0.02), thus mimicking the action of GppNHp. However, the n_H was further increased by GppNHp in the (+)-pentazocine-treated tissue (n_H = 0.76 $\pm$ 0.03). The results suggest that σ_1 receptor activation produces at least partial uncoupling of muscarinic M1 receptors from G-proteins, which may contribute to the heterologous desensitization of the muscarinic PPI response. Studies are in progress to determine if the effects of (+)-pentazocine on muscarinic receptor-effector coupling are mediated by protein kinases.

631.5

EFFECTS OF SIGMA LIGANDS ON THE NEURONAL ACTIVITY IN THE RAT MESOLIMBIC AND NIGROSTRIATAL DOPAMINERGIC SYSTEMS. B.Gronier* and G. Debonnel. Neurobiological Psychiatry Unit, Department of Psychiatry, McGill University, Montréal, Québec, Canada H3A 1A1.

The present studies were undertaken to investigate, *in vivo*, the effects of selective sigma (σ) ligands on the neuronal firing activity of dopaminergic (DA) neurons of the A_9 and A_{10} areas, as well as of neurons of the caudate and accumbens nuclei. Using extracellular unitary recording and microiontophoresis, we assessed the effects of σ ligands on the spontaneous firing activity, the NMDA-induced neuronal activation and the DA-induced suppression of firing activity. The selective σ_1 ligand S 21378, microiontophoretically applied, increased the spontaneous activity of both A_9 and A_{10} DA neurons as well as the NMDA-induced neuronal activity, whereas JO-1784, another σ_1 ligand, had no or little effect. DTG (20 µg/kg, i.v.), a non selective σ_1/σ_2 ligand, decreased the spontaneous activity of 30 % of A_{10} neurons. Microiontophoretic application and systemic administration (20 µg/kg, i.v.) of JO-1784 produced a marked increase of the NMDA-induced activation in the accumbens but not in the caudate. The σ_1 ligand (+)-pentazocine (30 µg/kg, i.v.) did not modify significantly the NMDA-induced activation of accumbens neurons, but potentiated slightly the NMDA response in the caudate. Microiontophoretic applications of JO-1784 increased significantly the suppressant effect of DA on NMDA-induced activation of accumbens neurons, but did not modify the suppressant effect of DA on A_9 and A_{10} neurons. These findings suggest that σ ligands differentially affect the glutamatergic activity in these two DA systems. The fact that the σ_1 ligands JO-1784, S 21378 and (+)-pentazocine had different modulatory effects further supports the existence of subtypes of σ_1 receptors, heterogeneously distributed. In addition, the fact that JO-1784 modulates the effect of DA in the accumbens suggests the existence of a functional interaction between σ and DA receptors in this area.

631.6

PHARMACOLOGICAL EVIDENCE FOR AN HALOPERIDOL-INSENSITIVE SIGMA RECEPTOR THAT MODULATES THE NMDA RESPONCE IN THE RAT CNS. F.P. Monnet*& E.E. Baulieu. DRC-DPIM Assistance Publique-Hôpitaux de Paris, CNRS-UPR2212 & INSERM-U33, F-94276 Kremlin Bicêtre, France.

The prototypic and high affinity sigma (σ_1 [(+)pentazocine, (+)PTZ] and σ_2 [1,3di(2)tolylguanidine, DTG] agonists are known to modulate selectively the neuronal response to *N*-methyl-D-aspartate (NMDA) in the rat CNS. Conversely, we have reported that the high affinity σ agonist (+)-cis-N-methyl-N-[2-(3,4-dichlorophenyl) ethyl]-2-(1-pyrrolidinyl) cyclohexylamine [BD-737] enhanced both NMDA and non-NMDA glutamatergic responses, suggesting that it may interact with an atypical σ site. The present study was thus undertaken to investigate the receptor by which BD-737 acts, using an *in vitro* model of release of [^{3}H]norepinephrine (NE) evoked by NMDA. Hippocampal slices (400 µm thick) from Sprague-Dawley rats were incubated with 0.1 µmol/l [^{3}H]NE for 30 min and superfused continuously with Mg^{++}-free Krebs' solution containing one of the selective and high affinity σ ligands DTG, (+)PTZ, BD-737 or its negative isomer BD-738 in the presence or absence of haloperidol, reduced haloperidol, BD-1063 [1[2-(3,4-dichlorophenyl)ethyl]-4-methyl piperazine]or*N*-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(1-pyrrolidinyl)ethylamine (BD-1008). Forty min later, the [^{3}H]NE overflow was evoked by NMDA (200 µM). BD-737 and (+)PTZ, from 10 nM to 1 µM, potentiated, while DTG (> 30 nM) decreased NMDA-evoked [^{3}H]NE overflow, BD-738 being less active than BD-737. Haloperidol and BD-1063 (> 30 nM) antagonized both effects of (+)PTZ and DTG but failed to reverse BD-737-induced potentiation. Conversely, reduced haloperidol and BD-1008 (> 10 nM) reversed the effect of BD-737, being much less active on both (+)PTZ- and DTG-related modulations. The present data constitute the first functionnal characterization supporting the notion that σ ligands, such as BD-737, reduced haloperidol and BD-1008, may act on an atypical σ receptor insensitive to haloperidol.

631.7

MODULATION OF NMDA NEURONAL RESPONSE BY SIGMA$_1$ AND SIGMA$_2$ LIGANDS. G. Debonnel*, R. Bergeron, B. Gronier, N. Lavoie., M.C Rettori* and B. Guardiola* Depart. Psychiatry, McGill University, Montréal, Qc, Canada and *Institut de Recherche International Servier, Paris, France.

Using *in vivo* extracellular recording and microiontophoresis, we have shown that low doses of various σ ligands potentiate selectively the NMDA response of CA_3 pyramidal neurons in the rat dorsal hippocampus. The present experiments were undertaken to assess in our electrophysiological paradigm and in radioligand binding studies the selective σ_1 and σ_2 ligands S 21377, S 21378, S 20955, S 21272, NE-100, FH-510, BD-1008, JO-5220 and SR-31742A. The affinity of these ligands was assessed for the σ_1 ([^{3}H]-(+)-pentazocine) and the σ_2 ([^{3}H]-DTG 1-150 nM in the presence of 500 nM cold (+)-pentazocine) subtypes. The order of potency for σ_1 binding sites was: BD-1008> NE-100 > FH-510> S 20955> S 21272> SR-31742A> JO-5220. The order of potency for σ_2 binding sites was: BD-1008> S 21272> NE-100> SR-31742A> S 20955> FH-510> JO-5220. S 21377 and S 21378 (1 to 2000 µg/kg) induced a selective dose-dependent potentiation of the NMDA response. S 21272 (5-25 µg/kg) also increased the NMDA response and, similarly to DTG, at higher doses, induced an epileptoid activity upon application of NMDA. All other σ ligands acted as antagonists, i.e. reversed the potentiating effects of σ agonists. S 20955 reversed the potentiating effects of S 21377 or (+)pentazocine, but not those of S 21378 and of JO-1784. NE-100 suppressed the potentiation of the NMDA response induced by DTG but not those induced by S 21378 or JO-1784. The epileptoid activity induced by either S 21272 or DTG was prevented by haloperidol, and BD-1008 suppressed S 21272-induced potentiation of the NMDA response.

Our results constitute another argument in favor of the existence of several subtypes of σ_1 receptors they also suggest that the epileptoid activity induced by some σ ligands could be mediated via σ_2 receptors.

631.8

THE POTENTIATION OF THE NMDA RESPONSE INDUCED BY SIGMA LIGANDS IS MARKEDLY REDUCED DURING PREGNANCY. R. Bergeron*, C. de Montigny and G. Debonnel. Neurobiological Psychiatry Unit, McGill University, Montréal, Québec, Canada H3A 1A1.

Some gonadal and adrenal steroids such as progesterone have high affinity for σ receptors. Low doses of σ ligands such as DTG and of DHEA act as agonists, *i.e.* potentiate the neuronal response to NMDA of pyramidal neurons in the CA_3 region of the rat dorsal hippocampus, whereas other σ ligands such as haloperidol act as antagonists, *i.e.* reverse this potentiation. We have shown that low doses of progesterone also reverse the potentiating effect of DHEA as well as those of several σ ligands. In late pregnancy, the levels of progesterone increase markedly. Thus, the present experiments were undertaken to determine if the potentiation induced by σ agonists is modified during pregnancy using an *in vivo* electrophysiological paradigm.

Glass micropipettes were used for extracellular recording of CA_3 pyramidal neurons in Sprague-Dawley rats. At day 18 of pregnancy, DTG (1 µg/kg, i.v.) did not modify the neuronal response to NMDA, whereas in control females this dose induced a three-fold increase of NMDA-induced activation. The potentiating effects of DHEA and of the σ agonist (+)pentazocine were also markedly attenuated in pregnant rats. At a dose of 10 µg/kg, DTG induced a two-fold increase of the neuronal response to NMDA in pregnant rats whereas, in control females, such a dose induced epileptoid responses to subsequent applications of NMDA. In contrast, a significantly greater potentiation of the NMDA response by DTGwas observed at post-partum day 5. At the post-partum days 10 and 15, the effect of DTG was back to normal.

These results suggest that the high levels of progesterone in late pregnancy dampen of the effect of σ receptor activation.

631.9

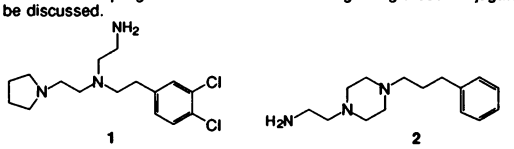
A NEARLY 2,000-FOLD AFFINITY-PURIFICATION OF *SIGMA* RECEPTORS SOLUBILIZED FROM RAT LIVER MEMBRANES: BIOCHEMICAL AND PHARMACOLOGICAL CHARACTERIZATION. Li.-I Tsao* and T.-P. Su. Molecular Pharmacology Section, DIR, NIDA/NIH, Baltimore, MD 21224.

σ receptors have been implicated in many physiological functions including learning and memory (Maurice *et al.*, *Pharmacol. Biochem. Behav.* 49: 859-869, 1994). However, σ receptors have not been sequenced or cloned. In this study, we continue to purify σ receptors from rat liver membranes. The liver was chosen since the σ receptor density is ten times higher in the liver than in the brain. σ receptors were solubilized from rat liver membranes by 3-[(3-cholamidopropyl)dimethylamino]-1-propanesulfonate (CHAPS) and subjected to an affinity chromatography utilizing a newly developed ligand DAPE (*N*-(2-[3,4-dichlorophenyl]ethyl)-*N*-(6-aminohexyl)-(2-[1-pyrrolidinyl])ethylamine). Solubilized σ receptors were adsorbed to the DAPE-coupled Sephadex G-25 resin and eluted with 10 mM haloperidol. This chromatography resulted in nearly 2,000-fold purification of σ receptors. The affinity-purified σ receptors were σ_1 receptors as they exhibited about 100-fold higher an affinity for (+)SKF-10047 than for (-)SKF-10047. Silver staining of the sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) of the affinity-purified σ receptors revealed a major diffuse band with a molecular mass of 31 kDa and minute amounts of a polypeptide near 65 kDa and a polypeptide larger than 97 kDa. CHAPS-solubilized σ receptors could be adsorbed to a wheat germ lectin Sepharose column and were eluted by *N*-acetyl-D-glucosamine. These results demonstrate a near-purity purification of σ receptors and show that highly-purified σ receptors retain appropriate pharmacological activity. The results also suggest that σ receptors in rat liver membranes are glycoproteins and that the main constituent of σ receptors in the rat liver membranes is a 31 kDa polypeptide.

631.10

CHARACTERIZATION OF NOVEL SIGMA LIGANDS FOR USE IN SYNTHESIS OF AFFINITY CHROMATOGRAPHY MATRICES AND BIOCONJUGATES. C. Torrence-Campbell*, Y. Zhang, W. Williams, B.R. de Costa and W.D. Bowen. Unit on Receptor Biochemistry and Pharmacology, Laboratory of Medicinal Chemistry, NIDDK, NIH, Bethesda, MD 20892.

Several primary amine derivatives, for example compounds **1** and **2** below, of the potent sigma ligands BD1008 (an aryl ethylene diamine) and BD1063 (an aryl piperazine) were synthesized and evaluated for sigma-1 and sigma-2 receptor affinity. The primary amine enables immobilization on column matrices or synthesis of bioconjugates. Since the ligands will be coupled via an amide linkage resulting from reaction of the primary amine with an appropriately activated carboxyl group in the acceptor molecule, the corresponding acetamide or N-Boc derivatives were synthesized and tested as well. The affinity of the primary amine for sigma-1 and sigma-2 receptors generally increased upon derivatization of the amine group, suggesting that there may be minimal loss of affinity in the conjugates. These compounds should be useful for the development of tools such as affinity chromatography matrices, biotinylated conjugates, and fluorescent probes for the further study of sigma receptors. Several compounds were coupled to CH-Sepharose-4B for affinity chromatography. Preliminary data also indicates successful coupling to imino-biotin. Results regarding these conjugates will be discussed.

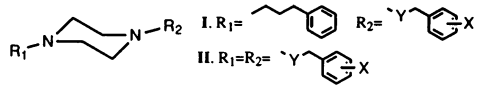


	1	2
sigma-1 K_i (nM):	40.7 ± 0.9	2.59 ± 0.09
sigma-2 K_i (nM):	135 ± 6	188 ±13

631.11

CHARACTERIZATION OF NOVEL N,N'-DISUBSTITUTED PIPERAZINES AS SIGMA RECEPTOR LIGANDS. Y. Zhang, W. Williams, K.C. Rice*, and W.D. Bowen. Laboratory of Medicinal Chemistry, NIDDK, NIH, Bethesda, MD 20892.

Recent studies have indicated that sigma receptors may be involved in a wide array of biochemical, physiological, and behavioral processes, including: regulation of motor behavior, neuroprotective and neurodegenerative effects, and modulation of NMDA, muscarinic, and dopamine receptor systems. Further progress will be enhanced by selective, high affinity ligands with agonist or antagonist activity. The N,N'-disubstituted aryl piperazine, BD1063 (1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine), has recently been shown to have antagonist activity in the drug-induced dystonia model (Matsumoto et al., *Soc. Neurosci. Abstr.* 19:421, 1993; *Eur. J. Pharmacol.*, 1995, in press). This suggests that other sigma-active substituted piperazines may also have antagonist activity. We have now synthesized a series of symmetrical and assymetrical N,N'-disubstituted piperazine compounds (I, II) to investigate the effect of N-substitution, aromatic substitution, and introduction of an amide function on receptor binding affinity. Both groups possess an alkylaryl ethylenediamine unit which, for this series, is the minimum structural requirement to display sigma binding activities based upon a previous observation (de Costa et al., *J. Med. Chem.* 36:2311-2320, 1993). The synthesis and binding affinities at sigma-1 and sigma-2 receptors will be presented.



631.12

Structural Requirements for σ_3 Receptor Binding and Function E.C.Bucholtz, C.E. Owens, P.C. Hooper, S.D. Wyrick*, R.G. Booth, R.B. Mailman, N.S. Kula, R.J. Baldessarini. Division of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599 and Harvard Medical School and McLean Hospital, Belmont, MA 02178

The 1-phenyl-3-amino-1,2,3,4-tetrahydronaphthalines (1-phenyl-3-aminotetralins, PATs) have previously been shown to stimulate tyrosine hydroxylase activity as well as dopamine synthesis in rat brain through interaction with the neuromodulatory σ_3 receptor. Experiments with resolved (±)-H_2-PAT indicate that the activity presides in the *trans* -(1R,3S)-(−) isomer of H_2-PAT. Structural requirements for binding were examined by altering the position and substitution pattern of the amino group and other functional groups on the tetralin moiety. A ligand based receptor model has been established using several PAT and non-PAT compounds. Two non-PAT compounds that exhibit high binding affinities are GBR-12909 and Ketanserin. These compounds have large N-alkyl substituents, that are well accommodated within the receptor model. Glennon has also reported that large N-phenylalkyl substituents on aminotetralins provide high affinity binding to the σ_1 receptor. To test how the σ_3 receptor might accommodate these large N-phenylalkyl substituents, we synthesized a series H_2 PAT analogs that include this moiety. Results indicate that this modification not only reduces affinity for the σ_3 receptor, but also increases affinity for σ_1 / σ_2 receptors negating the previous selectivity for σ_3. This data has been used to refine our receptor binding model. To further enhance the model, we have also undertaken the resolution of *cis*-(±)-H_2-PAT, an antagonist, which also has high affinity and selectivity for the receptor site, and to determine if these enantiomers follow the above noted stereoselective trend. This was accomplished by recrystallization techniques and the absolute configuration of *cis*-(+) H_2 -PAT was found to be (1R,3R) as determined by x-ray crystallography. [Support: URC Grant 5-44786, Pharmacy Foundation of NC #6-68379]

631.13

STRUCTURE-ACTIVITY RELATIONSHIPS FOR PHENYLAMINOTETRALINS AT [^{3}H]-PAT LABELED σ_3 RECEPTORS IN RODENT BRAIN. CE Owens*, EC Buchholtz, PC Hooper, AM Myers, NS Kula, RJ Baldessarini, RB Mailman, SD Wyrick, RG Booth. Division of Medicinal Chemistry, School of Pharmacy & Brain and Development Research Center, University of North Carolina, Chapel Hill, NC 27599 & Harvard Medical School and McLean Hosp., Belmont MA 02178.

We recently described a novel brain sigma (σ) receptor that is recognized by certain *trans*-1-phenyl-3-aminotetralin (PAT) derivatives and modulates dopamine biosynthesis. Functional and receptor binding and mapping studies in brain with [^{3}H]-(−)-*trans*-H_2-PAT indicate that the site has σ receptor characteristics inconsistent with known σ_1/σ_2 receptors, therefore the site was designated σ_3 (*Soc. Neurosci. Abstr.*, 1994, **20**, 748). We are further characterizing the biochemical pharmacology of the σ_3 receptor by synthesizing novel PAT analogs designed to elucidate the structure-activity relationship (SAR) of PATs at σ_3 receptors. Affinity ($K_{0.5}$) is determined using competition binding assays for [^{3}H]-PAT labeled σ_3 receptors in guinea pig brain membranes (for comparison, 3-*N*,*N*-dimethlyl-H_2-PAT $K_{0.5}$=0.36 nM). The steric and electronic nature of the nitrogen binding domain is assessed by altering the substitution pattern of the 3-amino group. 3-*N*-monomethyl-H_2-PAT shows decreased affinity ($K_{0.5}$=112 nM) suggesting that the two methyl groups are important for hydrophobic interactions. 3-*N*,*N*,*N*-trimethyl-H_2-PAT (the quaternary salt) also has reduced affinity ($K_{0.5}$=35 nM) indicating that the ability to form a hydrogen bond as well as an ionic bond is essential for high affinity. 3-*N*-methyl-*N*-phenethyl, -propyl, and -butyl substituents significantly reduces affinity ($K_{0.5}$=140, 332, and 212, respectively), consistent with earlier observations that increased steric bulk on the nitrogen reduces σ_3 binding affinity. The corresponding 3-*N*-normethyl-*N*-phenalkyl analogs are essentially inactive ($K_{0.5}$>2 μM). Ring-expanded 5-phenyl-7-amino-5,6,7,8-tetrahydrobenzocycloheptenes retain moderate affinity ($K_{0.5}$=40 nM) but altering the location of the nitrogen to the 2-position greatly reduces σ_3 binding affinity ($K_{0.5}$ *ca.* 1 μM). The 1-methyl substituted analog has moderate affinity ($K_{0.5}$ =34 nM) suggesting that added steric bulk is detrimental. The orientation and interactions (hydrophobic and electrostatic) of the appended phenyl ring with the receptor are currently being evaluated. These studies should lead to the design of potent, selective σ_3 receptor ligands. [Support: UNC-544786 and Pharmacy Foundation of NC]

631.14

Binding sites for sigma ligands in rat spleen: differential localization of three distinct sites. P. Saini, B.B. Whitlock, Y. Liu, R.H. Ho* and S.A. Wolfe, Jr. Departments of Molecular Genetics, Cell Biology/Neurobiology and Anatomy, Medical Microbiology and Immunology, Ohio State University, Columbus, OH 43210.

We have used in vitro receptor autoradiography to determine the distribution in rat spleen of binding sites for sigma receptor ligands. σ_1 sites were labeled with 5nM [^{3}H]pentazocine in the presence of 100μM naltrexone, and nonspecific binding was defined using 1μM haloperidol. σ_2 sites were labeled with 10nM [^{3}H]DTG in the presence of 1μM dextrallorphan and 100μM naltrexone, and nonspecific binding was defined using 2μM haloperidol. Novel binding sites for sigma ligands that we have recently described (see accompanying abstract) were labeled with 8nM-1μM [^{3}H]-(+)-3-PPP in the presence of 10-50μM haloperidol, and nonspecific binding was defined using 100μM naltrexone. σ_1 sites localized evenly through the outer (T cell-rich) regions of the periarterial lymphatic sheath of the white pulp. σ_2 sites were distributed in a smooth pattern including the red pulp and the marginal zones of the white pulp. Very little σ_2 labeling was seen in the lymphocyte-rich regions of white pulp. The novel sites localized with a coarse, punctate pattern to the marginal zones and red pulp. While the distribution of σ_2 sites is difficult to interpret, these results suggest that σ_1 receptors may be present primarily on T cells, while the novel sites may be quite dense on macrophages or dendritic cells.

631.15

Preliminary molecular characterization of novel, haloperidol- and DTG-inaccessible binding sites for sigma and opiate ligands in rat spleen. S. Chang, B.B. Whitlock, J. Moyers, S.A. Tjioe* and S.A. Wolfe, Jr. Departments of Medical Microbiology and Immunology, Pharmacology, Ohio State University, Columbus, OH 43210.

The present study is a preliminary molecular characterization of a novel drug binding site in rat and human immune tissues which fails to bind DTG or haloperidol, but does bind other sigma and opiate ligands including naltrexone, SKF10,047, pentazocine, 3-PPP, dextrorphan, dextromethorphan, and PCP. We labeled this site in rat splenic homogenates with 1μM [^{3}H]-(+)-3-PPP in the presence of 50μM haloperidol, and defined nonspecific binding using 10μM (+)-SKF10,047. Binding was obliterated by boiling or treatment with 100-200μg/ml phospholipase C, but was resistant to trypsin, chymopapain, pronase, neuraminidase and α-amylase. We failed to solubilize binding activity using CHAPS, Triton X-100, CTAB or sodium cholate. 0.025 - 0.05% SDS did solubilize binding activity, but higher concentrations destroyed all binding. Our conclusions are: (1) although these novel binding sites are protease resistant, their susceptibility to denaturation by boiling suggests they may be proteins; (2) binding sites can be solubilized by low concentrations of SDS; (3) the destruction of binding by higher concentrations of SDS and by phospholipase C suggests phospholipid components, or a lipid environment, are critical for function. Further work to more fully characterize this site is in progress.

631.16

T lymphocyte production of interleukin 2 (IL-2) is regulated by sigma-1 receptors. B.B. Whitlock and S.A. Wolfe, Jr.* Department of Medical Microbiology and Immunology, Ohio State University, Columbus, OH 43210

We have previously demonstrated sigma receptors (σ_1 and σ_2) in immune tissues and have shown that proliferation of T cells is suppressed by sigma agonists through interaction with σ_1 receptors. The objective of the current study was to determine whether this effect was due to suppression of the proliferation-supporting cytokine, IL-2. We measured σ_1 receptors in saturation binding assays using [^{3}H]-(+)-pentazocine, which bound mouse spleen and the IL-2 producing LBRM33 T cell line with K_d values of 10.1±1.0 and 10.7±0,3nM, respectively. LBRM33 cells displayed approximately 40,000 binding sites/cell. We confirmed the σ_1 identity of the labeled sites by determining that they bound haloperidol and DTG, and were selective for the (+) over the (-)-enantiomer of N-allylnormetazocine ($SKF_{10,047}$). We then determined that sigma agonists could suppress PHA-induced IL-2 production by LBRM 33 cells. The ability of sigma agonists to suppress IL-2 (EC_{50}) correlated well (r = 0.84) with their affinity at σ_1 receptors (K_i). A good correlation (r = 0.91) also existed between suppression of T cell proliferation and suppression of IL-2 production. Our conclusions are: (1) suppression of IL-2 production by sigma agonists is due to drug interactions with σ_1 receptors on T cells; (2) inhibition of T cell proliferation by sigma agonists in prior studies was due to this suppression of IL-2 production.

631.17

B lymphocyte proliferation is not regulated by sigma receptors. Y. Liu, C.C. Whitacre* and S.A. Wolfe, Jr. Department of Medical Microbiology and Immunology, Ohio State University, Columbus, OH 43210.

In contrast to their inhibitory actions on T cells, sigma agonists have been reported to potentiate B cell activities. In the present study, we tested the ability of sigma agonists to modulate proliferation of purified mouse B cells stimulated with antibody (anti-μ) directed against cell surface immunoglobulin. The sigma agonists DTG and (+)- and (-)-pentazocine had no effect, but haloperidol enhanced the proliferation of B cells and lowered the threshold of anti-μ antibody needed to trigger cell proliferation. Since haloperidol can also act as an antagonist at D_2 and 5-HT_2 receptors, several D_2 and 5-HT_2 antagonists were also tested. Spiperone, but not other D_2 and 5-HT_2 antagonists, potentiated B cell proliferation. Our conclusions are: (1) since the classical sigma agonists DTG and (+)- and (-)-pentazocine failed to show any effect, sigma receptors or our newly described, novel sites probably do not modulate B cell proliferation; (2) the enhancement of proliferation by haloperidol and spiperone is probably not mediated through D_2 or 5-HT_2 receptors; (3) at this time, the mechanism of haloperidol's and spiperone's actions on B cells is unknown.

CATECHOLAMINE RECEPTORS: ALPHA ADRENERGIC

632.1

ALPHA-2A- AND 2C-ADRENOCEPTOR- LIKE IMMUNOREACTIVITIES (-LIs) IN THE RAT CNS. C. Broberger, L. Stone†, A. Szallasi*, R. Elde† and T. Hökfelt. Department of Neuroscience, Karolinska Institutet, 17177 Stockholm, Sweden and †Department of Cell Biology and Neuroanatomy, Univ. of Minnesota, MN 55455, USA.

Adrenoceptors are grouped into α_1, α_2, β_1 and β_2 types. α_2-adrenoceptors can be further divided into A-, B- and C-types. mRNAs for these α_2-receptor subtypes have been detected and found to be differentially localized in the CNS by in situ hybridization and Northern blot. Immunohistochemistry and binding studies also confirm the heterogeneity of α_2-receptor subtypes in the CNS. In this study, antibodies were developed by immunizing rabbits with thyroglobulin-conjugated peptides corresponding to the C-terminal portion of the α_{2A}- and α_{2C}-adrenoceptors. Antibodies were utilized for immunohistochemistry on formalin-fixed sections of rat brain using the indirect immunofluorescence method. α_{2A}-adrenoceptor-LI was found in perikarya in many regions of the brain, e.g. the hypothalamus, amygdala and pons, and in fibers e.g in the forebrain and amygdala. α_{2C}-adrenoceptor-LI was detectable in cell bodies e.g. in the cerebral cortex, hypothalamus and pons, with fibers innervating large parts of the brain. Immunoreactivity for both receptors was seen in the dorsal horn of the spinal cord. Specificity of staining was controlled for by pre-absorbing the antibodies with their corresponding antigen. Also, antibodies raised in different rabbits against the same antigen showed the same staining patterns.

632.2

PARTIAL PURIFICATION OF RECOMBINANT HUMAN α_2C2-ADRENOCEPTORS FROM YEAST CELLS. S.Ala-Uotila, U. Petäjä-Repo, K. Keinänen, A. Marjamäki*, M. Scheinin and M. Jalkanen, Dept. of Pharmacology and Centre for Biotechnology, Univ. of Turku, PO Box 123, FIN-20521 Turku, Finland, VTT Biotechnical Laboratory, PO Box 202, FIN-02151 Espoo, Finland.

Recombinant human α_2C2-adrenoceptors were solubilized and partially purified from *Saccharomyces cerevisiae* cells. The α_2C2-construct expressed in yeast cells was modified in its N-terminus where the first nine amino acids of the human α_2C2-adrenoceptor were replaced by the peptide MDYKHHHHH. The B_{max} of the resulting clone FH-11 was 40 pmol/mg total cellular protein. Recombinant α_2C2-receptors were solubilized with the non-ionic detergent sucrose monolaurate. The solubilization yield was approximately 50%. The expressed histidine-tagged protein was partially purified by metal-affinity chromatography. The presence of α_2C2-adrenoceptors in the soluble fraction, in the pellet and in metal affinity chromatography fractions was visualized by immunoblotting with a rabbit polyclonal antibody. The yield of this step was 80-90% as determined by computer densitometer of SDS-PAGE immunoblots.

632.3

ACTIVATION OF THE HUMAN α_2A, α_2B, AND α_2C ADRENERGIC RECEPTOR SUBTYPES STIMULATES MAP KINASE ACTIVITY IN COS CELLS. W.D. Stamer, R.P. Gruener*, T.H. Burkey, and J.W. Regan. Depts of Pharm/Tox and Physiology, The University of Arizona, Tucson, AZ 85721.

Activation of α_2-adrenergic receptors (AR) modulates various cellular second messenger systems including adenylyl cyclase and mitogen-activated protein (MAP) kinase. In stably transfected CHO cells, it has been previously shown that the rat α_2A and α_2B subtypes couple to MAP kinase whereas the rat α_2C does not. The purpose of this study was to determine the subtype-specific coupling of the human α_2-ARs to MAP kinase in transiently transfected COS cells. COS cells were transfected with 50 μg of plasmid DNA encoding either the α_2A, α_2B or α_2C using DEAE-dextran/DMSO shock. Cells were incubated in the presence of serum for 48 hours and then were serum starved for 24 hours. Cells were stimulated with either phorbol myristate acetate (100 nM), epinephrine (10 μM), dexmedetomidine (100 nM), or dexmedetomidine (100 nM) plus yohimbine (10 μM) and lysates were prepared. MAP kinase activity was measured by the incorporation of γ^{32}P-ATP into myelin basic protein. Activation of all three human α_2-ARs by agonists resulted in a significant increase in the activity of MAP kinase over controls (unstimulated); e.g. dexmedetomidine yielded a 1.8 fold increase for the α_2A, a 1.7 fold increase for the α_2B and a 1.6 fold increase for the α_2C. Preicubation of cells for 30 minutes with yohimbine significantly reduced activation of MAP kinase by agonists. Western blot analysis further showed that the amount of immunoreactive protein was the same in both control and agonist treated cells which is consistent with an increase in the activity, rather than, the amount of MAP kinase. In conclusion, all three human α_2-AR subtypes are capable of stimulating MAP kinase in transiently transfected COS cells.

632.4

COTRANSFECTION OF α_2C10 ADRENERGIC RECEPTOR WITH G-PROTEIN ALPHA SUBUNITS DRAMATICALLY INCREASES AGONIST POTENCY IN PI AND R-SAT ASSAYS. D.W. Gil*, T. Messier, D. Eubanks, L.A. Wheeler and M.R. Brann, Biological Sciences, Allergan, Inc., Irvine, CA 92713-9534 and Receptor Technologies, Inc. Winooski, VT 05404.

The fact that G protein coupled receptors can activate multiple signaling pathways, often with substantially different EC50s, has led to the suggestion that different receptor conformations are necessary for activating various G proteins. We investigated this possibility by studying the activation of several signaling pathways by the α_2C10 receptor coexpressed with G protein alpha subunits. The α_2-C10 receptor transiently transfected into COS-7 cells mediated a weak phosphoinositide (PI) response. UK14304, for instance, elicited a 2-fold stimulation (EC50=155 nM) while the oxymetazoline (EC50=33 nM) response was only 25% over basal levels. Since α_2 receptors couple primarily to G_i, the receptor was coexpressed with $\alpha_{q/i5}$, a G protein alpha subunit chimera in which the five C-terminal amino acids of α_q are replaced with the corresponding residues of α_{i2} so that it mimics the receptor coupling of α_i (Conklin et al., Nature 363, 274-6). The PI signal was enhanced 3-6 fold and compounds were 10-fold more potent, approximating their potency at inhibiting adenylate cyclase. The results were even more dramatic in the Receptor Selection and Amplification Technology (R-SAT) assay, a high-throughput reporter gene assay that measures the G_q-dependent amplification of NIH-3T3 cells. The coexpression of $\alpha_{q/i5}$ enhanced the oxymetazoline response more than six-fold and shifted the EC50 from 2800 nM to 2.3 nM. In order to determine whether the potency shifts were due to more efficient coupling of $\alpha_{q/i5}$ or alpha subunit overexpression, the receptor was coexpressed with α_q resulting in a three-fold enhancement of the R-SAT signal and a similar increase in agonist potencies (oxymet. EC50=4.6 nM). Thus, large differences in an agonist's potencies for activating multiple signal pathways via a single receptor can be attributed to differences in G protein reserve and receptor/G protein coupling efficiency.

632.5

α_2C10 ADRENERGIC RECEPTOR WITH MINIMAL α_1A THIRD INTRACELLULAR LOOP ELICITS α_1A SIGNAL IN RESPONSE TO α_2 AGONISTS. K.M. Kedzie, D. Eubanks, E.P. Monaghan*, M.R. Brann and D.W. Gil. Allergan, Inc., Irvine, CA 92713-9534, University of Vermont, Burlington, VT 05405 and Receptor Technologies, Inc., Winooski, VT 05404.

The α_2 receptors are primarily coupled to inhibition of adenylate cyclase, which is a difficult response to measure in high throughput drug screening. We determined whether the signaling repertoire of the α_2C10 receptor could be rewired by replacing its third intracellular (i3) loop with portions of the α_1A receptor i3 loop that have been implicated in G-protein coupling. A chimeric receptor was constructed by replacing the i3 loop of the α_2C10 receptor with 22 and 20 amino acids of the amino and carboxyl ends, respectively, of the α_1A i3 loop. In phosphoinositide hydrolysis (PI) assays and the high-throughput R-SAT assays (patent pending), the α_2 agonist, UK14304, but not the α_1 agonist, phenylephrine, elicited responses that were blocked by the α_2 antagonist rauwolscine. Using both assays, the rank order of potency for a series of compounds was the same for activation of the α_2C10 and the α_2/α_1 receptors. Unlike the parent α_2C10 receptor, but like an α_1 receptor, the α_2/α_1 PI response was not sensitive to pretreatment with pertussis toxin. In an assay of cyclic AMP accumulation, using a cyclic AMP-responsive CAT reporter gene, the chimeric receptor no longer inhibited cyclic AMP levels. Interestingly, it retained the ability to stimulate cyclic AMP accumulation. Thus, replacement of the i3 loop of the α_2C10 receptor with two fragments from the α_1A i3 loop alters its signaling repertoire without changing its pharmacology.

632.6

ANTISENSE KNOCK-DOWN OF α_{2C}-ADRENOCEPTORS IN RAT STRIATUM. Lingen Lu* and Gregory A. Ordway. Departments of Psychiatry & Human Behavior and Pharmacology & Toxicology, University of Mississippi Medical Center, Jackson, MS 39126.

The predominate subtypes of α_2-adrenoceptors in the brain are α_{2A} and α_{2C}. The shortage of selective ligands for each of the α_2-adrenoceptor subtypes hampers the functional characterization of these receptors. We exploited an antisense strategy as an alternative pharmacological tool to study α_{2C}-adrenoceptors. In rat corpus striatal membranes, α_2-adrenoceptors were characterized using the subtype-nonselective antagonist [^{3}H]2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline ([^{3}H]RX821002). Specific [^{3}H]RX821002 binding was saturable and to a single class of high affinity sites (K_D = 0.45 ± 0.04 nM; Bmax = 159 ± 3 fmol/mg protein). Curves for the inhibition of [^{3}H]RX821002 binding by the α_{2C}-selective compound, prazosin, were fit best by a model assuming binding to two sites, (%R_H = 41%; %R_L = 59% ; $p < 0.005$), presumably reflecting binding to α_{2C}- and α_{2A}-adrenoceptors, respectively. A 15-mer phosphorothiate oligodeoxynucleotide (α_{2C}AS) complementary to α_{2C}-adrenoceptor mRNA, or a random sequence (RS), was administered to rats continuously for 4-5 days directly into the striatum. α_{2C}AS treatment significantly reduced the Bmax of [^{3}H]RX821002 in striatal homogenates (in fmol/mg protein, RS = 151 ± 10; α_{2C}AS = 98 ± 8; $p < 0.01$). Curves for the inhibition of [^{3}H]RX821002 binding by prazosin were fit best to a model assuming a single site interaction in α_{2C}AS-treated rats and to a model assuming two sites of binding in RS-treated rats. These results confirm the existence of α_{2A}- and α_{2C}-adrenoceptors in the rat corpus striatum and also demonstrate the feasibility of an antisense approach to studies of α_2-adrenoceptor pharmacology.

632.7

DISSOCIATION OF ANESTHETIC ACTIONS OF α_2 AGONISTS FROM A DECREASE IN CYCLIC AMP IN THE RAT. B. C. Rabin, T-Z. Guo, L. R. Poree*, and M. Maze. Stanford Univ. Sch. of Med., Stanford, CA 94305.

α_2 adrenergic agonists (α_2 AA) are used clinically for their anesthetic, analgesic and sympatholytic actions in surgical patients. All α_2 adrenergic receptors (α_2 AR), when activated by α_2 AA's, are able to inhibit adenylate cyclase. Correspondingly, elucidation of the role of adenylate cyclase in α_2 AR-elicited biologic responses is of principal importance to understanding the transduction mechanisms of these receptors.

In this study we have examined the α_2 AR-mediated anesthetic actions of dexmedetomidine, a highly selective α_2 AA, after pretreatment of the animals with rolipram, a cAMP-specific phosphodiesterase inhibitor. Cyclic AMP (cAMP) accumulation and monoamine turnover were measured in the locus coeruleus (LC) and hippocampus (Hc) following administration of rolipram (275 $\mu g \cdot kg^{-1}$ i.p.) and dexmedetomidine(100 $\mu g \cdot kg^{-1}$ i.p.). The hypnotic response to dexmedetomidine was also measured in these animals. In other experiments, rats were stereotaxically cannulated in the LC with an indwelling catheter, and after the second day, the tail flick analgesic response to dexmedetomidine (7 $\mu g \cdot 0.2 ul^{-1}$ LC) , following rolipram (275 $\mu g \cdot kg^{-1}$ i.p.) pretreatment, was assessed.

In the presence of elevated cAMP levels, the hypnotic, analgesic, and sympatholytic effects of dexmedetomidine persisted. These data suggest that adenylate cyclase activity does not mediate the cellular responses to α_2 AAs but instead may act in concert with other α_2 AR-coupled effector mechanisms to transduce the anesthetic actions of these agents.

632.8

A POSSIBLE EXPLANATION FOR THE LACK OF A PALMITOYLATION SITE IN THE CARBOXY TERMINUS OF THE α_2C ADRENERGIC RECEPTOR. A.C. Porter, H.E. Laird, II* and J.W. Regan. Dept. of Pharmacology and Toxicology, University of Arizona, Tucson, AZ 85721

Most adrenergic receptors (AR), including the α_2A and α_2B subtypes, have a carboxy terminal cysteine which is a potential site for post-translational modification by palmitoylation. Palmitoylation of this cysteine has been hypothesized to form a fourth intracellular loop which may be involved in receptor/G-protein coupling. The α_2C subtype, on the other hand, has phenylalanine instead of cysteine at this position. In these experiments, phenylalanine 452 in the α_2C was mutated to cysteine (F452→C) and was then evaluated in terms of its ability to be expressed, its second messenger coupling and its ability to bind [^{3}H]rauwolscine, an α_2 selective antagonist. Immunohistochemical analysis using an α_2C specific antibody directed against the third intracellular loop, indicates that the mutant receptor is expressed in COS-7 cells at levels which are comparable to the wild type receptor. In contrast, when transiently expressed in JEG-3 cells, the F452→C mutant was unable to inhibit the activity of a forskolin-stimulated cyclic AMP responsive reporter gene. This indicates that the mutant receptor is non-functional. The F452→C mutant also did not show any specific binding of [^{3}H]rauwolscine. However, prior treatment of the membranes with hydroxylamine, followed by washout, restored some specific binding of [^{3}H]rauwolscine. Since hydroxylamine can selectively hydrolyze thioester linkages between palmitic acid and cysteine, these results suggest that palmitoylation is not tolerated in the carboxy terminus of the α_2C AR.

632.9

α_2-ADRENERGIC RECEPTOR AGONIST ACTIVITY OF DESIPRAMINE IN GT1 CELLS. P.G.Guyenet*, D. Huangfu, Y. Zhu, and R. L. Stornetta. Dept. of Pharmacology and Neuroclinical Trials Center, Univ. of Virginia, Charlottesville, VA 22908.

Experiments were designed to test whether the tricyclic antidepressant desipramine (DMI) has agonist activity at α_{2A}-adrenergic receptors (α_{2A}-ARs), a receptor subtype involved in CNS sympathetic control. In the presence of blockers of dopamine and β-adrenergic receptors, α-methyl noradrenaline (MNE), clonidine and UK14304 dose-dependently inhibited the forskolin-stimulated (2 μM) accumulation of cAMP by GT1 cells ($\log_{10}ED_{50}$: -5.3, -6.8, -6.2; max inhibition: 38%, 25% and 32%, respectively). These inhibitions disappeared after pertussis toxin treatment and were blocked by either yohimbine or methoxyidazoxan (MOI 10μM). Yohimbine and MOI had no effect on cAMP accumulation by themselves. DMI (10^{-8} to 10^{-5} M) dose-dependently inhibited forskolin-stimulated cAMP production (50±3% at 10^{-5} M). The effect of DMI was abolished in pertussis toxin treated cells and was attenuated 70% by yohimbine or MOI. No evidence was found that DMI might have partial agonist activity at the α_{2A}-ARs of GT1 cells. Amitriptiline (AMI), another tricyclic antidepressant, also reduced cAMP accumulation (56±4% at 10^{-5} M) but this effect was reduced only 15% by MOI. The effect of 1μM AMI was eliminated by pertussis toxin treatment, while that of 10μM AMI was reduced by 40%. In conclusion, DMI is a full agonist at α_{2A}-ARs in GT1 cells. CNS α_{2A}-AR stimulation probably contributes to DMI's sympatholytic effect. AMI has little α_{2A}-AR agonist activity but it and DMI may also activate other G-protein coupled receptors negatively linked to adenylyl cyclase in GT1 cells (Grant HL-28785 to PGG).

632.10

MEDIAN PREOPTIC NUCLEUS (MnPO) NEURONS POSSESS FUNCTIONAL α1 AND α2 ADRENERGIC RECEPTORS. D. Bai* and L.P. Renaud. Neuroscience, Loeb Research Institute, Ottawa Civic Hospital and University of Ottawa, Ottawa, Ontario, CANADA K1Y 4E9

The MnPO is an important CNS site for hydromineral and cardiovascular homeostasis. In addition to local amino acid and peptidergic inputs, MnPO receives a prominent innervation from medullary catecholaminergic neurons. To define the nature of this adrenergic innervation, we utilized whole cell patch clamp recordings obtained in vitro from MnPO neurons in coronal and saggital slices of rat forebrain. Some 20% of cells responded to brief (10-30 sec) applications of noradrenaline (NA, 50μM) or phenylephrine with a TTX-resistant, prazosin-sensitive membrane depolarization; in some cells a reduction in membrane conductance was observed. Another 20% of cells responded to NA or the α2 agonist UK14,304 with a TTX-resistant yohimbine-sensitive membrane hyperpolarization accompanied by an increase in a membrane K^+ conductance. This diversity in catecholamine receptor neuropharmacology among MnPO cells supports functional heterogeneity in their responsiveness to diverse cardiovascular stimuli. Supported by MRC and the Heart & Stroke Foundation of Canada.

632.11

COMPARATIVE EXAMINATIONS ON SOME EFFECTS OF ALPHA$_2$-ADRENOCEPTOR AGONISTS WITH DIFFERENT LIPOPHILICITY (DEXMEDETOMIDINE AND ST-91) M. Szikszay, G. Horváth, G. Szabó*, G. Benedek Dept. Physiol, A. Szent-Györgyi Med. Univ, Szeged, Hungary

Alpha$_2$-adrenoceptor agonists have sedative and mydriatic effects that originate supraspinally. There analgesic effect is mediated predominantly at the level of the spinal cord. Because intrathecal (IT) administration of a lipophilic compound can cause supraspinal effects, it is necessary to compare a hydrophilic (ST-91) and a lipophilic (dexmedetomidine, DEX) alpha$_2$-adrenoceptor agonists given IT to evaluate whether the effects result from actions at spinal *vs* supraspinal sites. Additionally, we examined whether the enhanced urine flow induced by these drugs might be modulated by centrally and/or peripherally.

The IT administration of DEX led to dose-dependent analgesia (tail-flick, TF and hot-plate HP tests) and mydriasis, although the mydriasis appeared at subanalgetic dose (1 μg). The effective analgetic dose (9 μg), however, caused a visible sedation and motor impairment. The ST-91 (20 μg IT) caused dose-dependent analgesia only at HP test, without any mydriasis, sedation or motor impairment. Systemic administration of both agents caused an increased urine output volume, the intrathecally applied ST-91, however, failed to influence it, suggesting a peripheral site of action. Our results clearly proved the effects of the IT applied highly-lipophilic alpha$_2$-adrenoceptor agonist, DEX, may result from a rapid diffusion into the general circulation and than into the brain. These observed discrepancies suggest that consideration should be paid not only the route of administration, but also to the physico-chemical properties of a given compound.

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632.12

MODULATION OF RECOMBINANT HUMAN α_2-ADRENOCEPTORS BY SODIUM: EFFECTS ON AGONIST BINDING. M. Halme, B. Sjöholm and M. Scheinin*. Department of Pharmacology and Centre for Biotechnology, University of Turku, FIN-20520 Turku, Finland.

Sodium ions modulate agonist binding to several G-protein coupled receptors, presumably by interfering with a charge interaction of a conserved aspartate residue that participates in the maintenance of the active conformation of the receptor proteins. This study utilized recombinant human α_2-adrenoceptor subtypes (α_{2A}, α_{2B}, α_{2C}) expressed in S115 mouse mammary tumour cells. The osmolality of the binding medium was held constant using 35 or 75 mM N-methyl-D-glucamine$^+$Cl$^-$ buffer (pH 7.4). The affinities of all three α_2-adrenergic receptor subtypes for the α_2-antagonist radioligand [^{3}H]RX821002 were increased in the presence of 40 mM NaCl. The increase in affinity was 1.5-fold for α_{2A}, 3-fold for α_{2C} and 4-fold for α_{2B}. NaCl potently decreased the affinity of catecholamine agonists at α_{2A}-adrenoceptors, whereas the affinities of clonidine and dexmedetomidine were increased 3.5-fold. The potency order of monovalent cations was $Na^+ > Li^+ >> K^+$ for (-)-noradrenaline binding to α_{2A}-receptors. At α_{2C}-receptors, the affinities of (-)-noradrenaline and dexmedetomidine were clearly reduced by Na^+, whereas the affinity of clonidine was not influenced. At α_{2B}-receptors, the affinity of dexmedetomidine was reduced by Na^+, but the affinity of noradrenaline was only slightly decreased and clonidine binding was not affected. The results indicate that the effects of Na^+ on agonist affinity to α_2-adrenoceptors are complex and may depend on ligand structure. The concentration of Na^+ in the binding assay may critically affect the apparent receptor subtype selectivity of drugs.

632.13

HIGH AFFINITY AGONIST BINDING IN TRANSFECTED CLONED HUMAN α_2-ADRENOCEPTORS. A. L. KIRIFIDES* and E. E. CODD. Drug Discovery Research, The R.W. Johnson Pharmaceutical Research Institute, Welsh and McKean Roads, Spring House, PA 19477-0776.

Human α_{2A}-, α_{2B}- and α_{2C}-adrenergic receptors belong to the family of membrane bound G-protein coupled receptors regulated by mono and divalent cations and guanine nucleotides. Whereas many previously published radioligand binding assays used conditions conducive to formation of the agonist low affinity state of the receptor (Uhlen, JPET, 1994), the present study was performed using 50 mM Tris buffer in the presence of 5 mM $MgCl_2$ to promote formation of the agonist high affinity state. The cloned receptors were expressed in insect cells with a baculovirus expression vector (Biosignal). The α_2-antagonist [^{3}H]-MK-912 was used as the ligand due to its high affinity for α_2-adrenoceptors and low non-specific binding. Saturation studies fit a single site model with K_d values of 0.405 for α_{2A}- and 0.114 nM for α_{2C}-, similar to the results of Uhlen. The inhibition curve of the α_2-antagonist yohimbine fit a single site model with K_i values of 5.0 nM at the α_{2A}- and 5.69 nM at the α_{2C}-adrenergic receptor. However, the inhibition curve of the α_2-agonist clonidine was best fit by a two site model with K_i values of 22.9 and 328 nM (p= 0.023) for α_{2A}- and 160 and 1395 nM (p<0.0005) for α_{2C}-receptors. These and related data suggest that the present assay conditions distinguish agonist from antagonist binding to cloned α_2-adrenoceptors, greatly enhancing the usefulness of this assay as a pharmacological tool.

632.14

AGONIST SPECIFIC COUPLING OF A CLONED HUMAN α2A-ADRENERGIC RECEPTOR TO MULTIPLE SECOND MESSENGERS. P.D.Evans[1*], C.N.Airriess[1], L.S.Swales[1], T.R.Cheek[1] and J.M.Midgley[2]. [1]The Babraham Institute Laboratory of Molecular Signalling, Dept. Zoology, Univ. Cambridge, Cambridge, CB2 3EJ, U.K. and [2]Dept. Pharmaceutical Sciences, Univ. Strathclyde, Glasgow, G1 1XW, U.K.

Agonist-specific coupling of a cloned *Drosophila* octopamine/tyramine receptor, expressed in a Chinese hamster ovary (CHO) cell line, has been demonstrated by us (Robb *et al.*, 1994, EMBO J. 13:1325). Thus, octopamine and tyramine, which differ structurally by only a single side-chain hydroxyl group, differentially couple the receptor to cyclic AMP and Ca^{2+}-based second messenger systems. Here we report on the ability of octopamine, which is a naturally occurring ligand of sympathetic α-adrenergic receptors, to couple a cloned human α2A-adrenergic receptor (α-2AR) to multiple second messenger systems when expressed in a CHO cell line. The ability of agonists to inhibit or stimulate forskolin-stimulated cyclic AMP levels in this cell line was assessed in the presence or absence of pertussis toxin and their ability to change cytosolic Ca^{2+} levels was assessed using ratio imaging techniques with the dye fura 2. (-)*m*-Octopamine was more potent than either (+)*m*-octopamine or (-)*p*-octopamine in inhibiting adenylate cyclase activity in CHO cells expressing the α-2AR and both enantiomers of the positional isomers of octopamine were less potent than either (-)-epinephrine or (-)-norepinephrine. However, in contrast to the catecholamines, both positional isomers of (-)-octopamine did not stimulate adenylate cyclase activity in cells incubated with pertussis toxin, indicating that the α-2AR is coupled to G_i but not G_s when activated by octopamine. Another difference between *m*-octopamine and the catecholamines was the ability of very low concentrations (10^{-12} to 10^{-9}M) of the former, but not the latter, to initiate cytosolic Ca^{2+} signals. These studies suggest a functional role for the octopamine co-released with norepinephrine from sympathetic neurons in the down-regulation of adenylate cyclase activity. Further, they raise the potential for a specific functional role for octopamine mediated by changes in cytosolic Ca^{2+} levels through the activation of an α-adrenergic receptor.

632.15

ESTROGEN DECREASES THE LEVEL OF α_{2D}-ADRENERGIC RECEPTOR mRNA IN FEMALE RAT CORTEX. George B. Karkanias* and Anne M. Etgen. Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461

We previously demonstrated that estrogen increases the density of α_{2D}-adrenoceptor binding sites in female rat hypothalamus by ≈30% while causing a concomitant ≈16% decrease in cortical α_{2D}-adrenoceptors. The present experiments test the hypothesis that estrogen also regulates the level of α_{2D}-adrenoceptor (RG20) mRNA in the hypothalamus and cortex of female rats. To test this hypothesis we developed a quantitative RNAse protection assay for the measurement of RG20 mRNA. We found that ovariectomized female rats injected twice with 2 μg of estradiol benzoate 24 and 48 hrs prior to sacrifice demonstrated a 50% reduction in the level of cortical RG20 mRNA when compared to ovariectomized, oil-treated control animals. Estrogen treatment for 48 hrs did not alter the level of RG20 mRNA in female rat hypothalamus. These findings suggest that the estrogen-mediated decrease in cortical α_{2D}-adrenoceptor levels may result from hormone-dependent reductions in the mRNA encoding this receptor. Supported by HD29856, MH41414, RSDA MH00636 and T32 NS07183.

632.16

EFFECT OF SELECTIVE α_2-ADRENERGIC AND IMIDAZOLINE I_2 RECEPTOR DRUGS ON EARLY RESPONSE GENE AND GFAP MRNA IN RAT BRAIN. A.L. Gundlach* and T.C.D. Burazin. Dept. of Medicine, University of Melbourne, Austin and Repatriation Medical Centre, Heidelberg, Vic. 3084, Australia.

In brain, imidazoline drugs such as idazoxan are known to interact with both neuronal α_2-adrenoceptors and imidazoline I_2 receptors, thought to be predominantly located on glial cells. Previous studies have shown that chronic treatment with idazoxan, but not other α_2-adrenoceptor antagonists, increases the level of glial fibrillary acidic protein (GFAP) immunoreactivity in cerebral cortex and GFAP mRNA in cultured astrocytes. The imidazolines methoxyidazoxan (MeIDAZ) and 2-(4,5-dihydroimidaz-2-yl)-quinoline (BU224) are ≥ 5000-fold selective for α_2-adrenoceptors and I_2-receptors, respectively. This study examined the acute effects of these drugs on the level of mRNAs encoding the early response genes, c-*fos* and NGFI-A, and GFAP in rat forebrain. Male Sprague-Dawley rats (4 per group) were injected intraperitoneally with either MeIDAZ, BU224 (1 or 10 mg/kg) or vehicle and killed after 1 h. Brains were processed for *in situ* hybridization histochemistry with specific [^{35}S]-labeled oligonucleotides and x-ray film autoradiograms were generated and analyzed by computer-assisted densitometry (MCID). High basal levels of NGFI-A mRNA were present in regions such as cerebral cortex (especially layers IV & VI), hippocampal pyramidal cells, amygdala and caudate putamen. Basal levels of c-*fos* mRNA were low throughout the forebrain. After 1 h, both doses of MeIDAZ produced a marked induction of NGFI-A and c-*fos* mRNA in cortex (25-110%) and the dorsal caudate putamen (45-120%). BU224 also increased mRNA levels in these areas, but to a lesser extent (25-40%). These results confirm the constitutive, inhibitory effect of α_2-adrenoceptors on forebrain NGFI-A and c-*fos* expression and the weak α_2-adrenoceptor activity of BU224. In vehicle-treated rats, high levels of GFAP mRNA were present in the major white matter tracts such as the corpus callosum, fimbria, stria terminalis and optic tract, in the molecular layer of the hippocampus, and in the arcuate and interpeduncular nuclei. After 1 h, neither MeIDAZ or BU224 had any apparent effect on GFAP mRNA levels, suggesting that any specific glial effects of these drugs may occur over a longer time-course (≥ 3 h) and this possibility is currently under investigation.

632.17

EFFECT OF ACUTE DEXAMETHASONE INJECTION ON ADRENERGIC RECEPTOR SUBTYPE mRNA EXPRESSION IN THE THALAMUS OF ADULT RATS. S. K. McCune* and J. M. Hill. Dept. of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD 21287 and Lab. of Dev. Neurobiol., NICHD, NIH, Bethesda, MD 20892.

Adrenergic receptors mediate tissue responses to catecholamines. However, the physiologic and functional roles of multiple receptor subtypes in the CNS has yet to be well defined. It is thought that the multiplicity of homologous subtypes may allow for differential tissue responses to treatment with various ligands. In order to investigate this hypothesis, we treated animals with dexamethasone and studied the regional CNS effect on α-1A, α-1B and β-1 adrenergic receptor subtype mRNA expression. Dexamethasone, a glucocorticoid, has been shown to promote catecholaminergic activity in the CNS of the neonatal animal.

Adult male rats were injected with either dexamethasone 0.8 mg/kg or an equal volume of saline. The animals were sacrificed 2 hours after injection and the brains frozen at -80°C until use. *In situ* hybridization using oligonucleotide probes for each of the subtypes was performed followed by densitometric analysis.

The α-1A and β-1 subtypes were localized in the reticular nucleus of the thalamus while the α-1B subtype had a more widespread thalamic expression. Preliminary results showed that acute treatment with dexamethasone did not alter α-1A expression but caused a significant up-regulation of α-1B and β-1 subtype mRNAs in their respective areas of the thalamus.

These data suggest that multiple adrenergic receptor subtypes localized in the thalamus have differing responses to identical ligands. These unique responses may influence subtle alterations in thalamic integration.

632.18

STRUCTURE ACTIVITY RELATIONSHIPS OF A SERIES OF BUSPIRONE ANALOGS AT α_1-ADRENOCEPTORS. D.L. Saussy, Jr., A.S. Goetz, H.K. King* & R.J. Unwalla. Departments of Cellular Biochemistry and Structural Chemistry, Glaxo Res. Inst., Res. Tri. Park, NC 27709.

The activities of a series of busprione analogs at human α_{1B}, α_{1C}, and α_{1D} adrenoceptors were determined by radioligand binding assays using membranes prepared from Rat-1 fibroblasts expressing recombinant receptors with [^{125}I]I-HEAT as the radioligand. BMY 7378 (pK$_i$ α_{1B}, 7.25; α_{1C}, 6.80; α_{1D}, 9.39) and MDL 73005EF (pK$_i$ α_{1B}, 6.88; α_{1C}, 6.10; α_{1D}, 8.16) were found to have significant selectivity for the α_{1D} subtype. The corresponding butyl- linker compounds, 8-(2-[4-(2-methoxyphenyl)-1-piperazinyl]butyl)-8-azaspiro[4.5]-decane-7,9-dione dihydrochloride (pK$_i$ α_{1B}, 8.04; α_{1C}, 9.08; α_{1D}, 8.76) and MDL 72832 (pK$_i$ α_{1B}, 7.94; α_{1C}, 8.41; α_{1D}, 8.11) had substantially increased affinity for α_{1C} and α_{1B} and a slight decrease in the affinity for α_{1D}. Similarly, replacement of the bulky 8-azaspiro[4.5]decane-7,9-dione moiety with a planar phthalimido-group yielded compounds that had roughly equivalent affinities for all three subtypes for both the ethyl- linker compound, 1-(2-methoxy-phenyl)-4-[4-(2-phthalimido)ethyl] piperazine (pK$_i$ α_{1B}, 6.87; α_{1C}, 7.55; α_{1D}, 7.52) and butyl- linker compound, NAN-190 (pK$_i$ α_{1B}, 9.02; α_{1C}, 9.66; α_{1D}, 9.47) compounds. Molecular modeling studies were performed to map the important pharmacophores for the different subtypes and generate a hypothesis which offers the most plausible explanation for differences in their activity. This series of compounds should be useful for characterizing the structure, pharmacology, and tissue distribution of α_1 adrenoceptor subtypes.

632.19

COLOCALIZATION OF $\alpha_{1A/D}$ ADRENORECEPTOR mRNA WITH TYPE I AND TYPE II GLUCOCORTICOID RECEPTOR mRNA IN RAT HIPPOCAMPUS USING DOUBLE IN SITU HYBRIDIZATION. R.L. Petit*, A. M. Williams, and D. A. Morilak, Dept. Pharmacology, Univ. Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX, 78284-7764.

Noradrenergic transmission plays an important role in the hormonal response to stress, and also in the mechanisms of antidepressant drug action. An important component of adaptation to chronic stress, and perhaps also in antidepressant effects, is regulation of glucocorticoid receptors (GRs) in the hippocampus. We postulate a regulatory influence of α_1 adrenoreceptors on hippocampal GRs that may provide a link between stress, noradrenergic transmission, and antidepressant activity. In this study, we have identified hippocampal neurons that coexpress $\alpha_{1A/D}$ adrenoreceptor mRNA with either mineralocorticoid (Type I GR) or glucocorticoid (Type II GR) receptor mRNA using double in situ hybridization.

Fresh frozen 20 μm rat brain sections were mounted on poly-lysine coated slides and processed for combined in situ hybridization. Hippocampal sections were co-incubated with a digoxigenin-UTP-labeled riboprobe for the $\alpha_{1A/D}$ receptor mRNA, and ^{35}S-labeled riboprobes for either the glucocorticoid or mineralocorticoid receptor mRNA. Following visualization of the digoxigenin label via an alkaline-phosphatase reaction (Boehringer-Mannheim), the slides were dipped in nuclear track emulsion, exposed at 4°C and developed. We observed extensive colocalization of $\alpha_{1A/D}$ receptor mRNA with both glucocorticoid and mineralocorticoid receptor mRNA in the dentate gyrus and CA fields of the hippocampus. This colocalization, which was not observed in $\alpha_{1A/D}$ expressing cells in other brain regions, provides an anatomical substrate for potential regulatory interactions between noradrenergic transmission and GR regulation in stress and depression.

632.20

A POLYCLONAL ANTISERUM AGAINST THE RAT α_{1B} ADRENERGIC RECEPTOR PROTEIN: INITIAL CHARACTERIZATION AND IMMUNOREACTIVITY IN THE RAT HYPOTHALAMUS. A.M. Williams and D.A. Morilak* Dept. of Pharmacology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr., San Antonio, TX, 78284-7764

α_1 adrenergic receptors are now classified into several related subtypes. To address the specific functions of the different subtypes in such contexts as stress, arousal or depression, it would be useful to identify the specific subpopulations of neurons that express α_1 receptor subtypes in the brain. Toward this end, we have raised a polyclonal antiserum against the rat α_{1B} receptor protein, and demonstrated its utility in immunohistochemical applications. A bacterial TrpE-α_{1B} fusion protein was generated by subcloning a fragment of α_{1B} cDNA coding for a unique portion of the C-terminal into the inducable pATH-10 expression vector. After PAGE separation, excision and electroelution, the fusion protein was used to innoculate rabbits (HTI BioProducts). Serum from one rabbit produced a consistently high titer (>10^4) by ELISA. Dilutions of antiserum at >10^{-4} were able to detect 20 ng of antigen in dot-blots. Specificity of the antiserum is currently being characterized in COS7 cells transiently tranfected with an expression plasmid containing the entire coding region of the rat α_{1B} receptor (pcDVα_{1B}, J. Lomasney, Duke University). Controls include omission of primary antiserum, preadsorption with the fusion protein antigen, untransfected COS7 cells, and incubation with pre-immune serum in place of primary antiserum. In preliminary immunocytochemical experiments in rat hypothalamus, antibody dilutions from 1/500 to 1/2000 label a distinct population of cell bodies in the ventral-medial and the dorsal parvocellular paraventricular nucleus. A more extensive, punctate labelling, some of which was localized to cell processes, was seen throughout the PVNpc and other hypothalamic subnuclei, as well as in specific extrahypothalamic sites, including the dorsal thalamus.

CATECHOLAMINE RECEPTORS: BETA ADRENERGIC

633.1

MECHANISM OF ACTION OF CISSUS SICYOIDES WATERY EXTRACT ON MALE GUINEA PIG INTESTINAL MUSCLE. X. García*, L. Cartas, M. Lorenzana-Jimenez, and E. Gijón. Dept. of Physiology and Dept. of Pharmacology, School of Medicine. UNAM. México D.F. 04510. México.

Cissus sicyoides extract 1:10 w/v from dry leaves applied to the organ bath (10 ml) induces relaxation, biphasic or contraction responses dose dependent (0.01-1.6 ml). As relaxation or inhibitions from intestinal smooth muscle are induced by stimulation of adrenergic receptors we tested a beta adrenergic blocker, propranolol on Cissus sicyoides biphasic responses from intestine. Propranolol increased relaxation response without affecting contraction, did not change biphasic responses, or blockade relaxation without changing contraction responses, in a dose dependent form. This suggest that relaxation responses are mediated by beta adrenergic receptors.

633.2

PARTICIPATION OF VITAMIN B_{12} IN β-RECEPTOR MEDIATED REGULATION OF ADENYLYL CYCLASE SYSTEM. M. Watanabe*, S. Hatta[1], H. Ikeda, S. Toki, H. Ozawa, T. Saito, and N. Takahata Department of Neuropsychiatry and Pharmacology[1], School of Medicine, Sapporo Medical University, Sapporo, 060 Japan

It has been suggested that psychiatric and behavioral symptoms such as depression, schizophrenia, and dementia can be attributed to a deficiency of vitamin B_{12}. Low levels of vitamin B_{12} has also been reported in dementia of Alzheimer type and vitamin B_{12} deficiency is thought to be related to cognitive impairment in Alzheimer patients. Furthermore, several studies have indicated that methyl-vitamin B_{12} (mecobalamin) has clinical effects for non-24-hour sleep-wake syndrome and delayed sleep phase syndrome. On the other hand, vitamin B_{12} is known to be involved in the synthesis of S-adenosyl-L-methionine, which has been demonstrated to possess the anti depressive effect. Thus, there are several lines of evidence to suggest that vitamin B_{12} participates in the regulation of cellular responsiveness, especially in neuronal systems. The β-adrenergic receptor adenylyl cyclase (AC) system is one of the best-characterized hormonal signal transduction systems which mediate intracellular effects through the Gs protein. In this study, rats were fed with and without mecobalamin. Basal, GppNHp-, forskolin-, and $MnCl_2$-stimulated AC activities were significantly lower in mecobalamin-deficient rats than in controls. Activation of AC with isoproterenol in the presence of GppNHp was also significantly reduced in mecobalamin-deficient rats compared with that in controls. However, there was no difference in quantitative and qualitative estimation of G proteins between control and mecobalamin-deficient rats. Furthermore, mecobalamin restored the lowered level of AC activities in mecobalamin-deficient rats to control levels. These findings indicate that vitamin B_{12} play a dynamic role in the neuronal signal transduction cascade.

633.3

BETA ADRENERGIC RECEPTORS AS A TOOL FOR EVOLUTION STUDIES? A. Fernández-López*[1], V. Revilla[1], M.A. Candelas[1], R. Revilla[1] and A. Pazos[2]. [1]Dpt. Biología Celular. Facultad de Biología. Universidad de León. León Spain. [2]Dpt. Fisiología y Farmacología. Facultad de Medicina. Universidad de Cantabria. Santander. Spain.

An autoradiographic study of ß-adrenoceptors with [^{125}I] cyanopindolol was performed in avian brain from a number of representative orders. Several structures from different functional systems have been measured. Since some of the species studied are scarce, only injured animals discarded from a recuperation center were used in that case.

The following density's profiles were obtained in some of the different structures studied. Cerebellum: Passerifomes(100%) > Psittacifomes(75%) > Columbiformes(68%) > Falconiformes(54%) > Caprimulgiformes(53%) > Galliformes(48%)> Strigiformes(41%). Tectum opticum (layer 3) Passerifomes (100%) > Psittacifomes(82%) > Caprimulgiformes(68%) > Columbiforme(52%) > Falconiformes(47%) > Strigiformes(35%) > Gallifomes(26%). The results described here roughly correlate with the postulated cladistic relationships of avian from a number of synapomorphies. The similarity of the density's profile throughout these orders strongly supports the idea that ß-adrenoceptors could be an important tool to study the evolution in birds in order to establish phylogenetic relationships. However it must be must taken into account that a higher number of taxa, species and samples have still to be studied for further corroboration. This work has been supported by a the grant LE 10/94 from Junta Castilla-León. Spain.

633.4

EFFECTS OF PRENALTEROL ON BETA ADRENERGIC RECEPTORS EXPRESSED IN CHINESE HAMSTER OVARY CELLS. K. Zhang, Y. Li, F. Karim, R. Maloney* and J. M. O'Donnell. Dept. of Pharmacology, LSUMC, Shreveport, LA 71130

Prenalterol, although shown to be a beta-1 selective adrenergic receptor (AR) agonist in functional studies, has been suggested not to stimulate beta AR-linked adenylyl cyclase activity in rat cerebral cortex. In order to assess its activity at human beta ARs, the binding of prenalterol and its effects on adenylyl cyclase activity were studied in Chinese Hamster Ovary (CHO) cells that were permanently transfected either with human beta-1 AR cDNA or human beta-2 AR cDNA cloned into a plasmid pcDNAI. The binding of prenalterol to beta ARs were studied using a competition assay in membrane preparations. The ability of prenalterol to stimulate adenylyl cyclase activity through these receptors was studied using a cyclic AMP accumulation assay in intact cells. Prenalterol inhibited the binding of ^{125}I-pindolol to beta-1 and beta-2 ARs with IC_{50} values of 1.2 and 4.0 uM, respectively. The nonhydrolyzable GTP analog GppNHp (250 uM) shifted the competition curve for beta-1 ARs to the right about 1.5 fold, but had no effect on prenalterol binding to beta-2 ARs. Prenalterol stimulated cyclic AMP production dose-dependently in CHO cells transfected with human beta-1 ARs; this effect was antagonized by propranolol, a beta AR antagonist. By contrast, prenalterol did not increase cyclic production at all in CHO cells transfected with human beta-2 ARs at concentrations up to 100 uM. These results are in agreement with the cardioselectivity of prenalterol observed clinically, and suggest that lack of effect of prenalterol on beta AR-linked adenylyl cyclase activity in rat brain may either be due to its low intrinsic activity or to a species difference.

633.5

MODULATION OF β-ADRENERGIC RECEPTOR SUBTYPE DENSITY USING INDUCIBLE VECTORS: EFFECTS ON CATECHOLAMINE RESPONSIVENESS IN RAT C_6 GLIOMA CELLS. Shelly D. Wood*, Hongying Zhong and Kenneth P. Minneman. Dept. of Pharm., Emory Univ. Sch. of Med., Atlanta, GA 30322.

Receptor density increases during the phenomenon of supersensitivity. To better understand the impact of such changes, we studied the relationship of β-adrenergic receptor (AR) density and subtype ratio on catecholamine signaling. C_6 cells, which natively express β_1- and β_2-ARs, were stably transfected with an inducible vector containing the cDNA for the β_1- or β_2-AR, and receptor expression was modulated in a time and concentration-dependent manner by exposure to the nonhydrolyzable galactose analog, IPTG. Receptor number increased ~20- and ~7-fold for β_1- and β_2-ARs, respectively.

We studied catecholamine signaling by measuring: (1) whole cell cAMP accumulation and (2) membrane adenylyl cyclase (AC) activity. In whole cells, increases in β_1- AR expression resulted in parallel increases in agonist potency with no change in the maximum cAMP response whereas, increases in β_2-AR density enhanced agonist potency, but decreased the maximum by ~30%. Neither cell type showed a change in basal cAMP levels following receptor induction. In contrast, induction of β_1-ARs increased membrane AC basal activity ~2-fold relative to that of noninduced cells, and surprisingly, the maximum agonist stimulated response increased ~2-fold relative to the induced basal activity and therefore ~4-fold relative to the noninduced basal activity. In addition, induction of β_1-ARs increased agonist potency at least 5-fold. (Supported by NS 21325 and DA05677)

633.6

BINDING OF FLUORINATED CATECHOLAMINES TO ADRENERGIC RECEPTOR SUBTYPES M. Singh*, J. Wess, B.K. Kobilka[†], K.L. Kirk Lab. of Biochemistry, NIDDK, NIH, Bethesda, MD 20892, [†]Dept. Of Cardiology and Molecular and Cellular Physiology, Howard Hughes Medical Institute, Stanford CA 94305.

Introduction of fluorine into biologically active molecules often produces analogues with markedly altered behavior. The lack of steric interactions accompanying fluorine substitution coupled with the high electronegativity of this element are important factors that influence biological behavior. Our previous studies with ring-fluorinated catecholamines have revealed that fluorine substitution can dramatically affect interactions of adrenergic agonists with α- and β-adrenergic receptors. We have found that α–adrenergic receptors have a higher affinity for 6-fluoro-epinephrine (6FE) and 6-fluoro-norepinephrine (6FNE) than for the 2-fluorinated analogues (2FE and 2FNE). On the other hand, β–adrenergic receptors have a higher affinity for 2FE and 2FNE than for 6FE and 6FNE. Binding studies with cloned receptors confirmed these results originally obtained by the use of tissue preparations. To identify the receptor sites determining the selectivity of these compounds, their ability to bind to chimeric α_2/β_2-receptors transiently expressed in COS-7 cells was examined. Preliminary studies showed that more than one receptor domain is responsible for the observed selectivity profiles of the various fluorinated analogues.

633.7

DELINEATION OF LIGAND-SPECIFIC CONFORMATIONAL CHANGES IN THE β_2 ADRENERGIC RECEPTOR BY FLUORESCENT LABELING WITH AN ENVIRONMENTALLY SENSITIVE CYSTEINE REAGENT. **U. Gether*, Sansan Lin and B.K.Kobilka. Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305.**

Purified β_2-adrenergic receptor was fluorescently labeled with an environmentally sensitive cysteine reagent (IANBD) to directly monitor structural changes in the receptor in response to ligand binding. These studies represent the first attempt to correlate the conformational state of a purified G protein coupled receptor with the functional properties of different classes of receptor ligands. All tested compounds including 'neutral' antagonists surprisingly caused an apparent conformational change in the receptor as reflected in a change in the fluorescent emission from the covalently bound IANBD. The conformational change induced by full agonists was clearly distinguishable from that induced by inverse agonists (negative antagonists) and from the unoccupied receptor. Thus, full agonists induced a stereo-selective, dose-dependent, reversible decrease in fluorescence, whereas inverse agonists caused a relative increase in baseline fluorescence, which correlated with their negative intrinsic activity in biological assays. However, weak partial agonists induced a change in fluorescence that was more similar to the change induced by antagonists than by full agonists.These results cannot be simply explained by the current and most widely accepted two-state model for G protein coupled in which the receptors are assumed to exist in a dynamic equilibrium between two states; an inactive (R) and active conformation (R*). Rather our fluorescent data suggest that the two-state model may have to be extended to a model involving multiple conformational states induced or stabilized by different classes of ligands.

634.1

ß-CARBOLINES STIMULATE BENZODIAZEPINE-INDEPENDENT RECEPTOR MECHANISMS. H. Rommelspacher*, S. Sällström Baum, J.F. Klinker. *Dept. Neuropsychopharmacology, Free University, D-14050 Berlin, Germany.*

The function of the two ß-carbolines harman (H) and norharman (NH) has been investigated in various in vitro and in vivo systems. [^{3}H]norharman binds with high affinity to a specific membrane site in rat brain membranes (Pawlik and Rommelspacher, Eur. J. Pharmacol. 147, 163, 1988). We will present evidence that a high affinity site is coupled to G-proteins and causes an increase of inositol 1,4,5-triphosphate production in a neuronal cell line. The effect does not involve benzodiazepine-activated and u-opioid receptor mechanisms. A second binding site was detected with an affinity of about one magnitude less. Pharmacological characterization of the binding sites revealed that several ß-carbolines bind to both sites in contrast to benzodiazepines and those ß-carbolines, which are inverse agonists at the benzodiazepine receptor. The function of the new receptor sites was investigated in vivo using in vivo microdialysis techniques. The extraneuronal concentrations of dopamine, DOPAC, HVA, serotonin and 5-HIAA were determined in the nucleus accumbens following intraperitonal injection of various doses of H and NH in the freely-moving rat. Low doses of NH (e.g. 0.5 mg/kg) caused an increased concentration of dopamine whereas somewhat higher doses of NH reduced the level of DA. 9 mg/kg caused again an increase, which was prevented by flumazenil (Sällström Baum et al. Life Sci 56, 1715, 1995). The injection of H caused U-shape changes of dopamine concentrations in the nucleus accumbens as well. In conclusion, the findings demonstrate the existence of a new class of receptors, which are expressed in neuronal cell lines and in limbic structures of rat brain. - Supported by the DFG (AZ: He 916/7-2).

634.2

ENDOGENOUS GABA MODULATES HISTAMINE RELEASE FROM THE ANTERIOR HYPOTHALAMUS OF RAT. K. Okakura-Mochizuki, T. Mochizuki, Y. Yamamoto, A. Uno, Y. Kondo, M. Suzuki and A. Yamatodani*. Dept. of Medical Physics and Otolaryngol., Faculty of Med., Osaka Univ., Suita, Osaka 565 Japan.

Using a microdialysis method, we investigated the effects of GABA on *in vivo* release of histamine from the anterior hypothalamic area (AHy) of urethane anesthetized rats. Infusion of nipecotic acid (0.5mM), an uptake inhibitor of GABA, decreased histamine release to about 60% of the basal level. This effect was partly antagonized by both picrotoxin (0.1mM), an antagonist of GABA$_A$ receptors, and phaclofen (0.1mM), an antagonist of GABA$_B$ receptors. These results show that endogenous GABA modulate histamine release through GABA$_A$ and GABA$_B$ receptors.

When the tuberomammillary nucleus, where the cell bodies of the histaminergic neurons are localized, was electrically stimulated, the evoked release of histamine from the nerve terminals was enhanced by phaclofen, suggesting that GABA$_B$ receptors may be located on the histaminergic nerve terminals and presynaptically modulate histamine release. On the other hand, picrotoxin caused an increase of histamine release to about 170% of the basal level, and this increase was diminished by co-infusion of AP5 (0.1mM), an antagonist of NMDA receptors. Previously, we indicated that glutamate makes a tonic control of histamine release through NMDA receptors which are located on the histaminergic terminals in the AHy. These results suggest that GABA$_A$ receptors are located on glutamatergic nerve terminals and indirectly regulate basal release of histamine.

634.3

DEFECTIVE HERPES SIMPLEX VIRUS VECTORS FOR THE STUDY OF GLUTAMATE DECARBOXYLASE EXPRESSION IN CELLS OF THE CNS K. C. New*, M. R. Proctor, R. L. Martuza, K. Gale, S. D. Rabkin. Departments of Microbiology, Neurosurgery and Pharmacology, Georgetown Univ. Med. Cen., Washington, DC 20007.

Glutamate decarboxylase (GAD) exists in the brain as two isoforms, 65 or 67 kD, which are responsible for the synthesis of γ-aminobutyric acid (GABA). We have constructed defective herpes simplex virus vectors containing GAD65, GAD67 or ß-galactosidase (lacZ, control). These vectors were used to infect primary cultures of: a)cerebellar granule cell neurons; and b)cortical astrocytes. In both cultures, GAD vector infection resulted in a greater number of GAD and GABA-like immunoreactive cells compared to lacZ infected cultures. Further, infected granule cell cultures contained neurons expressing GAD65 or GAD67 in both their cell bodies and processes. Amino acid levels from infected cells (moi 1.0) were determined after OPA derivatization by HPLC and fluorescent detection. Intracellular GABA was (pmole/µg protein):

	GAD65	GAD67	lacZ
Neurons	10.8 ± 2.0 *	14.9 ± 2.2 *	0.5 ± 0.5
Astrocytes	25.0 ± 4.3 *	60.8 ± 12.6 *	1.0 ± 1.0

* p<0.01 compared to lacZ

Increased levels of extracellular GABA were detected after GAD65 infection of granule cell cultures. Intracellular glutamate levels in GAD infected cultures did not vary significantly compared to controls. We conclude that vector derived GAD65 and GAD67 proteins are able to synthesize GABA in neurons and astrocytes. Neurologic disorders that result from decreased GABA levels are potential targets for gene therapy using these vectors.

634.4

NULL MUTATIONS OF THE GLYCINE RECEPTOR ALPHA 1 SUBUNIT GENE IN MOUSE AND HUMAN. C.-M. Becker, W. Brune, C. Kling, R. Weber, B. Saul, H.-M. Meinck, K. Kellermann° (SPON: ENA). Neurologische Klinik, Zentrum für Molekulare Biologie, and Institut für Humangenetik, Universität Heidelberg, 69120 Heidelberg; °Städtisches Kinderkrankenhaus, 50335 Köln, Germany.

The adult isoform of the strychnine-sensitive glycine receptor, GlyR_A, comprizes ligand-binding α1 and structural β subunits. The *oscillator (spd^{ot})* mouse carries a microdeletion within the α1 gene *(Glra1)* that produces a frameshift affecting a C-terminal segment of the subunit. Although neonates appear normal, homozygous mutants develop a motor disorder leading to death at the age of ≈3 weeks. Western blots and immunoassays employing subunit-selective antibodies showed that the α1 polypeptide is completely absent from mutant CNS. Residual [^{3}H]strychnine binding can be attributed to the minor receptor isoforms. Thus, *oscillator* represents a functional null mutation of *Glra1* which becomes apparent in adult mice.

The human disorder hyperekplexia, which is characterized by neoanatal hypertonia and exaggerated startle responses persisting into adulthood, has been associated with point mutations of the α1 gene *(GLRA1)*. In a sporadic, recessive case of hyperekplexia, we identified an extensive deletion affecting *GLRA1*. Southern blots and PCR amplification of genomic DNA revealed the absence of exons 1 to 6. By FISH, the corresponding null allele was detectable in the child's genome as well as on parental chromosomes. In the human, the homozygous null mutation is phenotypically indistinguishable from dominant variants of the disease. These observations suggest a disparate regulation of glycine receptors in mice and humans.

634.5

ACTION OF THE VOLATILE ANESTHETIC ISOFLURANE ON NEURONAL & CLONED GLYCINE RECEPTORS. A.C. Hall*, D.L. Downie, R. Dickinson, S.L. Tomlin, N.P. Franks & W.R. Lieb. *Biophysics Section, Imperial College, London SW7 2BZ, UK.*

The effect of the volatile general anesthetic isoflurane was investigated on glycine-evoked Cl^- currents recorded from neonatal rat medullary neurons (whole-cell patch clamp) and from *Xenopus* oocytes co-injected with cDNAs encoding the human α_1 and rat β-subunits of the glycine receptor (two-electrode voltage clamp).

In the acutely dissociated neurons held at -40 mV, strychnine-sensitive Cl^- currents (demonstrated by appropriate reversibility) were evoked by exposure to glycine (3-300 µM) *via* a local perfusion system. Co-application of isoflurane (0.31 mM, a concentration corresponding to anesthesia in humans) with a low concentration of glycine (10 µM) resulted in a reversible, voltage-independent potentiation (by 82 ± 20%, mean ± sem, n = 10 cells) of the non-desensitising control response. Maximal potentiation (405 ± 94%, n = 6 cells) of the 10 µM glycine response was achieved with 1.52 mM isoflurane, while higher concentrations resulted in reduced potentiations. Isoflurane (0.61 mM) was always observed to potentiate responses to 3 and 30 µM glycine, while a maximal response (to 300 µM glycine) was either unaffected or slightly inhibited, indicating that isoflurane causes a leftward shift in the concentration-response relationship for glycine.

In experiments with oocytes, held at -50 mV, bath application of glycine (300 nM – 10 mM) evoked a concentration-dependent inward current response (EC_{50} = 216 ± 6 µM, Hill coefficient = 1.7 ± 0.1, n = 4). The response to glycine (300 µM) was virtually abolished by co-application of strychnine (100 nM), but was insensitive to picrotoxin (100 µM). Co-application of isoflurane (0.15 - 1.2 mM) with glycine (10 µM, 1% of maximum response) produced a concentration-dependent enhancement of the glycine current (up to 50 fold ■ 45% of max.). Examination of the effect of isoflurane (0.15 - 0.6 mM) on the glycine concentration-response relationship reveals a parallel leftward shift of the glycine curve, with no significant increase in the amplitude of the maximal response to glycine (EC_{50} = 84 ± 7 µM, Hill coefficient = 1.6 ± 0.2, n = 4, at 0.6 mM), mirroring the effect on the native receptors.

634.6

EXPRESSION OF GLYCINE RECEPTOR SUBUNITS IN GLIAL CELLS OF THE RAT SPINAL CORD. Frank Kirchhoff*,#, Cornel Mülhardt+, Andrea Pastor#, Cord-Michael Becker+ and Helmut Kettenmann#

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By electrophysiological methods it was shown that different glial cells, namely astrocytes, oligodendrocytes and precursor cells of the rat spinal cord respond to the inhibitory neurotransmitters glycine.

In this study the reverse transcription mediated polymerase chain reaction (RT-PCR) in combination with the patch-clamp technique was applied to individual glial cells to investigate subunit expression of the receptor. After electrophysiological recording in the whole-cell configuration the cytoplasm of those glial cells was harvested which displayed a response to glycine. After reverse transcription of cellular mRNA glycine receptor subunit specific DNA fragments were amplified by PCR and analyzed by agarose gel electrophoresis, Southern blotting and cDNA sequencing. The α1 subunit of the glycine receptor but not α2, α3 or α4 could be detected in all glial cell types investigated in rats of postnatal day 3 to 18. In addition, about half of the glial cells investigated expressed β subunit transcripts.

The detection of glycine receptor subunit mRNAs in glial cells confirms the electrophysiological studies showing glycine evoked inward currents and substantiates that glial cells themselves express the receptors. The physiological role of them may be controlling the microenvironment in the vicinity of neurons during synaptic transmission. The contribution of other neurotransmitter receptors known to be expressed in glial cells is conceivable.

634.7

CLONING OF A HUMAN P_2Y PURINERGIC RECEPTOR.

J. B. Schachter*, Q. Li, J. L. Boyer, R. A. Nicholas, and T. K. Harden, Dept. of Pharmacol., Univ. of North Carolina, Chapel Hill, NC 27599.

Extracellular adenine and uridine nucleotides interact with P_2 purinergic receptors to produce a broad range of physiological effects. One impediment to the development of selective drugs for these receptors is the lack of tissue sources known to possess single subtypes of nucleotide receptors. To facilitate the pharmacological analysis of one of these receptor subtypes, we have undertaken the cloning and expression of a human P_2Y purinergic receptor (P_2Y-R). A 702 base DNA probe was generated by PCR from rat genomic DNA using primers based upon the sequence of a P_2Y-R from turkey. This probe was used to screen a cDNA library prepared from human brain RNA. One positive clone was obtained from $4x10^6$ clones screened. Although incomplete at the 5' end, sequence analysis indicated that this clone contained an open reading frame of 972 bases that was 79 % homologous with a P_2Y-R cloned from turkey. A 300 base fragment of this clone was used to probe a human genomic library. Two genomic clones containing the cDNA sequence were obtained, both of which contained an ATG start site and an open reading frame of 1119 bases. The overall homology between the human sequence and that of the turkey was 74% at the nucleotide level and 82% at the amino acid level. The human receptor is being expressed in 1321N1 cells to compare its pharmacological profile with that of the P_2Y-R from turkey, which has also been expresssed in these cells.

634.8

CHARACTERIZATION OF P3 PURINOCEPTOR-LIKE PROTEIN PURIFIED FROM RAT BRAIN MEMBRANES. Y. Saitoh[1], Y. Sekino[2]*, and H. Nakata[1], [1]Dept. of Mol. & Cell. Neurobiol., Tokyo Metropolitan Inst. for Neurosci., Fuchu-shi, Tokyo 183, Japan, [2]PRESTO, JRDC, c/o Mitsubishi-kasei Inst. of Life Sci., Machida-shi, Tokyo 194, Japan.

Purinoceptors are traditionally classified into P1 and P2 on the basis of their pharmacological properties. We found a novel adenosine-binding protein in the rat brain which is not classified into the traditional purinoceptors. The protein showed the highest affinity to 5'-N-ethylcarboxamidoadenosine (NECA) among various adenosine agonists. The NECA binding activity was not inhibited by xanthines, antagonists of P1 purinoceptor. We developed the binding assay system of this protein, and determined tissue and subcellular distributions. It prominently distributed in the brain, and most of the binding activity was located to synaptosomal membranes. Detailed studies on the ligand binding specificity revealed that this adenosine-binding protein can bind to adenine nucleotides and adenosine with the same affinity. It was classified into neither the traditional purinoceptors, nor adenotin-like proteins. We purified it >10,000-fold from rat brain membranes using six chromatography steps. Recently, a new purinoceptor site (P3 purinoceptor) which is activated by both adenosine and ATP was proposed in the rat caudal artery (Shinozuka et al, 1988). These results suggest that this adenosine-binding protein is a new purinoceptor in the brain, probably P3 purinoceptor.

REGIONAL LOCALIZATION OF RECEPTORS AND TRANSMITTERS I

635.1

DEVELOPMENTAL LOCALIZATION OF D-SERINE IN RAT BRAIN ASTROCYTES. M.J. Schell*, O.B. Zand, and S.H. Snyder, Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Recent studies indicate that free D-serine is present in brain regions enriched in NMDA receptors. Because D-serine is a potent agonist at the NMDA receptor glycine site, it may act as an endogenous ligand for these sites in brain--instead of or in concert with glycine. In a previous study, using an antibody highly specific to D-serine coupled to glutaraldehyde, we localized D-serine to astrocytes of the gray and white matter of the adult rat telencephalon. We have now localized D-serine during the first three postnatal weeks, a period when NMDA receptor expression varies both regionally and temporally. D-Serine immunopositive glial cells were present at birth in the thalamus and brainstem . During the first postnatal week, large pools of small, round cells proliferated near the lateral ventricles and appeared to cluster over thalamic nuclei and migrate on top of white matter tracts. By day 7, most subcortical regions contained large numbers of these cells. During the second postnatal week, D-serine positive cells appeared in the cerebral cortex and hippocampus and elaborated immunopositive processes; staining in the cerebellar molecular layer also increased rapidly. By day 14, immunoreactivity began to diminish in the midbrain, brainstem and spinal cord, presumably reflecting the expression of D-amino acid oxidase in astrocytes of these regions. During week three, D-serine positive astrocytes became increasingly prominent and elaborate in the gray matter of the telencephalon and assumed an adult-like pattern by day 21. We conclude that, throughout the postnatal period, astrocyte-derived D-serine is well-positioned to be an endogenous modulator of NMDA receptors.

635.2

CHARACTERIZATION OF NEUROTROPHIN RECEPTOR CONTAINING-ENTERIC NEURONS OF THE RAT SMALL INTESTINE. J. Arakawa, R. De Giorgio*, D. Su, N.C. Brecha and C. Sternini, CURE:VA/UCLA Gastroent. Biol. Ctr., & Depts. Neurobiol. & Med., UCLA & VAMC-WLA, LA, CA 90073.

Neurotrophins (NT) and their high-affinity receptors (*trk*-A, -B and -C) are widely distributed in the developing and mature nervous system, suggesting that NT may have other effects in addition to support differentiation and survival. We have demonstrated that *trk*-C, the high-affinity receptor for NT-3, is expressed in a large population of neurons of the enteric nervous system (ENS). The aim of this study was to characterize *trk*-expressing enteric neurons using double labeling with *trk* antibody and markers for distinct types of enteric neurons. Many *trk*-immunoreactive (IR) enteric neurons, in both ganglionated plexuses, contained tachykinin-IR. A subpopulation of *trk*-IR submucosal neurons was positive for vasoactive intestinal polypeptide (VIP) -IR. In contrast, no apparent *trk* and VIP colocalization was observed in the myenteric plexus. Enteric neurons stained for NADPH-diaphorase, which was used to identify neurons synthesizing NO, appear to be distinct from those expressing *trk*. The presence of *trk*-IR in distinct subpopulations of enteric neurons supports the hypothesis that NT-3 is involved in the maintenance and function of different neuronal circuits in the mature ENS.

Supported by Morphology/Imaging Core DK41301 & VA Medical Funds

635.3

AUTORADIOGRAPHIC VISUALIZATION OF RECEPTOR-ACTIVATED G-PROTEINS USING [^{35}S]GTPγS BINDING. Laura J. Sim*, Dana E. Selley, Ruoyu Xiao and Steven R. Childers, Dept. of Physiology & Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157.

We have developed an autoradiographic technique to identify receptor-activated G-proteins in brain sections using agonist-stimulated binding of [^{35}S]GTPγS in the presence of excess GDP. In this study, [^{35}S]GTPγS binding was examined in response to specific opioid (mu, delta and kappa), cannabinoid and $GABA_B$ agonists in rat and guinea pig brain sections. Agonist-stimulated [^{35}S]GTPγS binding was shown to be receptor-mediated, since the effect was concentration-dependent and blocked by appropriate antagonists. The anatomical distribution of agonist-stimulated [^{35}S]GTPγS binding corresponded to known receptor distributions characteristic of each receptor system. For example, [^{35}S]GTPγS labeling in the cerebellum was stimulated by cannabinoid, kappa opioid and $GABA_B$ agonists, whereas [^{35}S]GTPγS binding in the striatum was stimulated by mu, delta and kappa opioid, and cannabinoid agonists. However, quantitative analysis of agonist-stimulated [^{35}S]GTPγS binding among these receptor systems revealed that the maximal stimulation of [^{35}S]GTPγS binding by agonists was not always correlated with the number of receptor binding sites: although the number of cannabinoid binding sites exceeded that of mu sites by tenfold, mu opioid-stimulated [^{35}S]GTPγS binding was higher than that of cannabinoid agonists. These data reveal potential differences in receptor coupling efficiency.

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635.4

GABAERGIC AND OTHER NON-CHOLINERGIC BASAL FOREBRAIN NEURONS PROJECT TO MESO- AND ISO-CORTICAL REGIONS IN THE RAT BRAIN. B.E. Jones*[1], L. Mainville[1], M. Mancia[1] and I. Gritti[2], [1]Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada H3A 2B4 and [2]Institute of Human Physiology II, University of Milano, Milano, Italy 20133.

In the present study in the rat, we investigated whether GABAergic basal forebrain neurons project to the cerebral cortex by employing retrograde transport of cholera toxin b subunit (CT) from the prefrontal cortex, a meso-cortical region, and from the parietal cortex, an iso-cortical region, presumed to receive input predominantly from cholinergic magnocellular basal forebrain neurons. Brains were processed by dual immunohistochemical staining for CT and glutamic acid decarboxylase (GAD) or choline acetyltransferase (ChAT) in adjacent series. Of a large number of cells that projected to the prefrontal cortex (~6800, mean, n=3) and the parietal cortex (~5100, mean, n=3), the proportion which were GAD-positive (~31% and ~35%, respectively) was found to be approximately equivalent to that which were ChAT+ (~19% and ~27%). Another equivalent or larger proportion (~50% and ~38%) would be neither GABAergic nor cholinergic. As evident in 3-dimensional reconstructions, the cortically projecting GABAergic, cholinergic and other non-cholinergic neurons were co-distributed through the basal forebrain and were commonly medium to large in size, thus collectively comprising neurons of the magnocellular basal cell complex. These results demonstrate that a significant number of GABAergic and other non-cholinergic magnocellular basal forebrain neurons project to the cerebral cortex and could thus serve in parallel with the cholinergic magnocellular neurons to modulate cortical activity. (Supported by MRC and ASSORN.)

635.5

SEMIQUANTITATIVE IMMUNOCYTOCHEMICAL ANALYSIS OF $GABA_A$ RECEPTOR SUBUNITS EXPRESSION IN THE RAT NEOSTRIATUM. H.J. Caruncho[1]*, I. Liste[1], C. Pesold[2], F. Impagnatiello[2], A. Guidotti[2] and J.L. Labandeira-Garcia[1] 1. Department of Morphological Sciences, University of Santiago de Compostela School of Medicine, Spain. 2. Center for Neuropharmacology, N.S. Kline Institute for Psychiatric Research, Orangeburg, NY 10962.

Different striatal regions receive afferents from different neocortical structures. In addition, striatal interneurons expressing a variety of neurotransmitters are unevenly distributed in different striatal areas. To assess the possible existence of specific $GABA_A$ receptor subtypes in the dorso-lateral, dorso-medial and ventro-medial striatum, we used an immunogold silver enhancement technique with $GABA_A$ receptor subunit specific antibodies. The results document a different abundance in the striatum of three groups of $GABA_A$ subunits: $\alpha 1$ and $\alpha 3$ (labeling density of less than 100 gold particles per 1000 μ^2), $\gamma 2$ and δ (labeling density between 100 and 200 gold particles per 1000 μ^2), $\alpha 2$ and $\beta 2/3$ (labeling density of more than 300 gold particles per 1000 μ^2). Analysis of the labeling density in different striatal regions shows that $\alpha 1$ and $\alpha 3$ subunits are more abundant in the dorsal than in the ventral striatum where the labeling density is 35% lower; $\beta 2/3$ and $\gamma 2$ subunits are more abundant in the medial than in the lateral striatum where the labeling density is 40% lower, while the $\alpha 2$ and δ subunits fail to show differences in regional expression. The present data is consistent with a probable existence of differences in $GABA_A$ receptor subtype expression in the three striatal regions mentioned above. Supported in part by DGICYT grant PB92-0378 (Spain) and NIH grant RO1-MH49486-01A3.

635.6

AMPA RECEPTOR SUBUNIT AND GAD-65/67 EXPRESSION PROFILE IN NOS POSITIVE NEURONS: A DOUBLE-LABELING STUDY. M.V. Catania[1], T. Tölle[2], P.H. Seeburg[1], R. Bernardini*[3] and H. Monyer[1]. [1]Center for Molecular Biology (ZMBH), INF 282, 69120 Heidelberg, FRG, [2]Max Plank Institute for Psychiatry, Kraepelin Straße 10, 80804 München, FRG and [3]Institute of Pharmacology, University of Catania, 95100 Catania, Italy.

NOS (nitric oxide synthase) positive neurons in the cortex, striatum and hippocampus represent a small population of scattered aspiny interneurons which contain a Ca^{2+}/calmodulin dependent constitutive NOS. The functional role of these neurons is not completely established. We studied the expression profile of AMPA receptor subunits in NOS-positive neurons of the adult rat cortex, striatum and hippocampus by a double-labeling approach, combining non-radioactive in situ hybridization and immunohistochemistry. We also examined the expression of GAD-65 and -67 isoforms in order to establish which of these is present in NOS positive neurons.

The majority of cortical and hippocampal NOS-positive neurons were characterized by a predominant expression of GluR-A and -D mRNAs and low or undetectable expression of GluR-B and -C mRNAs. In the striatum all AMPA receptor subunit mRNAs were expressed at low levels. A marked regional diversity of NOS-positive neurons emerged from our studies analyzing the expression of GAD-65 and -67 mRNAs: NOS positive neurons expressed high levels of GAD-65, but not -67 in the cortex, high levels of both GAD-65 and -67 in the hippocampus and low or undetectable levels of both mRNAs in the striatum.

Our results indicate that NOS positive neurons in the three regions examined share the common feature of low GluR-B expression, suggesting the presence of AMPA receptor channels with high Ca^{2+} permeability, regardless of the regional location. The region dependent variability found in the expression of the GluR-A and -D as well as in the expression of the GAD isoforms suggests a different functional role of this special type of interneurons within a distinct circuit.

635.7

DISTRIBUTION OF AMPA AND KAINATE RECEPTOR SUBUNIT MESSENGER RNA IN PRIMATE AND HUMAN BRAIN. J.H.Meador-Woodruff*, R.E. King, S.P. Damask. Mental Health Research Institute and Department of Psychiatry, University of Michigan, Ann Arbor MI

Four major families of glutamate receptors have been defined: three families of ionotropic receptors (the NMDA, kainate, and AMPA families), and one family of metabotropic receptors. Each of these glutamate receptor subfamilies contains multiple specific receptor members. The ionotropic receptor families are characterized by genes that encode receptor subunits with three or four putative transmembrane domains. These expressed subunits are assembled to form ligand-gated ion channels. On the other hand, the metabotropic glutamate receptors are members of the superfamily of the seven transmembrane domain, G-protein coupled receptors. We have initiated studies examining the anatomy and regulation of the mRNAs encoding these receptors and receptor subunits, and have primarily focused on the AMPA and kainate receptor subunits to date. The kainate family of receptors consists of two high (KA1, KA2) and three low (gluR5-gluR7) affinity kainate receptor subunits. The AMPA receptors are derived from four distinct subunits (gluR1-gluR4). These subunits can be expressed in a number of forms, due both to alternative splicing and to RNA editing. The AMPA receptors are widespread in the rat brain, especially in cortex and hippocampus; the kainate receptors may have a more limited distribution. Less is known, however, about the distributions of these mRNAs in primate and human brain. In this study, we have determined the distributions of the AMPA and kainate subunit mRNAs in old-world monkey and human brain by *in situ* hybridization using [^{35}S]-labeled riboprobes. These subunit mRNAs were found to be differentially distributed in monkey and human brain, and in many cases different from what has been reported in the rat brain. These data reflect the complexity of the mammalian glutamate systems, and prepare the way for our ongoing studies on the expression of glutamate receptors in postmortem brains of schizophrenics.

635.8

SUBCELLULAR LOCALIZATION OF CAM II KINASE-α AND GLUTAMATE RECEPTORS IN VB NUCLEUS OF RAT THALAMUS. X.-B. Liu*, R.M. Mansour and E.G. Jones. Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Low threshold somatosensory input to the ventrobasal thalamus (VB) is mediated by NMDA and non-NMDA receptors. Both NMDA and non-NMDA receptors are substrates for CAM II kinase which is specifically expressed by thalamic relay neurons. The precise location of immunoreactivity for the receptor proteins in relation to identified afferent axon terminals has not been established. Preembedding immunostaining (for receptor proteins) and postembedding immunogold labelling (for glutamate and GABA) revealed the relationship of CAM II kinase-α, glutamate receptor proteins and glutamatergic or GABAergic axon terminals in rat VB nucleus. Immunostaining for CAM II kinase-α, NMDAR1 and GluR2/3 labeled many neurons in VB. NMDAR1, GluR2/3 and CAM II kinase-α immunoreactivity was found within somata and dendrites of glutamatergic relay cells. Glutamatergic RL type terminals were associated with postsynaptic density immunoreactivity for all three in large dendrites. CAM II kinase-α, NMDAR1 and GluR2/3 immunoreactivity in distal dendrites was confined to postsynaptic densities and especially associated with glutamatergic RS terminals. GABAergic terminals were not associated with CAM II kinase-α, NMDAR1 or GluR2/3 immunoreactive synaptic densities. These results indicate: (1) CAM II kinase-α, NMDAR1 and GluR2/3 have similar distribution patterns in VB, and are colocalized within excitatory relay cells. (2) CAM II kinase-α and glutamate receptor proteins are localized postsynaptically and associated specifically with glutamatergic terminals.

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635.9

N-METHYL-D-ASPARTATE RECEPTOR LOCALIZATION, IDENTIFIED USING ANTIBODY IMMUNOCYTOCHEMISTRY, IS PRIMARILY POSTSYNAPTIC IN THE CAT SUPERIOR COLLICULUS R.R. Mize* and G.D. Butler Dept. of Anatomy and Neuroscience Center, Louisiana State University Medical Center, New Orleans, LA 70112

The n-methyl-d-aspartate receptor (NMDAR), an ionotropic glutamate receptor, is known to play an important role in long term potentiation and synapse plasticity. However, little is known about the subcellular distribution of this receptor in brain. We have used antibody immunocytochemistry to localize receptor sites for the NMDAR1 subunit of this receptor in the cat superior colliculus (SC). Lesions of areas 17-18 were made in 2 adult cats in order to identify cortical terminals within SC.

At the light microscope level, NMDAR1 receptor labeling was distributed throughout all layers of SC. The densest labeling was seen in the superficial gray layer (SGL). The antibody labeled both dendritic processes and selected cell bodies. At the electron microscope level, the NMDAR1 receptor subunit had a highly specific distribution. Label was identified at many synaptic sites, primarily at the postsynaptic densification. Internalized label was also seen occasionally along the membranes of mitochondria and on microtubules and neurofilaments within labeled dendrites. Presynaptic profiles opposite labeled dendrites were frequently retinal terminals with pale mitochondria (RTs) or cortical terminals (CTs), both of which contain round synaptic vesicles and are known to be glutamatergic. Labeled profiles receiving input from synaptic terminals with flattened vesicles were also seen occasionally. The types of profiles labeled by NMDAR1 included both large caliber conventional dendrites, and frequently, presynaptic dendrites containing loose accumulations of pleomorphic synaptic vesicles which are known to be GABAergic. By contrast, label was rarely if ever found in association with presynaptic axon terminals.

These results show that the NMDAR1 receptor subunit is selectively associated with the postsynaptic densities of synapses known to use glutamate. It also frequently labels the postsynaptic densities found on the membranes of GABAergic presynaptic dendrites. Supported by NIH EY-02973.

635.10

THE LOCALIZATION OF GLUTAMATE RECEPTOR SUBTYPE-1 (GluR1) mRNA EXPRESSION IN HUMAN POSTMORTEM BRAIN TISSUE. J. Logel*, C.R. Breese, C.E. Adams, and S. Leonard. Department of Pharmacology, University of Colorado Health Sciences Center, and Veterans Administration Medical Center, Denver, CO 80262.

Glutamatergic receptors have been localized throughout the mammalian central nervous system (CNS). Since glutamatergic neurotransmission plays a crucial role in normal CNS function, changes in glutamate receptor function may be important in the pathogenesis of a number of neurological disorders. A human cRNA probe to GluR1, was used for *in situ* hybridization to examine the anatomical and cellular localization of the GluR1 receptor subunit in various regions of human post-mortem brain tissue. Our results indicate that the highest levels of hybridization were to the hippocampal pyramidal cells of the CA1-CA3 region, and the granular cells of the dentate gyrus. Hybridization was also observed in medium to large neurons of the cingulate cortex, temporal lobe, septum, and amygdala, as well as in scattered neurons in the thalamus, cerebral cortex, and medulla. Light hybridization was also observed along the granular/molecular layer border of the cerebellum. These results closely correspond with relative GluR1 receptor levels observed in several of these brain regions examined using a western blotting protocol (Breese and Leonard, J Mol Neurosci 4:263-275, 1994), as well as for the observed and reported mRNA localization in the rat brain (Keinanen et al., Science 249:556-560, 1992). Studies are currently underway to map and compare the localization of other members of the AMPA family of glutamatergic receptors in the human brain. Examination of the glutamatergic receptor protein and mRNA expression in human postmortem brain tissue may provide information on the molecular basis of a variety of neurological disorders of the CNS.

635.11

EXPRESSION OF NADPH-DIAPHORASE IN THE CROTALINE SNAKE NERVOUS SYSTEM. S. Terashima* and P.-J. Jiang. Dept. of Physiol., Univ. of the Ryukyus Sch. of Med., Nishihara-cho, Okinawa 903-01 Japan.

As a specific method for detecting nitric oxide-producing neurons we used NADPH-diaphorase histochemistry. We found some discrete populations for NADPH-d in the brain, spinal cord, and ganglia of the crotaline snake *Trimeresurus flavoviridis*.

Nine snakes were anesthetized with halothane gas and with ketamine (n=7) or pentobarbital sodium (n=2) (no difference was observed between the results of the two groups), and paralyzed with pancuronium with artificial respiration. The snakes were perfused with freshly prepared fixative solution consisting of 2.5% paraformaldehyde and 1.5% glutaraldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The brain was postfixed in the same fixative for 1 hour. Serial frozen sections were cut at 30-50 μm coronally or sagittally and collected into 0.1 M PB. The free-floating sections were reacted for 1-3 hrs using conventional NADPH-d histochemical procedures. The reaction solution contained 100 mg β-NADPH (Kohjin Co., Japan), 20 mg nitroblue tetrazolium (Sigma), and 0.3% Triton-X 100 in 100 ml 0.1 M PB. Histochemical control experiments were reacted without NADPH.

Some populations were positive in NADPH-d labeling: 1) periglomerular cells of the accessory olfactory bulb, 2) supraoptic nucleus, 3) tall ependymal cells of the subcommissure organ, 4) neurons and their fibers in the descending nucleus of the trigeminal nerve, 5) large and small cells of the nucleus mesencephalicus n. trigemini, 6) nucleus loci coerulei, 7) columna viscero-motorius, 8) neurons in the trigeminal ganglion, 9) neurons in the nodose ganglion, and 10) fibers of the dorsal root. We could not find NADPH-d positivity in the cortex or the cerebellum.

In conclusion, NADPH-d positivity is very specific for some cells in the nervous system of the crotaline snake.

635.12

NADPH DIAPHORASE IN THE INSULAR CORTEX OF THE SYRIAN GOLDEN HAMSTER. R.G. Wehby*[1] and J.A. London[1,2], Center for Neurological Sciences[1] and Department of BioStructure and Function[2], University of Connecticut Health Center, Farmington, CT 06030.

NADPH diaphorase (NADPHd) activity in the insular cortex of the Syrian golden hamster was examined. Staining was found in both cell bodies and fibers (n = 7 animals). The cell body staining is of two types: (a) cells that are filled solidly with reaction product, or (b) cells that display granules of reaction product in the cytoplasm. The solid cells are: (1) found scattered singly, (2) mostly large, aspiny and multipolar, (3) mostly (>85% of cells, two animals, n = 622 cells) located in layers V and VI, and (4) mostly (>54% of cells, two animals, n = 622 cells) located rostral to and including the genu of the corpus callosum. In contrast, the cells with the granular cytoplasmic reaction product are: (1) very numerous and are grouped together, so that they appear to form a band or ribbon of labeled cells, (2) medium-sized, (3) found almost exclusively in layers II and III of the dysgranular and agranular insular cortex, and (4) found throughout the rostrocaudal extent of the insular cortex. Stained fibers are found largely in layers II, III, V, VI, and have a strong radial orientation in layers V and VI. The NADPHd procedure used in this study indicates nitric oxide synthase is present (Matsumoto et al., 1993). These data thus suggest that nitric oxide plays a role in the function of the insular cortex of the hamster.

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635.13

HISTAMINE H3 RECEPTORS AND DENERVATION SUPERSENSITIVITY. T. Watanabe*, J.H. Ryu, Y. Nakagawa[1], X.L. Zhao, H.W.M. Steinbusch[2] and K. Yanai. Depts. Pharmacology I and Ophthalmology[1], Tohoku Univ. Sch. of Med., Sendai 980, Japan, and [2]Dept. Psychiatry and Neurophysiology, Univ. of Maastricht, the Netherlands.

Histamine H3 receptors are not only presynaptic autoreceptors of histaminergic neurons but also heteroreceptors located at their postsynaptic neurons. We examined changes of the density of H3 receptors using 3 different types of neurotoxic and mechanical denervation by quantitative autoradiography with tritium sensitive phosphor imaging plates and [^{3}H](R)-α-methylhistamine as ligand. Changes in NADPH diaphorase histochemistry and NO synthase (NOS) immunostaining were examined in parallel. H3 receptor binding in the visually deprived superior colliculus, contralateral to the enucleated eye, was markedly increased 5-50 days after unilateral orbital enucleation of PVG rats, but NOS was not induced in the superior colliculus. H3 receptors were also highly up-regulated in the denervated striatum and substantia nigra of rats after unilateral intranigral injection of 6-hydroxydopamine. The distribution of H3 receptors was not changed in the dorsal motor nucleus of the vagus and the ambiguus nucleus of rats after unilateral vagotomy, although NADPH/NOS were highly increased in the vagal complex. These data showed that H3 receptors are markedly up-regulated at the postsynaptic sites of denervated neurons, whereas NOS is highly increased at the damaged neurons by neuronal injuries probably due to the increased cytosolic free calcium concentrations.

635.14

IMMUNOCYTOCHEMISTRY FOR ARGININE IN THE RAT CENTRAL NERVOUS SYSTEM. Petrusz P.*, Kharazia V.N., Grossman G., Weinberg R.J., Salt T.E.† Dept. of Cell Biology & Anatomy, U. of North Carolina, Chapel Hill, NC 27599, U.S.A.; †Institute of Ophthalmology, U. of London, U.K.

Activity in the thalamus may be modulated via multiple mechanisms. Stimulation of afferents to the ventrobasal thalamic complex triggers release of arginine (Do et al., 1994), suggesting a modulatory role for nitric oxide in thalamic mechanisms of somatosensation. We performed immunocytochemistry to study the regional and cellular distribution of arginine. Antibodies were prepared in rabbits against arginine, conjugated to keyhole limpet hemocyanin according to Aoki et al. (1991). As shown by ELISA these antibodies are highly sensitive and specific for L-arginine. Anesthetized Sprague-Dawley rats were perfused with aldehydes; brains were removed and postfixed in the same fixatives for 2-8 hours. Sections of paraffin-embedded material on slides, or free-floating Vibratome-cut material, were processed for immunocytochemistry. Staining in the thalamus was predominantly in endothelium and in cells recognizable for their appearance and distribution as neuroglia. Nuclei of many neurons were immunopositive and staining was in the cytoplasm of neurons in some extrathalamic structures, e.g raphe and septum. Post-embedding electron microscopic immunocytochemistry confirmed that staining is predominantly in endothelial cells and glia. These results extend and support previous information about the location of arginine in the central nervous system (Aoki et al., 1991). Arginine in the thalamus may be stored in glia whence it is supplied to terminals as substrate for the activity of nitric oxide synthase.

635.15

EXPRESSION OF THE INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR TYPE 1 (IP3R1) PROMOTER-*lacZ* FUSION GENE IN TRANSGENIC MOUSE BRAIN. D. Furutama[1,2], K. Shimoda[3], S. Yoshikawa[1], A. Miyawaki[1], T. Furuichi*[1], and K. Mikoshiba[1]
1: Dept. of Mol. Neurobiol., Institute of Med. Sci., Univ. of Tokyo,
2: First Dept. of Internal Medicine, Osaka Med. College,
3: Lab. of Animal Center, Sch. of Med., Keio Univ., Japan

To analyze the spatial and temporal pattern of IP3R1 expression in detail, we generated lines of transgenic mice carrying sequences upstream of mouse IP3R1 gene (5'-promoter regions; 1N region from -528 to +169 or 4N region from -4187 to +169) fused to E. coli β-galactosidase (β-gal) gene (*lacZ*). The cell type specificity of transgene expression was examined by tissue histochemistry for β-gal activity using a chromogenic substrate, X-gal. Two of ten lines obtained, both 1N lines and 4N lines, showed characteristic patterns of β-gal activity in brain as well as in non-neuronal tissues, almost consistent with the expression of the endogenous IP3R1 expression known thus far. The transgene expression in a 1N line showed segment-like pattern in the developing cerebellar Purkinje cells, but not in the 4N lines. These findings suggest that the short region from -528 to +169 is involved in the cell type-specific expression of IP3R1, and might confer the segment-like gene expression in the cerebellar Purkinje cells.

635.16

CORTICOTOPIC ORGANIZATION OF THE CHOLINERGIC PROJECTIONS FROM THE BASAL FOREBRAIN IN RATS: PARALLEL ELECTROPHYSIOLOGY AND MICRODIALYSIS-HPLC DATA. R.W. Dykes, M.E. Capdeville and A.A. Myasnikov* Dept. de physiol., Faculty de med., Univ. de Montreal, C.P. 6128, Succ. centre-ville, Montreal (Que.) H3C 3J7.

The acetylcholine (ACh) released from the somatosensory and either the visual or motor areas of cortex was measured simultaneously in acute experiments using microdialysis and high performance liquid chromatography (HPLC) in 23 adult male rats anesthetized with urethane or sodium pentobarbital. Electrical stimulation of different basal forebrain (BF) sites released different amount of ACh from different cortical sites suggesting some degree of corticotopic organization of the cholinergic projections from BF. The BF sites provoking the highest rates of release from the somatosensory and visual cortex were found at greater distances from one another than the sites in the BF capable of most effectively releasing ACh from the somatosensory and motor regions.

Stimulation of the BF also changed cortical neuronal activity recorded with a tungsten-in-glass electrodes. The magnitude, duration and delay of the multiunit responses in somatosensory cortex varied with the BF site stimulated. The nature of the response served as a predictor of the amount of ACh that would be released by stimulation of that BF site. Some BF sites provoked a long inhibition of spontaneous cortical neuronal activity and did not increase the ACh released while others provoked a marked enhancement of cortical activity and significant increases in ACh release. The changing relationship among the excitatory sites and the inhibitory sites suggests an elaborate control of sensory cortex by a BF mechanism involving an interaction of the gabaergic and cholinergic neurons found there.

(Supported by the Medical Research Council of Canada)

635.17

INVESTIGATION OF IL-1 RECEPTORS EXPRESSION IN THE RAT BRAIN

G. N. Luheshi*, C. Davies, N. J. Rothwell and S. Toulmond.
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Interleukin-1 (IL-1) is a potent endogenous pyrogen which acts directly on the central nervous system to induce fever. Our studies in rats show that intracerebroventricular injections of IL-1ß induce marked increases in body temperature, which is inhibited by the central injection of ALVA-42, an antibody raised against the human 68kDa Type-II IL-1 receptor, suggesting the involvement of this receptor in fever. Other groups have suggested that the type II receptor is not functional, but acts as a decoy receptor, and that although ALVA-42 binds to IL-1 type II receptor expressing cells (human B lymphocytes), it does not bind to the type-II receptor but instead to MHC class II antigen expressed on the same cells. While distribution of type II receptors in rat brain is unknown, MHC class II antigens are known not to be expressed by normal brain cells, but to be induced by inflammatory challenges or after brain lesion. We have therefore investigated and compared ALVA-42 binding and MHC class II antigen expression in both normal and injured (lateral fluid percussion trauma) rat brain. Immunocytochemical analysis (avidin-biotin method) was carried out on vibratome sections (80 µm) of perfused-fixed rat brain, with monoclonal mouse anti-human IL-1 type II receptor (ALVA-42; Genzyme) and monoclonal mouse anti-rat MHC class II antigen (OX6; Serotec) as primary antibodies. Results from these studies revealed positive ALVA-42 binding to astrocytes in identical areas of normal and injured brain (hypothalamus, hippocampus, corpus callosum). In contrast, MHC class II antigens were not detected in normal brain, while their expression by microglia-like cells was evident at the lesion site in the injured brain. MHC class II antigens were not detectable in any of the brain regions positive for ALVA-42 binding. These observations indicate that, in rat brain, ALVA-42 is binding to sites other than MHC class II antigens. Further experiments are required to confirm ALVA-42 binding to IL-1 type II receptors.

635.18

ESTROGEN RECEPTOR IMMUNOREACTIVE CELLS IN THE FOREBRAIN OF FEMALE RABBITS: EFFECT OF ESTRADIOL. M. Caba[1,2], C. Beyer[1], G. González-Mariscal[1*], and J.I. Morrell[3]. [1]CIRA: CINVESTAV-Universidad Autónoma de Tlaxcala and [2]Universidad Veracruzana(México); [3]Center for Molecular and Behavioral Neuroscience, Rutgers University (Newark NJ, USA).

Little is known about the distribution of estrogen receptors (ERs) in the female rabbit brain. Yet, as in many mammals, estradiol in female rabbits regulates many reproductive events (e.g., ovulation, scent-marking, sexual behavior, maternal behavior). Therefore, information on the distribution and regulation of ERs in the brain of female rabbits is crucial for understanding many aspects of the female rabbit's reproductive physiology. Rabbits from three different conditions were used: estrous (E), ovariectomized-non treated (OVX), and OVX-estrogen treated (OVX-E_2; 5 µg estradiol benzoate/day for five days). Subjects (Ss) were perfused with paraformaldehide and sections through the forebrain were incubated with the H222 antibody (Abbot labs.) and reacted with nickel-intensifed diaminobenzidine. Nuclear staining was observed in OVX does in: preoptic area (POA), ventromedial hypothalamus (VMH), arcuate nucleus (ARC), and lateral hypothalamus (LH). Neurons in the LH also showed immunoreactivity in the cytoplasm. The presence of estradiol, in both E and OVX-E_2 Ss, down-regulated ER-immunoreactivity in the POA and LH but only slightly decreased staining in the VMH and ARC. Thus, the effects of estrogen on ER-immunoreactivity in the female rabbit brain are apparently not uniform: while this steroid may selectively down-regulate ER-immunoreactivity in some brain regions, it seems to have little or no effect in other areas.

635.19

DISTRIBUTION OF PROGESTERONE RECEPTOR mRNA IN THE MOUSE BRAIN AS DETERMINED BY *IN SITU* HYBRIDIZATION.

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Kinsmen Laboratory of Neurological Research, Dept. of Psychiatry, University of British Columbia, Vancouver, B.C. Canada, V6T 1Z3.

Hormones exert profound effects upon behaviour. Many of these effects are mediated by classic intracellular hormone receptors, acting as ligand-dependent transcription factors. As part of an ongoing research program aimed at understanding the role of hormones in altering gene transcription in the brain, we have mapped the distribution of progesterone receptors in the mouse brain. To determine the distribution of progesterone receptor (PR) in the mouse brain, RT-PCR and in situ hybridization techniques were applied. A PCR product the size predicted from the cDNA for mouse PR was obtained from mouse uterine total RNA. The amplified product of this cDNA was subcloned, sequenced and consequently confirmed to be PR. *In situ* hybridization with riboprobes derived from the cloned PR PCR product indicate that this steroid receptor is highly expressed in the hypothalamus, cortex, amygdala, substantia nigra and the bed nucleus of the stria terminalis, and moderately expressed in the hippocampus and habenula. Similar expression patterns have been observed in previous mRNA localization studies performed on rats. These data demonstrate that the distribution of progesterone receptors is well conserved in rodent brains, and lays the foundation for studying progesterone receptor function in the mouse brain.

REGIONAL LOCALIZATION OF RECEPTORS AND TRANSMITTERS II

636.1

DIFFERENTIAL LOCALIZATION OF D_2 VERSUS D_3 mRNA IN MIDGESTATIONAL HUMAN FOREBRAIN BY *IN SITU* HYBRIDIZATION. A.S. Unis*, M.D. Roberson, K. Hill, M.W. Hamblin and D.M. Dorsa. Dept. of Psychiatry & Beh. Sci., and the Seattle VAMC, Univ. of Washington, Seattle, WA 98195-6560.

Dopamine receptors are expressed in human forebrain in the first trimester and increase approximately 10-fold in concentration by the second trimester. Because dopamine appears to induce neuronal differentiation when D_2 and D_3 receptors are transfected into mouse neuroblastoma cells, Todd et al. (1992) have suggested that dopamine may play a "morphogenic" role in brain development. Using *in situ* hybridization with ^{33}P-UTP-labeled antisense and sense riboprobes for D_2 and D_3 receptor mRNA's, we characterized the similarities and differences in dopamine D_2 and D_3 receptor gene expression in first and second trimester human forebrain.

Antisense D_3 receptor riboprobe hybridization could be clearly identified in the first trimester forebrain within the cerebral vesicles. At midgestation, intense hybridization signal localized to the periventricular germinal matrix and the cortical plate. Hybridization signal in the germinal matrix appeared to intensify from frontal to parietal coronal sections and attenuated in occipital regions until it was undetectable in sections containing striatal cortex. Some antisense hybridization signal could be seen in the caudate but was dorsomedial in its localization and weak in comparison to that seen in the germinal matrix. As expected, D_3 sense riboprobe controls failed to hybridize to any anatomic areas in corresponding sections. In marked contrast to the D_3 findings, D_2 antisense riboprobe hybridized at midgestation to caudate and midbrain, with no detectable hybrization signal in adjacent differentiating neuronal populations. Hybridization signal instensity appeared to follow a ventrolateral-to-medial gradient within brain sections and was higher when comparing brains from gestational week 24 to 20.

If dopamine plays a role in neuronal differentiation in the human brain, the prominent expression of D_3 receptors in extra-striatal, rapidly developing brain regions make this receptor a likely candidate to mediate dopamine's effects.

636.2

FLUORESCENCE COLOCALIZATION OF D1 AND D2 DOPAMINE RECEPTORS ON LIVING NUCLEUS ACCUMBENS MEDIUM SPINY NEURONS IN VITRO.

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In the striatal complex, it has been controversial as to whether D1-D2 interactions occur at the level of individual postsynaptic neurons. To address this issue, we visualized D1 and D2-like receptors on living postnatal rat nucleus accumbens (nAcc) neurons in culture. Previously, we have shown that rhodamine-NAPS (10 nM), a fluoroprobe derivative of the D2 antagonist spiperone, labels D2 receptors on living nAcc neurons (Rayport and Sulzer, *J. Neurochem.*, 1995). We now show that rhodamine-SCH23390 (Molecular Probes; 30 nM), a derivative of the D1 antagonist, labels D1-like receptors. Putative specific membrane labeling reached a plateau after about 20 min. Labeling was stereospecific, as it was unaffected by competition with 100 nM (-)butaclamol, while it was completely blocked by 100 nM (+)butaclamol (the butaclamol enantiomers show a ~10,000-fold differential affinity for cloned D1 receptors). In 14 cultures (n = 178 cells), we found that 52 ± 7% of nAcc medium spiny neurons showed D1 labeling. D1 labeling was also seen on larger putative cholinergic neurons as well as on presynaptic terminals abutting D1-labeled and unlabeled cell bodies. By sequential labeling of the cultures first with the rhodamine-SCH23390 and then rhodamine-NAPS, we found that 38 ± 6% of medium spiny neurons express both D1 and D2-like receptors. This indicates that D1-D2 interactions in the nAcc are likely to occur at the level of individual postsynaptic cells.

636.3

ENRICHMENT OF DOPAMINE TRANSPORTER, AND D_{1A} AND D_{2A} RECEPTORS IN STRIATAL PATCHES DURING ONTOGENY. B.J. Ciliax*, N.F. Ciliax, A.I. Levey. Department of Neurology, Emory University School of Medicine, Atlanta, GA 30322

Striatal patch and matrix compartments receive dopaminergic nigrostriatal afferents and send out striatonigral efferents at different times during ontogeny. Relative expression, however, of dopamine receptor molecular subtypes and dopamine transporter (DAT) in these striatal compartments and in substantia nigra has not been determined during pre- and postnatal development. We used characterized anti-fusion protein antibodies specific for dopamine D_{1A} and D_{2A} receptors, and DAT for light microscopic immunocytochemistry in developing rat brain. Each of these dopaminergic proteins were enriched in the striatal patch compartment by postnatal day 0. In rostral striatum, patches were located more ventrolaterally early and dorsomedial patches appeared later. Expression of these proteins in matrix occurred in a similar ventrolateral to dorsomedial sequence. In substantia nigra, D_{1A} was localized to puncta in pars reticulata, while D_{2A} and DAT were localized to neurons in pars compacta, similar to respective adult distribution patterns. These data indicate that dopaminergic connections in patch compartment mature more quickly than those of matrix. Moreover, since proteins responsible for dopaminergic signal transduction and termination are present early in development, dopamine neurotransmission is probably functional in perinatal basal ganglia. Supported by the APDA and the Stanley Foundation.

636.4

DISTRIBUTION OF DOPAMINE D_2-FAMILY RECEPTORS IN HUMAN POSTMORTEM BRAIN SECTIONS: COMPARISON OF RECEPTOR DENSITIES BETWEEN NORMALS AND SCHIZOPHRENICS OFF AND ON NEUROLEPTICS. R.A.Lahti*, R.C. Roberts, R. Conley and C.A. Tamminga. Maryland Psychiatric Research Center, Univ. of Maryland School of Medicine, Baltimore, MD 21228.

The D2, D3 and D4 receptors comprise the D2-family of receptors (Bunzow, 1988; Sokoloff, 1990; Van Tol 1991). Most neuroleptics are D2 selective yet have high affinity for the D4 receptor; clozapine is selective for the D4 receptor. Studies have found increases in the D4 density in striata of schizophrenic vs normal postmortem tissue (Seeman, 1992; Sumiyoshi, 1994; Murray, 1995). We have determined receptor distributions autoradiographically in sections from normal human postmortem tissue (Maryland Brain Collection). The ligands were [^{3}H]-raclopride for D2/D3; [^{3}H]-YM-09151-2 for D2/D3/D4; and [^{3}H]-(+)-7-OH-DPAT for the D3. The difference between [^{3}H]-raclopride and [^{3}H]-(+)-7-OH-DPAT is taken as D2 distribution, between [^{3}H]-YM-09151-2 and [^{3}H]-raclopride as the D4 distribution. D2 receptors are most dense in putamen, caudate and nuc. accumbens; D3 in nuc. accumbens; D4 in cortical areas, hippocampus, with little evident in the caudate-putamen. These receptor distribution patterns are similar to those found for their mRNAs (Meador-Woodruff, 1994, 1995; Landwehrmeyer, 1993). Numerous brain structures were analyzed for receptor densities in normal controls (n=9), and schizophrenic subjects off (n=5) and on (n=3) drug at the time of death. No statistically significant differences were found between normals and either group of schizophrenics using [^{3}H]-YM-09151-2, [^{3}H]-raclopride or the difference between the two ligands, the "presumed D4" receptor. Thus no evidence was found for an increase in any D_2-family receptor in schizophrenics off neuroleptics, including the D_4 receptor, as reported by others.

636.5

IN VIVO RECEPTOR BINDING BY A UNIQUE PET D-2 RADIOLIGAND: N-METHYL-[F-18]BENPERIDOL. S.M. Moerlein*, J.S. Perlmutter and M.J. Welch. Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, MO 63110.

Previous PET studies have shown that N-methyl-[F-18]benperidol ([F-18]NMB) is a high-affinity D-2 ligand that binds specifically to D-2 receptors *in vivo* (JCBFM 13: S235 [1993]). In this work we have further evaluated the D-2 receptor binding characteristics of this PET radioligand. In particular, the reversibility of binding, inertness to elevated dopamine levels, and internalization of the radioligand were studied in primates with use of PET. [F-18]NMB selectively localizes in control studies within D-2 receptor-rich tissues, resulting in striatum/cerebellum radioactivity concentration ratios as high as 15 after 3 h. Unlabeled D-2 antagonist eticlopride (4 mg/kg, i.v.) given 45 min after [F-18]NMB caused a rapid decrease in striatal activity to cerebellar levels, indicating that D-2 receptor binding was reversible. *In vivo* localization of [F-18]NMB in the striatum was unaffected by dextroamphetamine (1 mg/kg, i.v.) or levodopa/carbidopa (20 and 10 mg/kg, p.o.), showing that receptor binding by the radioligand was resistant to competitive displacement by synaptic dopamine. [F-18]NMB does not undergo agonist-mediated internalization, as demonstrated by studies involving sequential i.v. administration of [F-18]NMB, dextroamphetamine (1 mg/kg 30 min after [F-18]NMB), and eticlopride (4 mg/kg 68 min after [F-18]NMB). In this experiment, dextroamphetamine had no effect on striatal binding, yet subsequent administration of eticlopride completely displaced [F-18]NMB from D-2 receptors. The special combination of reversible D-2 binding by [F-18]NMB that is fully displaceable by D-2 antagonists yet resistant to physiologically-meaningful levels of dopamine makes this radioligand unique among all other PET radiopharmaceuticals.

636.6

WHOLE-BODY BIODISTRIBUTION OF THE PET D2 RADIOLIGAND N-METHYL-[F-18]BENPERIDOL IN PRIMATES. J. Giovanni, J.S. Perlmutter*, S.M. Moerlein, P.D. Cutler, M.M. Anderson and M.J. Welch. Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, MO 63110.

PET imaging studies of nonhuman primates have shown that N-methyl-[F-18]benperidol ([F-18]NMB) is a unique radioligand for noninvasive investigation of cerebral D2 receptors. Preceding application of this tracer in humans, we have estimated its absorbed radiation dosimetry via PET imaging of baboons injected with [F-18]NMB. Four scanning positions corresponding to different regions of the animals' bodies were imaged sequentially. Position 1 included the brain and eyes, position 2 the heart and lungs, position 3 the liver, gall bladder, upper intestines and kidneys, and position 4 the bladder, lower intestines and gonads. Accumulation of radioactivity accumulation in tissues other than these organs was uniform and minimal. Tissue attenuation was measured for each position via a transmission scan using rotating rod sources of [Ge-68]/[Ga-68]. After injection of [F-18]NMB, 5-min long scans were acquired for each position sequentially over a 4 h interval; for each position, 4-5 scans were obtained. For smaller organs, regions of interest (ROI) were defined that completely encompassed the target organ. For larger organs, multiple large representative ROIs were identified that included all local peaks of radioactivity. The average activity concentration then was calculated for the target organ and multiplied by the organ weights to give the radioactivity accumulation per organ as a function of time. The residence time of radioactivity in each organ and S-values were used to derive absorbed dose per unit cumulated activity. For reference man, the critical organ was the gall bladder (0.6-1.1 rad/mCi), with relatively high dose delivered to the heart wall (0.3 rad/mCi) as well. Gonadal dose was minimal (0.05 rad/mCi). The results suggest that ca. 5 mCi of [F-18]NMB can be safely administered to human subjects for PET study of cerebral D2 receptor binding *in vivo*.

636.7

DOPAMINE, NOREPINEPHRINE, AND SEROTONIN AXONS IN THE HUMAN AND MONKEY NUCLEUS BASALIS, AND IN ALZHEIMER'S DISEASE. J.F. Smiley* and M.-M. Mesulam. Northwestern University, Chicago, IL.

Physiology studies in the rat have shown that monoaminergic modulation of the nucleus basalis can affect cortical acetylcholine release. While anatomical studies in the rat have shown monoamine input to the nucleus basalis, similar evidence is lacking in primates. We used single- and double-labeling immunocytochemistry to visualize monoamine axons and their proximity to cholinergic Ch4 cells in the nucleus basalis. Norepinephrine axons, labeled with anti-dopamine-ß-hydroxylase (DBH), formed a bed of fine varicose axons that co-distributed with Ch4 neurons. Double labeling showed frequent contacts with the soma and dendrites of cholinergic cells. Tyrosine hydroxylase (TH-) immunoreactive axons, presumed to be mainly dopaminergic, were 10 to 20 times more abundant than DBH axons. While many TH axons were thick and non-varicose, there were also small varicose axons, which appeared in double labeled sections to make contacts with Ch4 neurons. Reliable serotonin-immunoreactivity was obtained only in the monkey. Fine serotonin-immunoreactive axons were abundant throughout the basal forebrain, and double labeling showed many axons in contact with Ch4 neurons. In summary, all three monoamine systems appear to innervate the cholinergic (and probably also non-cholinergic) neurons of the nucleus basalis. Preliminary measurements of DBH and TH axon density in Alzheimer's and non-Alzheimer nucleus basalis suggest that the density of these monoamine axons in diseased brains is within the range of density seen in controls. Supported by NS20285-11.

636.8

LOCALIZATION OF MULTIPLE DOPAMINE D1 RECEPTOR SUBTYPES IN AVIAN BRAIN USING *IN SITU* HYBRIDIZATION AND RECEPTOR AUTORADIOGRAPHY. L.L.Demchyshyn*, L.M. Dixon, J.N. Nobrega and H.B. Niznik. *Depts. of Psychiatry & Pharmacology, Univ. of Toronto and Lab. Mol. Neurobiology, Clarke Institute of Psychiatry, Toronto, Canada, M5T 1R8.*

To date, the avian dopamine D1 receptor subfamily is encoded by three distinct genes termed D1A, D1B, and D1D, which are distinguished on the basis of their pharmacological and structural profiles. Northern blot analysis has demonstrated the expression of all three receptors in brain. We assess here the distribution of these receptors by *in situ* hybridization of messenger RNA's encoding D1A, D1B and D1D receptors and by *in vitro* receptor autoradiography in chicken brain. At 1-2nM, [^{3}H]SCH23390 bound receptors primarily in 'striatal' regions, specifically the paleostriatum complex, as well as nucleus accumbens and olfactory tubercle. Non-specific binding was determined in the presence of 2µM (+)butaclamol. The D1A receptor mRNA visualized by non-overlapping [^{35}S-dATP] labelled oligonucleotide probes from unconserved 3' regions were strongly expressed within striatal regions. Preliminary observations indicate that the D1B and D1D receptors were also expressed to a lesser extent within these regions. Control slices were either RNase pre-treated or incubated in the presence of excess unlabelled oligonucleotides. The distribution of dopamine D1-like receptor mRNA's corresponded to receptor binding. Localization of D1 receptor subtypes in the paleostriatum coincide well with observations reported on the effects of dopaminergic agonists and antagonists on stereotypical and motor behaviour in birds. The analysis of these receptor subtypes may help to better understand the anatomical organization and how they further relate to the pharmacological and biochemical multiplicity of D1-mediated events.

636.9

INTERNALIZATION OF SIGMA(σ)-1 RECEPTORS INTO THE PRIMARY CULTURED NEURONAL CELLS. H Yamamoto[1], T. Yamamoto[1,2], A. Baba[1], E. Takamori[1], Y.L. Murashima[3]* and S. Okuyama[4]. [1]Dept. of Psychopharmacol., [3]Dept. of Neurophysiol., Tokyo Inst. Psychiatry, Tokyo 156, [2]Mol.Recog., Yokohama City Univ., Yokohama 236, [4]Taisho Pharmaceutical Co., Ltd., Saitama 330, Japan.

Bindings to the primary cultured neuronal cells were performed using [^{3}H]NE-100 or [^{3}H](+)pentazocine as selective σ-1 ligands. To assess the properties of σ-1 receptor, first, living cells in DMEM were incubated with [^{3}H]NE-100, [^{3}H](+)pentazocine or [^{3}H]TCP for 60 min at 4° and then 37°. Bounds of [^{3}H]NE-100 or [^{3}H](+)pentazocine at equilibrium were markedly higher at 37° than 4° and not dissociated by the addition of excess σ ligand. By contrast, bound of [^{3}H]TCP at 37° was not potently increased. When cells incubated at 37° were washed with acidic high sodium solution (pH 2.5) to wash out surface-bound radioactivity, a significant radioactivity was remained as a non-washable fraction. Second, incubating of cells with [^{3}H]NE-100 at 37°, but, in the presence of an endocytosis inhibitor, the binding was similar to that seen at 4°. Third, when cells were subjected to σ ligands at 37° for 30 min prior to binding, the non-washable bound was decreased in proportion to its affinity for σ-1 receptor. Although the results described here do not completely elucidate the question that the internalizaiton process of σ-1 ligand is due to ligand alone or ligand-receptor complex, the result which pre-exposure of cells to σ ligands induced reduced bound suggests that a time-and temperature-dependent increase of the selective σ-1 ligand binding to living cells is a consequence of receptor internalization.

636.10

THE EFFECTS OF AGING ON SPINAL NEUROCHEMISTRY IN THE RAT. M.L. Ko, J.L. Stafinsky and T. Crisp*. Departments of Pharmacology and Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

The purpose of this study is to assess the effects of aging on basal levels of norepinephrine (NE), dopamine (DA), serotonin (5-HT) and their respective metabolites in the spinal cord using high performance liquid chromatography (HPLC) and electrochemical detection. Young, mature and aged (5-6, 15-16 and 25-27 months old respectively) male Fischer 344 rats were used in all experiments. Dorsal and ventral halves of each vertebral section (cervical, thoracic, lumbar and sacral) were analyzed. In general, the results indicate that aged rats have less neurotransmitter/ug tissue than their younger cohorts with the main differences being in the levels of NE and 5-HT. An age-related increase in monoamine oxidase (MAO) activity may account for the observed decreases in biogenic amine levels with advanced age. Moreover, since NE and 5-HT are believed to play a role in the inhibition of primary nociceptive neurons in the spinal cord, a decrease in these neurotransmitters may diminish pain tolerance in aged animals.

636.11

Immunohistochemical localization of α_{2A}-adrenergic receptors in rat forebrain: light (LM) and electron microscopy (EM). D.L. Rosin,*[1] E.M. Talley,[1] T.A. Milner,[2] P.G. Guyenet[1] & K.R. Lynch,[1] Dept. of Pharmacol., Univ. of Virginia, Charlottesville, VA 23298[1] and Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., NY, NY 10021[2]

We have described previously the immunohistochemical localization of α_{2A}-adrenergic receptors (α_{2A}-ARs) in rat brainstem using a subtype-specific polyclonal antibody (Rosin et al., 1993; Guyenet et al., 1994) and now report the distribution of α_{2A}-ARs in di- and telencephalic nuclei. Light microscopic examination revealed discrete intracellular (dotted in appearance) and diffuse perikaryal α_{2A}-immunoreactivity (α_{2A}-LI) as well as neuropil labeling. Intense labeling was observed in the anterior olfactory nucleus, lateral septum, diagonal band, bed nucleus of the stria terminalis, fundus striatum and substriatal area, and various nuclei of the amygdala and hypothalamus. Labeling in the cortex and hippocampal formation displayed a clearly laminar distribution. Particularly striking was the intense neuropil labeling in the lacunosum molecular layer of CA1. The distribution of α_{2A}-LI in rat brain resembles closely the pattern of ^{3}H-*p*-aminoclonidine binding autoradiography reported by others. The dotted perikaryal α_{2A}-LI in the medial septum and in the CA1 region of hippocampus appeared by EM to correspond to peroxidase product associated with the Golgi apparatus and rough endoplasmic reticulum. In CA1 pyramidal cells, α_{2A}-LI was also found in large (0.5-1.0 μm in diameter) intracellular structures close to the nucleus that may be endosomal in nature but are clearly not lysosomal. Dendrites exhibited immunoreactivity associated with plasmalemmal surfaces both in terminals and at postsynaptic densities. Immunoreactivity (either by LM or EM) was eliminated by preadsorption of the antibody with excess antigen. In summary, α_{2A}-LI shows a wide pattern of distribution in rat forebrain consistent with other measures of receptor localization. Further ultrastructural analyses will help to define the cellular substrates of α_{2A}-AR function. *Supported by DA07216, DA08259, MH42834 and the Virginia Amer. Heart Assoc.*

636.12

DIFFERENTIAL DISTRIBUTION OF σ_2-ADRENOCEPTORS AND NORADRENERGIC NEURONS IN THE HUMAN LOCUS COERULEUS.

V. Klimek*, G.W. Cason, S.N. Luker, C.A. Stockmeier, G. Dilley, H.Y. Meltzer, J. Overholser, G.A. Ordway. Dept. Psychiatry & Human Behavior, University of Mississippi Med. Ctr., Jackson, MS 39216; Depts. Psychiatry & Psychology, Case Western Reserve University., Cleveland, OH 44106.

Morphometric changes in the human locus coeruleus (LC) have been described for normal aging, neurodegenerative diseases, and most recently, in major depression and in victims of suicide. Independently, changes in concentrations of σ_2-adrenoceptors (σ_2AR, noradrenergic autoreceptors) in the LC have been reported for normal aging and in victims of suicide. The relationship between neurochemical and cellular alterations in the LC has not been addressed. In this study, the quantitative autoradiography of p-[^{126}I]iodoclonidine ([^{126}I]PIC) binding to σ_2AR was assessed coordinately with a morphometric analysis of neuromelanin-containing cells (noradrenergic neurons) in tissue sections cut at 10-14 levels along the rostral-caudal axis of the LC in normal subjects (n = 7; ages 44-78). Both [^{126}I]PIC binding and the number of LC neurons were differentially distributed ($p < 0.01$) along the LC axis with almost identical topographic patterns. The highest concentrations of binding and the greatest number of noradrenergic cells per section occurred near the middle portion of the nucleus. There was a significant correlation between the number of noradrenergic cells per section vs. specific [^{126}I]PIC binding at any particular level of the LC ($r^2 = 0.59$, $p < 0.0001$). These data suggest that noradrenergic cell number determines the density of [^{126}I]-PIC binding to σ_2AR throughout the LC. Studies using the same measurements in suicide victims with retrospective diagnoses of major depression will also be presented. (Supported by MH46692 and MH45488).

636.13

DISTRIBUTION OF THE VESICULAR TRANSPORTER FOR ACETYLCHOLINE IN THE RAT CNS. R.H. Edwards*, A. Shirzadi, L.L. Butcher, and A. Roghani. Depts of Neurology and Psychology, UCLA, Los Angeles, CA 90024. Abundant evidence has implicated acetylcholine (ACh) in the pathogenesis of neurodegenerative disorders. ACh and other transmitters are stored into synaptic vesicles from which their release can be regulated in response to neural activity. This storage requires active transport from the cytoplasm. We recently found that vesicular monoamine transporters can protect against the neurotoxin MPP^+ and we used this phenomenon to clone two rat transporters(Cell 70, 539). Genetic studies in the nematode *C. elegans* recently identified a putative vesicular ACh transporter (*Unc-17*). This protein shows sequence homology to the vesicular amine transporters and its distribution supports a role in cholinergic transmission. Using RT-PCR, we have recently isolated homologous transporters, first from *Torpedo californica*, and then from rat spinal cord (rVAChT) (PNAS *91*, 10620). We also have demonstrated expression of high affinity vesamicol binding with this clone. In situ hybridization using labeled rVAChT RNA showed expression in all of known cholinergic nuclei in the central nervous system, including the basal forebrain, dorsal and ventral striata, mesopontine complex, motor and autonomic cranial nerve nuclei, and ventral and intermediolateral horns of the spinal cord. Hybridization with probes for choline acetyltransferase(ChAT) showed an identical pattern. We have also raised high titer antipeptide antibodies against the cloned transporter in rabbits. All the antisera recognize the VAChT protein on western blots of membrane preparations from VAChT-transfected cells. One of the antibodies stains apparent cholinergic neuronal cell bodies in all of known cholinergic nuclei identical to those we observed in our in situ hybridization studies. We also detect staining of cholinergic fibers and terminals in the cortex, basolateral amygdala, hippocampus, medial habenula, laterodorsal tegmental nuclei, thalamus, basal forebrain, superior colliculus, interpeduncular nucleus and dorsal and ventral striatum. The VAChT-positive cells often extend processes radiating in multiple directions with occasional branching, where many of these fibers are fine caliber and possess numerous puncta. Thus, the pattern of staining with antibodies to rVAChT strongly resembles that reported for the ChAT distribution in the rat CNS.

636.14

DISTRIBUTION OF MONOAMINES IN THE CORPUS CALLOSUM OF THE CAT. B. Jardon[1], F. Lepore[1], T.A. Reader[2], A.R. Ase[2], and C.F.P. Casanova[3]* *CRSN, Départements de Psychologie[1] et Physiologie[2], Université de Montréal, et Département d'Ophtalmologie[3], Université de Sherbrooke, (Québec) Canada.*

The aim of this work was to determine the presence of chemically-identified neurotransmitters in the corpus callosum (CC) of the cat. Brains from adult cats were rapidly frozen, sliced in coronal 3-4 mm thick sections, and the CC dissected over a cold plate under a binocular microscope. Three different portions of the CC were removed along a rostro-caudal axis, namely the anterior or *genu* (CCA), the middle or body (CCM) and the posterior or *splenium* (CCP). Samples from the visual cortex (VIS), or areas 17-18, as well as of the suprasylvian gyrus (SS) were also removed for comparison purposes. After extraction in 0.1 *N* monochloroacetic acid (pH 3.3) the samples were centrifuged (40,000 x *g* for 45 min at 4°C), and the supernatants assayed by HPLC with electrochemical detection (650 mV). The pellets were dissolved overnight in 1 *N* NaOH for protein assay. The catecholamines noradrenaline (NA), adrenaline (AD) and dopamine (DA) were present in all divisions of the CC; the greatest amounts were found in the CCA. There were also trace amounts of the NA metabolite 4-hydroxy-3-methoxyphenylglycol (MHPG), and of the DA metabolites 3,4-dihydroxyphenylglycol (DOPAC) and homovanillic acid (HVA). The indoleamine serotonin (5-HT) was measured in all the divisions of the CC, and was especially abundant in the CCP. Its main metabolite 5-hydroxyindole-3-acetic acid (5-HIAA) followed the same pattern of distribution. To confirm the identification of the monoamines, cats were treated with *p*-chlorophenylalanine (PCPA) or with α-methyl-*p*-tyrosine (αMPT) to block 5-HT or catecholamine synthesis, respectively. After PCPA, the indoleamine peaks (5-HT and 5-HIAA) were greatly reduced, while the DA and NA peaks decreased after αMPT. The results demonstrate the presence of monoamines in the cat CC. The data will be compared to anatomical studies of the CC using tracing methods, and to measurements obtained from animals that were callosotomized 6 months earlier. *[Supported by the MRC(C) and NSERC(C)]*

636.15

DISTRIBUTION OF THE α_{1A} ADRENOCEPTOR IN RAT CNS - COMPARISON OF *IN SITU* HYBRIDIZATION AND RECEPTOR AUTORADIOGRAPHY STUDIES. M.M. Durkin*, E.L. Gustafson, C. Forray, K.E. Smith, J.M. Wetzel, C. Gluchowski, T.A. Branchek. Synaptic Pharmaceutical Corporation, Paramus, NJ 07652.

In the present study we have employed two localization techniques to compare the anatomical distribution of the α_{1A} adrenoceptor (formerly α_{1C}) mRNA and binding sites in rat brain. *In situ* hybridization was performed to localize those cells which express the α_{1A} receptor RNA utilizing two sets of antisense/sense [^{35}S]dATP labeled oligoprobes designed to the coding region of the α_{1A} gene. *In vitro* receptor autoradiography using 1 nM [^{3}H]prazosin in the presence of 10 µM norepinephrine and 100 nM SNAP 5272, to selectively block the α_{1D} (formerly α_{1A}) and α_{1A} adrenoceptors, respectively, enabled us to evaluate the displacement of the ligand from its binding sites thereby indicating the presence of the α_{1A} receptor in the CNS. Nonspecific binding was determined using 10 µM phentolamine. The areas of highest gene expression were observed in the hippocampus and motor trigeminal nucleus, however, receptor binding in these areas was very low. Moderate levels of mRNA expression and binding sites were detected in the olfactory bulb, bed nucleus of the stria terminalis, hypothalamus, medial amygdala and horizontal diagonal band. Lower expression of message and number of binding sites were observed in all the layers of the cerebral cortex, caudate-putamen, nucleus solitarius, thalamus, central gray and in the brainstem. There is a high degree of correlation between the localization of the mRNA and the presence of binding sites for the α_{1A} adrenoceptor. The presence of α_{1A} receptor message, as well as the localization of binding sites to the same regions might imply a postsynaptic somatodendritic location for the receptor in these areas. These regions are all areas of known adrenergic innervation. However, a presynaptic localization cannot be excluded as suggested by the localization of mRNA in the hippocampus.

636.16

LOCALIZATION OF VASOPRESSIN RECEPTORS, BUT NOT OF OF OXYTOCIN RECEPTORS, IN PUTENDAL MOTOR NUCLEI IN BOTH MALE AND FEMALE RATS. E. Tribollet[1], G. Kato* and Y. Arsenijevic[1]. [1]Dept. of Physiol., Univ. Med. Center, 1211 Geneva 4 and Neurotech., 1228 Plan-les-Ouates, Switzerland.

Central administration of oxytocin (OT) facilitates lordosis in female rat and penile erection in male rat. This suggests that endogeneous OT present in the spinal cord may participate to the regulation of sexual behavior in the rat, possibly by acting on motor nuclei innervating pelvic muscles. In the present work, we have labelled OT and vasopressin (AVP) receptors throughout the spinal cord of adult male and female rats by using selective radioiodinated antagonists and in vitro autoradiography. In both sexes, AVP binding sites were detected in the segment L6, localized in the four pelvic motor nuclei, i.e. the dorso-medial, ventral, dorso-lateral and retrodorso-lateral groups of motoneurons. The density of binding was high in the ventral, dorso-lateral and retrodorso-lateral groups in both males and females, while in the sexually dimorphic dorso-medial group, binding was dense in males, but just detectable in females. In addition to L6 motor nuclei, AVP receptor binding sites were only found in the most lateral motor nucleus in segment C8; motor nuclei in other spinal segments were not labelled with the radioiodinated AVP antagonist. With the radioiodinated OT antagonist, low amounts of binding was found in laminae I and II at all spinal levels, as previously described; specific binding was never detected in motor nuclei, neither in L6, nor in other segments. These observations indicate that AVP rather than OT may be involved in the control of motor sexual behavior in the rat, at least at the spinal cord level.

636.17

THE EXPRESSION OF KAPPA OPIOID RECEPTOR MESSENGER RNA IN THE HIPPOCAMPUS AND THE ENTORHINAL CORTEX OF ALZHEIMER'S DISEASE PATIENT. M. Yamada*, N. Nakachi, K. Groshan, Mi. Yamada and E. Richelson, Mayo Foundation and Mayo Clinic Jacksonville, Jacksonville, FL 32224

We recently cloned the kappa opioid receptor from a human brain cDNA library. A northern blot analysis showed the presence of a single transcript of about 6 kB in size for mRNA prepared from several human brain regions, which hybridized to the human kappa opioid receptor cDNA. These regions included the amygdala, caudate nucleus, corpus callosum, hippocampus, hypothalamus, substantia nigra, subthalamic nucleus, and thalamus. Using *in situ* hybridization histochemistry techniques, with a ^{33}P-labeled antisense RNA probe complementary to this receptor cDNA, we studied the expression of this receptor in the hippocampus and the entorhinal cortex of post-mortem human brain from control and from Alzheimer's disease subjects. Kappa opioid receptor mRNA was present in high levels in the stratum granulosum of the dentate gyrus, the stratum pyramidale of region CA1-CA4, the subiculum and the entorhinal cortex. On the other hand, Alzheimer's disease brain had lower levels of mRNA for the kappa opioid receptor in these regions. It is proposed that the kappa opioid receptor expressed in the hippocampus and the entorhinal cortex may play an important role in the normal function of the limbic system and possibly in the pathophysiology of Alzheimer's disease. (Supported by USPHS Grant MH27692 and Mayo Foundation).

636.18

DEVELOPMENTAL PROFILE OF THE NEUROPEPTIDE Y Y_1 RECEPTOR SUBTYPE IN THE RAT BRAIN: A COMBINED IN SITU HYBRIDIZATION AND RECEPTOR AUTORADIOGRAPHIC STUDY. Y. Tong[1,2]*, Y. Dumont[1], J.G. Chabot[1], S.H. Shen[2] and R. Quirion[1] [1]Douglas Hospital Research Center, Dept. Psychiatry, McGill University, 6875 LaSalle Blvd. Montréal, Québec, Canada, H4H 1R3. [2]Biotechnology Research Institute NRCC, 6100 Royalmount Ave., Montréal, Québec, Canada H4P 2R2

The developmental profile of the neuropeptide Y (NPY) Y_1 receptor in the rat brain was assessed by in situ hybridization (ISH) and receptor autoradiography using a rat ^{35}S-cRNA probe (Eva et al., FEBS Lett. 271: 81-84, 1990) and a selective Y_1 radioligand ([^{125}I][Leu^{31},Pro^{34}]PYY; Dumont et al., J. Pharmacol. Expt. Ther. 272: 673-680, 1995), respectively. As early as by gestational day 12 (E12), low amounts of specific Y_1 receptor mRNA are detectable in few neuronal cells while Y_1/[^{125}I][Leu^{31},Pro^{34}]PYY binding sites failed to be detected at this early developmental stage. Specific Y1 mRNA ISH signals increased rapidly during the last week of gestation. At E15, a strong ISH signal is observed in the spinal cord and the striatum. By E18, the Y_1 receptor mRNA is widely distributed in the fetal brain including in the olfactory tubercle, neocortex, nucleus accumbens, thalamus, brainstem and spinal cord. Specific Y_1 mRNA ISH signals remained elevated early neonatally and up to post-natal day 12 to decline thereafter to reach adult levels by post-natal day 30. Overall, specific Y_1/[^{125}I][Leu^{31},Pro^{34}]PYY binding sites are distributed in a manner similar to that of the Y_1 receptor mRNA except that significant labelling was generally seen few days (2-3) after the detection of the specific mRNA suggesting the occurrence of a lag phase to allow for the accumulation of the translated receptor protein. Moreover, the very early embryonic expression of the NPY Y_1 receptor mRNA and its translated protein suggest that the Y_1 receptor subtype likely plays a regulatory role in normal brain development. Supported by the MRCC

BEHAVIORAL PHARMACOLOGY II

637.1

THE SEPTOHIPPOCAMPAL SYSTEM IS INVOLVED IN PREPULSE INHIBITION OF THE ACOUSTIC STARTLE RESPONSE IN RATS. M. Koch (SPON: European Neuroscience Association). Tierphysiologie, Universität Tübingen, Auf der Morgenstelle 28, D-72076 Tübingen, Germany.

The magnitude of the acoustic startle response (ASR) is reduced if a weak prepulse is presented 30 - 500 ms prior to the startle stimulus. This phenomenon is termed prepulse inhibition (PPI), and can be used as an operational measure for brain mechanisms that protect early stimulus processing routines from disruption by other stimuli. Deficient PPI has been observed in schizophrenic patients, a fact that has kindled much interest in understanding the neural basis of PPI. The present study investigated the role of the septohippocampal system in PPI. Lesions of the medial septal nucleus, made by local infusion of the glutamate receptor agonist AMPA (0.3 µl of a 0.015 M solution), did not affect PPI. Chemical stimulation of the medial septal nucleus by micro-injections of the glutamate receptor agonist kainate (0.8 - 1.2 nmol) led to a profound disturbance of PPI and also reduced the ASR amplitude in the absence of prepulses. The PPI deficit induced by intraseptal kainate was attenuated by systemic or intrahippocampal administration of the acetylcholine antagonist scopolamine. The present data suggest that stimulation of the septohippocampal system reduces PPI of the ASR, and that this deficit of response suppression is based upon activation of cholinergic receptors in the hippocampus.

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637.2

LOCOMOTION INDUCED BY SCOPOLAMINE: ROLE OF LATERODORSAL TEGMENTAL NUCLEUS (Ch6) CELLS. **S.LaViolette and J.S.Yeomans*. Dept. of Psychology, Univ. of Toronto, Toronto, Canada M5S 1A1.**

Cholinergic cells of the pedunculopontine (Ch5) and laterodorsal (Ch6) tegmental nuclei monosynaptically activate dopamine cells of the ventral tegmental area and substantia nigra. Microinjections of the antimuscarinic scopolamine (10-200 ug) in either cell group induces locomotion that resembles the locomotion induced by i.p. scopolamine (1-10 mg/kg). Either scopolamine-induced or spontaneous locomotion is blocked by carbachol (1-4 ug) in the dorsal tegmentum. In this study, ibotenate lesions of Ch6 cells attenuated locomotion induced by scopolamine (3 mg/kg) 7 days after the lesion as compared with pre-lesion scopolamine, without altering locomotion induced by the saline control. The difference between saline and scopolamine conditions was reduced by 70%. These lesions had no effect on scopolamine-induced stereotypy, however. When tested 28 days after the lesions, however, scopolamine-induced locomotion completely recovered. This recovery is consistent with the recovery of ChAT-labelled terminals in the ventral tegmental area and substantia nigra after ibotenate lesions of the Ch5 cell group. Therefore, Ch6 cholinergic cells appear to be important for scopolamine-induced locomotion, but other systems contribute and can take over following ibotenate lesions.

637.3

GLUTAMATERGIC-CHOLINERGIC INTERACTION ON MEMORY CONSOLIDATION IN MICE. C. Castellano°, A. Mele^, V. Cestari^* and A. Oliverio^. ^Università degli Studi di Roma "La Sapienza", Dipartimento di Genetica e Biologia Molecolare, P.le A. Moro, 5 - 00185 Roma, Italy. °Istituto di Psicobiologia e Psicofarmacologia del C.N.R., Via Reno,1 - 00198 Roma, Italy.

Post-training administration of the muscarinic agonist oxotremorine (0.02, 0.04, 0.06 mg/kg) dose-dependently improves retention of an inhibitory avoidance response in NMRI mice. In contrast, post-training administration of the muscarinic antagonist atropine (2, 3, 4 mg/kg) dose-dependently impairs retention in the same test. Moreover, the N-methyl-D-aspartate (NMDA) non-competitive antagonist MK-801 (0.1, 0.25, 0.5 mg/kg) dose-dependently impairs the performance in the retention test. In addition, oxotremorine (at per se ineffective dose of 0.02 mg/kg) antagonizes the impairing effects of MK 801 (0.25, 0.5 mg/kg) in the retention test, while atropine (at per se ineffective dose of 2 mg/kg) enhances the effects of two doses (0.1, 0.25 mg/kg) of MK 801.

The present results confirm previous data showing that cholinergic enhancing drugs improve learning and memory in a number of tasks, while memory impairments can be induced by drugs blocking cholinergic neurotransmission. Moreover, our results confirm previous ones showing the impairing effects of MK 801 in the same experimental conditions. Lastly, our results show a clear interaction between the cholinergic and glutamatergic systems in the modulation of memory consolidation, as it can be seen by the effects of the pretreatment with oxotremorine (antagonism) or atropine (enhancement) on the impairing effects of the treatment with MK 801.

637.4

ROLE OF $GABA_A$ RECEPTORS IN MEDIAL AMYGDALOID SUPPRESSION OF PREDATORY ATTACK BEHAVIOR FROM THE LATERAL HYPOTHALAMUS IN THE CAT. Y. C Han*, M. B. Shaikh, K.L. Schubert and A. Siegel. Lab. of Limbic System & Behavior, Dept. of Neurosciences, N.J. Medical School, and Graduate School of Biomedical Sciences, UMDNJ, Newark, N.J. 07103.

The medial amygdala (ME) suppresses quiet biting "predatory" attack behavior (QBA) via a disynaptic pathway involving an initial projection to the medial hypothalamus (MH) from ME and a second limb linking MH and the lateral hypothalamus (LH). This study tested the hypothesis that a GABAergic pathway from MH to LH provided the substrate for ME-induced suppression of QBA. Monopolar and cannula electrodes were implanted into ME and LH, respectively, for stimulation or drug infusion. Microinfusion of the $GABA_A$ antagonist, bicuculline (.015, .075, .15 nmole/.25µl) into LH significantly reduced ME-induced suppression of QBA ($p<.01$). Microinfusion of the GABA agonist, muscimol (10, 25,50 pmole/.25µl) into LH resulted in a significant increase in response latencies for QBA in a time- and dose- dependent manner. The results suggest the presence of an inhibitory GABAergic pathway between MH and LH that is activated following stimulation of ME, thus providing the basis for ME-induced suppression of QBA.

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637.5

GAMMA-HYDROXY-BUTYRATE PREVENTS THE STRESS-INDUCED ESCAPE DEFICIT IN RATS

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Gamma-hydroxy-butyrate (γ-OH-B) is a general anaesthetic used as a substitute of ethanol in the treatment of alcoholics. Given acutely to rats at doses of 50-100 mg/kg i.p., γ-OH-B increases the firing of dopaminergic neurons in the VTA, while decreasing it at anaesthetic doses (750 mg/kg). At high doses it also induces dopamine accumulation in the caudate-putamen and the limbic areas. Moreover, in animals made dependent on ethanol γ-OH-B completely substitutes for it. Its possible antidepressant effect was tested on a model of escape deficit, induced by exposing restrained rats to 80 electric shocks (1mA x 5 s, every 30 s) delivered through an electrode placed on the distal third of the tail (pre-test). Twenty-four hr after the pre-test, control animals, if exposed to escapable shocks, show an average of 2 ± 1 escapes out of 30 trials (escape deficit: ED); whereas, rats never exposed to previous shocks (naive) have 27 ± 2 successes. γ-OH-B injected acutely (50-100 mg/kg) 30 min before the pre-test did not prevent ED development. However, administered daily (75 mg/kg i.p. twice a day) during the 12 days preceding the pre-test, it completely prevented the occurrence of ED (20 ± 2 escapes). Moreover, the long-term γ-OH-B effectwas significantly antagonized by SCH 23390 (0.03 mg/kg s.c.), a selective D_1 dopamine receptor antagonist, given 20 min before the pre-test (6 ± 2 escapes), while pindolol (5 mg/kg i.p.), a rather specific 5-HT_{1A} receptor inhibitor, failed to modify it. It was concluded that γ-OH-B has a potential antidepressant activity, with an efficacy and a mechanism of action similar to those of imipramine.

637.6

AN ANALYSIS OF THREE SEDATIVE HYPNOTIC DRUGS IN RAT BEHAVIORAL MODELS OF SEDATION, SLEEP, ETOH INTERACTION, AND LEARNING AND MEMORY. T. Efferen, M. Grace, L. Rajachandran, A. Johnson, A. Enders, and J. V. Cassella*. Neurogen Corporation, Branford, CT 06405.

The ability to predict side effects as well as efficacy from animal models is an important part of drug discovery. For benzodiazepine-type sedative/hypnotic agents, interaction with ethanol and memory impairments are sometimes noted as side effects. Spontaneous Locomotor Activity, a rat model sensitive to sedative drug effects, was significantly reduced by temazepam (0.25 mg/kg, IV), lorazepam (0.03 mg/kg, IV), and zolpidem (0.25 mg/kg, IV). The latency to assume a sleep posture was significantly reduced by temazepam (2.5 mg/kg, PO), lorazepam (0.94 mg/kg, PO), and zolpidem (24 mg/kg, PO). All three drugs significantly potentiated the ETOH-induced loss of righting reflex (LRR): temazepam = 2.5mg/kg, PO; lorazepam = 0.16 mg/kg, PO; zolpidem = 16 mg/kg, PO). These compounds also produced significant retention deficits in the step-down passive avoidance (temazepam : 1.0 mg/kg, IV, lorazepam : 0.06 mg/kg, IV; zolpidem : 0.5 mg/kg, IV) as well as in the spatial water maze (temazepam: 5 mg/kg, PO; lorazepam 0.6 mg/kg, PO; zolpidem: 16 mg/kg, PO) models of learning and memory. (Note: all doses above represent the MED). These animal models may be useful in predicting amnestic and ETOH interaction effects, as well as efficacy, of potential sedative/hypnotic compounds. For between drug comparisons, various Therapeutic Indexes can be calculated.

637.7

Interactions between Neuropeptide Y and the discriminative stimulus and antinociceptive effects of morphine.

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Previous research has demonstrated that several effects of Neuropeptide Y (NPY) are naloxone reversible. The present study sought to further characterize interactions between NPY and the opioid system. Male Long-Evans hooded rats (n=8) were trained to discriminate 5.6 mg/kg morphine from saline using a standard two-lever, food-reinforced, drug discrimination procedure. Following training, cannulae were implanted in the right lateral ventricle of each animal. Across a range of doses (3, 5, & 10 µg), NPY failed to substitute for or antagonize the stimulus effects of the training dose of morphine. In addition, NPY (10 µg) failed to significantly alter the morphine dose-effect curve. The antinociceptive efficacy of NPY alone and in combination with morphine was examined using a warm water tail-withdrawal procedure. NPY (10 µg) failed to alter tail-withdrawal latencies when examined in 52° and 56° C water, whereas morphine produced a dose-related increase in latencies. This same dose of NPY also failed to alter morphine's antinociceptive efficacy. These results do not support a role for intraventricular administration of NPY in the discriminative stimulus and antinociceptive effects of morphine. (Supported by DA07327 and VA Merit Award).

637.8

EVIDENCE FOR INVOLVEMENT OF NMDA RECEPTORS IN MEDIAL HYPOTHALAMIC FACILITATION OF DEFENSIVE RAGE BEHAVIOR. K.L. Schubert*, M.B. Shaikh, Y. C. Han, K. Goldstein and A. Siegel. Lab. of Limbic System & Behavior, Dept. of Neurosciences, N.J. Medical School and Graduate School of Biomedical Sciences, UMDNJ, Newark, N.J. 07103.

Projections from the medial hypothalamus (MH) to the periaqueductal gray (PAG) serve as a substrate for defensive rage behavior (DR) in the cat. We have proposed that this pathway utilizes excitatory amino acids as a neurotransmitter that act upon NMDA receptors within the PAG.This study demonstrated that glutamate positive cells, present in MH, project to the PAG and that NMDA receptors are also present in the PAG. A retrograde axonal tracer, Fluoro-Gold, (4-8%/0.5 ul) was microinjected into DR sites within the PAG and the tissue was processed for labelling of glutamate positive cells. Double labeled cells for both glutamate and Fluoro-Gold were identified in the MH, indicating the presence of a glutamatergic pathway to the PAG from MH. Immunohistochemical and autoradiographic analyses of the tissue revealed the presence of $NMDA_{R1}$ receptors on dorsal PAG neurons.These data support the hypothesis that MH-induced DR is subserved by a descending pathway to the PAG whose functions are mediated by NMDA receptors.

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